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CONTENTS

1 Editorial

2 Disease
Diagnosis

9 Interpretation

10 Troubleshooting

11 Bouquet

12 Tulip News

Editorial

Influenza, commonly known as "the flu", is an infectious disease caused by influenza viruses. Symptoms range from mild to severe and often include fever, runny nose, sore throat, muscle pain, headache, coughing, and fatigue. These symptoms begin from one to four days after exposure to the virus (typically two days) and last for about 2–8 days. Diarrhea and vomiting can occur, particularly in children. Influenza may progress to pneumonia, which can be caused by the virus or by a subsequent bacterial infection. Other complications of infection include acute respiratory distress syndrome, meningitis, encephalitis, and worsening of pre-existing health problems such as asthma and cardiovascular disease.

There are four types of influenza virus, termed influenza viruses A, B, C, and D. Aquatic birds are the primary source of *Influenza A virus* (IAV), which is also widespread in various mammals, including humans and pigs. *Influenza B virus* (IBV) and *Influenza C virus* (ICV) primarily infect humans, and *Influenza D virus* (IDV) is found in cattle and pigs. IAV and IBV circulate in humans and cause seasonal epidemics, and ICV causes a mild infection, primarily in children. IDV can infect humans but is not known to cause illness. In humans, influenza viruses are primarily transmitted through respiratory droplets produced from coughing and sneezing. Transmission through aerosols and intermediate objects and surfaces contaminated by the virus also occur.

Frequent hand washing and covering one's mouth and nose when coughing and sneezing reduce transmission. Annual vaccination can help to provide protection against influenza. Influenza viruses, particularly IAV, evolve quickly, so flu vaccines are updated regularly to match which influenza strains are in circulation. Vaccines currently in use provide protection against IAV subtypes H1N1 and H3N2 and one or two IBV subtypes. Influenza infection is diagnosed with laboratory methods such as antibody or antigen tests and a polymerase chain reaction (PCR) to identify viral nucleic acid. The disease can be treated with supportive measures and, in severe cases, with antiviral drugs such as oseltamivir. In healthy individuals, influenza is typically self-limiting and rarely fatal, but it can be deadly in high-risk groups.

In a typical year, 5–15% of the population contracts influenza. There are 3–5 million severe cases annually, with up to 650,000 respiratory-related deaths globally each year. Deaths most commonly occur in high-risk groups, including young children, the elderly, and people with chronic health conditions. **INTERPRETATION** talks about various diagnostic platforms available to us under IMMUNOLOGIC TESTS.

TROUBLE SHOOTING highlights COVID 19 related issues in point of care devices. **BOUQUET** as usual does find a place.



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DISEASE DIAGNOSIS

INFLUENZA

Background

Influenza, one of the most common infectious diseases, is a highly contagious airborne disease that occurs in seasonal epidemics and manifests as an acute febrile illness with variable degrees of systemic symptoms, ranging from mild fatigue to respiratory failure and death. Influenza causes significant loss of workdays, human suffering, and mortality. **Although the seasonal strains of influenza virus that circulate** in the annual influenza cycle constitute a substantial public health concern, far more lethal influenza strains than these have emerged periodically. These deadly strains produced three global pandemics in the last century, the worst of which occurred in 1918. Called the Spanish flu (though cases appeared earlier in the United States and elsewhere in Europe), this pandemic killed an estimated 20 to 50 million persons, with 549,000 deaths in the United States alone. **Influenza also infects a variety of animal species.** Some of these influenza strains are species-specific, but new strains may spread from other animals to humans (see Pathophysiology). The term avian influenza, in this context, refers to zoonotic human infection with an influenza strain that primarily affects birds. Swine influenza refers to infections from strains derived from pigs. The 2009 influenza pandemic was a recombinant influenza involving a mix of swine, avian, and human gene segments (see H1N1 Influenza [Swine Flu]). **The signs and symptoms of influenza overlap with those of many other viral upper respiratory tract infections (URIs).** A number of viruses, including human parainfluenza virus, adenoviruses, enteroviruses, and paramyxoviruses, may initially cause influenzalike illness. The early presentation of mild or moderate cases of flavivirus infections (eg, dengue) may initially mimic influenza. For example, some cases of West Nile fever acquired in New York in 1999 were clinically misdiagnosed as influenza. **When influenza viruses are circulating in the community,** clinicians can often diagnose influenza on the basis of clinical criteria alone (see Presentation). Rapid diagnostic tests for influenza that can provide results within 30 minutes can help confirm the diagnosis. **It should be kept in mind, however, that these rapid tests have limited sensitivities and predictive values;** false-negative results are common, especially when influenza activity is high, and false-positive results can also occur, especially when influenza activity is low. Nevertheless, influenza virus testing may be considered if the results will change the clinical care of the patient (especially if the patient is hospitalized or has a high-risk condition) or influence the care of other patients. **The gold standard for confirming influenza virus infection** is reverse transcription-polymerase chain reaction (RT-PCR) testing or viral culture of nasopharyngeal or throat secretions. However, culture may require 3 to 7 days, yielding results long after the patient has left the clinic, office, or emergency department, and well past the time when drug therapy could be efficacious. **Prevention of influenza is the most effective strategy.** Each year in the United States, a vaccine that contains antigens from the strains most likely to cause infection during the winter flu season is produced. The vaccine provides reasonable protection against immunized strains, becoming effective 10 to 14 days after administration. Antiviral agents are also available that can prevent some cases of influenza; when given after the development of influenza, they can reduce the duration and severity of illness.

