SUPPLEMENT ARTICLE

Role of the Laboratory in Diagnosis of Influenza during Seasonal Epidemics and Potential Pandemics

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Laboratory diagnosis of influenza is critical to its treatment and surveillance. With the emergence of novel and highly pathogenic avian influenza viruses, the role of the laboratory has been further extended to include isolation and subtyping of the virus to monitor its appearance and facilitate appropriate vaccine development. Recent progress in enhancing testing for influenza promises to both improve the management of patients with influenza and decrease associated health care costs. The present review covers the technological characteristics and utilization features of currently available diagnostic tests, the factors that influence the selection of such tests, and the developments that are essential for pandemic preparedness.

Influenza virus belongs to the virus family *Orthomyxoviridae,* which includes the genera *Influenzavirus A, Influenzavirus B,* and *Influenzavirus C,* the former 2 of which cause most human infections. Influenza A viruses naturally infect humans, as well as such animals as birds, pigs, and horses, and they generally cause yearly epidemics and, potentially, pandemics. Infections with influenza B virus are generally restricted to humans and cause epidemics more rarely. Influenza A viruses, which are characterized by the antigenicity of their nucleocapsid and matrix proteins, are further classified into 16 H and 9 N subtypes according to their membrane glycoproteins (hemagglutinin [HA] and neuraminidase [NA]) and, finally, are identified as strains, such as the A/California/7/2004 strain, according to the time and place of their first isolation [1, 2]. The predominant influenza virus subtypes known to circulate among humans are H1N1, H2N2, and H3N2, but infection with novel subtypes has been documented as causing outbreaks associated with different clinical manifestations, including severe respiratory illness (H5N1), conjunctivitis (H7N7 and H7N3), and common influenza-like symptoms (H9N2) [3]. The potential for both common and novel subtypes to cause infection in humans underscores the importance of establishing a definitive laboratory diagnosis of influenza.

LABORATORY DIAGNOSIS OF INFLUENZA

Laboratory diagnosis of influenza has become a cornerstone of the prevention, containment, surveillance, and treatment of the associated illness. The emergence of novel, highly pathogenic avian influenza viruses, such as H5N1, has extended the role of the laboratory to include isolation and subtyping of the virus for disease surveillance and vaccine development. Because other respiratory viruses that cause similar nonspecific symptoms frequently cocirculate during influenza epidemics, establishing a diagnosis of influenza on the basis of the clinical presentation alone is problematic, with reported sensitivity ranging from 38% (for children) to 77% (for adults) [4, 5].

Laboratory testing for influenza has historically been of questionable value for the management of patients with influenza, because of limited test sensitivity, long turnaround times, and a lack of effective antiviral therapies. The development of more rapid and accurate tests for the detection of influenza now enables the laboratory to provide a prompt, definitive diagnosis, which allows clinicians to initiate antiviral therapy, limit

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the injudicious use of antibacterials, implement appropriate infection-control measures, decrease the duration of hospitalization, reduce ancillary testing, and decrease health care costs [6–8]. The present review describes the technological characteristics and utilization features of currently available diagnostic tests for influenza, with emphasis given to the use of such tests in the appropriate clinical context.

CURRENT DIAGNOSTIC TESTING OPTIONS

Laboratory diagnosis of influenza can be accomplished by the detection of (1) the virus or (2) the patient's immune response to the virus. Diagnostic approaches for the identification of the virus include viral isolation, detection of viral antigen by immunospecific assays, such as immunofluorescence microscopy, point-of-care (POC) testing (e.g., EIA or optical immunoassay), and detection of viral nucleic acid by use of amplification techniques (i.e., nucleic acid testing [NAT]). Antibody detection is usually accomplished by virus neutralization (virus NT) and hemagglutination inhibition (HI) tests conducted to monitor seroconversion to a specific virus strain or to determine immune status, for example, after vaccination [3, 9, 10].

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 error, both clerical and operational, and from suboptimal specificity of the test in question. Table 1 summarizes the advantages and disadvantages of individual tests.

Rapid Antigen (POC) Tests

Diagnosis of influenza by EIA has led to the development of easy-to-use, self-contained diagnostic kits that can provide results well within 1 h of the time of specimen collection. The World Health Organization has issued recommendations for the use of such kits [24]. Approval for some of these assays has been waived by the Clinical Laboratory Improvement Amendment, allowing for their use outside certified laboratories in POC settings. On-site diagnosis of influenza by POC tests has been shown to limit antimicrobial administration, requests for blood culture, and the use of chest radiography, and it ultimately has been shown to reduce patient costs [8]. Currently, most POC tests distinguish influenza A virus from influenza B virus, but their role in the identification of avian influenza virus subtypes is unclear, because most claims of detection of novel subtypes have yet to be confirmed in clinical studies. Table 2 describes the characteristics of representative POC tests.

