HTA REPORT

Rapid (bed-side) tests for influenza

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HTA REPORT

Rapid (bed-side) tests for influenza

Executive summary

One-liner

A quick diagnosis is necessary to identify influenza and target resources. We summarised the available evidence of performance and the impact of rapid tests (RTI).

Background

Influenza imposes a heavy morbidity and mortality burden on society, especially during periods of higher viral circulation. Influenza is difficult to distinguish clinically from other viral acute respiratory infections (influenza-like illness or ILI) and traditionally its diagnosis relied on results of lengthy viral culture or antibody titration in subjects with ILI symptoms. The development of quick diagnostic tests offers the promise of overcoming these hurdles to enable the rational prescribing of antivirals.

Aim

We aimed to assess the potential benefits of the RTI use by GPs in managing influenza appropriately and in epidemiological surveillance. We aimed also to assess the economic impact of RTI introduction in current practice and in particular, the potential savings during influenza seasons, due to identification of influenza A and/or B affected subjects.

Methods

We identified, assessed and synthesised all available evidence of diagnostic accuracy, possible harms, costs and effects of the use of rapid diagnostic tests for influenza.

We ran searches on three databases (PubMed MEDLINE, Embase and Cochrane Library) and included studies published or carried out since 1997, synthesised comparative evidence and single studies on the performance of quick tests. We extracted, quality assessed (using generic and QUADAS instruments) and synthesised the data. We included thirty-nine primary comparative studies and one systematic review. We could not find any economic evaluations and identified but could not retrieve four primary studies. We identified 24 potential studies in Japanese and 1 in Greek for later evaluation. Only nine of the 39 primary studies reached an acceptable level of quality. Nineteen studies (49%) used an appropriate reference standard, fifteen of which provided sufficient information to ensure replicability. Three studies (8%) reported sufficient data on influenza circulation, while only four (10%) assessed index test performance in the correct context (primary care or emergency department). The test performance data could not be aggregated given its low quality, diversity and absence of contextual variables. Sensitivity for the three best quality studies ranged from 85.5% to 88% and specificity from 75% to 100%.

Fourteen studies were sponsored by producers, but this did not influence the results.

We constructed two hypothetical utilisation scenarios describing the possible impact of the use of quick tests on the Italian health service delivery during periods of high and medium influenza circulation. One of these involves the test used by GPs and antiviral treatment (oseltamivir) of patients with influenza A and/or B; in the other symptomatic treatment of all patients with influenza symptoms (only potentially affected by influenza A and/or B) took place.

Results

Our research highlighted that performance of available RTI was similar. Their accuracy needed sometimes to be confirmed by RT-PCR or viral culture. Adverse events or problems of patient acceptability were not reported.

We found no original economic evaluation data on RTI, on their prices in the Italian market, nor disease prevalence in our context. We conducted an economic impact evaluation by simulating the two hypothetical scenarios above described.

In the first scenario the average direct cost per day of illness avoided is around € 183 with the three most used kits in the US, whereas in the second scenario the average direct cost for a day symptom relief of illness is around € 4,4.

Conclusions

Given the poor returns and high costs associated with community use of RTI (even under the most "favourable" conditions of a high viral circulation), we recommend that no publicly funded provision of RTI is made and no further studies on the topic be conducted with public funding.

SINTESI

Introduzione

L'influenza, in accordo con quanto definito dai CDC (Centers for Disease Control and Prevention) americani, è una patologia respiratoria che nel periodo invernale rappresenta una delle cause della cosiddetta sindrome influenzale (ILI – influenza-like illness) e risulta avere un impatto significativo sulla morbidità, sulla mortalità della popolazione e sulla società nel suo complesso.

È una malattia contagiosa causata da virus influenzali di diversa tipologia: i virus A e B che caratterizzano l'infezione epidemica stagionale, e il virus di tipo C che induce solo una lieve affezione respiratoria, ma non assume mai una connotazione epidemica. Nel periodo di media e alta circolazione virale, la frequenza con cui insorgono casi di influenza da virus A e/o B si aggira nella popolazione generale tra il 5-10%, raggiungendo nella fascia d'età 0-14 anni un' incidenza di circa il 15%.

Clinicamente si manifesta con i sintomi tipici della sindrome influenzale: febbre > 38°C, dolori muscolari, raffreddore, tosse, mal di gola e mal di testa.

La condizione clinica rende difficile distinguere l'influenza da virus A e/o B da altre infezioni acute respiratorie di origine virale o batterica. La sua diagnosi certa è basata, di norma, sui risultati (in ordine di affidabilita'):

- dell'isolamento del virus e coltura cellulare
- dei metodi molecolari RT-PCR (Reverse Transcriptase Polymerase Chain Reaction)
- della valutazione del titolo anticorpale (EIA, DFA, IFA)[1] o dell'antigene
- dei test sierologici (ELISA[2], fissazione del complemento).

Tali procedure di laboratorio, che costituiscono, ad oggi, lo standard di riferimento, forniscono risultati in un intervallo di tempo che va da 2 ore a settimane.

Nella sorveglianza epidemiologica è necessario che il medico effettui una diagnosi certa e rapida dell'influenza per adottare appropriate misure terapeutiche e limitare il rischio di diffusione della malattia in tempi ristretti spesso dettati dal tempo di permanenza del paziente a studio.

Nell'ultimo decennio sono stati immessi in commercio in Italia nuovi farmaci con azione antivirale attivi nella chemioprofilassi ed in grado di ridurre la durata della malattia di 30 -36 ore (Jefferson 2006); la loro azione si espleta attraverso l'inibizione della neuroaminidasi con conseguente rallentamento della propagazione del virus. Questi farmaci (Inibitori della Neuroaminidasi - NIs) sono attivi contro i virus influenzali appartenenti sia al tipo A che al tipo B, ma vanno assunti entro le 48 ore dall'insorgenza dei sintomi. L'intervallo di tempo necessario per ottenere risultati dalle attuali diagnosi di laboratorio (da circa 4 ore alle due settimane) limita notevolmente l'uso di tali medicinali.

1 EIA: Enzyme Immuno Assay , DFA: Immunofluorescenza diretta; IFA: Immunofluorescenza indiretta
2 ELISA: Enzyme - Linked Immunosorbent Assay

Per queste ragioni, di recente, sono stati sviluppati e commercializzati numerosi test rapidi per l'influenza (TRI) dei quali la Food and Drug Administration (FDA) ha approvato più di 10 differenti tipologie (tab.n.1 del report). I test, di facile impiego, forniscono il risultato entro 30 minuti, sono effettuati prevalentemente su prelievi con tampone nasale, ma presentano caratteristiche di esecuzione diversificate. Differente è anche l'accuratezza dell' identificazione dei virus A e B con limitata sensibilità (70-75%) ed elevata specificità (90-95%) (Call et al. 2005, WHO, 2005). Inoltre, il livello di circolazione virale tra la popolazione di riferimento influisce sui valori predittivi positivi (PPV) e negativi (NPV), mentre i falsi positivi sono più probabili nei periodi di bassa circolazione virale a differenza dei falsi negativi che prevalgono nei periodi di alta circolazione.

Obiettivo

La produzione di un report italiano di Health Technology Assessment (HTA) è scaturita dalla necessità di fornire ai decisori gli elementi per conoscere se l'utilizzo dei TRI, da parte dei medici di medicina generale (MMG) e/o dei pediatri di libera scelta (PLS), consenta di ottenere una diagnosi dell'influenza certa e rapida, tale da intervenire in maniera appropriata sia dal punto di vista terapeutico che nell'ambito della stessa sorveglianza epidemiologica. Inoltre, in termini di costo-efficacia è importante conoscere quali vantaggi potrebbero essere raggiunti in una politica di contenimento della spesa che deriva dalle epidemie influenzali che si verificano annualmente nel nostro paese.

A tale scopo è stato condotto lo studio sulle caratteristiche dei TRI disponibili e il possibile impatto economico/organizzativo del loro uso in Italia.

Metodi

È stata condotta una revisione sistematica delle evidenze destinata a valutare, in periodi di media ed alta diffusione del virus:

- l'accuratezza diagnostica dei TRI che indicano la presenza/assenza dei virus influenzali entro 30 minuti, identificando, analizzando e sintetizzando le evidenze della loro efficacia;
- le popolazioni target, effettuando una stratificazione delle evidenze per tipologia di popolazione;
- l'analisi dei costi e dell'efficacia dell'uso dei TRI.

La ricerca delle evidenze

Non sono stati rilevati report di HTA né revisioni sistematiche da aggiornare. Un'unica revisione sistematica (Call et al.) si è rivelata carente per le caratteristiche indagate.

In considerazione del fatto che le ricerche bibliografiche non hanno portato alla individuazione di alcuna revisione sistematica sull'accuratezza diagnostica dei TRI, ne è stata effettuata una mediante la valutazione delle evidenze degli studi primari.

La revisione sistematica sull'accuratezza dei TRI si è basata su una ricerca bibliografica, utilizzando parole-chiave (influenza, flu, ILI, influenza rapid test) senza restrizione di lingua ed è stata condotta su tre database (PubMed MEDLINE, Embase, Cochrane Library) a partire dal 1966.

La strategia di ricerca ha individuato 2566 studi potenzialmente utili dei quali, a seguito dell' applicazione dei criteri di inclusione (par. 4.1.2), 1020 sono stati esclusi perché relativi ad animali, 724 dopo verifica del titolo, e 736 con la lettura degli abstracts.

Successivamente, dei rimanenti 86 studi, sono stati esclusi 15 studi perché non pertinenti, 4 in quanto non revisioni sistematiche e 3 privi di valutazioni economiche contestualizzate; in questa fase sono stati trovati 5 studi correlati.

Dei 69 studi residui, 1 è risultato essere una revisione sistematica, 4 non sono stati recuperati, 1 era in greco e 24 erano in lingua giapponese; questi ultimi erano effettuati in un contesto di riferimento non compatibile con quello italiano e, pertanto, si è deciso di non valutarli.

In conclusione, la revisione sistematica è stata effettuata su 39 studi primari (fig.1 del report).

La sintesi delle evidenze

Al fine di avere disponibili tutti gli elementi di valutazione dei metodi diagnostici per l'influenza, sono state riassunte le caratteristiche operative sia degli standard di riferimento (comparatori) (App. 2a del report) che dei test rapidi (App.2b del report).

Per permettere una interpretazione uniforme dei risultati degli studi è stata utilizzata una matrice di estrazione dei dati per le revisioni sistematiche (App. 3 del report) ed una analoga matrice per gli studi singoli (App .4 del report), contenenti anche gli strumenti di valutazione della qualità (Quality Assessment – QA e Quality Assessment of Diagnostic Accuracy Studies - QUADAS).

Le evidenze di ciascuno studio sono state sintetizzate in una tabella strutturata sui punti-cardine della ricerca: stagione influenzale, popolazione target, tipologia di virus, tipo di campione, standard di riferimento, risultati, qualità degli studi.

Sono stati individuati criteri per la classificazione degli studi riguardo alla qualità metodologica (App. 11 del report).

RISULTATI

Non sono stati individuati trial clinici randomizzati, in quanto gli studi sono tutti di tipo comparativo trasversale.

Gli studi sono caratterizzati da un basso livello di qualità metodologica. Il 29% degli studi raggiunge un livello di qualità accettabile, il 49% prevede l'utilizzo di uno standard di riferimento appropriato e il 38% fornisce sufficienti informazioni per assicurare la replicabilità. Dalla valutazione degli studi in cui sono stati utilizzati uno o più TRI è emerso che essi rivelano una sensibilità medio-bassa ed una specificità alta. La sensibilità dipende dalle condizioni di esecuzione del test, dal livello di circolazione virale e dalla variabilità dei pazienti.

Da quanto è emerso, pur con la limitata disponibilità di evidenze, è possibile dedurre che la performance dei diversi TRI disponibili è da definirsi sovrapponibile.

Per quanto concerne la loro accuratezza diagnostica, invece, in presenza di risultati negativi talvolta è stata necessaria una conferma dei risultati con le metodiche standard, quali colture virali o RT-PCR.

Dal punto di vista della sicurezza non sono stati riportati eventi avversi associati al loro impiego in quanto i TRI prevedono procedure non invasive: per questa ragione è da ritenere anche che non vi siano problemi di accettabilità da parte dei pazienti, eccetto un limitato disagio durante il prelievo del campione.

Problemi di compliance possono invece verificarsi con la terapia con antivirali piuttosto che con il trattamento sintomatico.

In 14 studi è stata esplicitamente dichiarata la sponsorizzazione da parte dell'industria attraverso la fornitura gratuita dei kit, ma ciò non ha prodotto differenze di esito tra gli studi.

Non è stato possibile applicare metodiche metanalitiche, aggregando i dati di performance dei test, sia per la limitata qualità, sia per la diversità dei comparatori, sia per la scarsità o assenza di descrizione delle variabili di contesto essenziali a valutare la perfomance dei test (quali la descrizione del livello di circolazione virale nella popolazione di riferimento).

Non sono stati individuati studi con dati originali di valutazione di costo/efficacia dei TRI, dati sui loro prezzi unitari in Italia, né dati epidemiologici di prevalenza. Si è ritenuto di dover comunque sviluppare un ipotetico modello del loro eventuale utilizzo a carico del Servizio Sanitario Nazionale nel contesto italiano.

E' stato costruito uno scenario organizzativo semplice con due differenti percorsi diagnostico-terapeutici: un percorso prevede l'utilizzo da parte dei MMG e dei PLS del test rapido finalizzato al trattamento dei pazienti positivi per Influenza A e/o B con farmaci antivirali (oseltamivir), l'altro, in assenza di test diagnostico, ipotizza il trattamento dei sintomi di tutti i pazienti sintomatici (quindi solo presumibilmente affetti da virus A e/o B) (fig. 6 e tavv. 5-10 del report), prendendo in considerazione solo i costi diretti.

L'analisi che ne deriva, quindi, non può e non intende essere esaustiva.

