



Diagnostic tests

Rapid tests for the diagnosis of influenza

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Summary

Diagnosing influenza clinically is often difficult because of the variability of symptoms and the numerous other causes of 'influenza-like illness'. An accurate result from an influenza test performed at the bedside, or within hours of presentation, may assist in diagnosis and patient management. Rapid influenza tests based on viral antigen detection with point-of-care tests and immunofluorescence may be useful for primary care clinicians. However, it is important to know how to use these tests and to understand their limitations.

Key words: antigen detection, immunofluorescence, point-of-care testing.

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Introduction

Influenza is a contagious acute self-limiting infection caused by influenza A and B viruses. It is classically characterised by an abrupt onset of systemic symptoms, with fever, chills, headache, myalgia, malaise and anorexia, in addition to respiratory symptoms such as cough, pharyngitis and rhinorrhoea.

A reliable clinical diagnosis of influenza can be difficult, due to the variability of its presentation. There is also a multitude of other respiratory viruses in both children and adults which may cause a similar constellation of symptoms. Rapid diagnostic tests may assist the clinician to make a definitive diagnosis of influenza. Prompt diagnosis is important because antiviral therapy is most efficacious when commenced in the first 48 hours of illness. Furthermore, unnecessary investigations and antibacterial therapy (with the possible ramifications of increased antimicrobial resistance) may be avoided. Rapid diagnosis will also allow the early recognition of outbreaks in 'closed' environments such as nursing homes and schools.

Diagnosis

In patients presenting with cough and fever, testing for influenza is indicated when the clinical diagnosis is unclear, if antiviral therapy is a consideration, and in cases of suspected pandemic

influenza. A rapid laboratory diagnosis of influenza can be made by detection of influenza viral antigen or nucleic acid in respiratory tract samples (Table 1). Alternative laboratory methods include influenza viral isolation, which may take up to a week, and serological detection of influenza antibodies, which may take several weeks.

The choice of test depends on factors such as the duration of symptoms, prevalence of influenza in the community, the clinical setting and proximity to a laboratory.

Specimen collection

The type and quality of the specimen as well as the timing of its collection are all factors which may significantly affect the sensitivity of a test. Nasopharyngeal aspirates in young children and paired nasal and throat swabs (Fig. 1) in adults using specialised viral swabs are the most practical specimens to collect. Nasal washes and nasopharyngeal swabs are also appropriate. A good quality respiratory tract specimen is particularly important for rapid antigen detection tests, which rely on the presence of adequate numbers of infected respiratory epithelial cells.

Viral shedding peaks in the first 48–72 hours of illness, thus the sensitivity is greatest for specimens collected within this time period.

After collection, respiratory tract specimens should be transported to the laboratory promptly at 4°C.

Rapid antigen detection tests

These may take the form of 'point-of-care' tests or immunofluorescence assays.

Point-of-care tests

Point-of-care tests are usually immunochromatographic assays involving monoclonal antibodies directed against influenza A and B nucleoprotein or other conserved antigens impregnated on a strip or bound to a membrane.

The respiratory tract specimen is initially treated with an extraction buffer and then applied to either a filter paper or dipstick, depending on the test format. If influenza viral antigens are present, they react with the influenza-specific monoclonal antibodies which produces a visible colour change. Most kits

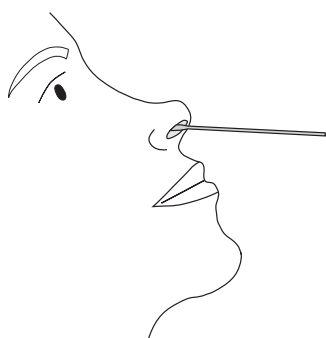
Table 1

Rapid tests for influenza ^{1,3}

Test	Turnaround time	Sensitivity	Specificity	Advantages	Disadvantages
Point-of-care test	15–30 minutes	59–93%	76–100%	<ul style="list-style-type: none"> Bedside test Fast Easy to perform No laboratory required 	<ul style="list-style-type: none"> Occasional false positives Limited kit shelf-life Lower sensitivity No viral isolate for vaccine studies Subtyping not possible
Immunofluorescence assays	2–4 hours	70–90%	More than 90%	<ul style="list-style-type: none"> Fast Assessment of specimen quality Inclusion of other respiratory viruses Swab can be used for virus isolation Subtyping of influenza A possible 	<ul style="list-style-type: none"> Labour intensive Laboratory and technical expertise required Less sensitive than nucleic acid tests
Nucleic acid test	24–48 hours	99%	99%	<ul style="list-style-type: none"> Highly sensitive Specimen quality less crucial Viable and non-viable virus detected Typing and subtyping of virus possible Batch testing possible 	<ul style="list-style-type: none"> High infrastructure requirements Expensive May be affected by viral genetic drift