The types of specimens used for POC testing, in decreasing order of sensitivity, are nasopharyngeal aspirates, nasopharyngeal swabs/washes, and throat swabs. The timing of specimen collection in relation to the onset of symptoms influences specimen sensitivity. Optimal sensitivity is achieved when specimens are collected within the first few days of illness, because viral shedding peaks within 48 h of the onset of symptoms, with children having the highest viral titers for the longest duration. In general, POC tests are contraindicated for patients who have had symptoms for >3 days.

Overall, POC tests vary greatly in their sensitivity and specificity. The reported ranges of sensitivity (57%–90%) and specificity (65%–99%) are influenced by the study population, the type of specimen, and the time of collection after presentation [39]. Detection of influenza after recent immunization with live attenuated viral vaccine, such as FluMist (MedImmune Vaccines), can confound a diagnosis [11]. Direct comparison of the sensitivity and specificity of multiple POC tests is challenging, because evaluations have been performed under variable conditions. Because the overall sensitivity of POC tests is lower than the overall sensitivities of immunofluorescence microscopy and isolation in cell culture, it is important that physicians who use POC tests have access to a reference laboratory to resolve ambiguous results and to ensure quality [5]. The clinical usefulness of these tests is associated with their positive and negative predictive values and is greatest during the peak influenza season, when false-positive results are less likely and the positive predictive value is high. Patients with a high pretest probability of infection and a negative POC result should undergo further laboratory testing. When influenza activity is low, false-positive results are likely, the positive predictive value is low, and the negative predictive value is high. Outside of the influenza season, POC tests must be used with caution, and their results must be confirmed by other tests [40, 41].

Immunofluorescence Microscopy

Detection of influenza virus by immunofluorescence microscopy, which is referred to as a "direct fluorescent antibody" (DFA) test or an "immunofluorescent antibody" (IFA) test, was first developed in the 1960s, and it remains a valuable method, despite its relatively extensive infrastructure requirements [13]. The DFA technology involves deposition of respiratory epithelial cells onto a welled slide, followed by staining with specific antibodies directly conjugated to a fluorescent dye. After drying and fixation, a monoclonal antibody conjugate is applied to slide wells and is incubated and washed before examination by fluorescence microscopy. Its sensitivity and specificity rely on the presence of an adequate number of infected cells, and they can vary according to specimen type. IFA technology involves

Table 1. Technologies used in diagnostic influenza tests and their associated characteristics.

Technology	In-house or commercial test	Laboratory requirement(s)	Time to final result	Advantage(s)	Disadvantage(s)	Reference(s)
Immunospecific assay for viral antigen detection						
Rapid antigen test	Multiple commercial tests[a]	BSL-2 (for HPAIV only)	<30 min	Fast; requires minimal technical expertise; requires minimal infrastructure; POC setting	Sensitivity is less in adults; specificity is suboptimal outside of the influenza season; cannot distinguish between H subtypes (e.g., H1, H3, and H5); sensitivity for HPAIV unknown	[5, 11, 12]
Immunofluorescence microscopy[b]	Commercial monoclonal antibody for influenza A and B viruses; noncommercial monoclonal antibody specific for H1, H3, H5, H7, and H9	BSL-2 for specimen preparation of HPAIV	~1–4 h	Fast and versatile; simultaneous diagnosis of multiple and alternative respiratory viruses; sensitivity greater than that of POC tests; detects viable and nonviable virus; identification of specific H subtypes in development	Currently cannot distinguish between H subtypes; requires technical expertise; requires fluorescence microscope; sensitivity for HPAIV unknown	[13, 14]
Nucleic acid testing[c]	Commercial kits available for detecting influenza A and B viruses; H5N1 sequences now publicly available	BSL-2 for sample preparation of HPAIV	4–6 h	Highly sensitive (detects ⩽1–10 infectious units); detects viable and nonviable virus; potential for high throughput	Requires technical expertise; primers may require updating because of antigenic drift, especially HPAIV; limited standardization between laboratories	[11, 15, 16]
Virus isolation						
Conventional culture	…	BSL-2; certified BSL-3 for HPAIV	3–14 days	Highly sensitive (~10 pfu/mL); provides viable virus for subtyping and antiviral resistance testing; detects all influenza A and B virus types, including HPAIV; detects other respiratory viruses	Delay in receiving results; requires viable virus; requires technical expertise	[11, 17]
Shell vial culture[d]	Commercial reagents available	BSL-2; certified BSL-3 for HPAIV	18–48 h	Faster than conventional culture; detects all influenza A and B virus types, including HPAIV; detects other respiratory viruses	Virus may be nonviable, making passage difficult; adequate viral RNA for RT-PCR	[18–20]

