Rapid Diagnosis of Influenza: State of the Art

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KEY POINTS

- Rapid influenza antigen tests have lower sensitivity compared to other methods, but newer assays control for some of the factors that may contribute to poor performance.
- Nucleic acid amplified tests are now available that allow for the identification of infection with influenza and other respiratory viruses with high sensitivity in as little 1 hour.
- The best way to clinically implement these assays remains unclear, and many different factors must be considered when choosing an optimal testing algorithm including: patient population tested, required turn-around-time, and testing-driven clinical interventions.
- To help guide both laboratory and provider decision making, studies are urgently needed to determine the clinical utility, impact on outcomes, and cost-effectiveness of rapid antigen and nucleic acid amplification tests for influenza and other respiratory viruses in different patient groups and clinical settings.

INFLUENZA VIRUSES

Influenza viruses are members of the family Orthomyxoviridae. Based on antigenic differences in the matrix (M) protein and the nucleoprotein (NP), influenza viruses are separated into 3 genera: Influenzavirus A, Influenzavirus B, and Influenzavirus C. Because influenza type C causes only mild illness, it is not further considered in this review. Influenza A is further classified into subtypes based on surface proteins hemagglutinin (HA) and neuraminidase (NA). Sixteen HA and 9 NA subtypes are now recognized. Strains have been identified within subtypes, and lineages or clades within strains. Aquatic birds are considered the reservoir of influenza A in nature.[1]

Influenza A and B genomes have 8 RNA segments encoding structural and nonstructural proteins. When 2 viruses infect the same cell, genetic reassortment can occur with generation of new strains or subtypes. Such reassortment between human and avian virus strains gave rise to the influenza A pandemics of 1957 (H2N2), 1968 (H3N2), and, to some degree, 2009 (H1N1). Swine can be infected with both human and avian viruses, and thus serve as a mixing vessel facilitating emergence of new subtypes that may or may not readily transmit. Some highly pathogenic avian viruses (H5N1 and H7N9) have been transmitted directly from birds to humans, resulting in high mortality but, fortunately to date, low transmissibility.[2] In addition, mutations occur during routine replication that can lead to antigenic change in both influenza types A and B.

The unique ability of influenza viruses to change their genetic and antigenic makeup leads to annual epidemics of illness, hospitalizations, and excess mortality, as well as the continual threat of a new pandemic, with potentially higher morbidity and mortality. For diagnostic laboratories, the challenge is providing assays that detect all circulating strains from year to year. Although tests typically target conserved M or NP genes or proteins, test performance should be validated annually, especially when new viruses emerge.

Pathogenesis

Influenza is transmitted primarily by droplets spread by sneezing and coughing but also by contact with infected surfaces and via small-particle aerosols. After entering the respiratory tract, influenza virions attach via HA envelope proteins to sialic acid receptors on ciliated columnar epithelial cells. After cleavage of the HA by cellular proteases, the virus is endocytosed and replication ensues. An essential step in viral release and infectivity is removal of sialic acid residues from the envelopes of new virions by viral neuraminidase. After an incubation of 1 to 4 days, virus shedding and symptoms appear. Viral shedding lasts for 5 to 10 days, but begins to decrease within 3 to 5 days after symptom onset.[3]

Clinical Presentation

Uncomplicated influenza is characterized by the abrupt onset of malaise, headache, myalgia, and fever, followed by sore throat and nonproductive cough. Children may also develop otitis media, nausea, and vomiting. Influenza typically causes a tracheobronchitis that resolves within a week, but cough and malaise can persist for weeks longer. Complications include febrile seizures in young children, sinusitis, viral pneumonia, secondary bacterial pneumonia, myocarditis, pericarditis, and encephalopathy. Older adults may not present with fever, but rather with decompensation of underlying cardiac or pulmonary conditions. The main focus of influenza management is prevention through vaccination, and annual vaccination is now recommended for all persons older than 6 months.[4]

Treatment

When indicated, treatment should be begun as soon as possible, ideally within 48 hours of onset of symptoms. Thus when suspicion is high, antiviral therapy should be administered without waiting for laboratory confirmation.[5] For severe disease, treatment may still be useful when initiated after 48 hours, and should not be withheld. The adamantanes, amantadine and rimantidine, block the influenza A M2 protein ion channel and thus prevent viral uncoating. However, because of widespread resistance, the adamantanes are no longer recommended for routine use. The neuraminidase inhibitors, oseltamivir and zanamivir, are active against both influenza A and

B. Although resistance has been reported with oseltamivir, most viruses are susceptible at present. Zanamivir is administered via inhalation and, owing to the risk of bronchospasm, is contraindicated in persons with underlying pulmonary disease. For the latest information on treatment of circulating strains, the reader is referred to the Centers for Disease Control and Prevention (CDC) Web site at http://www.cdc.gov/flu/professionals/antivirals/.

GENERAL PRINCIPLES OF LABORATORY DIAGNOSIS OF INFLUENZA INFECTION

For patients with influenza-like illness (ILI), clinical diagnostic efforts should be focused on detection of the virus rather than on antibody response. Samples should be collected ideally within 12 to 36 hours after onset of illness, to initiate antiviral therapy within the recommended 48-hour time frame, and within 72 hours for maximum detection. Factors affecting the performance of influenza diagnostic assays are briefly discussed in **Box 1**.

Viral diagnostic methods have evolved dramatically in the past 20 years, as summarized in **Table 1**. The traditional gold standard of viral diagnostics, conventional cell culture, can require up to 10-14 days to generate final results. Thus the introduction of rapid centrifugation culture, with results in 1 to 2 days, was a great advance. Viral antigen assays such as immunochromatography (IC) or direct immunofluorescence assays (DFA) are capable of yielding results in 10 minutes to 2 hours and are widely

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Box 1
Factors affecting detection of influenza viruses

Viral Factors

- Genetic variation: primer/probe mismatches; variations in antigens detected by rapid influenza diagnostic tests (RIDTs)
- Site of infection: lower respiratory tract specimens may be optimal for emerging viral strains

Sample Collection Factors

- Inadequate specimen: correct site, but few respiratory cells collected
- Improper specimen: nasopharyngeal swabs are recommended/approved, and other specimens may be suboptimal or nonvalidated
- Improper time of collection: collecting too early (<12 hours) or too late (>72 hours) after symptom onset

Storage and Transport Factors

- Incorrect transport medium: formulations vary, laboratory may have to reject if incorrect
- Improper transport or storage: freezing or prolonged storage reduces viral titers and can promote nucleic acid degradation
- Dilution: samples for RIDT should be minimally diluted in transport media

Testing Factors

- Test choice: different sensitivity of assay classes and assays within class
- Workflow: Storage of specimens before batch testing
- Workload: Suboptimal performance during periods of high testing intensity
- Interpretation: RIDT must be read at specific time for valid results; direct fluorescence assay requires subjective assessment

Table 1
Summary of methods for influenza diagnosis with advantages and limitations