Discussione

L'apparente copiosa disponibilità di evidenze sui TRI non offre una conseguente buona qualità per quanto attiene i requisiti minimi di interpretazione, di definizione delle caratteristiche operative e di generalizzabilità degli stessi test. Gli studi inclusi sono, nella quasi totalità, carenti di un background epidemiologico di riferimento. Anche quando condotti in maniera prospettica su popolazione selezionata come quella che si rivolge al Pronto Soccorso, forniscono dettagli insufficienti sulla circolazione virale nella comunità di riferimento. Inoltre, sono carenti le stime della prevalenza dell'influenza A e B, come anche sono carenti i criteri di selezione dei pazienti, un' accurata descrizione dei tipi di campioni, le procedure di esecuzione dei test e la loro durata.

Per quanto riguarda le caratteristiche operative, d'altro canto, test destinati per l'uso "bedside", a cura cioè di medici o infermieri che operano in strutture affollate, non dovrebbero richiedere di essere testati da laboratoristi che già effettuano, ogni giorno, numerose procedure diagnostiche.

La classificazione dei TRI è risultata difficoltosa sia per la scarsa qualità dei report sia per la mancanza di chiarezza circa il tipo di virus identificato dai test.

Ciò non è importante se il razionale dell'uso dei TRI è la prescrizione del farmaco antivirale, ma lo è per la sorveglianza, o la prescrizione di farmaci non attivi contro il virus B.

La scelta dei Reference Standard (RS) è sembrata confusa, come inopportuno è parso l'impiego di RS inappropriati o il non aver condotto studi in cieco.

Infine, gli scenari economici ipotizzati mostrano che nel percorso diagnostico-terapeutico che prevede l'utilizzo del TRI il costo medio per giornata libera da malattia è di ca. € 183,00, mentre in quello con diagnosi e trattamento sintomatici il costo per giornata libera da sintomi è di ca. €4,40.

Tenuto conto che l'uso dei TRI è associato ad alti costi per la comunità (anche nella condizione "favorevole" di alta circolazione virale) e della limitatezza delle evidenze si ha il dubbio se condurre o meno studi rigorosi quali Randomised Control Trials (RCT).

Raccomandazioni

Non vi sono evidenze affinché i TRI siano rimborsati a carico del SSN, né affinchè siano condotti ulteriori studi con oneri pubblici.


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1. Background

1.1 Influenza: disease/clinical problems and population

Influenza is a viral infection that affects mainly the nose, throat, bronchi and, occasionally, lungs. Infection usually lasts for about a week, and is characterised by sudden onset of high fever, aching muscles, headache and severe malaise, non-productive cough, sore throat and rhinitis (www.who.int/topics/influenza/en/[1]).

According to the US Centres for Disease Control: "Influenza" (the flu) is a contagious respiratory illness caused by influenza viruses. There are three types of influenza viruses: A, B and C. Influenza A and B viruses cause seasonal epidemics of disease. Influenza type C infections cause a mild respiratory illness and are not thought to cause epidemics[2].

Influenza A viruses are divided into subtypes based on two proteins on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). There are 16 different hemagglutinin subtypes and 9 different neuraminidase subtypes. Influenza A viruses can be further broken down into different strains. The current subtypes of influenza A viruses found in people are A (H1N1) and A (H3N2). Influenza B viruses are not divided into subtypes. Influenza B viruses also can be further broken down into different strains.

Influenza viruses are constantly changing through a process called 'antigenic drift/shift'.

Over the course of a flu season, different types (A & B) and subtypes of influenza A viruses can circulate and cause illness (www.cdc.gov/flu/[2]).

In periods of medium and high viral circulation, influenza-like illness (ILI) imposes a heavy morbidity and mortality burden on society. In Italy, during the winter months, the incidence of ILI can be up to 5-10% of the general population, reaching 15% in the age group 0-14. (www.flu.iss.it[3]). Influenza causes a variable proportion of ILI, but estimates vary from season to season and week to week. ILI are difficult to distinguish from influenza on a clinical basis i.e. without laboratory identification of the causal agent (Call[4]).

There are different options for minimising the burden of influenza. In the last decade a new generation of antivirals (a class known as neuraminidase inhibitors or NIs) have become available and are effective for chemoprophylaxis and treatment of influenza. (Details of effectiveness profile are in Appendix 1).

NIs are specific against influenza, as they act on one of the two key antigens on the viral envelope (neuraminidase) and they are active only if taken within 48 hours of symptoms developing. NIs also have a chemoprophylaxis role. However, they have little effect on other viral acute respiratory infections (ILI). A presumptive diagnosis is then necessary for appropriate use of antivirals. Traditionally a certain diagnosis relied on results of lengthy viral culture or antibody titration in subjects with ILI symptoms. The length of time (often weeks) needed to reach a laboratory diagnosis severely limits the clinical value of NIs and impedes a reliable real-time surveillance system.

This is important as real-time knowledge of locally circulating influenza A or B viruses heightens the clinical index of suspicion and makes clinical diagnosis more accurate (Call[4]). The development of quick diagnostic tests, if reliable, offers the possibility of overcoming these hurdles.

2. Technology, procedures and alternatives

Recently a growing number of quick tests for influenza have been released on the market. The U.S. Food and Drug Administration has approved more than 10 different tests (WHO 2005[6]). Rapid tests for influenza (RTI) provide a result within thirty minutes. Identified RTIs are listed, in alphabetical order of the manufacturer, in Table 1. Further technical information on RTIs is available at Appendix 2b.

Table 1: Rapid Diagnostic Tests for influenza (as at May 2008) (*)

Manufacturer	Device	Virus type detected (***)
Becton Dickinson and Company	Directigen Flu A+B	A+B
Becton Dickinson and Company	Directigen Flu A	A
Becton Dickinson and Company	Directigen EZ Flu A+B	A+B
Binax Inc.	Binax NOW Influenza A & B	A+B
BioStar Inc.	FLU OIA A/B	A/B
Coris BioConcept	Influ-A&B RespiStrip	A+B
Coris BioConcept	Influ-A Respi-Strip	A
Daiichi Pure Chemicals Co.	RapidTesta FLU AB	A+B
Denka Seiken Co. Ltd.	Quick S-Influ A/B "Seiken"	A+B
Fujirebio Corp.	Espline Influenza A&B-N	A+B
Genzyme Diagnostics	OSOM Influenza A&B	A+B
Inverness Medical Inc.	Clearview Exact Influenza A & B	A+B
Inverness Medical Inc.	Clearview Flu A/B	A+B
Meridian Bioscience Inc.	ImmunoCard STAT! Flu A&B	A+B
Quidel Corporation	Quick Vue Influenza A+B	A+B
Quidel Corporation	Quick Vue Influenza Test	A+B
Remel Inc.	Xpect Flu A & B	A+B
Rockeby biomed	Influenza A antigen test	A
SA Scientific Inc.	SAS Influenza A Test	A+B
Tauns Co. Ltd.	Capilia FluA,B	A+B**
ZymeTx Inc.	ZstatFlu Test	A/B

(*) The list may not include all test kits approved by the U.S. Food and Drug Administration
(**) Two devices in the same kit (one for A and one for B virus).
(***): A, the test detects only virus A;
A+B, the test distinguishes between virus A and virus B;
A/B, the test NOT distinguishes between virus A and virus B.
Source: WHO 2005[6], manufacturer website (see Appendix 2b)

RTIs are essentially based on nasopharyngeal swabbing but have different operating characteristics and differ in their accuracy to identify A or B viruses. Their sensitivity and specificity vary

respectively between 70-75% and 90-95% (Call[4], WHO 2005[6]). Moreover the positive and negative predictive values (PPV and NPV) are sensitive to the level of viral circulation in the reference population. False positives are more likely in periods of low viral circulation and false negatives are more likely in periods of high viral circulation (http://www.cdc.gov/flu[2])

No evidence-based documents on the properties of quick tests for influenza have been produced since the systematic review by Call[4] (which did not fully assess accuracy of quick tests), and a narrative WHO document containing a descriptive review of available tests.

2.1 Reference standard - existing procedures

Laboratory diagnosis of influenza virus infection is based on the following methods:

Viral isolation and culture. This is the gold standard as culture confirms the infectivity of the isolated virus. Culture is a highly sensitive method if clinical specimens have been sampled, collected, transmitted and stored correctly. Influenza viruses can be isolated on chicken embrionated eggs (preferentially) or cell culture such as Madin-Darby canine kidney cells (MDCK) and the primary rhesus monkey kidney (pRhMK).

Molecular methods. Polymerase Chain Reaction (PCR) is very sensitive technique for direct detection of the presence of viral genomes even at low concentrations. As the viral genome is made up of a single-strand RNA, a DNA copy (cDNA) must first be created before undertaking PCR. This is known as Reverse Transcriptase-PCR (RT-PCR) which can be carried out using standard methods (endpoint) or real-time.

Serological tests. These may be used to identify recent influenza infections when direct agent identification is not possible. Serological diagnosis is carried out only in cases when no culture is possible and consists of comparing two serum samples, one taken during the acute phase and one in convalescence phase at least 2-3 weeks apart. A fourfold or greater increase of antibody titre is considered diagnostic. Inhibition of haemogglutination is the preferred method. Other techniques include complement fixation, and the enzyme-linked immunosorbent assay (ELISA). Serological tests are the most time consuming and are used to confirm the diagnosis but have no role in the clinical management of influenza.

Direct detection of viral antigen. This is carried out using either immune enzymatic methods such as Enzyme ImmunoAssay (EIA), Direct Fluorescent Antibody tests (DFA) or Indirect Fluorescent Antibody tests (IFA) with commercially available monoclonal antibodies against influenza virus antigen. These are quick and sensitive tests carried out on respiratory epithelial cells that can identify viral types and subtypes.

Table 2 summarises methods and characteristics of reference standards (RS) for influenza diagnosis. See Appendix 2a for further details.

Table 2: Influenza diagnosis. Reference standards (*)

Diagnostic method	Characteristics
Viral isolation	- Embrionated chicken eggs - Cell culture (cellule MDCK, pRhM)
Molecular techniques	- RT-PCR
Antigen identification	- ELISA - DFA or IFA
Serology	- Inhibition of heamoagglutination - Complement fixation - Microneutralisation - ELISA

Abbreviations:
MDCK: Madin-Darby Canine Kidney; pRhM: primary rhesus monkey; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; ELISA: Enzyme-linked immunosorbent assay; EIA: Enzyme ImmunoAssay; DFA: Direct ImmunoFluorescence; IFA: Indirect ImmunoFluorescence
(*) WHO 2002[6] ; U.S. Department of Health & Human Services[7] ; CDC 2002[8]

2.2 Rapid and laboratory procedures for diagnosis of influenza

Rapid tests for the diagnosis of influenza (RTIs) are tests that can be read within 30 minutes from the beginning of the procedure. RTIs have the potential to help clinicians in the diagnosis and management of influenza-like illness by indicating the likelihood that the patient's symptoms may be due to influenza A or B viruses. RTI performance, like that of all tests, is heavily dependent on numerous variables such the setting of the test, viral circulation levels and the type and handling of the specimen collected. Standard laboratory diagnostic procedures such as RT-PCR or serology take a lot longer to perform (from 2 hours to many weeks) and are far more complex multi-step procedures. This complexity may affect their performance and in any case make them unlikely bed-side aids for busy clinicians. The comparison of new Index ("Itndex") tests with existing standard tests ("reference" standards) is the standard way of determining the performance of index tests and must be carried out in contexts and conditions that make their results reliable.

There are many variables that could affect reliability of results. The following table (Table 3) shows the relationship between the ITs assessed in studies, their reference standards and a synthesis of sources of variability between comparators.

Table 3: Relationship between ITs and RS and sources of variability.

Index test	Reference standard	Sources of variability
Rapid Diagnostic Tests for influenza (RTI) More than ten different types approved by the US FDA	**Laboratory tests:** *Virus culture* • Chick embryo eggs • Cell culture (MDCK, pRhM cells) *RT-PCR* *Antigen detection* • ELISA • Direct (DFA) or indirect IFA) Immunofluorescence *Serology* • Hemogglutation inhibition • Complement Fixation • Microneutralization • ELISA	**Each comparison must take into account:** • Viral circulation (high, medium, or low level) • Target population • Test setting (where the test is carried out) • Test conductor (who carries out the test) • Viral type (A and/or B) • Type of specimen • Operating characteristics and recommendations on test procedure (as indicated by the manufacturer) • Operating characteristics of reference tests (e.g. appropriateness and reproducibility).

2.3 Marketing status of RTI in Italy

There are no evidence-based documents offering guidance on the use of quick tests for the Italian NHS. We contacted the Italian Association of Producers and Distributors of Medical Devices (ASSOBIOMEDICA) and obtained a list of potential RTI distributors operating in Italy. We contacted them individually to obtain information on the distribution, costs and types of available RTIs. We have recevied no responses. However, from a series of informal interviews we know that RTIs are not yet widely available in Italy and are mainly used to screen samples in a few laboratories.

3. Report's objectives: policy question and research questions

3.1 Policy questions

Our policy questions were:

- What are the potential benefits of RTI use by GPs in managing influenza disease appropriately and in carrying out epidemiological surveillance?
- What is the economic impact of RTI introduction in current practice?
- What are the potential savings during influenza seasons, due to identification of influenza A and/or B affected subjects?

3.2 Research questions

Our research questions were:

- What are the characteristics of available RTIs?
- What would be the economic and organisational impact of using RTIs in Italy?


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4. Assessing the available evidence

4.1 Methods

4.1.1 Evidence searches

We ran searches on three databases: PubMed MEDLINE, Embase and Cochrane Library (see Appendix 3) using key words as influenza, flu, ILI, influenza rapid test etc.

We searched all identifiable websites of manufacturers, affiliates and marketing companies of influenza rapid tests as well as public health bodies to identify further background or unpublished evidence.

4.1.2 Inclusion criteria

We included all studies published or unpublished carried out on humans from 1997 (the last decade has seen the birth and development of RTIs) in any language presenting evidence of the performance of RTI for the diagnosis of influenza compared to a RS (or gold standard, as defined in the primary studies).