Viral antibody detection						
Virus NT	…	Cell culture equipment for virus NTs; BSL-2 for epidemic virus and BSL-3 for pandemic virus	Several weeks needed to obtain paired serum samples; a single convalescent-phase serum sample may confirm infection with novel subtypes	Highly specific and sensitive; may be superior for HPAIV strains	Labor intensive and time consuming	[21]
HI	…	HI equipment, plates, and diluters	Several weeks needed to obtain paired serum samples; a single convalescent-phase serum sample may confirm infection with novel subtypes	Simpler than NT but with equivalent sensitivity	Labor intensive; time consuming; suboptimal for HPAIV, (except if horse RBCs are used)	[10, 22]
EIA	In-house assays using commercially available anti–human antibody conjugates	…	Several weeks needed to obtain results for paired serum samples; a single convalescent-phase serum sample may confirm infection with novel subtypes	…	Require age-matched negative control serum samples	[17, 23]
Complement fixation	Commercial reagents difficult to locate	…	Several weeks needed to obtain results for paired serum samples	Measures seroconversion to influenza A and B viruses	Time consuming and seldom performed; likely inadequate for HPAIV	[10, 22]

NOTE. BSL, biosafety level; DFA, direct fluorescent antibody test; HI, hemagglutination inhibition; HPAIV, highly pathogenic avian influenza virus; NT, neutralization test; POC, point of care; RBCs, red blood cells; RT-PCR, reverse-transcription polymerase chain reaction.

[a] See table 2.
[b] DFA or IFA.
[c] RT-PCR and nucleic acid sequence–based amplification.
[d] Single cell line and mixed cell line; enable cultivation of other respiratory viruses.

Table 2. Examples of specific rapid antigen tests.

Name of test (manufacturer)	Basis of technology	Influenza virus(es) detected	Sensitivity,[a] %	Specificity,[a] %	Laboratory requirement(s)	Reference(s)
Directigen Flu A (Becton-Dickinson)	EIA[b]	A (including H5N1, H7N2, and H7N3)	24–78	91.6	Certification	[5, 25–28]
Directigen Flu A+B (Becton-Dickinson)	EIA[b]	A and B; distinguishes between the two	44–85	99.74	Certification	[5, 25, 27, 28]
FLU OIA (Thermo Electrón)	OIA[b]	A and B; does not distinguish between the two	…[c]	…[c]	Certification	[25, 29–32]
FLU OIA A/B (Thermo Electrón)	OIA[b]	A and B; distinguishes between the two	88.4[d]	69.4[e]	Certification	[5, 25]
XPECT Flu A&B (Remel)	Lateral flow[b]	A and B; distinguishes between the two	94.4		Certification	[5, 25]
NOW Influenza A&B (Binax)	EIA[b]	A and B; distinguishes between the two	75[f]; 50[g]	100[h]	Certification	[5, 33, 34]
QuickVue influenza test (Quidel)	Lateral flow	A and B; does not distinguish between the two	…[c]	…[c]	CLIA waived; may be performed in an office that requires waiver or certification	[5]
QuickVue influenza test A&B (Quidel)	Lateral flow	A and B; distinguishes between the two	70–95	82.6–98	CLIA waived; may be performed in an office that requires waiver or certification	[5, 25, 29, 35–37]
ZstatFlu (ZymeTx)	Chemiluminescence[i]	A and B; does not distinguish between the two	65–88	83–92	CLIA waived	[5, 36, 38, 39]

NOTE. CLIA, Clinical Laboratory Improvement Amendment; OIA, optical immunoassay.

[a] Compared with viral isolation.
[b] Nucleoprotein.
[c] Not assessed in peer-reviewed literature.
[d] For influenza A virus in nasal aspirates.
[e] For influenza B virus in nasal aspirates.
[f] For influenza A virus.
[g] For influenza B virus.
[h] For influenza A and B viruses.
[i] Neuraminidase.

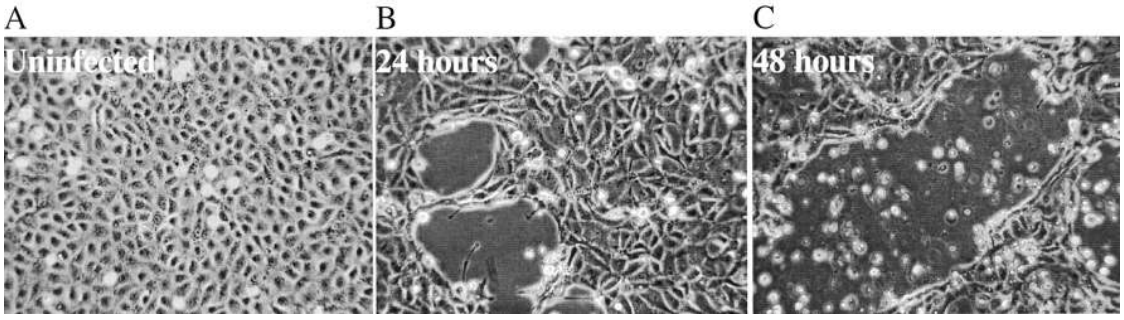

Figure 1. Cytopathic effect in Madin-Darby canine kidney cell cultures at 0, 24, and 48 h after infection