	Technique	Assay Time	Advantages	Limitations
Viral isolation	Conventional culture	1–14 d	Allows isolation of many viruses; can detect unexpected or novel viruses; more sensitive than antigen detection	Requires expertise to interpret CPE and maintain cell cultures; some viruses do not grow in routine cultures; biosafety concerns for zoonotic and emerging viruses
	Rapid culture	1–5 d	Most results in 1–2 d; requires less training to interpret IF staining than CPE; use of mixed-cell cultures allows detection of multiple viruses in a single vial	Requires cell culture and IF expertise; detects only targeted viruses; less sensitive than conventional culture; biosafety concerns
Antigen detection	DFA	1–2 h	Can be done on demand as samples arrive in the laboratory; reagents available for 8 respiratory and 4 herpes viruses; can assess sample quality	Requires substantial expertise for accurate results; manual and labor-intensive; requires an adequate number of target cells for valid results
	IC	<30 min	Requires no equipment and little expertise; simply add sample and set timer; approved for use at point of care	Less sensitive than other methods; limited test menu
NAAT	General comments		Most sensitive method; detects viruses that do not grow in culture; more rapid than culture; safer than culture because pathogens are inactivated and disrupted before testing; potential for automation and quantification	Requires specialized equipment and expertise; results variable across laboratories; inhibitors can prevent amplification; cross-contamination leads to false positives; can detect clinically irrelevant viruses; genetic variability can lead to false negative results; few FDA-approved assays
	End-point PCR	5–9 h	Uses inexpensive conventional thermocyclers; less affected by genome variability; highly multiplexed respiratory pathogen assays commercially available	Prone to carryover contamination from amplified products because tube is opened after amplification; slower than real-time methods; expensive and complex detection methods for multiplexed assays
	Real-time PCR	0.5–5 h	Faster, less prone to cross-contamination, readily quantified; laboratory-developed assays can be readily updated; more commercial kits becoming available, including walk-away tests	More prone to falsely negative or low values owing to genetic variations in viral strains; lack of standardization; limited capacity to multiplex

Abbreviations: CPE, cytopathic effect; DFA, direct immunofluorescence assay; FDA, Food and Drug Administration; IC, immunochromatography; IF, immunofluorescence; NAAT, nucleic acid amplification technique; PCR, polymerase chain reaction.

used. Molecular tests are now available that provide high sensitivity with a turnaround time (TAT) of approximately 1 hour. Thus, advancing technology has allowed clinicians to redefine what is considered as rapid testing. Although IC assays are most frequently referred to as rapid flu tests, in this article rapid testing is defined as those assays capable of providing a result in less than 3 hours. This time frame was selected based on recently released nucleic acid amplified tests (NAATs) that can, theoretically, be performed round the clock by core laboratory staff. In considering such assays it will be important to establish their performance characteristics in reference to the gold-standard methods of conventional culture and conventional reverse transcriptase–polymerase chain reaction (RT-PCR) assays.

SAMPLE COLLECTION AND TRANSPORT

Laboratories should provide collection guidelines appropriate to the tests that they offer, including sample type and volume, proper container, transport media or stabilizers if needed, transport temperature, and other special instructions, especially for commercial kits. For optimal results, sample-collection instructions should be strictly followed.

A variety of sample types have been studied for influenza testing (**Box 2**), but nasopharyngeal (NP) swabs were recommended by a recent consensus conference on respiratory virus testing.[6] Increased sensitivity may be seen with NP aspirates and

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Box 2
Types of specimens for influenza testing

Laboratories validate assays for specific specimen types. Deviation from recommended specimens may lead to test cancellation.

Nasopharyngeal (NP) Swabs

- Insert swab deep into nasopharynx past the point of resistance to collect ciliated respiratory epithelial cells
- Most widely accepted specimen; approved for all assays cleared by Food and Drug Administration (FDA)
- Less expertise to collect, but inadequate specimens not uncommon
- Swab type (flocked or unflocked) and transport media vary by institution

Nasal Washes/Aspirates

- Requires equipment and expertise to collect; more uncomfortable for patient
- Approved for use with some FDA-cleared assays
- Increased sensitivity compared with NP swabs
- May be required for young children

Lower Respiratory Specimens (Bronchoalveolar Lavage, Bronchial Brushing, Induced Sputum)

- Invasive techniques requiring specialized expertise and equipment
- Can help establish cause of pneumonia
- No commercially available FDA-approved tests; requires local validation

Others (Sputum, Throat Swab, Nares Swab)

- Much less well studied
- Variable sensitivity compared with NP swabs/washes/aspirates

NP washes, but these are relatively more invasive and require more expertise to collect.[7] Other specimen types are less widely used.

Once collected, swabs and tissues are usually placed into transport media that may vary with the test method or kit used. Factors associated with specimen transport and storage are summarized in **Box 1**. For laboratories performing tests that have undergone regulatory approval, manufacturers' guidelines for sample type, collection device, and transport should be followed.

DIAGNOSTIC METHODS
Viral Culture

Conventional viral culture has been the traditional gold standard for influenza diagnosis, and remains the comparator method for many commercial assays. Rapid culture techniques have been widely applied for respiratory viruses.[8–10] Culture techniques are discussed in **Box 3**, and have been recently reviewed.[11]

Viral Antigen Detection

Antigen-detection methods do not amplify the virus and are thus less sensitive than culture or NAAT. In addition, assay performance for influenza viruses can vary from year to year because of antigenic variation in circulating strains.[12–14]

Immunofluorescence

For DFA, cells are affixed to glass slides, stained with antibodies coupled to fluorophores, and examined under a fluorescence microscope to visualize viral proteins in infected cells. Cytospin preparation of slides improves results.[15] For respiratory

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Box 3
Viral culture methods

Conventional Culture

- Multiple cell lines inoculated to increase number of viruses detected
- Examined for 10 to 14 days for the presence of viral cytopathic effect (CPE)
- Time to CPE, CPE morphology, cell line(s) infected suggest potential virus
- Viral identification, usually by immunofluorescence (IF)
- Advantages: Traditional gold standard, sensitive for cultivatable viruses, comprehensive, can detect unexpected or unknown viruses, isolate obtained for further testing (eg, subtype or strain identification, antiviral susceptibility)
- Disadvantages: Requires expertise, potentially long turnaround time, some viruses are noncultivable in common cell cultures, some BSL-3 or BSL-4 pathogens may be inadvertently grown in culture

Rapid Culture

- Mixture of cell lines in a shell vial to increase viruses detected
- IF staining at 24 to 48 hours to assess for cells infected by virus present in specimen
- Pool of IF reagents → up to 8 respiratory pathogens
- Advantages: Detect viruses before CPE is apparent, less expertise than conventional culture
- Disadvantages: Limited number of viruses, requires some expertise, requires cell culture facility and IF microscope, some BSL-3 or BSL-4 pathogens may be inadvertently grown in culture

viruses, a pool of antibodies to several different pathogens can be used to screen a single cell spot for multiple viruses including 7 or 8 respiratory viruses (respiratory syncytial virus [RSV], influenza A and B, parainfluenza types 1, 2, 3, adenovirus, and human metapneumovirus).[16] For samples that screen positive, additional testing is required to identify infecting viruses.