Pathophysiology

Influenza viruses are enveloped, negative-sense, single-stranded RNA viruses of the family Orthomyxoviridae. The core nucleoproteins are used to distinguish the three types of influenza viruses: A, B, and C. Influenza A viruses cause most human and all avian influenza infections. The RNA core consists of eight gene segments surrounded by a coat of 10 (influenza A) or 11 (influenza B) proteins. Immunologically, the most significant surface proteins include hemagglutinin (H) and neuraminidase (N). **Hemagglutinin and neuraminidase are critical for virulence,** and they are major targets for the neutralizing antibodies of acquired immunity to influenza. Hemagglutinin binds to respiratory epithelial cells, allowing cellular infection. Neuraminidase cleaves the bond that holds newly replicated virions to the cell surface, permitting the infection to spread. **Major typing of influenza A occurs through identification of both H and N proteins.** Seventeen H and nine N types have been identified. All hemagglutinins and neuraminidases infect wild waterfowl, and the various combinations of H and N yield 144 potential subtypes of influenza. **The hemagglutinin and neuraminidase variants are used to identify influenza A virus subtypes.** For example, influenza A subtype H3N2 expresses hemagglutinin 3 and neuraminidase 2. The most common subtypes of human influenza virus identified to date contain only hemagglutinins 1, 2, and 3 and neuraminidases 1 and 2. H3N2 and H1N1 are the most common prevailing influenza A subtypes that infect humans. Each year, the trivalent vaccine used worldwide contains influenza A strains from H1N1 and H3N2, along with an influenza B strain. The quadrivalent vaccine contains an additional influenza B strain. **Because the viral RNA polymerase lacks error-checking mechanisms,** the year-to-year antigenic drift is sufficient to ensure that there is a significant susceptible host population each year. However, the segmented genome also has the potential to allow reassortment of genome segments from different strains of influenza in a coinfecting host. Influenza A is a genetically labile virus, with mutation rates as high as 300 times that of other microbes. Changes in its major functional and antigenic proteins occur by means of two well-described mechanisms: antigenic drift and antigenic shift. **Antigenic drift is the process by which inaccurate viral RNA polymerase frequently** produces point mutations in certain error-prone regions in the genes. These mutations are ongoing, and they are responsible for the ability of the virus to evade annually acquired immunity in humans. Drift can also alter the virulence of the strain. Drift occurs within a set subtype (eg, H2N2). For example, AH2N2 Singapore 225/99 may reappear with a slightly altered antigen coat as AH2N2 New Delhi 033/01. **Antigenic shift is less frequent than antigenic drift.** In a shift event, influenza genes between two strains are reassorted, presumably during coinfection of a single host. Segmentation of the viral genome, which consists of 10 genes on eight RNA molecules, facilitates genetic reassortment. Because pigs have been susceptible to both human and avian influenza strains, many experts believe that combined swine and duck farms in some parts of Asia may have facilitated antigenic shifts and the evolution of previous pandemic influenza strains. **The reassortment of an avian strain with a mammalian strain** may produce a chimeric virus that is transmissible between mammals; such mutation products may contain H or N proteins that are unrecognizable to the immune systems of mammals. This antigenic shift results in a much greater population of susceptible individuals in whom more severe disease is possible. **Such an antigenic**

shift can result in a virulent strain of influenza that possesses the triad of infectivity, lethality, and transmissibility and can cause a pandemic. Three major influenza pandemics have been recorded:

- The Spanish influenza pandemic of 1918 (H1N1)
- The pandemic of 1957 (H2N2)
- The pandemic of 1968 (H3N2)

Smaller outbreaks occurred in 1947, 1976, 1977, and 2009.

Transmission and infection

Transmission of influenza from poultry or pigs to humans appears to occur predominantly as a result of direct contact with infected animals. The risk is especially high during slaughter and preparation for consumption; eating properly cooked meat poses no risk. Avian influenza can also be spread through exposure to water and surfaces contaminated by bird droppings. **Influenza viruses spread from human to human via aerosols created** when an infected individual coughs or sneezes. Infection occurs after an immunologically susceptible person inhales the aerosol. If not neutralized by secretory antibodies, the virus invades airway and respiratory tract cells. **Once the virus is within host cells, cellular dysfunction and degeneration occur**, along with viral replication and release of viral progeny. As in other viral infections, systemic symptoms result from release of inflammatory mediators. **The incubation period of influenza ranges from 1 to 4 days.** Aerosol transmission may occur 1 day before the onset of symptoms; thus, it may be possible for transmission to occur via asymptomatic persons or persons with subclinical disease, who may be unaware that they have been exposed to the disease.

Viral shedding

Viral shedding occurs at the onset of symptoms or just before the onset of illness (0-24 hours). Shedding continues for 5 to 10 days. Young children may shed virus longer, placing others at risk for contracting infection. In highly immunocompromised persons, shedding may persist for weeks to months.

H5N1 avian influenza

To date, avian influenza (H5) remains a zoonosis. The vast majority of cases of avian influenza have been acquired from direct contact with live poultry, with no sustained human-to-human transmission. Hemagglutinin type 5 attaches well to avian respiratory cells and thus spreads easily among avian species. However, attachment to human cells and resultant infection is more difficult. **Avian viruses tend to prefer sialic acid alpha(2-3) galactose**, which, in humans, is found in the terminal bronchi and alveoli. Conversely, human viruses prefer sialic acid alpha(2-6) galactose, which is found on epithelial cells in the upper respiratory tract. Although this results in a more severe respiratory infection, it probably explains why few, if any, definite human-to-human transmissions of avian influenza have been reported: infection of the upper airways is probably required for efficient spread via coughing and sneezing. **Most human deaths from bird flu have occurred in Indonesia.** Sporadic outbreaks among humans have continued elsewhere, including China, Egypt, Thailand, and Cambodia. **In theory, however, mutation of the hemagglutinin protein** through antigenic drift could result in a virus capable of binding to upper and lower respiratory epithelium, creating a strain that is easily transferred from human to human and thus could cause a worldwide pandemic.

Etiology

Influenza results from infection with one of three basic types of influenza virus: A, B, or C. Influenza A is generally more pathogenic than influenza B. Epidemics of influenza C have been reported, especially in young children. Influenza viruses are classified within the family Orthomyxoviridae. **In the United States, as of early January 2020,** influenza B/Victoria was the predominant strain during the 2019-2020 flu season, followed by A(H1N1)pdm09. Influenza A(H3N2) and B/Yamagata viruses were reported to be circulating at very low levels. **Avian influenza (ie, human infection with avian H5N1 influenza virus)** is transmitted primarily through direct contact with diseased or deceased birds infected with the virus. Contact with excrement from infected birds or contaminated surfaces or water are also considered mechanisms of infection. Close and prolonged contact of a caregiver with an infected person is believed to have resulted in at least one case. Other specific risk factors are not apparent, given the few cases to date.

Epidemiology

In tropical areas, influenza occurs throughout the year. In the Northern Hemisphere, the influenza season typically starts in early fall, peaks in mid-February, and ends in the late spring of the following year. The duration and severity of influenza epidemics vary, however, depending on the virus subtype involved.

The WHO estimates that, 1 billion influenza cases, 3 to 5 million severe cases, and 290,000 to 650,000 influenza-related respiratory deaths occur each year worldwide (refer image on page 4).