staining dried, fixed cells with a monoclonal antibody to the respective viruses and then with an antibody conjugate to a mouse immunoglobulin. IFA provides greater consistency when testing for multiple respiratory viruses is done, because only a single conjugate is used, regardless of the number of virus-specific monoclonal antibodies used. IFA usually is more sensitive than DFA, but the latter is more popular because of its shorter turnaround time, and both tests allow simultaneous detection of other respiratory viruses (e.g., respiratory syncytial virus, parainfluenza virus, and adenovirus) [14]. However, the ability of commercial monoclonal antibodies to detect avian influenza virus subtypes has not been well described, and they cannot be recommended at this time. Although not commercially available, monoclonal antibodies specific for H1, H3, H5, and H7 can be obtained and will prove to be invaluable for subtype identification when multiple H subtypes are cocirculating [42]. Overall, DFA is a valuable diagnostic test for influenza because it provides fast, relatively accurate results and it is an excellent choice for confirming POC test findings.

Virus Isolation: Culture Techniques

Influenza virus was first isolated in 1933, by inoculation of specimens into the amniotic cavity of 10–12-day-old embryonated chicken eggs. Although high yields of virus can be harvested after 3 days of incubation, this approach is no longer routinely used in the diagnosis of influenza. It is, however, used by reference laboratories to achieve a high sensitivity for detection and to obtain high-titer virus stocks [1].

Conventional culture. The time-honored technique of conventional culture, introduced in the 1940s, involves inoculation of the patient specimen into a cell culture that is then monitored for the development of cytopathic effect, for manifestation of hemadsorption after the addition of erythrocytes, or for the presence of influenza antigen, as demonstrated by specific antibody staining, shown in figure 1*B* and 1*C*, figure 2*A*, and figure 3, respectively. The development of cytopathic effect, however, can be caused by a number of respiratory viruses, and the cytopathic-effect characteristic of influenza may not always be observed in infected cell lines; hence, viral infection must be confirmed by immunofluorescence microscopy or by hemadsorption performed using guinea pig erythrocytes [17]. Immunofluorescence microscopy is also used to identify the isolated virus as influenza A or B virus [17]. Virus isolation is effective only if the cell culture system is sensitive to the inoculated virus, and not all host cells are universally permissive to all influenza A viruses [16]. Isolation of influenza A and B

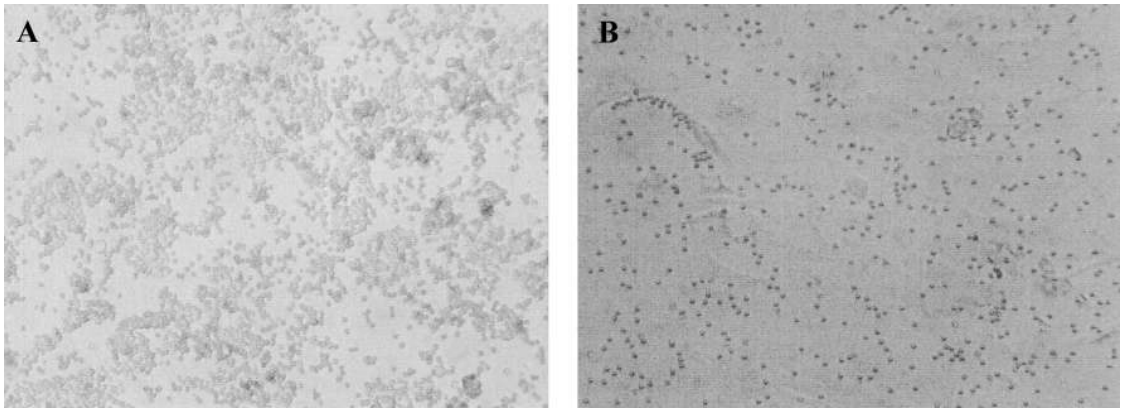

Figure 2. *A,* Presence of hemadsorption in rhesus monkey kidney (RhMK) cells. *B,* Absence of hemadsorption in RhMK cells.