Types of studies

We included systematic reviews (only the most up to date) and single studies either published after the reviews' end of search date or included in the reviews but for which we required additional information not available from the review.

Types of participants

We only included studies on specimens taken during naturally occurring influenza seasons.

Types of intervention

Rapid influenza test with time duration less than or equal to 30 minutes.

Type of comparator

Standard methodologies of laboratory diagnostics.

Types of outcome measures

Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), Likelihood Ratio (LR) of influenza rapid tests.

Clinical

Cases and diagnosis of influenza (A, B, A&B, A+B) in patients with or without symptoms of influenza.

4.1.3 Application of inclusion criteria

We initially identified 2566 potential studies. The application of the inclusion criteria was done in two phases:

1st phase: Using Procite software – ISI ResearchSoft (version 5 Windows 2000/98/95/NT) to manage the bibliography, we excluded all studies published before 1997 including animal studies (1020 studies). Of the 1546 remaining studies, 724 were excluded by reading the title and 736 excluded by reading the abstract.

2nd phase: 86 studies were positively identified and the full text was read. Fifteen of these studies were excluded for various reasons (e.g. not comparative, conducted on animals, not assessing rapid test, 4 were not systematic reviews and 3 were not economic evaluations) (see Appendix 6). In this phase, 5 linked studies were found.

The evidence progression is described and presented in flow chart format (figure 1).

In all, 69 studies were considered for inclusion in our systematic review. With the exception of one systematic review (Call[4]), the rest were all comparative primary studies evaluating the effectiveness of using rapid tests. Of these, 24 were in Japanese and 1 in Greek (Appendix 7). A Japanese reviewer was contacted to extract the information using a data extraction sheet (Appendix 4 and 5). The reviewer described a decision-making context and indications for the use of RTIs which were different from those in any possible Italian context. For this reason we decided to assess Japanese studies at a later date. We were unable to retrieve 4 of the remaining 43 studies (Madej-Pilarczyk[9], Rothberg[10], Schweiger[11], Umeda[12])

The list of included studies in our systematic review is reported at appendix 8.

4.1.4 Evidence synthesis

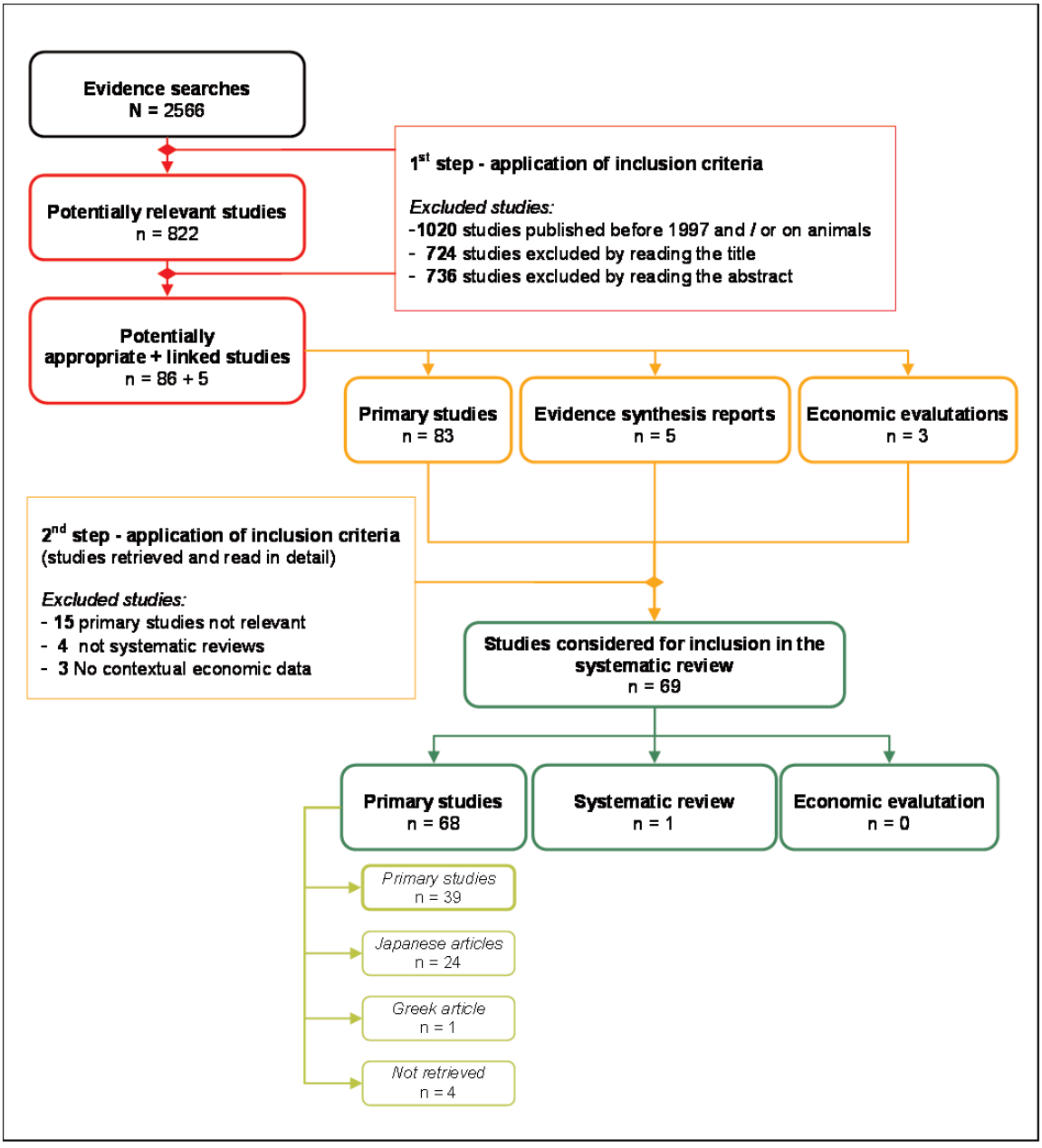
We applied inclusion criteria, extracted data and carried out appraisal of methodological quality in duplicate. We summarised the operating characteristics of each test (Appendix 2b) and comparator (Appendix 2a). We used the data extraction matrix shown in Appendix 4 for systematic reviews and the matrix shown in Appendix 5 for single studies. The evidence has been presented by type of quick test.

4.2 Assessment of diagnostic accuracy

4.2.1 What is the diagnostic accuracy of RTIs?

Our searches did not find published HTA reports or systematic reviewson diagnostic accuracy. The only systematic review is that by Call[4] in which the assessment of the operating characteristics of a RTI is a secondary objective. It briefly describes primary studies that are reported in a table not clearly constructed and showing aggregate data for patients and specimens. In the results section only one primary study is discussed (Rodriguez[13]).

Figure 1. Flow of studies into the review.



We assessed the evidence from primary studies, carrying out our own review of diagnostic accuracy. The table of synthesis of the evidence from primary studies (see Appendix 9) was structured criticality emphasising the following points:

- Influenza season.
- Target population: adult and paediatric population.
- Index test (IT): the performance of the index test is closely linked to contextual variables such as levels of viral circulation, setting and operator experience. These must be reported exhaustively to enable readers to assess test performance.

- Virus type (detected by IT): the type of virus detected must be reported and care taken with comparisons by the potential of the test. Some tests can identify differences between the two types of virus A and B. If the results are not reported by virus we marked it as "Not Reported" (NR).
- Specimen type: in practice there are numerous specimen typologies. Each manufacturer states the ideal typology to use in IT. For eah IT we reported the exact methods used by the testers and compared these to those recommended by the producer. When these were different we annotated it as not acceptable by the manufacturer. We also annotated whether a fresh specimen was taken during the study or whether the authors used thawed specimens (gathered/preserved for other purposes).
- Reference standard (RS): there is a hierarchical order of diagnostic accuracy for laboratory tests (see Appendix 10).
- Results: sensitivity, specificity, PPV, NPV, LR.
- Quality of the study and notes: we assessed the quality using a generic instrument for Quality Assessement (QA) and the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) instrument (see Appendix 5). The answers to the questions of the instruments were annotated as Yes (Y) No (N) or Unclear (UC). We extracted the information as follows: selection criteria (Yes/No/Not Reported/Unclear - Y/N/NR/UC), virus circulation (Y/NR/UC), disaggregate results for specimen (Y/NR/UC), disaggregate results for virus (Y/NR/UC), appropriate RS: (Y/NR/UC), replication of RS (data sufficient/NOT sufficient).

4.2.2 Description of studies

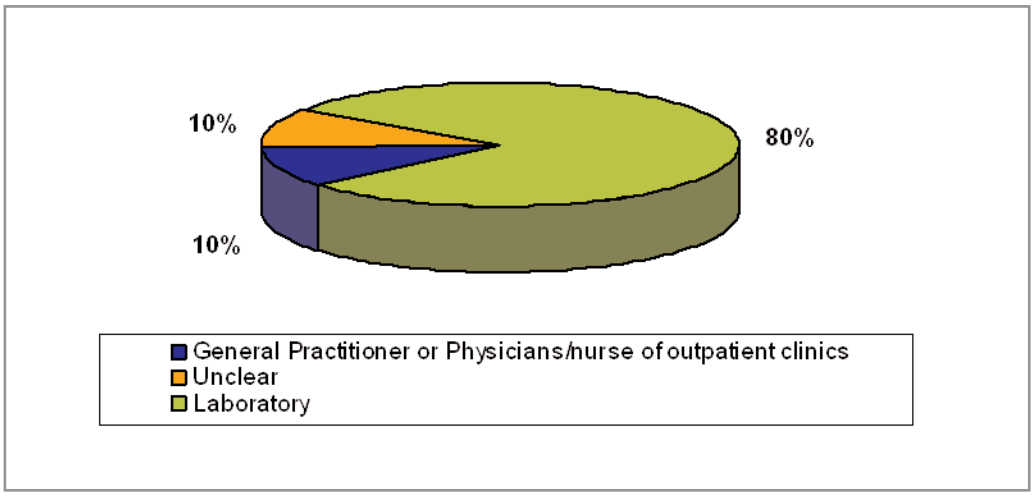
With the exception of the systematic review by Call[4], all studies included in our review were cohort studies in which the performance of one or more index tests were compared with one or more reference standards (RS).

Fifty one percent (20/39) of studies were carried out in the USA (Agoritsas[14], Cazacu[15], Cazacu[16], Covalciuc[17], Cruz[18], Drinka[19], Fader[20], Hamilton[21], Hindiyeh[22], Hulson[23] Landry[24], Landry[25], Magauran[26], Mehlmann[27], Noyola[28], Poehling[29], Rahman[30], Rahman[31], Rodriguez[13], Weinberg[32]), 18% (7/39) in Europe (Grondal[33], Harnden[34], Herrmann[35], Pregliasco[36], Rashid[37], Reina[38], Schultze[39]) and the remainder had been carried out in a variety of different countries.

Thirty six studies (92%) specified the time frame of the study in relation to the influenza season, 3 did not (Herrmann[35], Landry[24], Landry[25]). However, only 8% of studies (3/39) (Cruz[18], Rashid[37], Simmerman[40]) report (with different levels of clarity) information on the epidemiology and viral circulation during the study period. Eighty eight percent of studies do not report any epidemiological information.

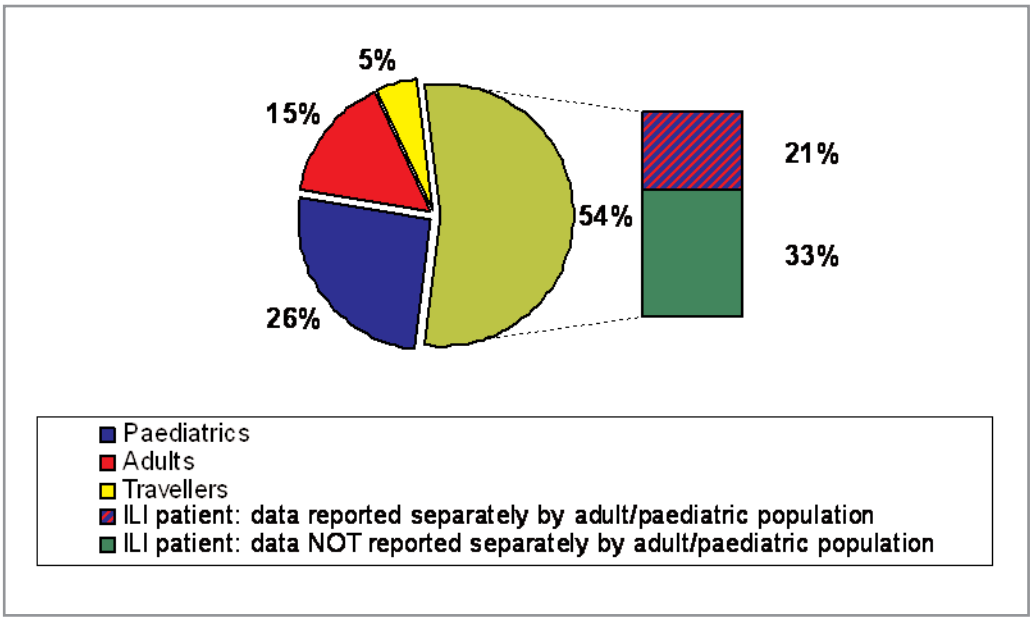
Only ten percent (4/39) of studies report carrying out the rapid tests in a primary care or outpatient clinic setting (Boivin[41], Harnden[34], Pregliasco[36], Simmerman[40]), in another 10% (4/39) the location of execution of the test is either not reported or unclear (Alexander[42], Dunn[43], Hurt[44], Mehlmann[27]). The remaining 80% of studies report carrying out the index test in a laboratory environment (see Figure 2)

Figure 2: Where the test was carried out - *(Total articles: 39)*



Fifty four percent (21/39) of studies carried out tests with samples from paediatric or adult patients with ILI (Boivin[41], Booth[45], Cazacu[15], Covalciuc[17], Cruz[18], Drinka[19], Herrmann[35], Hulson[23], Hurt[44], Landry[24], Magauran[26], Mehlmann[27], Poehling[28], Rahman[30], Rahman[31], Reina[38], Rodriguez[13], Ruest[46], Schultze[39], Smit[47], Weinberg[32]). Only 8 of these studies report data by age group (Cruz[18], Landry[25], Poehling[28], Rahman[30], Rahman[31], Reina[38], Ruest[46], Schultze[39]). Twenty six percent of the studies (10/39) were carried out on children with ILI (Agoritsas[14], Alexander[42], Cazacu[16], Chan[48], Fader[20], Grondal[32], Hamilton[21], Harnden[34], Noyola[28], Pregliasco[36]), while the remaining 20% were carried out on adults (Bellei[49], Landry[24]), on travellers (Rashid[37], Simmerman[40]) or do not report the study population (Dunn[43], Hindiyeh[22], Quach[50]) (see figure 3).