Prognosis

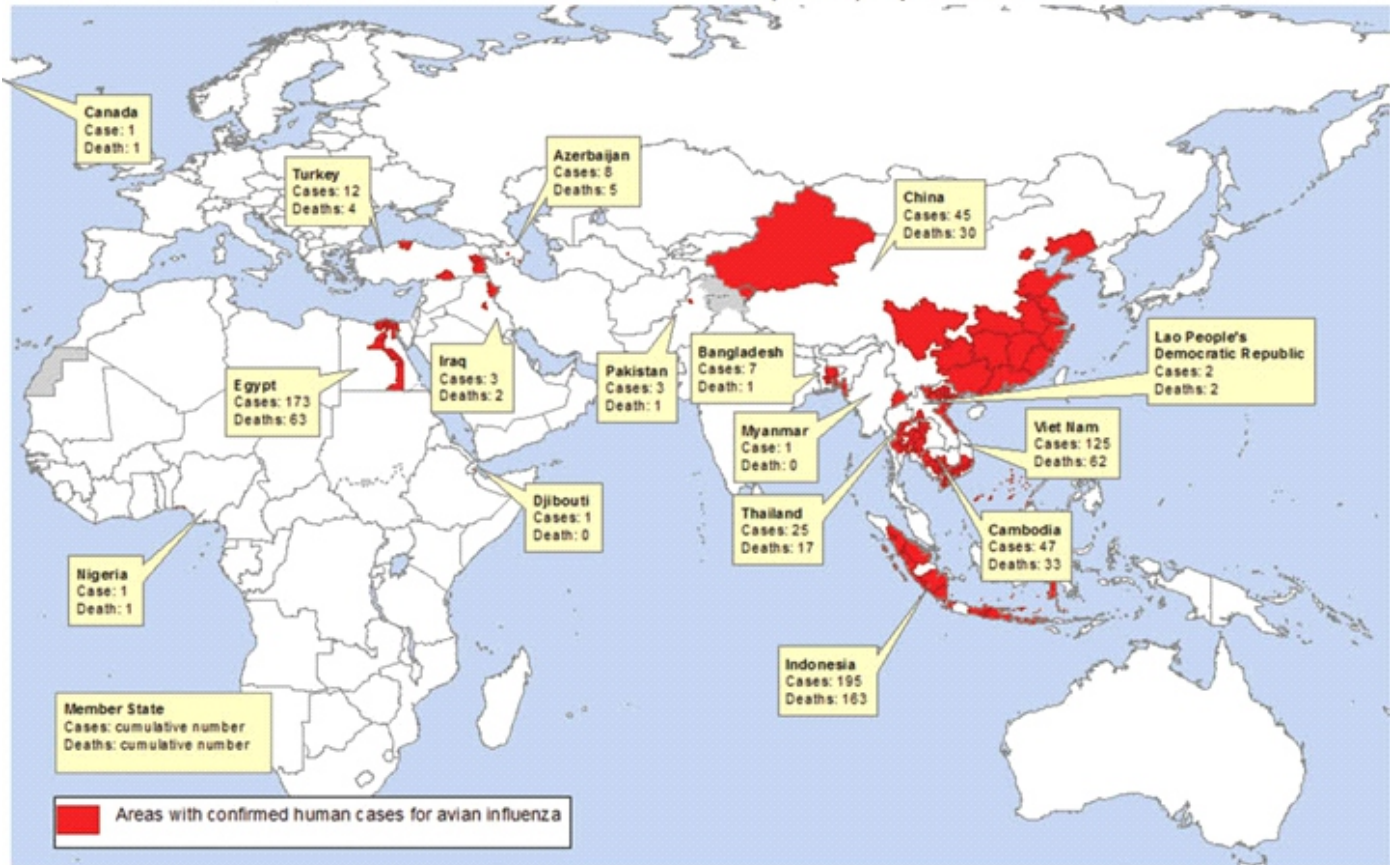
In patients without comorbid disease who contract seasonal influenza, the prognosis is very good. However, some patients have a prolonged recovery time and remain weak and fatigued for weeks. Mortality from seasonal influenza is highest in infants and the elderly. **The prognosis for patients with avian influenza is related to the degree and duration of hypoxemia.** Cases to date have exhibited a 60% mortality; however, Wang et al suggest that this may be an overestimate stemming from the underreporting of mild cases. **The risk for mortality from avian influenza depends on** the degree of respiratory disease rather than on the bacterial complications (pneumonia). Mortality is significantly lower among patients cared for in more-developed nations. Little evidence is available regarding the long-term effects of disease among survivors. **Diabetes increases the risk for severe flu-related illness.** In a cohort study of 166,715 individuals in Manitoba, Canada, Lau and colleagues found that adults with diabetes were at significantly greater risk for serious illness related to influenza compared with those without diabetes; this justifies guideline recommendations for influenza vaccination in this population. After controlling for age, sex, socioeconomic status, location of residence, comorbidities, and vaccination, adults with diabetes had a significant increase (6%) in all-cause hospitalizations associated with influenza ($P = .044$). Only 16% of the patients with diabetes in the cohort and 7% of the patients without diabetes had been vaccinated.

CLINICAL PRESENTATION

History

The presentation of influenza virus infection varies; however, it usually includes many of the symptoms described below. Patients with influenza

Areas with confirmed human cases for avian influenza A(H5N1) reported to WHO, 2003-2013*



*All dates refer to onset of illness
Data as of 24 January 2014
Source: WHO/GIP
Geographic coverage: World

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Countries where avian influenza has been reported. Image courtesy of the World Health Organization.

who have preexisting immunity or who have received vaccine may have milder symptoms. **Onset of illness can occur suddenly over the course of a day**, or it can progress more slowly over the course of several days. Typical signs and symptoms include the following (not necessarily in order of prevalence):

- Cough and other respiratory symptoms
- Fever
- Sore throat
- Myalgias
- Headache
- Nasal discharge
- Weakness and severe fatigue
- Tachycardia
- Red, watery eyes

Cough and other respiratory symptoms may be initially minimal but frequently progress as the infection evolves. Patients may report nonproductive cough, cough-related pleuritic chest pain, and dyspnea. In children, diarrhea may be a feature. **Fever may vary widely among patients, with some having low fevers** (in the 100°F range) and others developing fevers as high as 104°F. Some patients report feeling feverish and feeling chills. **Sore throat may be severe and may last 3 to 5**

days. The sore throat may be a significant reason why patients seek medical attention. **Myalgias are common and range from mild to severe**. Frontal or retro-orbital headache is common and is usually severe. Ocular symptoms develop in some patients with influenza and include photophobia, burning sensations, or pain upon motion. Some patients with influenza develop rhinitis of varying severity, but it is generally not the chief symptom. **Weakness and severe fatigue may prevent patients** from performing their normal activities or work. Patients report needing additional sleep. In some cases, patients with influenza may be bedridden. **The incubation period of influenza averages 2 days but may range from 1 to 4 days in length**. Because of aerosol transmission, and the possibility (albeit less likely) of transmission by asymptomatic persons and contaminated surfaces, the patient may be unaware of exposure to the disease.