Compared with lateral flow IC (see later discussion), DFA is more sensitive, allows for an assessment of sample adequacy (ie, sufficient numbers of target cells), and can detect multiple viruses in a single test.[17] However, application of DFA is limited by technical requirements (eg, a fluorescence microscope, dark room, and technical expertise),[14] and assay time is one to two hours, which is longer than simpler rapid tests.[18]

Lateral flow IC

IC assays are widely used for the detection of influenza A and B, and separate assays are available for RSV. When used for influenza, these are referred to as rapid influenza diagnostic tests (RIDT), some of which are approved as point-of-care tests. These assays are simple to perform and amenable to round-the-clock testing by laboratory generalists. Samples are minimally manipulated, added to the test kit, and read at 10 to 20 minutes (Fig. 1). RIDTs have reduced sensitivity in comparison with other techniques. Factors contributing to reduced test performance are outlined in Box 1.

Performance of RIDTs

In a seminal article from the onset of the 2009 H1N1 influenza A pandemic, Ginocchio and colleagues[12] reported the real-world performance of 2 RIDTs compared with DFA, rapid culture, and xTag RVP. The sensitivity of the RIDTs for influenza A was 18%, DFA
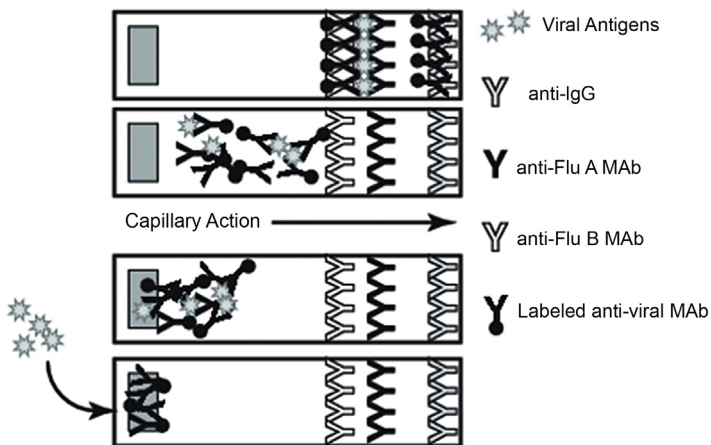
Fig. 1. Lateral flow immunochromatography for the detection of influenza A and B. (*Bottom left*) The patient specimen is applied to a defined area that contains antiviral antibodies labeled with a detection molecule. Next, labeled antibodies with or without bound antigen are drawn along the test strip through capillary action. Antiviral monoclonal antibody and anti–immunoglobulin G (IgG) are immobilized at the distal end of the test strip in well-demarcated areas corresponding to influenza A or influenza B. Viral antigens mediate the retention of labeled antiviral antibodies at the test strip, and anti-IgG binds residual labeled antibodies present. (*Top right*) Visible or fluorescent lines appear at both the test and control locations when viral antigens are present (positive test), or the control location only when antigens are absent (negative test).

was 47% sensitive, and R-mix (Diagnostic Hybrids/Quidel Corp) rapid culture was 89% sensitive compared with RVP. Several factors could have contributed to the reduced sensitivity of RIDT and DFA in this study, including: (1) antigenic variation in viruses; (2) high workload leading to suboptimal performance and interpretation; (3) poor sample quality owing to collection by inexperienced staff; and (4) inherent insensitivity of the assays.

To address the contribution of antigenic variation to analytical sensitivity of RIDTs, the CDC coordinated a study of 11 different RIDTs available in the United States.[19] The CDC prepared stocks of influenza A and B strains recently circulated in the United States. At high concentrations (a 1:10 dilution of stock), all viruses were detected by most of the RIDTs tested, but on further dilution performance quickly fell off such that less than half of the RIDTs were positive for 6 of 23 viruses tested at approximately a 1:30 dilution. At 1:100, some viruses were not detected by any of the 11 RIDTs. This study revealed the marked variability in RIDT detection of different influenza subtypes and strains, and confirmed the need for annual assessment of RIDT performance against circulating viruses.

These differences were further emphasized when the CDC examined the performance of RIDT for the influenza A H3N2v that emerged in the summer of 2012.[20] Several of the assays were able to detect all of the strains tested, but RIDT performance was highly variable.

Three meta-analyses of RIDTs have recently been published (Table 2).[21–23] Among these, Chartrand and colleagues[23] looked at 159 studies comparing the performance of 26 different RIDTs with either RT-PCR or culture as the gold standard, and specifically pulled out several different factors from the included studies to perform a comprehensive analysis of RIDT sensitivity and specificity.

Higher sensitivity was seen in studies of children (66.6%) in comparison with adults (53.9%, $P<.001$). Sensitivity was lower for studies using RT-PCR as the gold standard (53.9%) than those using culture (72.3%, $P<.001$). Factors that did not significantly affect RIDT sensitivity were specimen type or testing at the point of care. In reviewing studies in which the duration of patient symptoms were tracked, the highest sensitivity was seen for patients tested between 1 and 3 days from the time of symptom onset.

Two studies performed during the 2009 pandemic found substantially lower specificities than expected.[24,25] However, specificity increased during the course of the flu season, as the nonlaboratory staff performing and interpreting the tests gained expertise.

Table 2
Summary of recent meta-analyses of rapid influenza diagnostic test (RIDT) performance

Authors,[Ref.] Year	Time Period (% Studies)	No. of Studies	No. of RIDTs Studied	Pooled Sensitivity (95% CI) (%)	Pooled Specificity (95% CI) (%)	Reference Method(s)
Babin et al,[21] 2011	2009[a] (100)	14	7	67.5 (66.2–68.9)	80.7 (80.0–81.4)	RT-PCR[b]
Chu et al,[22] 2012	2009[a] (100)	17	7	51 (41–60)	98 (94–99)	RT-PCR[b]
Chartrand et al,[23] 2012	2009[a,c] (35) Non-2009[c] (65)	159	26	62.3 (57.9–66.6)	98.2 (97.5–98.7)	RT-PCR or Culture

Abbreviations: CI, confidence interval; RT-PCR, reverse transcriptase–polymerase chain reaction.
[a] Predominantly pandemic H1N1 influenza A.
[b] RT-PCR was the predominant method.
[c] Study period not specified in 44 studies.

Several manufacturers have developed systems that automate the reading of RIDTs (Table 3). Both the Sofia Influenza A + B fluorescent immunoassay (FIA) (Quidel) and the 3M Rapid Detection Flu A + B test use antibodies coupled to fluorescent compounds, whereas the BD Veritor System uses a proprietary enhanced colloidal-gold particle for detection. All 3 devices standardize interpretation among personnel, but the BD Veritor does not have a walk-away function, such that reading time could still vary. Several independent studies of the Sofia,[26–29] Veritor,[30] and 3M Rapid Detection system[12,31,32] have been published.

Taken together, these studies confirm, to a high degree of certainty, what had previously been reported, namely that both the clinical and analytical sensitivities of RIDTs are less than those of other methods including DFA, culture, and RT-PCR. These studies did not address the clinical utility or cost-effectiveness of RIDTs. Because of the low sensitivity of RIDTs, there has been an impetus to develop molecular assays that can provide both high sensitivity and a relatively rapid TAT.

Nucleic Acid Detection

Molecular methods for virus detection have gained favor because (1) their sensitivity is equal to or exceeds that of culture, (2) they can be quantitative, (3) they can detect viruses that are otherwise unculturable, (4) their TAT is a day or less, (5) multiplexed methods allow for the detection of multiple analytes in a single test, and (6) extraction renders the virus noninfectious. NAATs have been historically restricted to larger academic centers and reference laboratories, but the recent development of kits approved by the Food and Drug Administration (FDA) and less complex assays has allowed for these highly sensitive methods to become more widely used.