Figure 3: Study population types



Only eight percent of studies report clear inclusion criteria for participants (Boivin[41], Hulson[23], Rashid[37]) while all other studies either do not report criteria (Dunn[43], Fader[20], Hamilton[21], Harnden[34], Hurt[44], Landry[24], Landry[25], Magauran[26]), or do so in an unclear manner.

Twenty eight percent of studies (11/39) report carrying out the study on thawed collections of specimens, taken previously, with different aims, over several influenza seasons (Bellei[49], Boivin[41], Cazacu[15], Cazacu[16], Chan[48], Dunn[43], Hamilton[21], Hulson[23], Hurt[44], Landry[25], Weinberg[32]). In the remainder the specimens were taken ad hoc (fresh specimens) during the influenza season.

Sixty two percent of the FDA-registered RTIs (13/21) were the object of formal diagnostic accuracy assessment studies (see appendix 2b - table 1).

Seventy four percent (28/39) of studies were carried out on a single RTI:

- 9 on QuickVue Influenza A+B (Quidel Corp.) (Agoritsas[14], Bellei[49], Harnden[34], Mehlmann[27], Poehling[28], Pregliasco[36], Quach[50], Rashid[37], Simmerman[40]);
- 7 on Directigen Flu A+B EIA (Becton Dickinson) (Alexander[42], Chan[48], Drinka[19], Grondal[32], Landry[24], Rahman[30], Reina[38]);
- 5 on FLU OIA (BioStar, Inc.) (Boivin[41], Covalciuc[17], Herrmann[35], Hindiyeh[22], Schultze[39]);
- 4 on Binax Now Flu A & Flu B Test (Binax Inc) (Cruz[18], Fader[20], Magauran[26], Rahman[31])
- 1 on ImmunoCard STAT! Flu A and B (Meridian Bioscience INC) (Weitzel[51]);
- 1 on Xpect Flu A/B (Remel Inc.) (Cazacu[15]);
- 1 on ZstatFlu (Zymetx Corp.) (Hulson[23]);

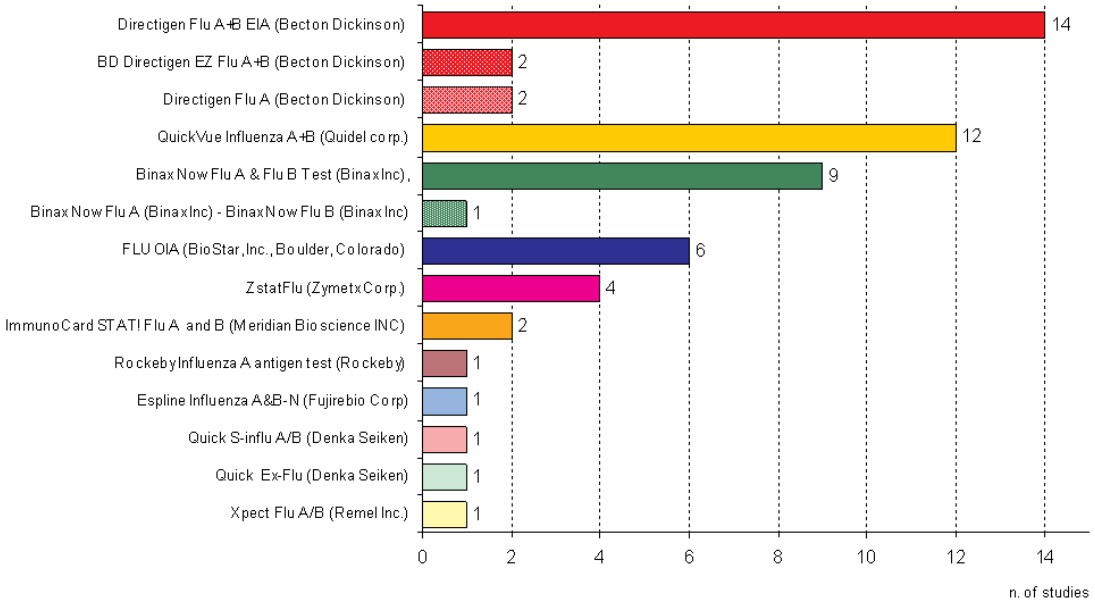
The remaining 11 studies assess more than one test adding Quick S-influ A/B (Denka-Seiken, Espline Influenza A&B-N (Fujirebio, Japan), Directigen EZ Flu A+B (Becton-Dickinson,USA), Influenza A Antigen Test (Rockeby, Singapore), Directigen FluA (Becton-Dickinson), Binax NOW Flu A - Binax NOW Flu B (Binax Inc., Portland, Maine).

The breakdwon of included studies by type of RTI assessed is shown at Figure 4.

Twenty three percent of studies (9/39) report using a specimen type recommended by the manufacturer (Chan[48], Covalciuc[17], Drinka[19], Fader[20], Grondal[32], Harnden[34], Rashid[37], Reina[38], Schultze[39]). In the remainder, specimens used are not mentioned in manufacturers' recommendations.

Only one study reports using a RTI (ZstatFlu - Zymetx Corp.) which does not identify viral type (Hulson[23]), while the tests assessed in the remaining 38 studies either identify viral type (A or B). Result data by viral type are not reported in 47% of the studies(18/38) (Agoritsas[14], Bellei[49], Boivin[41],Covalciuc[17], Cruz[18], Drinka[19], Harnden[34], Herrmann[35], Hindiyeh[22], Magauran[26], Mehlmann[27], Poehling[28], Pregliasco[36], Quach[50], Rahman[30], Rahman[31], Schultze[39], Simmerman[40]) or their identification is only partially reported (6/38 studies) (Cazacu[15], Chan[48], Fader[20], Hamilton[21], Poehling[28], Rodriguez[13]). Thirty seven percent of studies (14/38) report viral type in an exhaustive manner (Alexander[42], Booth[45], Cazacu[16], Dunn[43], Grondal[32], Hurt[44], Landry[24], Landry[25], Rashid[37], Reina[38] , Ruest[46], Smit[47], Weinberg[32], Weitzel[51]).

Figure 4: Number of evaluated studies broken down by rapid test type.



Seventy two percent of studies (28/39) used a single comparator (see Appendix 9) as follows:

Viral culture

A1 type 6/28 (Bellei[49], Chan[48], Covalciuc[17], Landry[25], Rahman[30], Reina[38]);

A2 type 7/28 (Cazacu[15], Cazacu[16], Cruz[18], Fader[20], Hamilton[21], Magauran[26], Noyola[28]);

A3 type 3/28 (Drinka[19], Hulson[23], Rodriguez[13]).

RT-PCR

B3 type 2/28 (Grondal[32], Rashid[37]);

B4 type (Harnden[34]).

Antigen Detection

C type (Landry[24]).

Mixed

A1+A2 type 4/28 (Hurt[44], Pregliasco[36], Quach[50], Smit[47]);

A1+B2 type (Weitzel[51]);

A1+C type (Schultze[39]);

A2+B1 type (Poehling[28]);

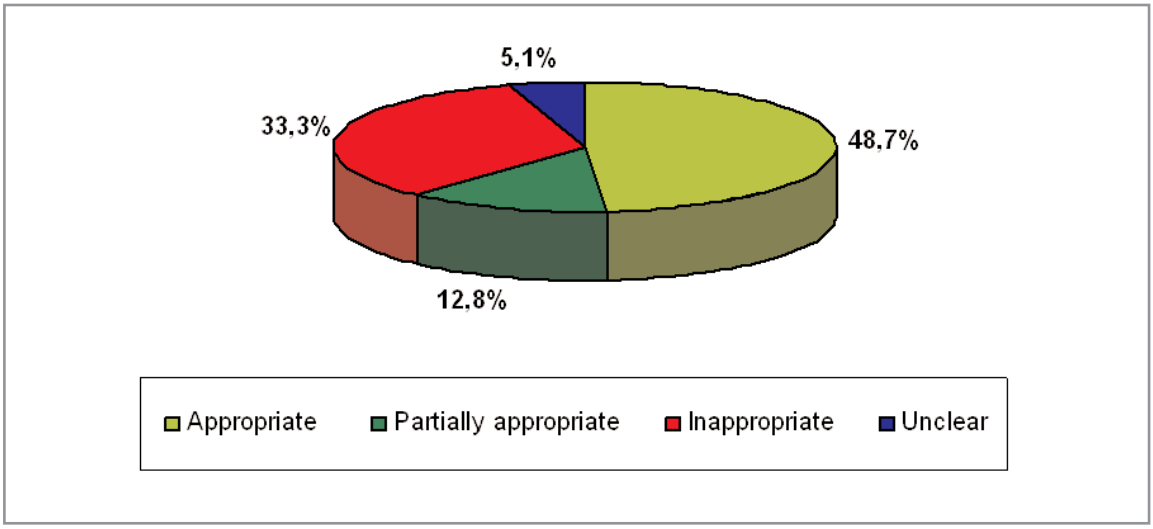
A2+B3 type (Weinberg[32]).

The remaining 28% of studies (11/39) used more than one comparator within the same study reporting disaggregate results by RS type.

The range of multiple comparators per study is 2-4 with a RS combination of 13. We found variable evidence of reproducibility and appropriateness of RS choice as follows (see figure 5).

Forty nine percent of studies (19/39) report using an appropriate RS. Seventy nine percent of studies (15/19) are replicable (Bellei[49], Boivin[41], Chan[48], Covalciuc[17], Grondal[32], Herrmann[35], Hurt[44] Landry[25], Pregliasco[36], Rahman[30], Rahman[31] Rashid[37], Ruest[46], Simmerman[40], Smit[47]) while the remaining (4/19) do not report sufficient data to ensure replication of the test (Harnden[34] Quach[50], Reina[38] Weitzel[51]); thirteen percent of studies (5/39) used a partially appropriate RS, 3 of which (Dunn[43], Hindiyeh[22], Mehlmann[27]) are reproducible while 2/5 do not report sufficient data for the RS to be reproduced (Alexander[42], Booth[45]); thirty three percent of studies (13/39) uses an inappropriate RS, 6 out of which are reproducible (Agoritsas[14], Fader[20], Landry[24], Noyola[28], Schultze[39], Weinberg[32]) and 7 do not report sufficient data to allow test reproduction (Cazacu[15], Cazacu[16], Cruz[18], Drinka[19], Hamilton[21], Magauran[26], Poehling[28]); five percent of studies (2/39) do not report sufficient data to assess the appropriateness of RS and are not reproducible (Hulson[23], Rodriguez[13]).

Figure 5: Breakdown of studies by appropriateness of RS used - (Total articles 39)



In seven studies (most of which used an appropriate RS), the assessment of the comparison between IT and RS could not be interpreted as they were performed on two different specimens from the same person (Boivin[41], Harnden[34], Poehling[28], Pregliasco[36], (only for the first season), Rashid[37], Simmerman[40], Weitzel[51])

Thirty one percent of studies (12/39) reported types of outcome measures within 95% confidence intervals (Cazacu[15], Covalciuc[17], Cruz[18], Harnden[34], Mehlmann[27], Pregliasco[36], Quach[50], Rahman[30], Ruest[46], Schultze[39], Weinberg[32], Weitzel[51]). All other studies do not report inferential data.

4.2.3 Study methodological quality

There were nine good quality studies (Bellei[49], Chan[48], Covalciuc[17], Grondal[32], Hurt[44], Landry[25], Pregliasco[36], Rahman[30], Smit[47]). For further detail on study assessment see Appendix 11.

4.2.4 Description of included studies by rapid test

Table 4 summarises the index tests assessed in each included study. For a detailed description of each included study see Appendix 9.

Table 4: List of included studies by main IT assessed

Rapid test	Study
QuickVue Influenza A+B (Quidel corp.)	Agoritsas 2006, Bellei 2003, Mehlmann 2007, Cazacu 2003, Poehling 2002, Quach 2002, Rashid 2007, Pregliasco 2004, Simmerman, 2006, Ruest 2003, Rodriguez 2002, Hurt 2007
Directigen Flu A+B EIA (Becton Dickinson)	Cazacu 2003 , Hamilton 2002 , Landry, 2004, Landry, 2003, Drinka 2006, Alexander 2005, Chan 2002, Grondal 2005, Smit 2006, Rahman 2007, Ruest 2003, Reina 2002 , Dunn 2003, Weinberg 2005
Directigen Flu A (Becton Dickinson)	Rodriguez, 2002, Noyola 1999
BD Directigen EZ Flu A+B (Becton Dickinson)	Hurt 2007, Weinberg 2005
FLU OIA (BioStar, Inc., Boulder, Colorado)	Rodriguez, 2002, Covalciuc 1999, Hindiyeh 2000, Herrmann 2001, Schulltze 2001, Boivin 2001
Binax Now Flu A & Flu B Test (Binax Inc),	Landry, 2004, Smit 2006, Booth 2006 , Cruz 2006, Rahman 2007 bis, Magauran, 2007, Fader 2005, Hurt 2007, Weinberg 2005
Binax Now Flu A (Binax Inc) - Binax Now Flu B (Binax Inc)	Smit 2006
ImmunoCard STAT! Flu A and B (Meridian Bioscience INC)	Booth 2006 , Weitzel 2007
Xpect Flu A/B (Remel Inc.)	Cazacu 2004
ZstatFlu (Zymetx Corp.)	Noyola 1999, Hamilton 2002 , Rodriguez, 2002, Hulson 2001
Quick Ex-Flu (Denka Seiken)	Hurt 2007
Quick S-influ A/B (Denka Seiken)	Dunn 2003
Espline Influenza A&B-N (Fujirebio Corp)	Hurt 2007
Rockeby Influenza A antigen test (Rockeby)	Hurt 2007

4.3 Systematic review results

4.3.1 Diagnostic accuracy

RTIs overall have on average low sensitivity and high specificity. Sensitivity is however relative to conditions of test execution, the level of viral circulation and patient variables. The included studies were generally of a low level of methodological robustness. Only 9 of the 39 primary studies reached an acceptable level of quality and nineteeen studies (49%) used an appropriate RS.. Fifteen of these provided sufficient information to ensure replicability. Three studies (8%) reported sufficient data on influenza circulation, while only 4 (10%) assessed IT performance in the correct context (primary care or emergency department). We could not aggregate the test performance data because of their low quality, heterogeneity and absence of contextual variables.