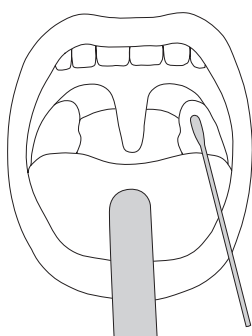
Fig. 1

Collecting specimens from the nose and throat



Nasal swab

1. Tilt patient's head back gently and steady the chin
2. Insert sterile swab into nostril and rub firmly against the turbinate (to ensure swab contains cells as well as mucus)
3. Insert swab into collection tube, break off shaft of swab and recap tube



Throat swab

1. Ask patient to open mouth and stick their tongue out
2. Use tongue spatula to press the tongue downward to floor of the mouth
3. Swab the posterior pharynx and the tonsillar area on both sides, without touching the sides of the mouth
4. Insert swab into same collection tube containing nose swabs, break off shaft and recap tube

distinguish between influenza A and B viruses, but do not allow further subtyping.

The point-of-care tests are generally simple to perform and interpret, and results are available within 15–30 minutes. For optimal results, some training is desirable in collecting respiratory specimens and performing point-of-care tests. As these tests can be performed outside of a laboratory setting they may have a role in doctors' surgeries and emergency departments, remote settings, or in outbreak situations where a rapid test result can significantly impact on clinical decision making.

The sensitivity of point-of-care tests is about 70% (59–93%)¹ depending on the test kit, the age of the patient (young children tend to shed higher viral titres for longer periods of time) and the timing of specimen collection (maximal sensitivity is achieved in early illness and falls significantly after day five of illness). The sensitivity of point-of-care tests is higher with influenza A compared to influenza B, and limited data suggest that they have reduced sensitivity for human cases of influenza A H5N1 infection (avian influenza). The specificity of point-of-care tests ranges from 76% to 100%.²

Point-of-care tests are most useful during the influenza season when the prevalence of influenza in the community is high, and the positive predictive value of the test is greatest.³ A positive test result in this context is highly suggestive of influenza infection. Patients with suspected influenza who have negative point-of-care tests during the influenza season should undergo further testing with more sensitive methods. During periods of low influenza activity, point-of-care tests have a low positive predictive value, and a false positive result is more likely.³ These tests are therefore recommended only during periods of high influenza activity.

The main drawbacks of point-of-care test kits are their expense and limited shelf-life (1–2 years). Poor specimen collection technique and misinterpretation of test strips by inexperienced staff can give inaccurate results. They do not provide a live isolate of the influenza strain needed for surveillance and annual vaccine design.

Immunofluorescence assays

These assays are based on the same principle as point-of-care tests (that is, detecting an interaction between viral antigen and specific antibodies) but are performed in a laboratory. Direct immunofluorescence assays involve placing the respiratory tract specimen onto a slide and staining with specific monoclonal antibodies conjugated to a fluorescent dye. Indirect immunofluorescence assays have an additional staining step with a second conjugated antibody, which increases the sensitivity of the test at the expense of an increased turnaround time.³ Slides are examined with a fluorescent microscope to detect nuclear and cytoplasmic fluorescence staining. The

quality of the sample can be assessed by observing the number of respiratory epithelial cells present. A repeat specimen can be collected if a poor quality sample leads to a negative test result.

Influenza immunofluorescence assays have a rapid turnaround time of 2–4 hours. Screening for other respiratory viruses (such as parainfluenza, respiratory syncytial virus and adenovirus) can be performed simultaneously, thereby enabling an alternative diagnosis or detection of viral co-infection. These assays distinguish between influenza A and B viruses. Specific monoclonal antibodies for H1, H3 and H5 viral antigens ('avian' influenza) are available and allow subtyping of influenza A viruses.

The sensitivity of influenza immunofluorescence assays is 70–90% and their specificity is over 90%.¹ Immunofluorescence assays need a specialised laboratory, fluorescent microscope and technical expertise, and are more labour intensive than point-of-care tests. Their use is therefore often restricted to working hours which may delay results.