Conventional PCR

Conventional PCR consists of 3 steps: extraction and purification of nucleic acid, amplification of target sequences using specific primers and DNA polymerase, and detection of amplified fragments (Box 4, Fig. 2). The 3 steps must be performed in separate spaces with unidirectional workflow so as to limit cross-contamination and false-positive results. For RNA viruses such as influenza viruses, viral RNA must first be reverse transcribed to cDNA before PCR amplification (ie, RT-PCR). Assays in which amplified products are analyzed independently of the amplification step are

Table 3
Performance of RIDTs with automated readers[a]

Assay	Comparator	Flu A Sensitivity[b] (95% CI) (%)	Flu A Specificity[b] (95% CI) (%)	Flu B Sensitivity[b] (95% CI) (%)	Flu B Specificity[b] (95% CI) (%)	Walk-Away
Quidel Sofia Influenza A + B	Culture	97 (91–99)	95 (93–96)	90 (83–95)	97 (95–98)	Yes
BD Veritor	RT-PCR	81.3[c] (70.0–88.9)	97.4[c] (94.4–98.8)	85.6[c] (76.8–91.4)	99.0[c] (96.5–99.7)	No
3M Direct Detection Flu A+ B	Culture	80.3 (68.7–89.1)	96.6 (94.8–97.9)	58.3 (27.7–84.8)	98.0 (96.6–99.0)	Yes

[a] Data derived from product inserts accessed from company Web sites, October 2013.
[b] Sensitivity and specificity shown for nasopharyngeal swabs.
[c] Positive percent agreement and negative percent agreement shown on product insert.

Box 4
Conventional nucleic acid amplification steps

Extraction: Isolate nucleic acids and remove inhibitors

- Automated extractors commonly used for high-volume testing
- Multiple parameters vary: extracted volume, washing, elution volume, DNA, RNA, or total nucleic acid recovery
- Extraction protocols may vary among laboratories using same assay, thus must interpret literature carefully

Amplification: Increase amount of target nucleic acids to facilitate detection

- Polymerase chain reaction is the most common technique
- Sensitivity and specificity are strongly affected by primers used
- Targeted genes can be specific for subtypes (eg, influenza A hemagglutinin) or broadly reactive (eg, influenza A matrix)
- Primer mismatches and falsely negative results may occur in evolving/mutating viruses

Detection: Detect amplified nucleic acids

- Agarose gel electrophoresis with ethidium staining or dot-blot hybridization
- New highly multiplexed conventional methods use novel detection methods

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end-point PCR assays, whereas those whereby amplification and detection occur simultaneously are said to be real-time PCR assays. The advantages and disadvantages of these assay formats are listed in **Table 1**. Laboratories have generally moved from end-point to real-time PCR assays, but several highly multiplexed commercial kits for the detection of respiratory viruses use end-point methods.

Real-time PCR
Real-time PCR methods have had a major impact on diagnostic testing by combining amplification and detection into one step (see **Fig. 2**). This combination shortens assay
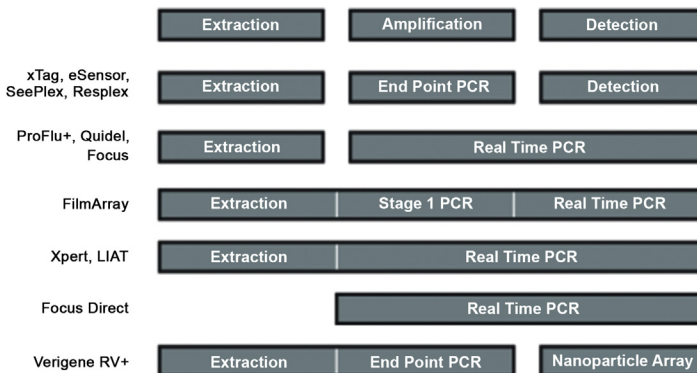
Fig. 2. Workflow of commercially available influenza virus nucleic acid amplification test. Proprietary detection methods are used by xTag, eSensor, SeePlex, and Resplex assays. Black boxes indicate steps performed on a single instrument, white spaces indicate requirement to move samples to new instrument, and white lines indicate discrete processes occurring on a single instrument. Processes are not drawn to scale. PCR, polymerase chain reaction.

time, reduces amplicon cross-contamination because the reaction tube is not opened, and allows visualization of amplification results as they are unfolding in real time. All steps can be performed in one room. Real-time PCR methods use nonsequence specific fluorescent DNA binding dyes such as SYBR Green or sequence-specific fluorescent DNA probes. Thus, real-time assays require the use of thermocyclers with built-in light sources, filters, and detectors.

Assays using SYBR dyes gain their specificity through a melt-curve analysis wherein fluorescence is monitored with increasing temperature after completion of a certain number of cycles. The melting of double-stranded DNA occurs in a sequence-specific manner, and this is associated with a change in detectable fluorescence. One of the most common real-time PCR assays uses hydrolysis probes (eg, TaqMan probes) that contain a reporter fluorophore and quencher in close proximity. When the probe is intact, fluorescence from the reporter is quenched, but when the probe binds to a DNA segment undergoing amplification, the 5′-3′ nuclease activity of Taq polymerase degrades the probe, releasing the reporter from the quencher and leading to detectable fluorescence. For assays using this design, specificity is determined by both primer and probe sequences, and although a product may be amplified, mismatches in the probe might lead to failed hydrolysis and no detectable fluorescence. Other real-time assay designs circumvent this problem by incorporating the fluorescent reporter and quencher into the primer itself.

Several different thermocyclers are commercially available. These devices differ in the mechanism by which temperature cycling occurs (eg, metal blocks, heated air), reaction vessels (eg, 96-well plates, capillary tubes, proprietary cartridges), and fluorescent channels available, among other parameters. These factors determine how quickly reactions can take place, the number of analytes that may be detected, and compatibility with commercial assays.

Multiplex methods

Multiplexing refers to the detection of more than 1 analyte in a sample in a single test reaction. Real-time PCR instruments contain several different filters and/or light sources that allow for the detection of up to 6 different fluorophores, but given the need for internal controls and a reference dye, these instruments are limited to only 3- or 4-plexing. There are several different assays commercially available capable of detecting influenza A, influenza B, and RSV, as well as an internal control.[13,14]

Several manufacturers have developed novel methods to multiplex up to 20 different targets. For respiratory pathogens, BioFire, Luminex, and GenMark have FDA-cleared highly multiplexed assays. Other companies including Seegene, Qiagen, and Nanosphere have highly multiplexed respiratory pathogen panels that may be available for in vitro diagnostics (IVD) or as research-use only (RUO) tests depending on laboratory location. The BioFire FilmArray and Nanosphere Verigene are discussed herein, and recent reviews in Clinics in Laboratory Medicine discussed the Luminex and Genmark systems.[33]

RAPID NAAT FOR THE DETECTION OF INFLUENZA VIRUSES

TAT for current molecular assays can vary from 30 minutes to 9 hours from start to finish, and many real-time PCR assays can go from sample to result in approximately 5 hours. However, this does not likely reflect true clinical TAT because the assay workflow is not compatible with random access/on-demand processing and testing. Extraction and amplification/detection instruments are often not designed to be used for a single specimen, and laboratory protocols often require several controls for each assay run, leading to rapidly escalating reagent costs for each single sample

run. Because of this, most laboratories perform NAAT for influenza viruses in batches with the frequency of runs determined by staffing, workload, and clinical need. This strategy delays clinical TAT beyond the actual time required to perform the assays.