4.3.2 Safety

None of the included studies reported harms related to the use of RTIs. However, the apparent medium-low sensitivity of RTIs would be reflected in low NPVs with the creation of many false negatives. For these reasons many of our included studies contained recommendations for the confirmation of the results of RTIs with laboratory methods such as RT - PCR or viral culture.

4.3.3 Patient's acceptability

We do not believe that there would be problems in patient acceptability by administration of a non-invasive test except, perhaps, with discomfort during sample taking. Acceptability issues may be linked mainly to therapy rather than diagnosis, with a refusal to accept antiviral treatment rather than symptomatic treatment. In this case there may be a delicate trade-off between benefit from the reduction of symptoms and the shortening of illness (by 1.14 days) and the risks associated with antiviral use. Acceptability is also influenced by the recommendations of the family doctor and the information that the patients are supplied with.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5. Context specific analysis

5.1 Scenario and Cost Analysis

We encountered many difficulties in finding data on availability of RTIs on the Italian market. Therefore we were unable to calculate RTI costs used to diagnose influenza and identify periods of high viral circulation.

Furthermore we found no economic studies of family doctors, patients and the specific epidemiological context in Italy.

5.1.1 Existing economic evidence

We found no studies with original data evaluating the cost effectiveness of RTs for influenza relevant to our study. We developed a hypothetical organisational scenario for the introduction of RTs within the Italian context.

5.1.2 Assumptions

We constructed a simple scenario (see figure 6) with two different therapeutic pathways for the diagnosis and treatment of influenza.

We considered only the healthy population. We excluded children under 2 years as there are no trials on the effectiveness of antivirals in children. In addition we excluded the elderly population as complications in this age group are difficult to identify by agent, leading to an overestimation of the impact of influenza (Matheson[52]). For the antiviral to be effective, patients need to present themselves to the GP within 48 hours of symptom onset. Our choice of NIs for the scenarios was Oseltamivir (as it is easier to administer than Zanamivir). Oseltamivir should be prescribed after certain diagnosis as it is not effective against influenza-like illness (see Appendix 1).

We constructed two different scenarios comparing the use of RTIs with antivirals or with symptomatic treatment. We considered including a scenario with testing by RT - PCR as the most likely alternative test to RTIs. However given the time frame involved in RT-PCR specimen collection, processing and answer, its high costs (over 300 Euros), the time window for the use of NIs for treatment and the fact that RT-PCR is not a real alternative to RTI as it is carried out in a laboratory and not in GP office, we excluded this scenario as unrealistic. We assigned relevant management pathways (see Table 5) to the remaining two options. The scenarios were constructed on a 1000 hypothetical resident population from which we excluded the elderly (above 65 years) and children (under 2 years) (see Table 6). To estimate cost variables in our scenario, the healthy population was further subdivided into paediatric patients (2 - 14 years) under the care of a primary care paediatrician, and adults (15 - 65 years) under the care of GPs. The costs of oseltamivir therapy were divided by age groups (2 -13 years and 14 years and older).

We did not assess the effect on complications of influenza as these are rare in healthy people. For example a systematic review of the evidence reported a hospitalization rate for influenza ranging from 5.769/100.000 in the 0-5 years age group (denominator 52) to 32/100.000 in people aged up to 16 years (denominator 150.000) (Bueving[53]).

Figure 6: Scenario for diagnosing and treating influenza like illnesses in healthy people aged from 2 -65

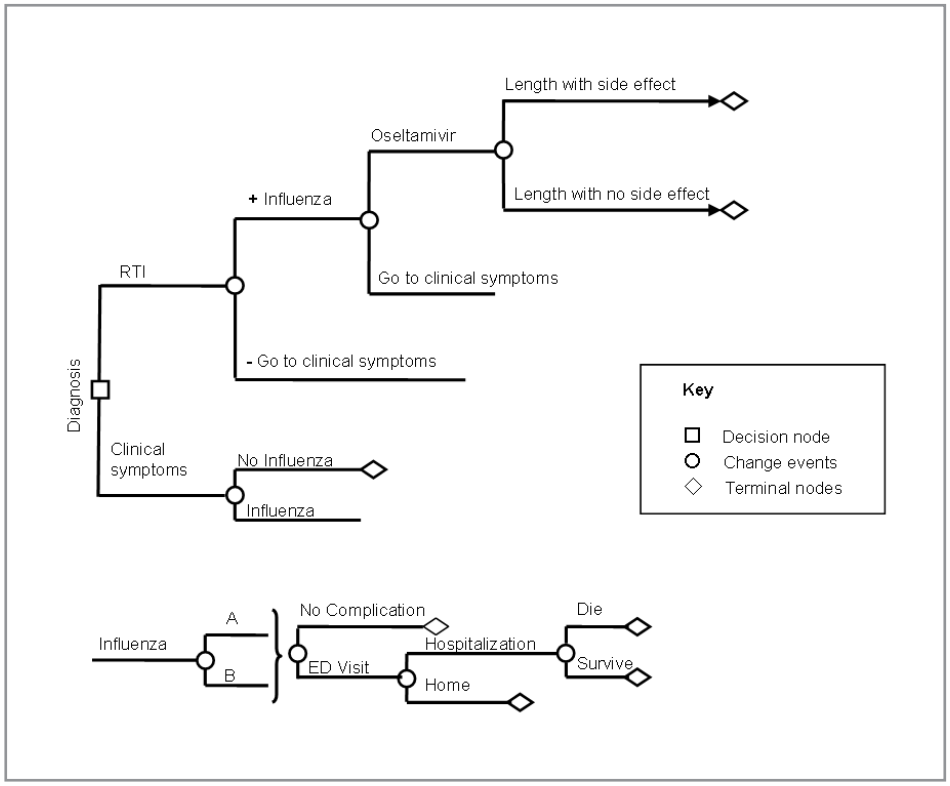

Table 5: Treatment pathway

Treatment pathway	Diagnosis	Treatment 1	Treatment 2
Pathway 1	RTI	Oseltamivir	Symptomatic treatment
Pathway 2	Symptomatic diagnosis	-	Symptomatic treatment

Table 6: Resident population as at 1st January 2007 by age group

Age group (Years)	Population		Population in the scenario* (n)
	N	%	
0-1	1.109.850	1.88	19
2-65	46.868.868	79.26	793*
2-14	7.212.050	12.20	122*
15-65	39.656.818	67.07	671*
66+	11.152.569	18.86	188
TOT.	59.131.287	10.00	1000

Source: based on Italian National Institute of Statistics (ISTAT) data

Diagnosis

Rapid test

We incorporated estimates of performance from tests assessed in the three highest quality studies. These are:

1. Quick Vue Influenza A+B (Quidel Corp) (Bellei 2003[49] – Level of accuracy: I b);
2. Directigen Flu A+B (Becton Dickinson) (Rahman 2007[30] – Level of accuracy: I-b);
3. Flu OIA (Biostar) (Covalciuc 1999[17] - Level of accuracy: I-c)

(see appendices 9 and 11).

Clinical symptoms

We used the WHO definition of ILI (see Introduction)

Treatment

Oseltamivir

The choice of Oseltamivir was made as it is indicated for the use in paediatrics (older than 1 year of age) and adults, whereas Zanamivir (less prescribed) is approved for treatment of influenza in adults and children more than 7 years of age (Moscona[54]). Oseltamivir is prescribed for five days subdivided in different dosage in adults (13+) and children (1-13 years) (see Table 7).

Table 7: Oseltamivir treatment

Age breakdown (years) (*)	Weight (KG)	Dosage	Treatment days
2-3	< 15	Oral suspension powder 30 g	5
4-8	15-23	Oral suspension powder 30 g	5
9-13	24-40	Oral suspension powder 30 g	5
14	> 40	capsule 75 mg	5
15-65	> 40	capsule 75 mg	5

(*) Calculated from the weight/age growth curves and the Italian National Formulary 2007 (AIFA, Agenzia Italiana del Farmaco[59]).

Symptomatic treatment

We considered the use of over the counter remedies as they are most frequently used in ILI cases.

5.2 Costs

5.2.1 Material and methods.

We took into consideration the social perspective of using RTIs in the Italian NHS. We considered unit costs of a visit to the family doctor, the cost of the RTI kit and the cost of a five-day course of antivirals and the cost of symptomatic treatment. We then calculated the costs per day of symptoms relieved by treatment with NIs. We considered non-use of RTIs and symptomatic treatment as standard care. However the costs and effects of this option are not directly comparable with those of option 1 because all trials included in the Cochrane reviews of NIs used placebo as a comparator (with no symptomatic treatments allowed in the protocol). Option 2 however provides a baseline to gauge the magnitude of difference to the INHS of introducing RTIs and a consequent therapy with NIs.

Table 8 shows the types of costs stratified by treatment pathway.

We tested the robustness of our findings by constructing the two treatment pathways, stratified by three hypothetical periods of influenza virus circulation: low (1%) – medium (5%) and high circulation (10%).

To estimate the population likely to have influenza correctly identified by RTI by viral circulation level, we used the steps synthesised at Table 9.

Treatment pathway 1.

The strategies with RTI include the costs of the diagnostic kits and the costs of the visit with administration costs, nurse's time, the cost of the actual RTI strip or reagent and materials (i.e., gloves and equipment used to take the sample). We estimated the costs of RTI by accessing a US distributor website (Table 8) (http://www.fishersci.com[57]) as we had no information on the costs in Italy. In addition we included the costs of antivirals and of symptomatic treatment by payer (the Italian NHS and patient). We further estimated the unit costs of antiviral therapy by dosage and by age group using weight/age growth curves (see Table 7). Symptomatic treatment costs were estimated from the study by Sessa et al.[55] which include over the counter remedies (borne by patients) and antibiotic therapy (borne by the INHS). We then proceeded to convert USD into Euros at an exchange rate of USD 1.6 for 1 Euro (www.borse.it[58] accessed 10th July 2008).

Treatment pathway 2.

The second pathway, symptomatic treatment, includes only physician and treatment costs which are the same as in pathway 1.

5.2.2 Results

The results reported below are stratified by three periods of virus circulation (see Table 10). From the remaining population we calculated the number of patients attending the GP or the family pediatrician. We then applied test sensitivity and specificity data from the three best quality studies included in our review (Bellei[49], Covalciuc[17] and Rahman[30]). From these we derived the number of patients proceeding to the remainder of pathways 1 and 2 and we then calculated the positive and negative likelihood ratios of the patient being infected with influenza viruses. Our calculations of the total costs in 2007are in Euros by type of RTI, by level of viral circulation, by the number of days of illness avoided and by cost per day of illness avoided in our hypothetical situation are in Table 10. As shown, the differences between the tests in terms of cost per day of illness avoided, are minimal and the results are insensitive to differing levels of viral circulation. The cost of symptomatic therapy per natural day of illness (estimated as 5 days) is around 22 Euros for the whole illness.

Table 8: Treatment pathways and direct costs by diagnostic unit

Treatment pathways	Cost /Unit cost (€)
Pathway 1	
Rapid Test	17.37 - 20.92 (a)
Cost/Person-hours	
Physicians office	
Family doctor attendance (we assumed no administrative costs)	12.49 (b)
Organisational cost	
Disposal of RTI, consumables (e.g. gloves -)	0.01 (c)
Oseltamivir Cost (2 – 13 years)	0.714 - 1.428
Oseltamivir Cost (14 – 65 years)	35.70
Cost of symptomatic therapy	
Cost to NHS/ Cost to Patients	9.58 (b)
Pathway 2	
GP/Family paediatrician attendance	12.49 (b)
Cost of symptomatic therapy	
Cost to NHS/ Cost to Patients	9.58 (b)

(a) Fisher HealthCare: (http://www.fishersci.com[57], accessed 10th July 2008)
(b) Sessa A et al.[55] Lo studio 606. L'influenza ai raggi X. Rivista SIMG numero 2. 2002 .
(c) Vasara F et al[56], Screening del cancro del colorettale. Valutazione dei costi. Quaderno n. 9, Gennaio 2005. CPO Piemonte.