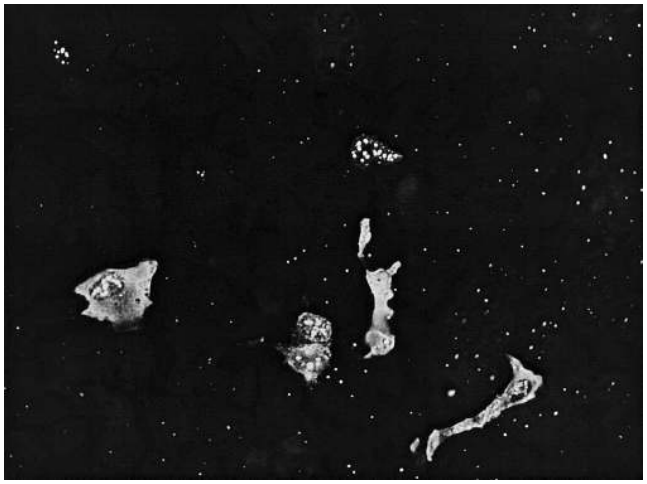

Figure 3. Immunofluorescence microscopy of influenza-infected R-Mix culture.

viruses and other respiratory viruses is conventionally performed on monolayers of either primary cell cultures, such as rhesus monkey kidney (RhMK) or African green monkey kidney (AGMK) cells, as well as established cell lines, including Madin-Darby canine kidney (MDCK), mink lung epithelial cell line (Mv1Lu), rhesus monkey kidney (LLC MK2), and buffalo green monkey kidney (BGMK). MDCK cells are especially useful for isolation of influenza B virus, and Mv1Lu cells have been reported to be more sensitive than MDCK or RhMK cells for rapid detection of influenza virus in culture [43, 44].

Rapid shell vial culture. Shell vial culture uses single or mixed cell lines and has the unique feature of enhancing sensitivity and shortening the time to detection through enhancing the viral infectivity of the cells by centrifugation. Detection of other respiratory viruses can be facilitated by using monolayers of 2 different cell types in a single vial (e.g., R-Mix). Diagnosis by shell vial culture is more rapid than diagnosis by conventional culture (time to diagnosis, 24 h vs. >3 days), with sensitivity equivalent to that of conventional tube cultures and greater than that of DFA [18–20]. Although cell lines used for seasonal influenza also support the growth of highly pathogenic avian influenza viruses, isolation of these viruses is restricted to appropriately certified biosafety level 3 (BSL-3) laboratories. Figure 1 shows the presence and absence of cytopathic effect in MDCK cell cultures; figure 2, the presence and absence of hemadsorption in RhMK cells; and figure 3, positive fluorescent staining for influenza virus in R-Mix culture.

NAT

The most common NAT used for the 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 the most sensitive, specific, and versatile tests for the diagnosis of influenza and are replacing viral isolation as the reference standard [15]. 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 even the strain by sequence analysis. The viral genotype 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. Most RT-PCR assays for influenza A and B viruses use primers complementary to the relatively stable gene 7, which encodes the conserved matrix protein, and can successfully detect all viral strains observed to date [16]. HA-specific RT-PCR with primers targeting gene 4 allows identification of the H subtype of influenza A virus.

Compared with isolation in cell culture, sensitive PCR assays can more readily identify influenza viruses in immunosuppressed transplant recipients and persons with chronic lung diseases for whom frequent lower respiratory tract infections are often associated with low viral levels. Rapid, accurate identification of respiratory pathogens in these vulnerable populations is critical to timely treatment and limitation of the nosocomial spread of infection [45].

Serological Testing for Influenza

Serological tests, including virus NT, HI, complement fixation, EIA, and indirect immunofluorescence microscopy, are based on the presence of influenza-specific antibodies that first appear ~2 weeks after initial infection and that reach peak levels 4–7 weeks after initial infection. These tests are not widely available and are rarely used for patient management, but they may be indicated for retrospective diagnosis of and disease surveillance for novel subtypes [2]. A ⩾4-fold increase in the influenza antibody titers noted between the acute-phase and convalescent-phase (3–4 weeks after initial infection) serum samples obtained from patients is diagnostic of infection. In adults who have sustained multiple influenza virus infections, increases in the strain-specific antibody titer must be interpreted with caution, because a response to the infecting virus strain may be accompanied by parallel responses to previously encountered strains [46]. However, in patients infected with a novel subtype, detection of its specific antibody is diagnostic. Finally, serological testing allows for quantification of responses to influenza vaccination, even though those responses often are not as robust as those resulting from virus infection [22].