Description of the Systems

In the past 3 years, 5 NAATs designed to be random-access and capable of giving results in less than 3 hours have been cleared by the FDA for the detection of influenza A and B (Table 4). These assays use several unique modifications to the traditional extraction/amplification/detection workflow required for conventional NAATs (see Fig. 2). Most test systems are not amenable to the implementation of laboratory-developed tests (LDTs) because of their proprietary disposables. However, all are classified as moderately complex, allowing for performance by a much broader spectrum of laboratory personnel, and hands-on requirements are minimal regarding both time and manipulation.

Focus Diagnostics released an FDA-cleared highly complex multiplexed real-time RT-PCR assay for the detection of influenza A, influenza B, and RSV in 2011 that required a separate extraction step, but Focus has subsequently received FDA clearance for a Flu A/B & RSV Direct (no extraction) assay that uses a larger sample input volume than is possible on its previous assay. Focus has several other FDA-cleared assays available for this platform, and several analyte-specific reagent assays are available for bacterial and viral targets. The platform is amenable to implementation of LDTs including fully home-brew assays, but adapting existing assays to new platforms may not be straightforward.

The GeneXpert Flu assay components are found in a self-contained cartridge in which a series of chambers house appropriate reagents and are used for different assay functions. There are several other assays available for use on the GeneXpert system, including viral, bacterial, and other tests.

The FilmArray uses a series of different chambers in a mylar pouch to separate extraction, first-stage amplification, and second-stage amplification/real-time detection of respiratory pathogens. It uses a nested PCR approach whereby products from a first-stage PCR reaction that amplifies targets from several pathogens are diluted into a pathogen-specific second-stage PCR array. During second-stage PCR, amplification is detected in real time through a target-independent DNA-binding dye, and amplification specificity is confirmed by melt-curve analysis. Throughput is limited to 1 specimen per instrument. A highly multiplexed assay for the detection of bacterial and fungal pathogens from blood cultures was recently FDA-cleared on the BioFire instrument.

The Verigene system uses gold nanoparticles as a novel means to detect amplified nucleic acids. Samples undergo extraction, RT-PCR, and incubation with slide-immobilized gold nanoparticles in a single-use cartridge in the Verigene processor. Slides are then removed from the processor, and the presence of analytes is detected by the reader. A processor is occupied continuously by a specimen, potentially limiting throughput. A highly multiplexed respiratory virus assay with an additional 9 respiratory pathogens is currently an RUO test. Verigene has other clinical diagnostics assays, for both microbiology and human genetics/pharmacogenetics applications, all FDA-cleared on this platform.

Like the GeneXpert, the iQuum LIAT influenza A/B assay is performed in a self-contained test cartridge, and the influenza A/B assay is the only test currently available on this platform. Like the FilmArray and Verigene, each LIAT analyzer can handle only 1 test at a time, potentially limiting throughput.

Table 4
Rapid nucleic acid amplification test for influenza (TAT <3 hours)

Assay	Manufacturer	Pathogens Detected	Unique Instrumentation (Other Assays)	TAT (h)	Throughput	Refs.
Flu A/B/RSV Direct	Focus	Influenza A, influenza B, RSV	3M Integrated Cycler (Yes)	1.25	Up to 8 samples/ instrument/run	34
Xpert Flu	Cepheid	Influenza A, influenza A 2009 H1, influenza B	Gene Xpert (Yes)	1.25	Variable[a]	35–39
FilmArray	BioFire	Influenza A (H1, H3, 2009 H1), influenza B, adenovirus, parainfluenza 1, 2, 3, 4, RSV, hMPV, rhinovirus/ enterovirus, coronaviruses HKU1, NL63, 229E, OC43, *Bordetella pertussis, Chlamydophila pneumoniae, Mycoplasma pneumoniae*	FilmArray (Yes)	1.25	1 sample/instrument/run[b]	31–33,40–46
Respiratory Virus Plus	Nanosphere	Influenza A (H1, H3, 2009 H1), influenza B, RSV (A & B)[c]	Verigene Processor & Reader (Yes)	2.5	1 sample/processor/run[b]	46–48
LIAT	IQuum	Influenza A, influenza B	LIAT Analyzer (No)	0.5	1 sample/instrument/run[b]	None

Abbreviations: hMPV, human metapneumovirus; RSV, respiratory syncytial virus; TAT, turnaround time.
[a] Modular instrumentation with 1, 4, 16, or 80 possible positions.
[b] Instruments with small footprints to facilitate placement of multiple instruments to increase throughput.
[c] Oseltamivir call-out is available outside the United States.

Assay Performance

These newly developed assays have been available for only a short time, and comparison studies are limited. All have been subjected to FDA review, and the publicly available results from their FDA-clearance documents and product inserts suggest sensitivity and specificity consistent with many NAATs. However, for regulatory approval only the GeneXpert Flu was compared with a NAAT, and all others were compared with culture. NAATs are generally more sensitive than culture, thus this would lead to an overestimation of the sensitivity of these assays. Finally, all data from FDA trials are likely obtained under ideal circumstances and may not represent real-world performance. Thus, the sensitivities and specificities determined during FDA trials may not be borne out by subsequent postmarketing studies using NAAT as the gold standard (see later discussion).

The performance of the FilmArray has been the most thoroughly assessed, with at least 10 different comparison studies published.[35–39,42–46] Among these, only Van Wesenbeeck and colleagues[39] compared 2 assays with a TAT of less than 3 hours, the FilmArray and Verigene RV+. Five of the studies compared the FilmArray with other highly multiplexed assays including the Luminex RVP and RVP Fast, Qiagen ResPlex II, and GenMark eSensor. Others used LDTs or the Prodesse ProFlu+ as their comparator method. Not all studies differentiated among influenza A subtypes. The sensitivity of the influenza A assay ranged from 90.2% to 100%, and specificity was 100% in all studies. Detection of influenza B was between 77.3% and 100% sensitive, with 100% specificity across all studies. Performance of the FilmArray for influenza A subtypes was variable. Nearly all of these reports also compared the performance of the other analytes contained in the FilmArray panel either through comparison with other highly multiplexed tests, other commercial assays, or LDTs.

The Xpert Flu assay has been examined in several studies in comparison with the xTag RVP, several different LDTs, the ProFlu+, and/or culture.[34,47–50] These studies found sensitivity between 78.8% and 100% with 99.4% to 100% specificity for influenza A, and sensitivity between 76.5% and 100% and specificity of 100% for influenza B. However, the original Xpert Flu assay was released in 2011, and a reformulated version of the assay with an additional primer pair for influenza A was released in late 2012/early 2013; the published studies do not specify which version of the assay was used.