2009-2010 H1N1 influenza pandemic

In the 2009-2010 H1N1 influenza pandemic, initial symptoms included the following:

- High fever
- Myalgias
- Rhinorrhea
- Sore throat

- Nausea and vomiting
- Diarrhea

Physical Examination

The general appearance varies among patients who present with influenza. Some patients appear acutely ill, with some weakness and respiratory findings, whereas others appear only mildly ill. Upon examination, patients may have some or all the following findings:

- Fever of 100-104°F; fever is generally lower in elderly patients than in young adults
- Tachycardia, which most likely results from hypoxia, fever, or both
- Pharyngitis - Even in patients who report a severely sore throat, findings may range from minimal infection to more severe inflammation
- Eyes may be red and watery
- Skin may be warm to hot, depending on core temperature status; patients who have been febrile with poor fluid intake may show signs of mild volume depletion with dry skin
- Pulmonary findings may include dry cough with clear lungs or rhonchi, as well as focal wheezing
- Nasal discharge is absent in most patients
- Fatigued appearance

Acute encephalopathy has been associated with influenza A virus infection.

Complications

Primary influenza pneumonia is characterized by progressive cough, dyspnea, and cyanosis after the initial presentation. Chest radiographs show bilateral diffuse infiltrative patterns, without consolidation, which can progress to a presentation similar to acute respiratory distress syndrome (ARDS). [Risks for viral pneumonia involve complex host immune responses and viral virulence.](#) Persons in the third trimester of pregnancy are at higher risk, as they are for other complications of influenza A and B. The elderly, especially nursing home patients, and those with cardiovascular disease, usually are the groups at highest risk; however, particular influenza strains may target younger persons. For example, in the 1918-1919 epidemic, many young adults died of a pneumonia that some experts believe was caused directly by the virus. [Secondary bacterial pneumonia can occur from numerous pathogens](#) (eg, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*). The most dreaded complication is staphylococcal pneumonia, which develops 2 to 3 days after the initial presentation of viral pneumonia. Patients appear severely ill, with productive bloody cough, hypoxemia, an elevated white blood cell (WBC) count, and multiple cavitory infiltrates on chest radiography. [A study from Israel found an increase in *S pneumoniae* bacteremia during regular influenza seasons;](#) in addition, during the 2009-2010 H1N1 influenza pandemic, there were higher rates of *S pneumoniae* bacteremia among children (but not among adults) and higher rates of *S aureus* and *Streptococcus pyogenes* infections in all age groups. [Methicillin-susceptible *S aureus* \(MSSA\) and methicillin-resistant *S aureus* \(MRSA\) pneumonias](#) have occurred after influenza pneumonia. MRSA pneumonia may be severe and difficult to treat, and deaths have occurred within 24 hours of presentation of pneumonia symptoms. [S pneumoniae or H influenzae pneumonia](#), if occurring as a complication,

usually develops 2 to 3 weeks after the initial symptoms of influenza. These cases can be managed as a community-acquired pneumonia, in accordance with standard antibiotic and admission-discharge guidelines. [Pulmonary Aspergillosis has also been increasingly recognized as a complication of influenza in critically ill adults](#), occurring relatively early in the course of severe influenza infection. Risk factors leading to disease include damage to the respiratory epithelium, immunomodulatory agents, and use of neuraminidase inhibitors. [Myositis is a rare complication.](#) This group of patients may develop frank rhabdomyolysis, with elevated creatine kinase levels and myoglobinuria. Myocarditis and pericarditis have been associated with influenza infection. [A review of avian influenza cases in four countries](#) found that the clinical course progressed to ARDS and respiratory failure in 70-100% of patients. The mean time to the development of ARDS was 6 days. Lymphopenia at presentation is a significant predictor of the progression to ARDS and death. [Severe cases of avian influenza may progress to multiorgan failure.](#) In a study of 12 hospitalized patients with confirmed H5N1 influenza, 75% had respiratory failure, 42% had cardiac failure, and 33% had renal failure.

DIFFERENTIAL DIAGNOSIS

Diagnostic Considerations

Accurately diagnosing influenza A or B infection solely on the basis of clinical criteria is difficult because of the overlapping symptoms caused by the various viruses associated with upper respiratory tract infection (URI). Viruses that may initially cause influenzalike symptoms include adenoviruses, enteroviruses, and paramyxoviruses. [The early presentation of mild or moderate cases of flavivirus infections](#) (eg, dengue) may initially mimic influenza. For example, some cases of West Nile fever acquired in New York in 1999 were clinically misdiagnosed as influenza. [Like influenza, URIs from these viruses are more common in the winter.](#) As a result, during the winter, clinics and emergency department waiting rooms fill with patients who have influenza or other URIs. [Influenza pneumonia must be differentiated from other forms of viral pneumonia](#), bacterial pneumonia, and noninfectious causes of respiratory distress, such as heart failure, chronic obstructive pulmonary disease, pulmonary edema, and aspiration pneumonitis.

H5N1 avian influenza

Risk factors or features that should raise the index of suspicion of H5N1 avian influenza include the following :

- Recent (within the preceding 2 weeks) travel to or location in a country with known avian influenza cases in animals or humans
- Unusual comorbidities such as encephalopathy or diarrhea
- History of exposure to birds, especially living in close proximity to birds, contact with sick or dying birds, or consumption of incompletely cooked bird meat
- History of exposure to individuals with known avian influenza, especially family, or to sick people in a country with known human cases of avian influenza

The situation can be complicated during outbreaks of severe respiratory disease other than avian influenza. The first case of laboratory-confirmed avian influenza infection was documented during the severe acute respiratory syndrome (SARS) outbreak of 2002-2003 and was mistakenly misdiagnosed as SARS. [Cases of avian influenza in which respiratory disease was limited or not apparent](#) (with even normal chest

radiography findings) have been described, though they account for only a small percentage of cases overall. The primary presenting illness has been encephalitis or diarrhea.