Virus NT. The NT is the definitive serological method of identifying a specific strain of influenza virus or antibody to this virus, and it is particularly useful for the identification of highly pathogenic avian influenza viruses [42]. Because the test involves the use of infectious virus, its use is restricted to appropriately certified BSL-3 laboratories when these avian viruses

are handled. In this assay, 100 infectious units of test virus are added to serial dilutions of the serum, and, after incubation, the mixtures are applied to respective cell monolayers, which are then monitored for cytopathic effect. As the antibody becomes diluted below the level of protection, viral growth becomes detectable [21, 47].

HI assays. These are labor-intensive and time-consuming assays that require several controls for standardization. Their advantages include inexpensive, readily available reagents; sensitivity greater than that associated with complement fixation; and high specificity in identifying strains [48]. HI is used to determine the immune response to influenza in surveillance and vaccine studies [10, 22]. The technology involves the addition of 4 hemagglutinating units of virus to serial dilutions of serum; incubation; and, finally, the addition of washed chicken, turkey, human, or guinea pig erythrocytes to each dilution [47]. The highest dilution of serum that inhibits hemagglutination is designated as the HI titer. HI may not be as sensitive as NT for the detection of an immune response to avian influenza viruses, but reference laboratories have reported enhanced sensitivity with horse erythrocytes [49]. When the isolates are typed, the reference antisera to a number of strains must be included, because strains may differ by only one or more epitopes. The reference antiserum with the highest HI titer identifies the strain of the isolate [17]. Titers of at least 1:40 or serum neutralizing titers of $\geq$1:8 have been associated with protection.

Complement fixation. This test measures the antibody response to nucleoprotein, conserved among influenza A virus strains [17]. It has been mostly supplanted by more time-efficient EIAs, for which reagents are more readily available [10].

EIA. EIA is used largely for investigational studies, although EIA and Western blot analysis have been shown to be effective in detecting an immune response to avian influenza in children [17, 23]. Testing for antibody to influenza by use of these type-specific approaches is performed mainly by in-house assay, because commercial kits have yet to be well validated. Age-matched negative control serum samples, which may be difficult to locate, are necessary to establish the appropriate signal-to-noise ratio.

FACTORS INFLUENCING THE SELECTION OF TESTS

Test selection is governed by multiple factors outlined in table 3. The size and capacity of the laboratory have a major impact. Small laboratories associated with physician offices or small hospitals are usually restricted to the use of rapid POC tests, which require minimal infrastructure and can be performed by staff with limited knowledge of virology. Their comparatively high cost per test is compensated by low infrastructure expenses. Despite the seeming simplicity of Clinical Laboratory Improvement Amendment–waived testing, health care personnel must be vigilant about good laboratory practices, undergo adequate training, have access to quality control testing, and be appropriately supervised [41].

Large hospital and reference laboratories that are staffed with trained technologists have many options for influenza testing, including the use of POC tests. DFA, with its higher sensitivity and potentially lower associated cost per test, is often most appropriate in these settings. Isolation in cell culture is generally used as a reflex test after a negative DFA result, as a confirmatory test for quality assurance, or to amplify the virus for additional subtyping or resistance testing. The HI test may be used to monitor seroconversion and to subtype viral isolates. Increasingly, large laboratories are adopting nucleic acid–based technologies for the diagnosis of influenza and infection with other respiratory pathogens. Its implementation requires complex, expensive infrastructure; highly trained technologists; and space that minimizes amplicon contamination. Simpler and less expensive platforms for the nucleic acid–based diagnosis of influenza are currently under development.

INFLUENZA TESTING DURING A PANDEMIC: LABORATORY DEMANDS AND ANTIVIRAL SUSCEPTIBILITY TESTING

Laboratory demands for influenza testing will most likely increase substantially in the prepandemic stages and be compounded in early pandemic stages by the need for appropriately certified BSL-3 laboratory facilities. In pandemics, rapid diagnosis requires adequate surge capacity. NAT, accompanied by high-volume automated nucleic acid extraction, can be scaled up without a proportional demand on the technologist's time. More important, the RT-PCR assay can be used initially to detect all influenza viruses and subsequently can be reflexed to a more specific RT-PCR assay with HA-specific primers for the identification of a pandemic strain. Last, NAT is the least likely approach to be adversely affected by supply problems in the event of increased demands during a pandemic; except for primers and probes, virtually all reagents have broad applications and are readily available.

The maximum burden on the laboratory is likely to occur during pandemic stage 4, when the virus has evolved to allow human-to-human transmission but its clinical profile is not yet well established. During this stage, clinicians will likely rely heavily on laboratories to rule out avian influenza viruses in patients presenting with respiratory symptoms, especially during the winter months, when seasonal epidemic influenza virus strains are cocirculating. Under these circumstances, the laboratory will not have a strong basis for triage and, hence, will be required to process increasing numbers of specimens.