Three studies have examined the performance of the Verigene RV+ in comparison with other NAATs including the extracted Focus Flu A/B & RSV and ProFlu+, among others.[39,51,52] Sensitivity in 2 studies exceeded 96.6% with 100% specificity for influenza A, but a third study found sensitivity of only 84.7% with a high invalid rate.[39] Performance of the RV+ for influenza B was only assessed in one study, and was 100% sensitive and 99.4% specific.[52]

No studies examining the performance of the iQuum LIAT have been published, and only one study of the Focus Flu A/B & RSV Direct kit has been published. Woodberry and colleagues[53] found only an 86.4% positive agreement for influenza A and 36.8% for influenza B, but the investigators suspected a thermocycler malfunction could have negatively affected assay performance, especially that of influenza B. Two studies compared the performance of the 96-well Focus Flu A/B & RSV kit without extraction, and found much higher sensitivities and specificities for both influenza A and B.[54,55] However, Alby and colleagues[51] used the FDA-cleared extracted protocol and assay, and found sensitivities of 82.8% and 76.2% for influenza A and B, respectively.

These studies are of varying quality and sample size, mixture of prospective and retrospective designs, and performance during years with limited circulating strains.

Nonetheless, they demonstrate the potential real-world performance of assays. With only a few exceptions, these studies fail to address an important question: how does the performance compare among influenza NAAT with rapid TAT? Few head-to-head comparisons of rapid NAATs have been performed, and these studies are needed to help laboratories make informed decisions about assay selection.

Limitations and Future Developments

There are no currently available NAATs capable of delivering high-order multiplexing, TAT of less than 3 hours, and high throughput. Reagent and instrumentation costs are also substantial for many of these assays. Furthermore, influenza test volumes are fairly seasonal, and instrumentation dedicated to only influenza testing will likely sit idle for many months of the year. Most of the testing platforms discussed here have limited test menus that may not have assays with sufficient volumes to be performed year-round. Conversely, instruments with appropriate demand throughout the year may be unable to handle the increased volume associated with influenza season.

The GeneXpert, Focus Diagnostics, and LIAT assays all rely on real-time PCR, and the degree of multiplexing available on these platforms is intrinsically limited to the ability of their instruments to detect multiple fluorophores. Changing the viruses detected is possible (eg, RSV instead of 2009 H1 influenza A), but this would require new regulatory approval. By contrast, the Verigene and FilmArray systems are capable of high-order multiplexing for the simultaneous detection of 15 to 20 respiratory pathogens and/or pathogen subtypes, but throughput can be limiting on these instruments.

FACTORS TO CONSIDER

In selecting an assay, it is essential to identify why testing will be performed and whether multiplexed testing may be appropriate (Table 5). Several studies have been performed to assess the clinical impact of rapid influenza testing, and these

Table 5
Considerations for adoption of rapid respiratory virus testing

Testing Rationale	Focused Influenza Testing	Multiplexed Testing
Limit unnecessary testing	Known pathogen capable of causing severe disease Evidence to support	Theoretically better to detect more pathogens Unclear significance of coronaviruses, rhinoviruses No data to support
Patient cohorting	Need sensitive test May miss other pathogens requiring precautions	More pathogens detected limiting nosocomial spread Detection of coinfections Unclear how to respond to coronaviruses, rhinoviruses
Limit antibiotics	Known pathogen capable of causing severe disease Evidence to support	Theoretically better to detect more pathogens Unclear how to respond to coronaviruses, rhinoviruses No data to support
Targeted anti-influenza therapy	Theoretical benefit Empiric/clinician-guided therapy may be more cost-effective	No benefit

are split between asking 2 related, but different questions: (1) does the immediate availability of a rapid influenza test affect care, or (2) does a positive rapid influenza test affect care?

Several randomized studies compared triage-based protocol testing (ie, test results were available before the patient was seen) with standard care, and, although the results of these trials were mixed, they suggest that the availability of a rapid influenza test can decrease diagnostic evaluation, antibiotic utilization, and both the length and cost of visit in the emergency room.[56–61] Results were most pronounced for individuals testing positive for influenza, but not all studies performed this analysis, and trials were not necessarily powered to make these comparisons. All of these studies looked at only pediatric patients in an emergency room/urgent care setting. A recent meta-analysis of 5 studies recently concluded that RIDT use can reduce some diagnostic testing, but larger adequately powered studies are needed to fully address this issue.[62]

Several nonrandomized studies included adults and inpatients and used chart review to compare individuals testing positive for influenza with those testing negative.[63–67] These studies found decreased antibiotic usage and reduced length of visit/length of stay among individuals testing influenza positive. In one study among hospitalized adults with cardiopulmonary disease, a positive RIDT led to reduced antibiotic use and increased antiviral therapy. However, several influenza-positive adults continued to receive antibiotics, leading the investigators to conclude that better tools are needed to exclude bacterial infections and further reduce antibiotic utilization.[64]

Very few studies assessed whether rapid influenza testing availability or result affected the prescription of antivirals, which is likely due to the number of studies that examined only children. Two studies showed that antiviral prescriptions were appropriately increased among patients testing positive for influenza.[59,64] No studies examined the impact of rapid influenza testing on bed-management decisions beyond admission to hospital or discharge from the emergency department in a systematic manner.

All of the previously cited studies used either an RIDT or a 7-virus DFA for rapid testing, and studies of molecular tests are limited. Oosterheert and colleagues[68] examined the contribution of a PCR panel for viral and atypical bacterial pathogens on hospitalized adults, and although PCR increased the diagnostic yield and cost of care, there was no difference in antibiotic utilization for patients with available PCR results. The clinical impact of the BioFire FilmArray has been reported in one study that found decreased TAT compared with previous years, and timely prescription of oseltamivir.[69]

Many of the arguments put forth to rationalize rapid influenza testing also hold true for highly multiplexed assays, but this has not been studied in depth. Testing specifically for adenovirus by DFA affected the differential diagnosis, diagnostic evaluation, and management of hospitalized children.[70] Byington and colleagues[65] found that results of RSV testing in a 7-virus DFA also affect patient care. For well-characterized respiratory pathogens, identification of a potentially causative agent could be beneficial, but sensitive multiplex assays lead to higher rates of identification of coinfection and infection with agents associated with the common cold such as rhinovirus or human coronaviruses. In the former cases, it can be difficult to determine the virus responsible for the patient's current presentation. Rhinoviruses and coronaviruses can cause more severe disease, especially in immunocompromised hosts, yet may be disregarded as normal flora by some providers. Alternatively, symptoms may be ascribed to these viruses when other processes may be contributing to disease.

SUMMARY

It is now possible to identify infection with influenza and other respiratory viruses with high sensitivity in as little 1 hour. Manufacturers are also developing more advanced point-of-care IC assays that seek to minimize the known limitations of many RIDTs. Thus, laboratories and institutions have a wide variety of assays and platforms from which to choose when implementing rapid influenza testing.

The best way to clinically implement these assays remains unclear, and many different factors must be considered when choosing an optimal testing algorithm. The use, interpretation, and impact of rapid respiratory virus assays vary among children, adults, outpatients, inpatients, the immunosuppressed, and so forth. The patient population served is among the most important considerations when deciding whether to test, and clinician guidance and education are needed if optimal interventions are to occur. To help guide both laboratory and provider decision making, studies are urgently needed to determine the clinical utility, impact on outcomes, and cost-effectiveness of rapid antigen and NAATs for influenza and other respiratory viruses in different patient groups and clinical settings.