Table 9: Estimate of the probability of influenza infection

Population (§)	Sensitivity (a)	Specificity (b)	Estimates					
			LR + (c)	Pre test probability (d)	Pre test odds (e)	Post test odds (f)	Post test probability (g)	Pop with influenza diagnosis (h)
793	As reported by Bellei[48], Covalciuc[17] and Rahman[30]	(a)/(100-(b))	0.0100	0.010101	(c) * (e)	(f)/(1 + (f))	(g) * (§)	
			0.0500	0.052632				
			0.1000	0.111111				

(§) see table 6
(d) According to low (1%), medium (5%) and high (10%) viral circulation
(e) Pre test odds = (d)/(1 - (d))
Key. LR = likelihood ratio

Table 10: Total costs (in 2007 Euros), total day symptoms avoided and cost per day avoided

Technology	Viral circulation								
	High			Medium			Low		
	Total cost	Total day symptoms avoided	Cost per day symptoms avoided	Total cost	Total day symptoms avoided	Cost per day symptoms avoided	Total cost	Total day symptoms avoided	Cost per day symptoms avoided
Rapid test									
Quick Vue	50642,59	275,14	184,0612	28054,46	152,42	184,0602	6141,19	33,36	184,0884
Directigen	99409,87	538,02	184,7698	65711,61	355,64	184,7700	17703,13	95,81	184,7733
Flu OIA	45196,88	248,4	181,9520	24631,99	135,38	181,9470	5308,56	29,18	181,9246
Symptomatic therapy									
	1750,15			875,08			175,02		

5.2.3 Conclusions

Influenza has a variable impact on the health service, reflected in its costs. Our scenarios however were constructed in a simple manner due to the absence of good evidence and the scarcity of data on the RTI costs in Italy. Our analysis for these reasons did not include some of the cost variables such as indirect costs (loss of productivity) which impact directly on absenteeism, or indirectly through forcing parents to stay at home to care for sick children. Despite these caveats we conclude that although our evidence base is small the differences in performance between the tests are minimal from a clinical point of view. It is doubtful whether the costs of RTIs and antiviral therapy and their relative benefit are likely to have a major impact on the management of influenza-like illness.

6. Discussion

We found a plentiful evidence based on RTIs which did not turn out to have the minimum quality requisites for interpretation, definition of the operative characteristics of the RTIs or generalisation. Included studies lacked almost completely an epidemiological reference background. Even when they were carried out prospectively on selected populations such as Emergency Room attenders, insufficient details of viral circulation in the reference community were provided. In addition such vital study design components as patient selection criteria and accurate description of specimen type, methods of test execution, test duration and extraction times were often not reported. The latter are even more important when types of specimens not recommended by the manufacturer are used. Of note was the inappropriate or partially appropriate setting of the majority of studies. We cannot accept that tests which were devised and marketed for bed-side use, i.e. for use by physicians or nurses in busy clinics can be adequately tested by laboratory workers who are used to carry out thousands of such tests every day. Such a finding in our view, further limits the generalisability of our data set. Under these circumstances we thought that carrying out any kind of data pooling would be at best nonsensical and at worst misleading. The only certain point in our analysis was the Cochrane meta-analytical estimates of effect of antivirals, which however do not shed a light on the operating characteristics of RTIs. Fourteen studies were sponsored by the producers, at least in part through the provision of free kits, however we found no obvious differences in quality between industry and non industry-sponsored studies.

We found the classification of RTIs very difficult because of poor quality reporting and lack of clarity as to which influenza viruses the RT could identify. A lack of classification may not be important if the rationale for use of the RT is the prescription of neuraminidase inhibitors. However, if the rationale for the test is viral surveillance, or the reason for carrying out RT is the possible prescription of antivirals witch have no effect against influenza B viruses (adamantanes), then viral type identification is important. This lack of clarity in the aims of the studies is another indication of the fuzzy nature of the studies included. The choice of RS in the included studies also left us confused, as the choice of a RS with variable sensitivity implies variability of study results. Use of a RS with low sensitivity (not recommended by any international agency) provides as consequence a spuriously high sensitivity of the index test. This is especially so for open studies, i.e. studies in which operators had not been blinded. Lack of blinding may results in observer bias.

Our simple scenarios show that the average direct cost per day of illness avoided is around €183,00 whereas a symptom relief for a natural days of illness is around € 4,40 per day.

Given the poor returns and high costs associated with the community use of RTI (even under the most "favourable" conditions of a high viral circulation) we doubt whether carrying out rigorous publicly funded studies such as a randomised controlled trials would yield higher estimates of diagnostic accuracy.


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7. Recommendation

We recommend that RTI should not be used in the community or reimbursed from the public purse and no further studies should be undertaken.


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9. Competing interests declaration

The authors declare that they will not receive either benefits or harms from the publication of this report. None of the authors have or have held shares, consultancies or personal relationships with any of the producers of the devices assessed in this document.


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Glossary

Antibody titer

A measurement of how much antibody an organism has produced that recognizes a particular antigen.

Antigen (or immunogen)

A molecule that stimulates an immune response. Antigens are usually proteins or polysaccharides, include parts of bacteria, viruses, and other micro-organisms (coats, capsules, cell walls, flagella, and toxins).

Cochrane Library (CLIB)

A collection of databases, published on disk, CD-ROM and the Internet and updated quarterly, containing the Cochrane Database of Systematic Reviews, the Cochrane Controlled Trials Register, the Database of Abstracts of Reviews of Effectiveness, the Cochrane Review Methodology Database, and information about the Cochrane Collaboration and other information.

Cochrane Review

A Cochrane Review is a systematic, up-to-date summary of reliable evidence of the benefits and risks of healthcare. Cochrane Reviews are intended to help people make practical decisions. For a review to be called a "Cochrane Review" it must be in the Parent Database maintained by the Cochrane Collaboration. The Parent Database is composed of modules of reviews submitted by Collaborative Review Groups (CRGs) registered with the Cochrane Collaboration. The reviews contributed to one of the modules making up the Parent Database are refereed by the editorial team of the CRG, as described in the CRG module. Reviewers adhere to guidelines published in the Cochrane Reviewers' Handbook.

Gold standard

A gold standard test (or criterion standard test) is a diagnostic test or benchmark that is regarded as definitive. This can refer to diagnosing a disease process, or the criteria by which scientific evidence is evaluated. A hypothetical ideal gold standard test has a sensitivity, or statistical power, of 100% (it identifies all individuals with a disease process; it does not have any false-negative results) and a specificity of 100% (it does not falsely identify someone with a condition that does not have the condition; it does not have any false-positive results). In practice, there are no ideal gold standard tests. As new diagnostic methods become available, the gold standard test may change over time but before widespread acceptance of any new test, the former test retains its status as the gold standard.

Likelihood Ratios (LRs)

Positive Diagnostic Likelihood Ratios

Diagnostic likelihood ratios (DLR), can be a valuable tool for comparing the accuracy of several tests to the gold standard, and they are not dependent upon the prevalence of disease.

The positive PDLR represents the odds ratio that a positive test result will be observed in an infected population compared to the odds that the same result will be observed among a noninfected population.

Negative Diagnostic Likelihood Ratios

The negative NDLR represents the odds ratio that a negative test result will be observed in an infected population compared to the odds that the same result will be observed among a noninfected population.

Monoclonal antibodies

Antibodies that are identical because they are produced by one type of immune cell that are all clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance.

Odds ratio (OR)

Both the odds ratio and the relative risk compare the likelihood of an event between two groups.

Optical depth

A measure of transparency defined as the fraction of radiation (or light) that is scattered or absorbed on a path.

PCR

A polymerase chain reaction is a technique used in molecular biology to exponentially amplify a fragment of DNA by in vitro enzymatic replication. PCR permits amplification of a single or few copies of a piece of DNA.

Positive and Negative Predictive values (PPV and NPV)

The positive predictive value of a test is the probability that the patient has the disease when restricted to those patients who test positive. This term is sometimes abbreviated as PPV.

If the prevalence of the disease in a given situation is different from the prevalence of the disease in a research study under examination, it is possible to use likelihood ratios to estimate the PPV.

The negative predictive value of a test is the probability that the patient will not have the disease when restricted to all patients who test negative.

If the prevalence of the disease in your situation is different from the prevalence of the disease in the research study you are examining, then you can use likelihood ratios to estimate the NPV.

Relative risk (RR)

In statistics and mathematical epidemiology, relative risk (RR) is the risk of an event (or of developing a disease) relative to exposure. Relative risk is a ratio of the probability of the event occurring in the exposed group versus the control (non-exposed) group.

Sensitivity analysis

An analysis used to determine how sensitive the results of a study or systematic review are to changes in how it was done. Sensitivity analyses are used to assess how robust the results are to uncertain decisions or assumptions about the data and the methods that were used.

Sensitivity

The sensitivity of a test is the probability that the test is positive when given to a group of patients with the disease. Sensitivity is sometimes abbreviated Sn.

A large sensitivity means that a negative test can rule out the disease.

Specificity

The specificity of a test is the probability that the test will be negative among patients who do not have the disease. Specificity is sometimes abbreviated Sp.

A large specificity means that a positive test can rule in the disease.

Strain

A genetic variant (or subtype) of a virus or bacterium. For example, a "flu strain" is a certain biological form of the influenza or "flu" virus.

Virus culture

Virus culture in the diagnostic laboratory, virus can grow only in a cell culture because it can replicate themselves only by infecting a host cell.


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Appendix 1

The evidence of antiviral efficacy and effectiveness

A Cochrane review in healthy adults identified four prophylaxis, 13 treatment and four post-exposure prophylaxis (PEP) trials. In prophylaxis compared to placebo, NIs have no effect against ILI (RR 1.28, 95% CI 0.45 to 3.66 for oral Oseltamivir 75 mg daily; RR 1.51, 95% CI 0.77 to 2.95 for inhaled Zanamivir 10 mg daily). The efficacy of oral Oseltamivir 75 mg daily against symptomatic influenza is 61% (RR 0.39, 95% CI 0.18 to 0.85), or 73% (RR 0.27, 95% CI 0.11 to 0.67) at 150 mg daily. Inhaled Zanamivir 10 mg daily is 62% efficacious (RR 0.38, 95% CI 0.17 to 0.85). Neither NI has a significant effect on asymptomatic influenza. Oseltamivir induces nausea OR 1.79, 95% CI 1.10 to 2.93). Oseltamivir for PEP has an efficacy of 58.5% (15.6% to 79.6) for households and of 68% (34.9 to 84.2%) to 89% in contacts of index cases. Zanamivir has similar performance. The hazard ratios for time to alleviation of influenza symptoms were in favour of the treated group 1.33 (1.29 to 1.37) for Zanamivir and 1.30 (1.13 to 1.50) for Oseltamivir. Viral nasal titres were significantly diminished by both. Oseltamivir 150 mg daily prevented lower respiratory tract complications (OR 0.32, 95% CI 0.18 to 0.57)[4].

Another Cochrane review in children included three trials involving 1500 children with a clinical case definition of influenza, of whom 977 had laboratory-confirmed influenza (Matheson[52]). Overall, trial quality was good. Oseltamivir reduced the median duration of illness by 26% (36 hours) in healthy children with laboratory-confirmed influenza (P value less than 0.0001). The reduction was only 7.7% (10 hours) in "at risk" (asthmatic) children, and this did not reach statistical significance (P value = 0.54). Zanamivir reduced the median duration of illness by 24% (1.25 days) in healthy children with laboratory-confirmed influenza (P value less than 0.001). No data in "at risk" children were available. Only Oseltamivir produced a significant reduction in the complications of influenza (particularly otitis media), although there was a trend to benefit for Zanamivir. We identified one randomised, controlled trial of Oseltamivir for the prevention of influenza transmission in households, reporting data from 222 paediatric contacts. Where index cases had laboratory-confirmed influenza, a protective efficacy of 55% was observed, but this did not reach statistical significance (P value = 0.089). The adverse events profile of Zanamivir was no worse than placebo, but vomiting was more common in children treated with Oseltamivir.

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Appendix 2a

Diagnostic laboratory tests for influenza

Diagnosis of influenza virus infection by laboratory tests is based on the detection of antigen followed by the detection of the immune response.

Diagnostic laboratory tests for influenza are of four main types:

- virus culture (conventional and shell-vial);
- detection of viral nucleic acid (by molecular methods like Polymerase Chain Reaction, PCR);
- serology;
- detection of virus antigen (by fluorescent specific antibody).

Virus culture

Virus isolation represents the "gold standard" for influenza diagnosis since it confirms that the virus is infective. This method is highly sensitive with good quality clinical samples (nasal washouts, nasopharyngeal aspirates, nasopharyngeal and pharyngeal swabs, tracheal aspirates, bronchoalvelolar washouts), collected before 72 hours from the onset of symptoms, and transported as soon as possible in the lab by appropriate transport media. If the sample is properly stored at 2-4 °C, virus particles survive 24 hours about.

One of the main advantages of virus isolation is its immunological and genetic identification that allows the monitoring of new circulating influenza subtypes and strains and for vaccine formulation.

Influenza virus culture may be performed using embrionated chicken eggs (primary choice) or other cell cultures (e.g. Madin-Darby canine kidney cells, MDCK or primary rhesus monkey kidney, pRhMK).

However, traditional virus isolation and identification is time consuming and requires safety class 2 labs during pandemic alarm and safety class 3 labs when a highly pathogenic strain is suspected (e.g. H5N1). In addition, embryonated chicken eggs are mainly used for surveillance, rather than diagnostic purposes, and are uncommon in the diagnostic laboratory setting.

Since results are generally available in 4–5 days, the impact on patient care is very limited.

Isolation of influenza by rapid shell-vial culture represents an improvement over conventional culture in terms of speed and simplicity. Results are available in 18-40 hours.