Nucleic acid tests

There are a variety of commercial and in-house molecular assays for detecting influenza virus nucleic acid, either directly from the clinical specimen or from the viral isolate. Different nucleic acid tests may detect and characterise the influenza virus by type (A or B), usually by targeting the conserved matrix protein, or by subtype, using primers directed against the haemagglutinin or neuraminidase genes. The most common format involves a reverse transcriptase polymerase chain reaction.

After extraction of nucleic acid from the clinical sample, a set of enzyme primers are used to amplify a specific influenza nucleic acid region. A number of different methods exist for subsequent detection of the amplified gene product. A real-time polymerase chain reaction format simultaneously amplifies nucleic acid and detects product, and can significantly reduce turnaround time to 4–6 hours. Some assays can detect a number of different respiratory viruses in addition to influenza A and B.

Nucleic acid tests are the most sensitive diagnostic tests for influenza^{1,3}, with sensitivity and specificity approaching 100%.¹ Due to their high sensitivity and ability to detect both viable and non-viable virus, the quality and timing of specimen collection is less important than with antigen detection techniques. Nucleic acid tests are less labour intensive than immunofluorescence assays because they are automated and large numbers of specimens can be tested simultaneously. Although results can take six hours, transporting the specimen to the laboratory and the need for batch testing within working hours can delay results by 24–48 hours. Nucleic acid tests are also more expensive because technical expertise and specialised equipment are required.

Alternative tests

Viral isolation techniques are available in a limited number of laboratories. Standard influenza viral culture takes several days to a week, although rapid shell-vial viral culture techniques can reduce the turnaround time to 48 hours. Here, the clinical specimen is centrifuged directly onto a cell monolayer, which accelerates infectivity. Specific monoclonal antibodies can detect viral antigen after 24–48 hours. This negates the need to look for cytopathic effects of the virus, which may take up to a week, as in standard viral culture. Culture-based methods provide a viral isolate for surveillance purposes, detailed subtyping, antiviral resistance testing and annual vaccine development.

Serology offers a retrospective diagnosis of influenza, as it relies on detecting a rise in antibody titres between acute (within one week) and convalescent (four weeks) blood samples. Therefore, it is not useful in making an acute diagnosis of influenza.

Conclusion

The public health benefits stemming from a rapid diagnosis of influenza cannot be underestimated. Prompt detection of influenza is important not only for the individual, who may benefit from early commencement of antiviral drugs, but also for the community (including 'closed' environments such as households, nursing homes, schools and military facilities) by reducing transmission of the virus. Outbreaks of influenza may be prevented by treating individuals when they are most contagious, and by considering antiviral prophylaxis for exposed individuals at highest risk of complications from influenza.

References

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3. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. *J Infect Dis* 2006;194 Suppl 2:S98-110.

Further reading

For more information on interpreting diagnostic tests, see Attia J. Diagnostic tests. Moving beyond sensitivity and specificity: using likelihood ratios to help interpret diagnostic tests. *Aust Prescr* 2003;26:111-13.

Professor Dwyer has participated in laboratory evaluations of various commercial 'point-of-care' tests for influenza, and in clinical trials of various anti-influenza drugs.

Self-test questions

The following statements are either true or false (answers on page 87)

1. Viral subtyping is usually possible with point-of-care testing.
2. Nucleic acid tests are the most sensitive test for detecting influenza.



NPS RADAR update

The latest issue of *NPS RADAR* reviews hydromorphone, lanthanum and teriparatide listed on the Pharmaceutical Benefits Scheme on 1 May 2009.

Hydromorphone is a strong opioid that is approximately five times more potent than morphine. The once-daily tablets are available in 8 mg, 16 mg, 32 mg and 64 mg strengths. The 32 mg and 64 mg tablets equate to about 160 mg and 320 mg oral morphine respectively and so would be suitable only for patients who are highly opioid tolerant. *NPS RADAR* reminds prescribers of the risks of toxicity with inappropriate use or accidental overdose.

Lanthanum is a rare earth element that reduces serum phosphate concentration. It is listed as an authority

prescription for adults with chronic kidney disease who are on dialysis.

Teriparatide is a recombinant human parathyroid hormone given as a daily subcutaneous injection using a pre-filled multidose delivery device (pen). Unlike antiresorptive agents, which inhibit bone loss, teriparatide stimulates bone formation. *NPS RADAR* discusses where teriparatide fits among the options for osteoporosis.

For more information about hydromorphone, lanthanum and teriparatide, see the complete reviews on the *NPS RADAR* website (www.npsradar.org.au).

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