Demands for laboratory testing and testing algorithms may change as the pandemic evolves. Possible testing algorithms for

Table 3. Factors that influence selection of test.

Test	Specimen type	Duration of specimen acceptability[a]	Most appropriate patients	Most appropriate laboratory setting	Level of influenza virus activity detected	Laboratory requirement(s)	Reference(s)
Rapid antigen tests (POC)	NP swab, throat swab, NP wash, and nasal aspirate	<72 h	All, especially children who have higher viral titers; patients with a high pretest probability of infection; travelers from areas of endemicity; patients with link to outbreaks	Office-based clinics; small laboratories; large laboratories outside of the influenza season	Epidemic and sporadic; some tests are effective for detection of H5 strains	Minimal	[5][b]
Immunofluorescence microscopy	NP swab or wash, bronchial wash, and nasal and endotracheal aspirates	Best at <72 h	All	Larger laboratories, including hospital, academic, reference, and public health laboratories	Sporadic and epidemic; pandemic, if reagents are available	BSL-2	[13, 50]
Viral culture[c]	NP swab, throat swab, NP or bronchial wash, NP and endotracheal aspirate, and sputum	Best at <72 h; acceptable for symptomatic patients for >72 h	All; acceptable for patients with decreasing levels of viral shedding	Hospital, academic, reference, and public health laboratories; only laboratories with appropriate BSL-3 certification for pandemic strains	Sporadic and epidemic; pandemic only in certified BSL-3 laboratories	BSL-2 laboratories for sporadic and epidemic influenza; BSL-3 laboratories with appropriate enhancements for HPAIV	[15, 17, 51]
NAT[d]	NP swab, throat swab, nasal or bronchial wash, nasal or endotracheal aspirate, and sputum	Best at <72 h; acceptable for symptomatic patients at ⩾72 h	All; acceptable for patients with decreasing levels of viral shedding	Hospital, academic, reference, and public health laboratories	Sporadic, epidemic, and pandemic	BSL-2	[15, 45, 51]
HI and virus NT	Whole-blood specimens; paired acute- and convalescent-phase specimens obtained 14–21 days apart	Acute-phase specimens at presentation of illness; convalescent-phase specimens 2–3 weeks later	Outbreak investigations	Public health, academic, and reference laboratories	Sporadic, epidemic, and pandemic	HI: BSL-2; Virus NT: BSL-3	[17]

NOTE. BSL, biosafety level; HI, hemagglutination inhibition; HPAIV, highly pathogenic avian influenza virus; NAT, nucleic acid testing; NP, nasopharyngeal; NT, neutralization test; POC, point of care.

[a] From the onset of symptoms.
[b] See table 2.
[c] Conventional or shell vial.
[d] Reverse-transcription polymerase chain reaction and nucleic acid sequence–based amplification.