Differential Diagnoses

- Acute Respiratory Distress Syndrome (ARDS)
- Adenovirus
- Arenaviruses
- Cytomegalovirus (CMV)
- Dengue
- Echovirus Infection
- Hantavirus Pulmonary Syndrome
- HIV Infection and AIDS
- Legionnaires Disease
- Human Parainfluenza Viruses (HPIV) and Other Parainfluenza Viruses

WORKUP

Approach Considerations

The gold standard for confirming influenza virus infection is reverse transcription-polymerase chain reaction (RT-PCR) or viral culture of nasopharyngeal or throat secretions. Rapid diagnostic tests for influenza are available and are becoming more widely used. These tests have high specificity but only moderate sensitivity. [Findings of standard laboratory studies, such as a complete blood count \(CBC\) and electrolyte levels](#), are nonspecific but helpful in the workup of influenza. Leukopenia and relative lymphopenia are typical findings. Thrombocytopenia may be present. In severe cases of influenza, the patient is likely to have hypoxemia, and the alveolar-arterial (A-a) gradient may be increased (>35 mm Hg). Patients with physical examination findings compatible with meningitis should undergo lumbar puncture.

Rapid Diagnostic Tests

The FDA waived federal Clinical Laboratories Improvement Act (CLIA) requirements and cleared for marketing seven rapid influenza diagnostic tests that directly detect influenza A or B virus-associated antigens or enzyme in throat swabs, nasal swabs, or nasal washes. These tests can produce results within 30 minutes. [A meta-analysis examining the accuracy of rapid influenza diagnostic tests](#) found a pooled sensitivity of 62% and specificity of 98%. The tests tended to be more sensitive in children (67%) than in adults (54%) and better at detecting influenza A (65%) than influenza B (52%). [The accuracy of these tests depends in part on the collection technique](#) and skill of the person performing the test. Nasal swabs must be deeply inserted and then swirled to attach the influenza virus.

The following three rapid diagnostic tests are considered of low complexity and may be used in physicians' offices:

- QuickVue Influenza A+B test (Quidel)
- ZstatFlu (ZymeTx)
- QuickVue Influenza test (Quidel)

The QuickVue tests provide results in 10 minutes or less; the ZstatFlu test provides results in 20 minutes. Genetic and antigenic changes in the virus can adversely affect diagnostic test performance; thus, these tests should be monitored annually. [In June 2014, the FDA also approved the](#)

[Alere i Influenza A & B Test](#), a new point-of-care influenza test that delivers highly accurate molecular results in less than 15 minutes. The test, which extracts and analyzes DNA and RNA strands to detect sequences associated with bacterial and viral infections, has a sensitivity of greater than 90% for both influenza A and B. Although other influenza detection tests that produce results in about 15 minutes are already on the market, those tests rely on antigen detection and their sensitivity ranges from 50% to 70%.

Viral Culture and Polymerase Chain Reaction Testing

RT-PCR testing or viral culture of nasopharyngeal or throat secretions is the criterion standard for confirming influenza virus infection. Culture may require 3 to 7 days, yielding results long after the patient has left the clinic, office, or emergency department and well past the time when drug therapy could be efficacious.

Viral culture

For viral culture, nasopharyngeal samples are obtained with Dacron swabs and sent in appropriate viral transport media (eg, multimicrobe [M4] transport media) to the laboratory to be cultured in several lines of cells. A laboratory diagnosis of influenza is established once specific cytopathic effect is observed or hemadsorption testing findings are positive. For example, staining the infected cultured cell lines with fluorescent antibody confirms the diagnosis.

Polymerase chain reaction tests

Most laboratories and hospitals now offer nucleic acid (PCR)-based studies. A nasal swab is submitted in special transport media to the laboratory, and results can be available within 24 hours. Sensitivity for influenza is greater than 90%. These tests may be offered as respiratory panels that provide information on the presence of other viruses, such as respiratory syncytial virus (RSV) and adenovirus. These can Internationally RT-PCR kits have been used with good results.

- Identify and distinguish between influenza A and B viruses
- Classify influenza A viruses by subtype
- Detect highly pathogenic avian influenza A (H5N1) virus infection in human respiratory tract specimens

Direct Immunofluorescent Tests and Serologic Testing

Some laboratories offer direct immunofluorescent tests on fresh specimens, but these tests are labor- and personnel-intensive and are less sensitive than culture methods. To overcome the expensive and time-consuming obstacle of culturing, several serologic tests have become available. Many of these are not actually bedside tests; generally, 30 to 60 minutes are required to perform the test's multiple steps. Test sensitivities generally range from 60-70%. [A study by Haran et al suggests that cytokine markers may help distinguish influenza from bacterial pneumonia or other viral respiratory infections](#). In this study, differences were observed between the bacterial pneumonia group, on one hand, and all other viral infections grouped together, on the other, with regard to interleukin (IL)-4, IL-5, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interferon gamma levels. However, IL-10 concentrations were uniquely elevated in patients with influenza (88.69 pg/mL) as compared with all other groups combined (39.19 pg/mL; P = .003).

Testing for Avian Influenza

The standard commercially available rapid influenza A tests do not

detect H5N1 avian influenza. A rapid test from nasopharyngeal swab specific to H5N1 influenza (Arbor Vita Corporation) was approved by the FDA in 2009. **A CBC may be more clinically useful in avian influenza than in seasonal influenza.** Leukopenia (WBC count 454-4900/ μ L), especially lymphopenia, is common and is observed in 50-80% of patients. In at least one study, lymphopenia at presentation (absolute lymphocyte count < 1500/ μ L) was a significant predictor of the progression to acute respiratory distress syndrome. More than half of patients will have mild-to-moderate thrombocytopenia. **In addition to thrombocytopenia,** some patients with severe disease will develop disseminated intravascular coagulation (DIC), as shown in coagulation studies. **Liver function tests (LFTs) may be useful in differentiating avian influenza** from other febrile tropical diseases. Aminotransferase levels are elevated in more than half of all patients with H5N1 infection. **A basic metabolic panel is generally required** in the care of all seriously or critically ill patients. Abnormalities in renal function may herald the progression to organ failure. **According to expert recommendations,** clinicians should attempt to specifically identify avian H5N1 influenza in patients with all of the following characteristics:

- Severe illness that necessitates hospitalization or is fatal
- Documented temperature of 38°C (100.4°F) or higher in the past 24 hours or a history of feverishness during that time
- Radiographically confirmed pneumonia, acute respiratory distress syndrome (ARDS), or other severe respiratory illness for which an alternative diagnosis has not been established
- At least one potential exposure within 7 days of symptom onset

Testing may be considered in discussion with public health authorities in patients who have only some of these characteristics; all testing should be discussed with local public health departments.