Figure 1: Petri dishes for conventional virus culture

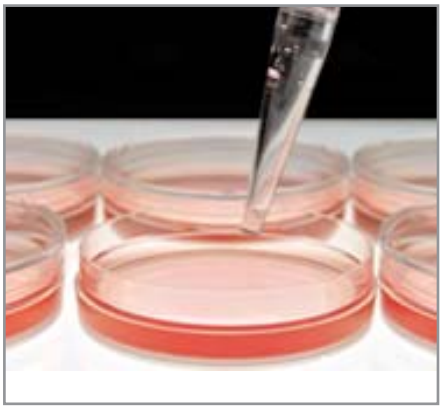
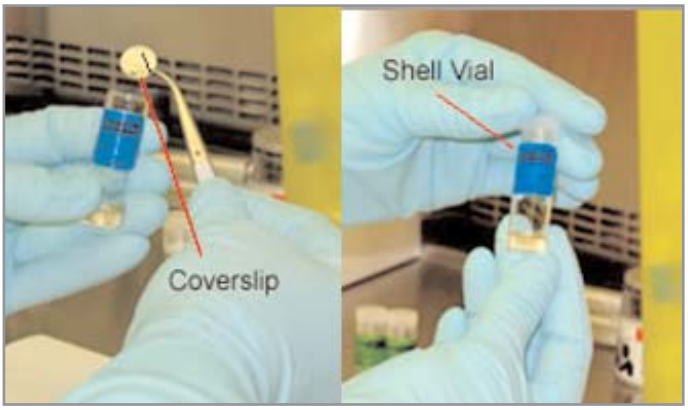


Figure 2: Rapid shell-vial culture



Detection of viral nucleic acid

The extreme genetic variability of influenza viruses is a challenge for the design of molecular-based diagnostic tests. However, a number of promising molecular based techniques have been developed. Polymerase Chain Reaction (PCR) is a very sensitive technique for the detection of viral genome even if present in low amounts. Influenza virus genome presents single chain RNA and thus a copy of DNA (cDNA) have to be synthesised before PCR: in this case the proper term is RT-PCR (reverse transcriptase-PCR). In this process several amplification cycles produce crescent amount of viral genome. False positive and false negative results (due to improper samples or genome degradation) have to be considered. Recently, Real-Time RT-PCR, in which amplification and detection occurs in the same reaction tube, decrease the risk of sample contamination. However, molecular techniques are the most expensive of the diagnostic tests for influenza and require 48 hours, considerable skill and expertise to perform and must be integrated into safety class 2 lab workflow. Further, in the event of large antigenic drift in influenza, new strains may not be detected and diagnosis may depend on traditional virus culture which will continue to play a major role in global epidemiologic influenza surveillance and vaccine strain selection.

Detection of virus antigen

One of the most common methods for the detection of viral antigens is direct immunofluorescence antibody (DFA). This method is rapid and sensitive thanks to the several specific antibodies available on the market. DFA tests use monoclonal antibodies against influenza virus antigen for the detection of influenza. Results are often available in 2–4 hours. However, the accuracy of DFA testing is heavily dependent on specimen quality (lack of adequate numbers of respiratory epithelial cells in the specimen could be a problem). Further, DFA tests require additional equipment and reagents (cyto-centrifuge, fluorescence microscope and monoclonal antibodies), are complex and technically demanding to perform and interpret. Detection of viral antigen is performed in safety class 2 labs.

Serology

Serologic diagnosis of influenza infection is based on the detection of a rise (four-fold or greater) in specific antibody titer in serum samples collected in the acute (as soon as possible after the onset of illness) and convalescent (2-3 week after) phase. The hemagglutination inhibition is the method of choice, followed by other serologic assays such as complement fixation, microneutralisation tests, enzyme-linked immunosorbent assay (ELISA). Patient vaccination history has to be considered for avoiding alteration of results. The need for paired serum samples makes serology a retrospective diagnostic tool and limits its clinical utility. The main value of this technology lies in epidemiology, as a research tool or when sample collection and viral isolation cannot be performed. Serologic assays require safety class 2 labs.

Table 1: Diagnostic laboratory tests for influenza

Method		Advantages	Disadvantages	Time for results	Relative cost
Virus culture	**Cell culture**	Sensitive and specific. Can detect viruses other than influenza. Current gold standard. Vital for surveillance and vaccine formulation	Labour intensive. Results not available in clinically relevant timeframe	3–10 d	$$
	Shell-Vial culture	Specific. Single specimen can be tested for viruses other than influenza	Quicker than culture but still too slow to influence treatment	1–3 d	$$
Serology		Sensitive and specific. Can detect culture-negative infection. Important research and surveillance tool	Purely retrospective diagnosis	2–4 wk	$
RT-PCR		Sensitive and rapid. Can detect non-culturable virus	Relatively complex, requires expertise, additional reagents and equipment. Requires adequate specimen. May miss new strains	4–48 h	$$$$
Antigen detection		Specific. Same-day test. Single specimen can be tested for multiple pathogens. Can be performed directly on clinical specimen	Relatively complex, requires expertise, additional reagents and equipment. Requires adequate specimen	2–4 h	$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Appendix 2b

Rapid (bed-side) tests for influenza

Operating principles of rapid diagnostic test for influenza

Diagnostic test for influenza A and B virus infections are defined "rapid" if they can provide results within 30 minutes. Despite the great number of rapid diagnostic tests for the diagnosis of influenza commercially available, there are few operating principles that permit the detection of viral antigen in about 30 minutes:

- EIA: Enzyme/Immuno Assay uses enzyme-bound antibodies or marked antibodies to detect antigen. The colour reaction can be enzymatic or chromatographic. Tests of this type can be realised with two main configurations (lateral flow or through-flow);
- OIA: Optical ImmunoAssay (or Solid-phase Assay) uses variations in the optical thickness of an antibody-coated surface that binds the antigens in the specimen. These variation alter the path of reflected light;
- VEA: Viral-encoded Enzyme Assay uses colour changes to detect chemical reactions catalysed by a viral enzyme.

Tests based on EIA technology

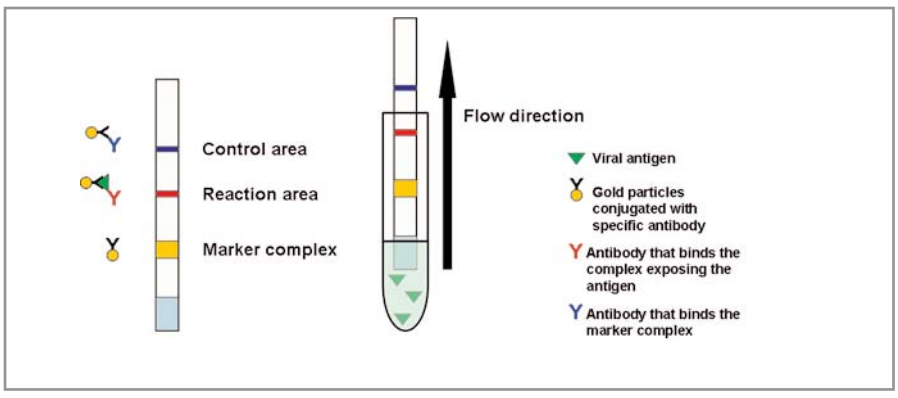
This type of tests are the most common for the diagnosis of influenza A and B virus infection.

EIA lateral flow technology

Generally, they are characterised by an adsorbent strip (usually a nitrocellulose pad) which is dipped into the solution obtained from the specimen. Due to capillary action the antigens obtained from the chemical disruption of viral particles move along the strip length and firstly react (bind) with specific marker particles (usually a gold-antibody conjugate) then with specific antibody (that binds such complex) and finally with control antibody (that binds the gold-antibody conjugate only).

Instead of gold particles, some devices use enzyme-bound antibodies as marker particles and instead of an adsorbent strip they can have a card-like shape. However the operating principle of the test is the same.

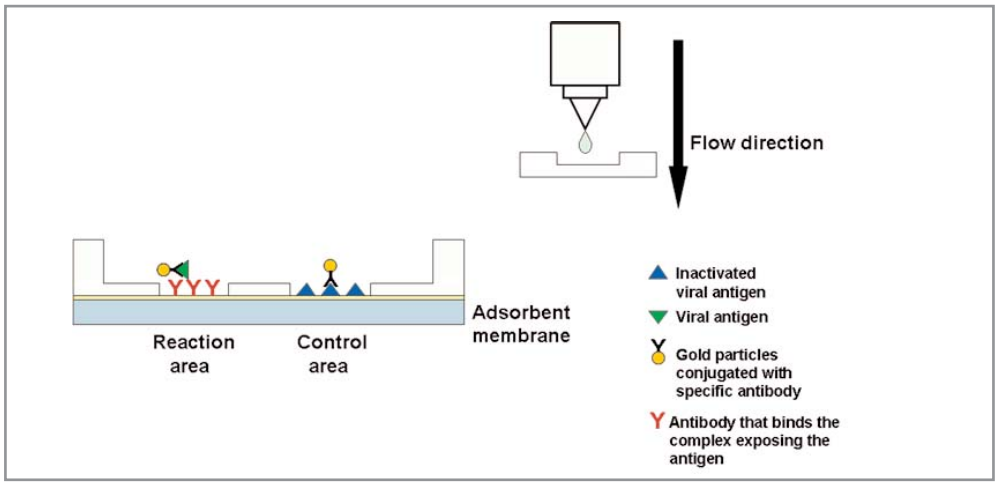
Figure 1: Schematisation of the EIA lateral flow technology



EIA through-flow technology

Generally, these devices are characterised by an adsorbent pad integrated in a well-like structure. A specific complex (gold-antibody conjugated) is added to the specimen solution containing viral antigens. This solution flows through a porous membrane and then into the adsorbent pad. A reaction and control area are present. The reaction area presents a specific antibody, fixed into the membrane, that binds to the complex formed by a gold particle, the antibody and antigen. The control area presents control particles (e.g. inactivated influenza virus particles) fixed to the membrane that bind to the complex formed by a gold particle and the antibody.

Figure 2: Schematisation of the EIA though-flow technology

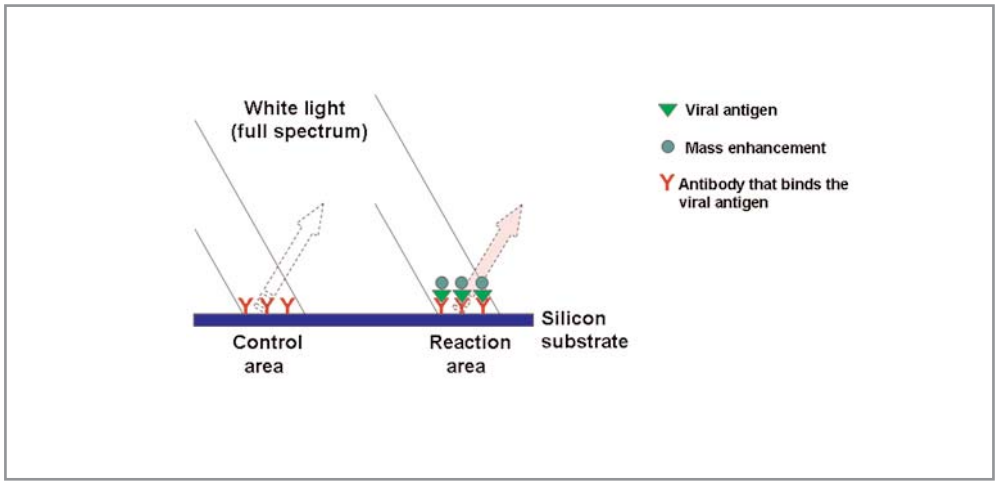


Tests based on OIA technology

Among the rapid diagnostic tests for influenza A and B commercially available, only one is based on this technology, also called Solid-Phase Assay technology.

Specific antibodies for A and B influenza virus are fixed onto an optical surface (silicon wafer) and bind the viral antigens in the specimen solution. After washing and the addition of a substrate that act as "mass enhancement", the path of the reflected light is altered (and results in a colour change).

Figure 3: Schematisation of the OIA technology

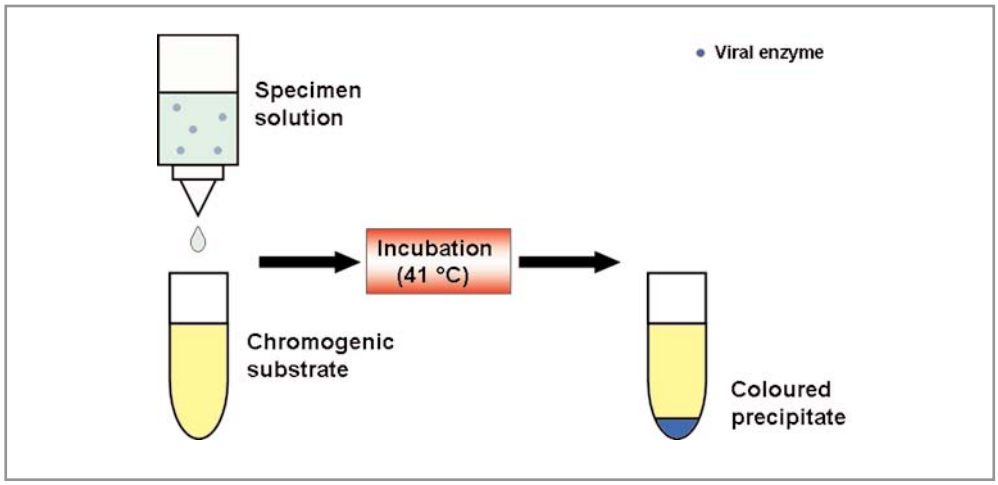


Tests based on VEA technology

Among rapid diagnostic tests for influenza A and B commercially available, only one is based on this technology.

The specimen is mixed with a chromogenic substrate able to recognise a viral enzyme (neuraminidase). After incubation at 41 °C, the solution containing the precipitate formed by the viral enzyme and the chomogenic substrate is transferred to a supported filter that collects the coloured precipitate.

Figure 4: Schematisation of the VEA technology



State of the Art

The devices are listed in the order of Table 1 (alphabetical order of the manufacturer).

Directigen Flu A
Directigen Flu A+B (through-flow) - Becton, Dickinson and Company

The Directigen Flu A+B antigen detection test is an immunomembrane filter assay to detect influenza A or B antigens extracted from suitable specimens of symptomatic patients.

Total test time is less than 15 min with reactivity determined by visual colour development.

The extracted specimen is expelled through a filter assembly into each of two wells of the test device. Influenza A or B antigens present in the specimen are non-specifically bound in a triangular shape to the membrane surface in the A and B wells as the specimen passes through the flow controller. Detection of antigen captured on the membrane is initiated after a membrane wash step.

Monoclonal antibody conjugates specific for influenza A nucleoprotein antigen are added to the upper A well of the test device. Monoclonal antibody conjugates specific for influenza B nucleoprotein antigen is added to the lower B well of the test device. The monoclonal antibody conjugates are bound to trapped antigen following their addition to the membrane.

The chromogen is then added after washing the membrane and allowed to incubate for 5 minutes.

Development of a purple triangle on the membrane in either the A well or the B well of the test device indicates a positive test for Flu A or for Flu B, respectively.