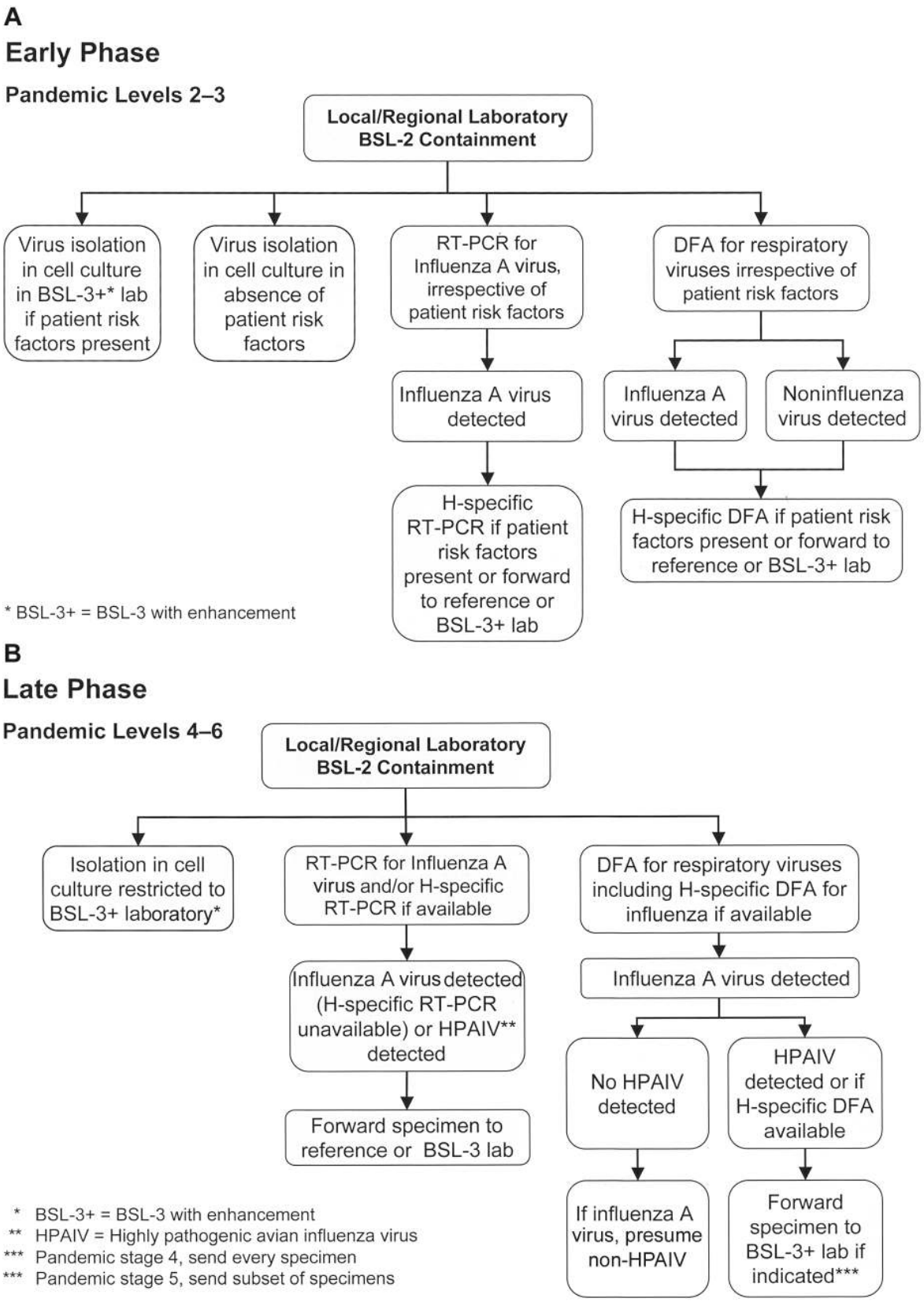

Figure 4. *A,* Possible testing algorithm during early pandemic stages. *B,* Possible testing algorithm during late pandemic stages. BSL, biosafety level; DFA, direct fluorescent antibody test; RT-PCR, reverse-transcription polymerase chain reaction.

early- and late-stage pandemic periods are shown in figure 4. Physicians will likely rely less on laboratory diagnosis as the unique clinical features of infection with the pandemic strain become apparent. However, there may be an increase in the demand for serological confirmation of influenza and evidence of immunity, as well as for testing for resistance to antiviral drugs, such as M2 and the NA inhibitors. As the pandemic progresses, documentation of immunity from a mild form of infection could be used to determine the need for antiviral drugs for individuals, such as medical personnel, who are working under a high risk of infection and are vital to the delivery of health care.

The susceptibility of a virus isolate to antiviral agents can be established by genotypic and phenotypic testing [52]. Testing for resistance to amantadine is currently accomplished by sequence analysis of the M2 open reading frame. Specific mutations in the NA gene have been shown to correlate with resistance to oseltamivir [52]. Although this approach can be applied to determine resistance to NA inhibitors, conventional inhibition assays in cell culture remain necessary, because knowledge of mutations leading to this resistance remains incomplete. The chemiluminescent NA enzyme assay is currently being used to monitor the appearance of clinical isolates that are resistant to zanamivir and oseltamivir [53]. However, comprehensive testing for antiviral resistance by use of inhibition of virus growth will likely be restricted to appropriately certified BSL-3 laboratories. Finally, assessment of the response to an NA inhibitor in very ill patients may be possible by determining the decrease in the viral load within days after initiation of treatment [52].

FUTURE DEVELOPMENT OF DIAGNOSTIC TESTS FOR INFLUENZA

Although our current diagnostic technologies are reasonably effective for sporadic and epidemic influenza, new developments are in progress to enhance diagnostic capability. Two new promising developments include technologies based on microarray chips and the Luminex X-Map. These methods are based on amplification of the viral genomic nucleic acid after hybridization to target probes, followed by monitoring by an automated reader [53, 54]. Other new developments will most likely include more sensitive and more specific POC tests, which would provide real-time laboratory diagnosis in clinical practice, improving the management of both sporadic cases and institutional outbreaks.

In the future, applications of microarray technologies will be expanded for NATs, providing same-day results for most respiratory viruses in larger laboratory settings [55]. The increasing use of genome sequence analysis is expected to allow more-rapid detection of new strains, which is critical both for viral surveillance and for the vaccination-development program. If POC tests can be developed to diagnose novel subtypes, they will play an important role in a pandemic setting. These new developments promise to improve the management of sporadic and epidemic influenza in both individual patients and communities, as well as to address the need for high-volume testing in the event of a pandemic.

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