The experts define potential exposure as follows:

- History of travel to a country where highly pathogenic avian influenza (HPAI) H5N1 virus has been documented in poultry, wild birds, or humans, with potential exposure to the virus during the visit
- Close contact (approach within ~6 ft) with an ill person who was under investigation for possible HPAI H5N1 virus infection
- Working with live HPAI H5N1 virus in a laboratory

If avian influenza is suspected, cultures should not be ordered without guidance from a public health laboratory. Many laboratories are not equipped to deal with the isolation needed to safely contain avian influenza (biosafety category 3+ containment, which is higher than that used for HIV). If a sample is accidentally handled, the laboratory may have to be shut down for decontamination. **Samples from patients with suspected avian influenza** should be sent to a dedicated central reference laboratory. **The best specimens are material collected with oropharyngeal swabs,** material from bronchoalveolar washes, or tracheal aspirates. Specimens from nasopharyngeal swabs are acceptable, but they may contain a low quantity of the virus. The experts recommend obtaining multiple respiratory specimens from different sites on at least 2 consecutive days, as soon as possible after illness onset—ideally, within the first 7 days. **Pneumatic tubing is not recommended for transport;** hand transport using a leakproof specimen bag is preferred. The specimen should be clearly labeled as “suspected AI,” and the person who transports the specimen should use appropriate protective equipment. **The standard commercially available rapid influenza A tests** do not detect H5N1 avian influenza. **A CBC may be more clinically useful in avian influenza than in seasonal influenza.**

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- Close contact (approach within ~6 ft) with an ill person who was under investigation for possible HPAI H5N1 virus infection
- Working with live HPAI H5N1 virus in a laboratory

If avian influenza is suspected, cultures should not be ordered without guidance from a public health laboratory. Many laboratories are not equipped to deal with the isolation needed to safely contain avian influenza (biosafety category 3+ containment, which is higher than that used for HIV). If a sample is accidentally handled, the laboratory may have to be shut down for decontamination. **Samples from patients with suspected avian influenza** should be sent to a dedicated central reference laboratory. **The best specimens are material collected with oropharyngeal swabs,** material from bronchoalveolar washes, or tracheal aspirates. Specimens from nasopharyngeal swabs are acceptable, but they may contain a low quantity of the virus. The CDC recommends obtaining multiple respiratory specimens from different sites on at least 2 consecutive days, as soon as possible after illness onset—ideally, within the first 7 days. **Pneumatic tubing is not recommended for transport;** hand transport using a leakproof specimen bag is preferred. The specimen should be clearly labeled as “suspected AI,” and the person who transports the specimen should use appropriate protective equipment.

Testing for H7N9

The recommendations include the following :

- Clinicians should consider H7N9 testing by reverse-transcription polymerase chain reaction (RT-PCR) assay for patients who meet

both clinical criteria (new onset of acute respiratory infection that is severe enough to require hospitalization, and lack of identification of an alternative infectious etiology) and exposure criteria (travel within 10 days of symptom onset to areas with known human cases of H7N9 infection or to areas where H7N9 viruses are circulating in animals, or close contact with confirmed human cases of H7N9 infection)

- In cases in which human H7N9 infection is suspected on the basis of current screening recommendations, respiratory specimens should be collected using infection precautions for novel virulent influenza viruses; the swab or aspirate should be placed in viral transport medium, and the state or local health department should be contacted to arrange transport to the appropriate health department for testing (viral culture should not be performed in these cases)
- Rapid influenza diagnostic tests may not identify H7N9 in respiratory specimens, and a negative test result does not exclude H7N9 infection; in addition, a positive test result for influenza A is unable to distinguish between influenza A virus subtypes, so it cannot confirm avian influenza virus infection; respiratory specimens should be obtained and sent for RT-PCR assay at a state public health laboratory when rapid influenza diagnostic tests are positive for influenza in patients suspected of having novel influenza A virus infection

Chest Radiography

In elderly or high-risk patients with pulmonary symptoms, chest radiography is indicated to exclude pneumonia. Early radiographic findings include no or minimal bilateral symmetrical interstitial infiltrates. Later, bilateral symmetrical patch infiltrates become visible. Focal infiltrates indicate superimposed bacterial pneumonia. [With avian influenza, pulmonary infiltrates are seen in almost all patients.](#) The widely varied radiographic characteristics range from diffuse or patchy infiltrates to lobar multilobar consolidation. Effusions and lymphadenopathy are also observed, as well as cystic changes. [In avian influenza, the severity of radiologically apparent disease](#) is a good predictor of mortality, including findings consistent with ARDS, such as a diffuse, bilateral ground-glass appearance.

TREATMENT AND MANAGEMENT

Approach Considerations

Prevention is the most effective management strategy for influenza. International organisations recommend routine annual influenza vaccination for all persons aged 6 months or older, preferably before the onset of influenza activity in the community. The experts also recommend on the use of antiviral agents for prevention and treatment of influenza. [Public health measures are effective in limiting influenza transmission in closed environments.](#) Enhanced surveillance with daily temperature taking and prompt reporting with isolation through home medical leave and segregation of smaller subgroups decrease the

spread of influenza. In one study, symptomatic illness attributable to influenza decreased from 12% to about 4% with the use of these measures. [Patients with influenza generally benefit from bed rest.](#) Most patients with influenza recover in 3 days; however, malaise may persist for weeks. [Patients most often require hospitalization when influenza exacerbates underlying chronic diseases.](#) Some patients, especially elderly individuals, may be too weak to care for themselves alone at home. On occasion, the direct pathologic effects of influenza may necessitate hospitalization. Most commonly, this is influenza.

Prevention

Prevention of influenza is the most effective management strategy. Influenza A and B vaccine is administered each year before flu season. The CDC analyzes the vaccine subtypes each year and makes any necessary changes for the coming season on the basis of worldwide trends. [Traditionally, the vaccine was trivalent](#) (ie, designed to provide protection against three viral subtypes, generally an A-H1, an A-H3, and a B). The first quadrivalent vaccines, which provide coverage against an additional influenza B subtype, were approved in 2012 and were made available for the 2013-2014 flu season. For the 2021-2022 influenza season, all flu vaccines are expected to be quadrivalent. [The FDA has approved a vaccine for H5N1 influenza.](#) It is available only to government agencies and for stockpiles. The following are influenza vaccine recommendations by the Advisory Committee on Immunization Practices:

- In the Northern Hemisphere, all persons aged 6 months or older should receive influenza vaccine annually by the end of October, if possible. Influenza vaccination should not be delayed to procure a specific vaccine preparation if an appropriate one is already available.
- The approved age indication for the cell culture-based inactivated influenza vaccine, cclIV4 [Flucelvax Quadrivalent], has been lowered to children ≥ 2 years
- Those with a history of egg allergy who have experienced only hives after exposure to egg should receive influenza vaccine. A previous severe allergic reaction to any egg-based IIV, LAIV, or RIV of any valency is a precaution to administration of cclIV4. A previous severe allergic reaction to a previous dose of any egg-based IIV, cclIV, or LAIV of any valency is a precaution to administration of RIV4.
- Regardless of allergy history, all vaccines should be administered in settings in which personnel and equipment for rapid recognition and treatment of anaphylaxis are available.