REFERENCES

1. Altmar RL, Lindstrom SE. Influenza viruses. In: Versalovic J, Carroll KC, Funke G, et al, editors. Manual of clinical microbiology. 10th edition. Washington, DC: ASM Press; 2011. p. 1333–46.
2. Yu H, Cowling BJ, Feng L, et al. Human infection with avian influenza A H7N9 virus: an assessment of clinical severity. Lancet 2013;382(9887): 138–45.
3. Hayden FG, Palese P. Influenza virus. In: Richman DD, Whitley RJ, Hayden FG, editors. Clinical virology. Washington, DC: ASM Press; 2009. p. 943–76.
4. Centers for Disease Control and Prevention (CDC). Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices—United States, 2013-2014. MMWR Recomm Rep 2013;62(RR-07):1–43.
5. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children—diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin Infect Dis 2009;48(8):1003–32.
6. Ginocchio CC, McAdam AJ. Current best practices for respiratory virus testing. J Clin Microbiol 2011;49(Suppl 9):S44–8.
7. Debyle C, Bulkow L, Miernyk K, et al. Comparison of nasopharyngeal flocked swabs and nasopharyngeal wash collection methods for respiratory virus detection in hospitalized children using real-time polymerase chain reaction. J Virol Methods 2012;185(1):89–93.
8. Fong CK, Lee MK, Griffith BP. Evaluation of R-Mix FreshCells in shell vials for detection of respiratory viruses. J Clin Microbiol 2000;38(12):4660–2.
9. Huang YT, Turchek BM. Mink lung cells and mixed mink lung and A549 cells for rapid detection of influenza virus and other respiratory viruses. J Clin Microbiol 2000;38(1):422–3.
10. Weinberg A, Brewster L, Clark J, et al. Evaluation of R-Mix shell vials for the diagnosis of viral respiratory tract infections. J Clin Virol 2004;30(1): 100–5.
11. Leland DS, Ginocchio CC. Role of cell culture for virus detection in the age of technology. Clin Microbiol Rev 2007;20(1):49–78.

12. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol 2009;45(3):191–5.

13. Centers for Disease Control and Prevention. Performance of rapid influenza diagnostic tests during two school outbreaks of 2009 pandemic influenza A (H1N1) virus infection—Connecticut, 2009. MMWR Morb Mortal Wkly Rep 2009;58(37):1029–32.

14. Landry ML, Ferguson D. Cytospin-enhanced immunofluorescence and impact of sample quality on detection of novel swine origin (H1N1) influenza virus. J Clin Microbiol 2010;48(3):957–9.

15. Doing KM, Jerkofsky MA, Dow EG, et al. Use of fluorescent-antibody staining of cytocentrifuge-prepared smears in combination with cell culture for direct detection of respiratory viruses. J Clin Microbiol 1998;36(7):2112–4.

16. Landry ML, Ferguson D. SimulFluor respiratory screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. J Clin Microbiol 2000;38(2):708–11.

17. Chan EL, Brandt K, Horsman GB. Comparison of Chemicon SimulFluor direct fluorescent antibody staining with cell culture and shell vial direct immunoperoxidase staining for detection of herpes simplex virus and with cytospin direct immunofluorescence staining for detection of varicella-zoster virus. Clin Diagn Lab Immunol 2001;8(5):909–12.

18. Landry ML. Developments in immunologic assays for respiratory viruses. Clin Lab Med 2009;29(4):635–47.

19. Centers for Disease Control and Prevention. Evaluation of 11 commercially available rapid influenza diagnostic tests—United States, 2011-2012. MMWR Morb Mortal Wkly Rep 2012;61(43):873–6.

20. Balish A, Garten R, Klimov A, et al. Analytical detection of influenza A(H3N2)v and other A variant viruses from the USA by rapid influenza diagnostic tests. Influenza Other Respir Viruses 2013;7(4):491–6.

21. Babin SM, Hsieh YH, Rothman RE, et al. A meta-analysis of point-of-care laboratory tests in the diagnosis of novel 2009 swine-lineage pandemic influenza A (H1N1). Diagn Microbiol Infect Dis 2011;69(4):410–8.

22. Chu H, Lofgren ET, Halloran ME, et al. Performance of rapid influenza H1N1 diagnostic tests: a meta-analysis. Influenza Other Respir Viruses 2012;6(2):80–6.

23. Chartrand C, Leeflang MM, Minion J, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. Ann Intern Med 2012;156(7):500–11.

24. Likitnukul S, Boonsiri K, Tangsuksant Y. Evaluation of sensitivity and specificity of rapid influenza diagnostic tests for novel swine-origin influenza A (H1N1) virus. Pediatr Infect Dis J 2009;28(11):1038–9.

25. Sambol AR, Abdalhamid B, Lyden ER, et al. Use of rapid influenza diagnostic tests under field conditions as a screening tool during an outbreak of the 2009 novel influenza virus: practical considerations. J Clin Virol 2010;47(3):229–33.

26. Lee CK, Cho CH, Woo MK, et al. Evaluation of Sofia fluorescent immunoassay analyzer for influenza A/B virus. J Clin Virol 2012;55(3):239–43.

27. Lewandrowski K, Tamerius J, Menegus M, et al. Detection of influenza A and B viruses with the Sofia analyzer: a novel, rapid immunofluorescence-based in vitro diagnostic device. Am J Clin Pathol 2013;139(5):684–9.

28. Rath B, Tief F, Obermeier P, et al. Early detection of influenza A and B infection in infants and children using conventional and fluorescence-based rapid testing. J Clin Virol 2012;55(4):329–33.