Treatment

In the United States, the following prescription antiviral drugs have been approved for treatment and/or chemoprophylaxis of influenza and are active against recently circulating subtypes of influenza:

- Baloxavir marboxil
- Oseltamivir
- Peramivir
- Zanamivir

INTERPRETATION

IMMUNOLOGIC TESTS

Immunologic tests use one of the following:

- **Antigen** to detect antibodies to a pathogen in the patient's specimen
- **Antibody** to detect an antigen of the pathogen in the patient's specimen

Specimen handling varies, but if testing is to be delayed, the specimen should typically be refrigerated or frozen to prevent overgrowth of bacterial contaminants.

Agglutination tests

In agglutination tests (eg, latex agglutination, coaggregation), very small particles (latex beads, gelatin particles, bacteria) are coupled to a reagent antigen or antibody. The resulting particle complex is mixed with the specimen (eg, cerebrospinal fluid, serum); if the target antibody or antigen is present in the specimen, it cross-links the particles, producing measurable agglutination.

If results are positive, the body fluid is serially diluted and tested. Agglutination with more dilute solutions indicates higher concentrations of the target antigen or antibody. The titer is correctly reported as the reciprocal of the most dilute solution yielding agglutination; eg, 32 indicates that agglutination occurred in a solution diluted to 1/32 of the starting concentration.

Usually, agglutination tests are rapid but less sensitive than many other methods. They can also determine serotypes of some bacteria.

Complement fixation

Complement fixation measures complement-consuming (complement-fixing) antibody in serum or cerebrospinal fluid. The test is used for diagnosis of some viral and fungal infections, particularly coccidioidomycosis.

The specimen is incubated with known quantities of complement and the antigen that is the target of the antibody being measured. The degree of complement fixation indicates the relative quantity of the antibody in the specimen.

The test can measure IgM and IgG antibody titers or can be modified to detect certain antigens. It is accurate but has limited applications, is labor intensive, and requires numerous controls.

Enzyme immunoassays

Enzyme immunoassays use antibodies linked to enzymes to detect antigens and to detect and quantify antibodies. Examples are

- Enzyme immunoassay (EIA)
- Enzyme-linked immunosorbent assay (ELISA)

Because sensitivities of most enzyme immunoassays are high, they are usually used for screening. Titers can be determined by serially diluting the specimen as for agglutination tests.

Test sensitivities, although usually high, can vary, sometimes according to patient age, microbial serotype, specimen type, or stage of clinical disease.

Precipitation tests

Precipitation tests measure an antigen or antibody in body fluids by the degree of visible precipitation of antigen-antibody complexes within a gel (agarose) or in solution. There are many types of precipitation tests (e.g., Ouchterlony double diffusion, counterimmunoelectrophoresis), but their applications are limited.

Usually, a blood specimen is mixed with test antigen to detect patient antibodies, most often in suspected fungal infection or pyogenic meningitis. Because a positive result requires a large amount of antibody or antigen, sensitivity is low.

Western blot test

The Western blot test detects antimicrobial antibodies in the patient's sample (eg, serum, other body fluid) by their reaction with target antigens (e.g., viral components) that have been immobilized onto a membrane by blotting.

The Western blot typically has good sensitivity, although often less than that of screening tests such as ELISA, but generally is highly specific. Thus, it is usually used to confirm a positive result obtained with a screening test.

Technical modifications of the Western blot are

- The line immunoassay (LIA)
- The recombinant immunoblot assay (RIBA), which uses synthetic or recombinant-produced antigens
- Immunochromatographic assays, which can rapidly screen specimens for specific microbial antigens or patient antibodies

Of the three, the immunochromatographic assay is easiest to do and the most commonly used—eg, to detect Shiga toxin-producing microorganisms, *Cryptococcus neoformans* capsular antigen, and influenza virus.

TROUBLESHOOTING

Point-of-care immunodiagnostic tests for COVID-19

Scientific Brief

In response to the growing COVID-19 pandemic and shortages of laboratory-based molecular testing capacity and reagents, multiple diagnostic test manufacturers have developed and begun selling rapid and easy-to-use devices to facilitate testing outside of laboratory settings. These simple test kits are based either on detection of proteins from the COVID-19 virus in respiratory samples (e.g. sputum, throat swab) or detection, in blood or serum, of human antibodies generated in response to infection.

WHO applauds the efforts of test developers to innovate and respond to the needs of the population.

However, before these tests can be recommended, they must be validated in the appropriate populations and settings. Inadequate tests may miss patients with active infection or falsely categorize patients as having the disease when they do not, further hampering disease control efforts. **At present, based on current evidence, WHO recommends the use of these new point-of-care immunodiagnostic tests only in research settings. They should not be used in any other setting, including for clinical decision-making, until evidence supporting use for specific indications is available.**

WHO continues to evaluate available immunodiagnostic tests for COVID-19 and will update this scientific brief when necessary.

Rapid diagnostic tests based on antigen detection

One type of rapid diagnostic test (RDT) detects the presence of viral proteins (antigens) expressed by the COVID-19 virus in a sample from the respiratory tract of a person. If the target antigen is present in sufficient concentrations in the sample, it will bind to specific antibodies fixed to a paper strip enclosed in a plastic casing and generate a visually detectable signal, typically within 30 minutes. The antigen(s) detected are expressed only when the virus is actively replicating; therefore, such tests are best used to identify acute or early infection.

How well the tests work depends on several factors, including the time from onset of illness, the concentration of virus in the specimen, the quality of the specimen collected from a person and how it is processed, and the precise formulation of the reagents in the test kits. Based on experience with antigen-based RDTs for other respiratory diseases such as influenza, in which affected patients have comparable concentrations of influenza virus in respiratory samples as seen in COVID-19, the sensitivity of these tests might be expected to vary from 34% to 80%.

Based on this information, half or more of COVID-19 infected patients might be missed by such tests, depending on the group of patients tested. These assumptions urgently require further study to understand whether they are accurate. Additionally, false-positive results – that is, a test showing that a person is infected when they are not – could occur if

the antibodies on the test strip also recognize antigens of viruses other than COVID-19, such as from human coronaviruses that cause the common cold. If any of the antigen detection tests that are under development or commercialized demonstrate adequate performance, they could potentially be used as triage tests to rapidly identify patients who are very likely to have COVID-19, reducing or eliminating the need for expensive molecular confirmatory testing.

With the limited data now available, WHO does not currently recommend the use of antigen-detecting rapid diagnostic tests for patient care, although research into their performance and potential diagnostic utility is highly encouraged.

Rapid diagnostic tests based on host antibody detection

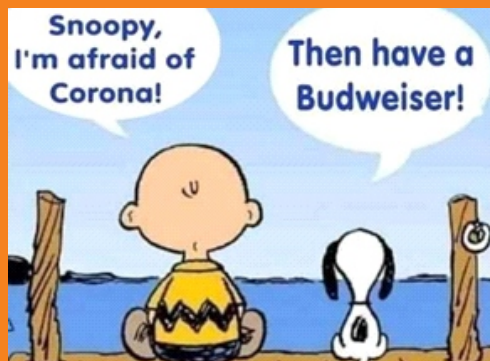
There is another, more common type of rapid diagnostic test marketed for COVID-19; a test that detects the presence of antibodies in the blood of people believed to have been infected with COVID-19. Antibodies are produced over days to weeks after infection with the virus. The strength of antibody response depends on several factors, including age, nutritional status, severity of disease, and certain medications or infections like HIV that suppress the immune system. In some people with COVID-19, disease confirmed by molecular testing (e.g. reverse transcription polymerase chain reaction: RT-PCR), weak, late or absent antibody responses have been reported. Studies suggest that the majority of patients develop antibody response only in the second week after onset of symptoms. This means that a diagnosis of COVID-19 infection based on antibody response will often only be possible in the recovery phase, when many of the opportunities for clinical intervention or interruption of disease transmission have already passed. Antibody detection tests targeting COVID-19 may also cross-react with other pathogens, including other human coronaviruses and give false-positive results. Lastly, there has been discussion about whether RDTs detecting antibodies could predict whether an individual was immune to reinfection with the COVID-19 virus. There is no evidence to date to support this.

Tests to detect antibody responses to COVID-19 in the population will be critical to support the development of vaccines, and to add to our understanding of the extent of infection among people who are not identified through active case finding and surveillance efforts, the attack rate in the population, and the infection fatality rate. For clinical diagnosis, however, such tests have limited utility because they cannot quickly diagnose acute infection to inform actions needed to determine the course of treatment. Some clinicians have used these tests for antibody responses to make a presumptive diagnosis of recent COVID-19 disease in cases where molecular testing was negative but where there was a strong epidemiological link to COVID-19 infection and paired blood samples (acute and convalescent) showing rising antibody levels.

Based on current data, WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and epidemiologic research.

BOUQUET

In Lighter Vein



A boy met a girl in Metro.
Girl: Every time you smile, I feel like inviting you to my place.
Boy: Awwww... Are you single?
Girl: No, I am a Dentist!

Son:

Dad, why doesn't law allow us to marry more than once.?



Father:

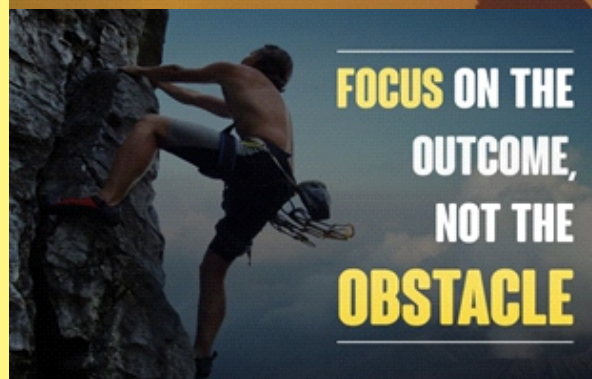
Son, when you grow old you will understand that they are protecting us..!



Wisdom Whispers



***PUSH YOURSELF,
 BECAUSE NO ONE ELSE
 IS GOING TO DO IT
 FOR YOU.***



**FOCUS ON THE
 OUTCOME,
 NOT THE
 OBSTACLE**

Brain Teasers

- How often does one need to take Influenza vaccine?
 - Once in a life time
 - Once in ten years
 - Once in a year before the influenza season starts
 - Once every quarter
- How many types of Influenza viruses are there?
 - 1
 - 2
 - 3
 - 4
- Which antiviral agent is available for influenza?
 - Oseltamivir
 - Penciclovir
 - Peramivir
 - Atripla
- What is the acronym used for diseases that resemble Influenza clinically?
 - ILD
 - ILF
 - ILI
 - ILS

ANSWER: 1:C, 2:D, 3:A, 4:C

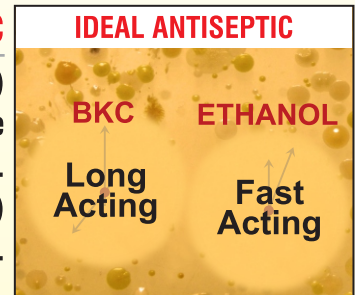
INJECTA™

Antiseptic for SAFE PHLEBOTOMY



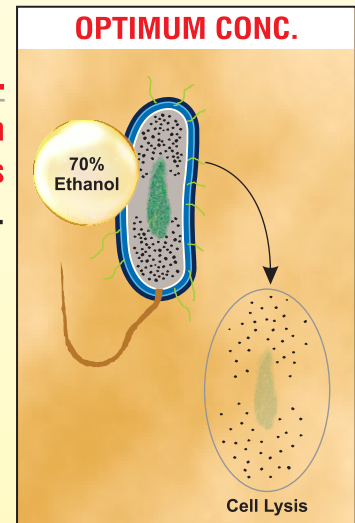
IDEAL ANTISEPTIC

- **BENZALKONIUM CHLORIDE (0.5% w/v)** long acting biocide with effective residual activity and cleansing action.
- **ETHANOL (70% v/v)** Fast acting antiseptic.



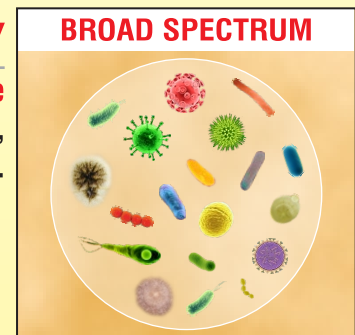
OPTIMUM CONCENTRATION OF ALCOHOL

- **ETHANOL (70% v/v)** Diffuses through the cell membranes for effective antiseptic activity.



BROAD SPECTRUM ACTIVITY

- Effective against wide spectrum of bacteria, viruses and fungi.



Effective Skin Preparation

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