29. Leonardi GP, Wilson AM, Zuretti AR. Comparison of conventional lateral-flow assays and a new fluorescent immunoassay to detect influenza viruses. J Virol Methods 2013;189(2):379–82.
30. Peters TR, Blakeney E, Vannoy L, et al. Evaluation of the limit of detection of the BD Veritor™ system flu A+B test and two rapid influenza detection tests for influenza virus. Diagn Microbiol Infect Dis 2013;75(2):200–2.
31. Ginocchio CC, Lotlikar M, Falk L, et al. Clinical performance of the 3M Rapid Detection Flu A+B Test compared to R-Mix culture, DFA and BinaxNOW Influenza A&B test. J Clin Virol 2009;45(2):146–9.
32. Dale SE, Mayer C, Mayer MC, et al. Analytical and clinical sensitivity of the 3M rapid detection influenza A+B assay. J Clin Microbiol 2008;46(11):3804–7.
33. Buller RS. Molecular detection of respiratory viruses. Clin Lab Med 2013;33(3):439–60.
34. Salez N, Ninove L, Thirion L, et al. Evaluation of the Xpert Flu test and comparison with in-house real-time RT-PCR assays for detection of influenza virus from 2008 to 2011 in Marseille, France. Clin Microbiol Infect 2012;18(4):E81–3.
35. Hammond SP, Gagne LS, Stock SR, et al. Respiratory virus detection in immunocompromised patients with FilmArray respiratory panel compared to conventional methods. J Clin Microbiol 2012;50(10):3216–21.
36. Renaud C, Crowley J, Jerome KR, et al. Comparison of FilmArray Respiratory Panel and laboratory-developed real-time reverse transcription-polymerase chain reaction assays for respiratory virus detection. Diagn Microbiol Infect Dis 2012;74(4):379–83.
37. Couturier MR, Barney T, Alger G, et al. Evaluation of the FilmArray® Respiratory Panel for clinical use in a large children's hospital. J Clin Lab Anal 2013;27(2):148–54.
38. Popowitch EB, O'Neill SS, Miller MB. Comparison of the Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP fast multiplex assays for detection of respiratory viruses. J Clin Microbiol 2013;51(5):1528–33.
39. Van Wesenbeeck L, Meeuws H, Van Immerseel A, et al. Comparison of the FilmArray RP, Verigene RV+, and Prodesse ProFLU+/FAST+ multiplex platforms for detection of influenza viruses in clinical samples from the 2011-2012 influenza season in Belgium. J Clin Microbiol 2013;51(9):2977–85.
40. Legoff J, Kara R, Moulin F, et al. Evaluation of the one-step multiplex real-time reverse transcription-PCR ProFlu-1 assay for detection of influenza A and influenza B viruses and respiratory syncytial viruses in children. J Clin Microbiol 2008;46(2):789–91.
41. Liao RS, Tomalty LL, Majury A, et al. Comparison of viral isolation and multiplex real-time reverse transcription-PCR for confirmation of respiratory syncytial virus and influenza virus detection by antigen immunoassays. J Clin Microbiol 2009;47(3):527–32.
42. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 2011;49(7):2449–53.
43. Loeffelholz MJ, Pong DL, Pyles RB, et al. Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens. J Clin Microbiol 2011;49(12):4083–8.
44. Pierce VM, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. J Clin Microbiol 2012;50(2):364–71.

45. Hayden RT, Gu Z, Rodriguez A, et al. Comparison of two broadly multiplexed PCR systems for viral detection in clinical respiratory tract specimens from immunocompromised children. J Clin Virol 2012;53(4):308–13.

46. Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP assay for detection of respiratory viruses in pediatric patients at a cancer hospital. J Clin Microbiol 2012;50(7): 2282–8.

47. Sambol AR, Iwen PC, Pieretti M, et al. Validation of the Cepheid Xpert Flu A real time RT-PCR detection panel for emergency use authorization. J Clin Virol 2010; 48(4):234–8.

48. Popowitch EB, Rogers E, Miller MB. Retrospective and prospective verification of the Cepheid Xpert influenza virus assay. J Clin Microbiol 2011;49(9):3368–9.

49. Novak-Weekley SM, Marlowe EM, Poulter M, et al. Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. J Clin Microbiol 2012;50(5): 1704–10.

50. Li M, Brenwald N, Bonigal S, et al. Rapid diagnosis of influenza: an evaluation of two commercially available RT-PCR assays. J Infect 2012;65(1):60–3.

51. Alby K, Popowitch EB, Miller MB. Comparative evaluation of the Nanosphere Verigene RV+ assay and the Simplexa Flu A/B & RSV Kit for detection of influenza and respiratory syncytial viruses. J Clin Microbiol 2013;51(1):352–3.

52. Boku S, Naito T, Murai K, et al. Near point-of-care administration by the attending physician of the rapid influenza antigen detection immunochromatography test and the fully automated respiratory virus nucleic acid test: contribution to patient management. Diagn Microbiol Infect Dis 2013;76(4):445–9.

53. Woodberry MW, Shankar R, Cent A, et al. Comparison of the Simplexa FluA/B & RSV direct assay and laboratory-developed real-time PCR assays for detection of respiratory virus. J Clin Microbiol 2013;51(11):3883–5.

54. Ko SY, Jang JW, Song DJ, et al. Evaluation of the Simplexa Flu A/B and RSV test for the rapid detection of influenza viruses. J Med Virol 2013;85(12): 2160–4.

55. Hindiyeh M, Kolet L, Meningher T, et al. Evaluation of Simplexa™ Flu A/B & RSV for the direct detection of influenza viruses (A, B) and respiratory syncytial virus in patient clinical samples. J Clin Microbiol 2013;51(7):2421–4.

56. Poehling KA, Zhu Y, Tang YW, et al. Accuracy and impact of a point-of-care rapid influenza test in young children with respiratory illnesses. Arch Pediatr Adolesc Med 2006;160(7):713–8.

57. Abanses JC, Dowd MD, Simon SD, et al. Impact of rapid influenza testing at triage on management of febrile infants and young children. Pediatr Emerg Care 2006;22(3):145–9.

58. Doan QH, Kissoon N, Dobson S, et al. A randomized, controlled trial of the impact of early and rapid diagnosis of viral infections in children brought to an emergency department with febrile respiratory tract illnesses. J Pediatr 2009;154(1):91–5.

59. Bonner AB, Monroe KW, Talley LI, et al. Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. Pediatrics 2003;112(2):363–7.

60. Iyer SB, Gerber MA, Pomerantz WJ, et al. Effect of point-of-care influenza testing on management of febrile children. Acad Emerg Med 2006;13(12): 1259–68.

61. Esposito S, Marchisio P, Morelli P, et al. Effect of a rapid influenza diagnosis. Arch Dis Child 2003;88(6):525–6.
62. Doan Q, Enarson P, Kissoon N, et al. Rapid viral diagnosis for acute febrile respiratory illness in children in the Emergency Department. Cochrane Database Syst Rev 2012;(5):CD006452.
63. Benito-Fernández J, Vázquez-Ronco MA, Morteruel-Aizkuren E, et al. Impact of rapid viral testing for influenza A and B viruses on management of febrile infants without signs of focal infection. Pediatr Infect Dis J 2006;25(12):1153–7.
64. Falsey AR, Murata Y, Walsh EE. Impact of rapid diagnosis on management of adults hospitalized with influenza. Arch Intern Med 2007;167(4):354–60.
65. Byington CL, Castillo H, Gerber K, et al. The effect of rapid respiratory viral diagnostic testing on antibiotic use in a children's hospital. Arch Pediatr Adolesc Med 2002;156(12):1230–4.
66. Mintegi S, Garcia-Garcia JJ, Benito J, et al. Rapid influenza test in young febrile infants for the identification of low-risk patients. Pediatr Infect Dis J 2009;28(11): 1026–8.
67. Barenfanger J, Drake C, Leon N, et al. Clinical and financial benefits of rapid detection of respiratory viruses: an outcomes study. J Clin Microbiol 2000; 38(8):2824–8.
68. Oosterheert JJ, van Loon AM, Schuurman R, et al. Impact of rapid detection of viral and atypical bacterial pathogens by real-time polymerase chain reaction for patients with lower respiratory tract infection. Clin Infect Dis 2005;41(10): 1438–44.
69. Xu M, Qin X, Astion ML, et al. Implementation of FilmArray respiratory viral panel in a core laboratory improves testing turnaround time and patient care. Am J Clin Pathol 2013;139(1):118–23.
70. Rocholl C, Gerber K, Daly J, et al. Adenoviral infections in children: the impact of rapid diagnosis. Pediatrics 2004;113(1 Pt 1):e51–6.