Directigen EZ Flu A+B (through-flow) - Becton, Dickinson and Company

The Directigen EZ Flu A+B test is a chromatographic assay to qualitatively detect influenza A and B viral antigens in samples processed from respiratory specimens.

When specimens are processed and added to the test device, influenza A or B viral antigens bind to anti-influenza antibodies conjugated to visualising particles in the corresponding A and B test strips. The antigen-conjugate complex migrates across the test strip to the reaction area and is captured by the line of antibody on the membrane.

Test results are interpreted after 15 minutes. A positive result for influenza A is visualized as a reddish purple line at the Test "T" position and the Control "C" position in the Flu A read window. A positive result for influenza B is visualised as a reddish purple line at the Test "T" position and the Control "C" position in the Flu B read window.

Binax NOW Influenza A & B (lateral flow) - Binax Inc.

The Binax NOW Influenza A & B Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in nasopharyngeal specimens. These antibodies together with a control antibody are immobilized onto a membrane support as three distinct lines and combined with other reagents/pads to construct a test strip. This test strip is mounted inside a cardboard, book-shaped hinged test device.

Test results are interpreted after 15 minutes based on the presence or absence of pink-to-purple coloured "Sample Lines". The blue "Control Line" turns pink in a valid assay.

Flu OIA (optical immunoassay) - BioStar Inc.

The OIA FLU A/B test is based on the detection of a protein antigen unique to influenza A or B. The Optical ImmunoAssay technology enables the direct visual detection of a physical change in the optical thickness of molecular thin films. This change is a result of antigen-antibody binding on an optical surface (silicon wafer). When extracted specimen is placed directly on the optical surface, the immobilised specific antibodies capture the antigen. After washing, the substrate is added, increasing the thickness (mass enhancement) of the molecular thin film. This change in thickness alters the reflected light path and is visually perceived as a change in colour.

A positive result appears as a purple spot on the predominant gold background.

Influ-A Respi-Strip
Influ-A&B RespiStrip (lateral flow) - Coris BioConcept

These immunochromatographic tests allow the detection, within 15 minutes, of the Influenza-A and B viruses in nasopharyngeal samples diluted in the provided dilution buffer. It is a one-step test using colloidal gold particles and two specific monoclonal antibodies. When the immunochromatographic strip is dipped into the diluted solution, the sample and rehydrated gold conjugate migrate by capillarity action, past the test and control areas, which contain immobilised antibodies. Pink/purple lines develop at sites of the immobilised antibodies if the corresponding antigen has been detected.

RapidTesta FLU AB (through-flow) - Daiichi Pure Chemicals Co.

The RapidTesta FLU AB is a flow-through immunoassay for rapid detection of influenza A and B viral antigens.

Influ AB Quick

Probably out of commerce. The Quick S-Influ A/B "Seiken" is the improved version.

Quick S-Influ A/B ‡Seiken· (through-flow) - Denka Seiken Co. Ltd.

The Quick S-Influ A/B "Seiken" is a through-flow immunoassay. The test principle involves a flow of fluid containing the analyte through a porous membrane and into an absorbent pad. To detect viral antigens, the corresponding analyte is bound as a spot on the membrane. This reagent "captures" the analyte as it flows through the membrane. If the specimen is positive a pink spot appears either in the A or B well.

Espline Influenza A&B-N (lateral flow) - Fujirebio Inc.

The Espline Influenza A&B-N is an immunochromatography test using enzyme immunoassay for rapid diagnosis of influenza A and B. In this assay system, monoclonal antibodies for viral antigens of influenza were divided into two parts, one for the capture line on the nitrocellulose membrane and the other for labelling with an enzyme.

When a specimen containing the corresponding viral antigen was dropped onto the kit, a sandwich complex was formed at the judgment line and reacted with the substrate.

The test indicated influenza A or B positive results when blue lines were formed on the A or B judgment lines.

OSOM Influenza A&B (lateral flow) - Genzyme Diagnostics

The OSOM Influenza A&B Test is an in vitro diagnostic immunochromatographic assay intended for the qualitative detection of influenza A and influenza B viral nucleoprotein antigens from nasal swab specimens in symptomatic patients.

The OSOM Influenza A&B Test consists of a test stick that separately detects influenza A and B. The test procedure requires the solubilisation of the nucleoproteins from a swab by mixing the

swab in "Extraction Buffer". The test stick is then placed in the sample mixture, which then migrates along the membrane surface. If influenza A and/or B viral antigens are present in the sample, it will form a complex with monoclonal antibodies to influenza A and/or B nucleoproteins conjugated to colloidal gold. The complex will then be bound by another anti-influenza A and/or B antibody coated on the nitrocellulose membrane. A pink to purple control line must appear in the control region of the stick for results to be valid. The appearance of a second and possibly a third light pink to purple line will appear in the test line region indicating an A, B or A and B positive result.

Wampole Clearview Flu A/B
Clearview Exact Influenza A & B (lateral flow) - Inverness Medical Inc.

The Clearviev Exact Influenza A & B test is an immunochromatographic membrane assay that utilizes sandwich immunoassay technology for the detection of influenza A and B viral antigens. The test consist of a dipstick device containing a membrane strip that has separate regions with immobilised influenza A and B specific monoclonal antibodies and a coloured gold conjugate that also consists of specific influenza A and B antibodies.

Test results are interpreted after 15 minutes based on the presence or absence of red/pink coloured lines in the influenza A and/or B test regions.

ImmunoCard STAT! Flu A&B (lateral-flow) - Meridian Bioscience Inc.

ImmunoCard STAT! Flu A&B is a rapid, qualitative, lateral-flow immunoassay for detecting both influenza A and influenza B viral antigens in human nasal wash, nasopharyngeal aspirate and nasal and nasopharyngeal swab samples. The chromatography strip is housed in a plastic frame. At the "TEST line" there are monoclonal anti-influenza A and B antibodies fixed on the membrane and goat anti-mouse antibodies at the "CONTROL line". The strip also contains colloidal gold conjugated to monoclonal anti-influenza A and B as detection antibodies.

ImmunoCard STAT! Flu A & B uses specific monoclonal antibodies directed towards the nucleoproteins of influenza A or influenza B as the capture and detector antibodies. Monoclonal influenza A and monoclonal influenza B are immobilized on the membrane of the test device at the reaction site marked "FLU A" and "FLU B", respectively. Monoclonal influenza A and influenza B conjugated to colloidal gold are suspended within the membrane. To perform the test, the sample (nasal wash, nasopharyngeal aspirate, nasopharyngeal swab, nasal swab) is first diluted with "Sample Diluent", then added to the sample port of the test device. Influenza A or influenza B antigens in the sample bind the conjugate detector antibodies as the sample migrates through the device. The influenza A-gold conjugate complex will bind at the window site marked "FLU A" producing a visible pink-red line. Similarly, a pink-red line will appear when the influenza B-gold conjugate complex binds at the window site marked "FLU B".

Quick Vue Influenza Test
Quick Vue Influenza A+B (lateral flow) - Quidel Corporation

The QuicVue Influenza Test is a lateral-flow immunoassay using highly sensitive monoclonal antibodies that are specific for influenza antigens. The patient specimen is placed in the "Extraction Reagent Tube", the virus particles in the specimen are disrupted, exposing internal viral nucleoproteins.

After extraction, the test strip is placed in the "Extraction Reagent Tube" where nucleoproteins, contained in the specimen, react with the reagents in the test strip.

If the specimen contains influenza antigen, a pink-to-red "Test Line" along with a blue "Control Line" will appear on the test strip indicating a positive result.

Xpect Flu A & B (lateral flow) - Remel Inc.

The Xpect Flu A & B is a lateral flow chromatographic immunoassays. Results can be read after 15 minutes of incubation at room temperature. Two black-coloured bands, one in the test region and one in the control region, indicated a positive result.

Rockeby Influenza A Antigen Test (lateral flow) - Rockeby biomed

The Rockeby Influenza A Antigen Test kit is a qualitative, one step chromatographic immunoassay to selectively detect the Influenza A virus.

The sample is absorbed through an absorbent membrane and allowed to migrate through the membrane. As the sample proceeds through the membrane, the colored conjugate (colloidal gold conjugate), which was pre-dried on the test strip, migrates with the sample. The sample and the conjugate move through the capture region, precoated with immobilised monoclonal antibody to Influenza A virus, and through the control band region, and then to the end of the membrane.

Test result can be read after 10 minutes. The bound antibody-antigen complexes are detected by giving a pinkpurplish color. The format provides a clear read out for positive (two lines) and negative (one line) specimens.

SAS Influenza A Test (through-flow) - SA Scientific Inc.

The SAS Influenza A Test utilises monoclonal antibodies against Influenza Type A viral nucleoproteins. The test begins with an extraction of Type A nucleoproteins. Extracted specimens are then added to the test device. If Type A nucleoproteins are present, they bind to the antibody-gold conjugate in the test membrane and form a complex. This complex migrates through the membrane and is captured by Type A antibody. In the presence of Influenza Type A nucleoproteins a pink coloured line develops in the specimen zone of the test device.

Capilia FluA,B (lateral flow) - Tauns Co. Ltd.

(two devices in the same kit: one for A and one for B virus).

The Capilia FluA,B is a rapid diagnostic kit for the detection and identification of influenza virus A and B, using the rapid immunochromatographic method. The identification was based on the monoclonal antibodies specific for the nucleoprotein of either influenza A or B.

The test plate is composed of three parts, namely sample pad, reagent pad and reaction membrane. The whole strip is contained inside a plastic plate. The reagent membrane contains the colloidal-gold together with the monoclonal antibodies for either influenza virus A or B; the reaction membrane contains the secondary antibodies for either virus A or B, and the antibodies for the mouse globulin, which are pre-immobilized on the membrane.

ZstatFlu-II test

ZstatFlu Test (viral-encoded assay) - ZymeTx Inc.

The ZstatFlu Test for Influenza Types A and B Virus is based upon the reaction between a viral enzyme (neuraminidase) from influenza and a chromogenic substrate that precipitates upon reaction. The chromogenic substrate consists of a recognition portion for the viral neuraminidase and a reporter portion that precipitates upon cleavage. Throat swab specimens from patients infected with influenza types A or B virus are added to the reconstituted reagents and incubated at 41 °C for 20 minutes. The resulting reaction mixture is then transferred into a collection device and the coloured precipitate is collected on a supported filter. Positive specimens are identified by blue colour.

Table 1: Rapid Diagnostic Tests for influenza (May 2008) (°)

Manufacturer	Device	Technology	Virus type
Becton, Dickinson and Company	Directigen Flu A+B (**)	Through-flow	A+B
Becton, Dickinson and Company	Directigen Flu A (**)	Through-flow	A
Becton, Dickinson and Company	Directigen EZ Flu A+B (**)	Through-flow	A+B
Binax Inc.	Binax NOW Influenza A & B (**)	Lateral flow	A+B
BioStar Inc.	FLU OIA A/B (**)	OIA	A/B
Coris BioConcept	Influ-A&B RespiStrip	Lateral flow	A+B
Coris BioConcept	Influ-A Respi-Strip	Lateral flow	A
Daiichi Pure Chemicals Co.	RapidTesta FLU AB	Through-flow	A+B
Denka Seiken Co. Ltd.	Quick S-Influ A/B “Seiken” (**)	Through-flow	A+B
Fujirebio Corp.	Espline Influenza A&B-N (**)	Lateral flow	A+B
Genzyme Diagnostics	OSOM Influenza A&B	Lateral flow	A+B
Inverness Medical Inc.	Clearview Exact Influenza A & B	Lateral flow	A+B
Inverness Medical Inc.	Clearview Flu A/B	Lateral flow	A+B
Meridian Bioscience Inc.	ImmunoCard STAT! Flu A&B (**)	Lateral flow	A+B
Quidel Corporation	Quick Vue Influenza A+B (**)	Lateral flow	A+B
Quidel Corporation	Quick Vue Influenza Test (**)	Lateral flow	A+B
Remel Inc.	Xpect Flu A & B (**)	Lateral flow	A+B
Rockeby Biomed	Influenza A antigen test (**)	Lateral flow	A
SA Scientific Inc.	SAS Influenza A Test	Through-flow	A+B
Tauns Co. Ltd.	Capilia FluA,B	Lateral flow	A+B*
ZymeTx Inc.	ZstatFlu Test (**)	VEA	A/B

* two devices in the same kit (one for A and one for B virus).
Notes: OIA, optical immuno-assay; VEA, viral-encoded enzyme assay; A, the test detects only virus A; A+B, the test distinguishes between virus A and virus B; A/B, the test NOT distinguishes between virus A and virus B.
** rapid test retrieved in the study included in systematic review
(°) Source: WHO 2005, manufacturers websites

Bibliography

Call, S. A, Vollenweider, M. A, Hornung, C. A, Simel, D. L., and McKinney, W. P. Does this patient have influenza?, JAMA. 2005; 293(8):987-97.

Gavin, P. J., Richard, B., Thomson, Jr. Review of rapid diagnostic tests for influenza, Clinical and Applied Immunology Reviews, 4 (2003) 151–172.

Uyeki, T. M., Influenza diagnosis and treatment in children: a review of studies on clinically useful tests and antiviral treatment for influenza, Paediatric Infections Disease Journal, 2003; 22: 164–77.

http://www.who.int/csr/disease/avian_influenza/guidelines/rapid_testing/en/

http://www.bd.com

http://www.binax.com

http://www.biostar.com

http://www.corisbio.com

http://www.denka-seiken.co.jp

http://www.fujirebio.co.jp

http://www.genzyme.com

http://www.meridianbioscience.com

http://www.quidel.com

http://www.remelinc.com

http://www.clearview.com

http://www.zymetx.com

http://www.sascientific.com

http://www.daiichichem.jp

http://rockeby.com/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Appendix 3

Search strategy

We searched EMBASE in any language from 1966 using the following strategy:

#2. 'influenza'/exp/dm_di/mj AND [humans]/lim AND [embase]/lim

#4. binax OR 'directigen flu a' OR 'directigen flu a+b OR 'flu oia' OR 'quickvue influenza' OR 'quickvue a + b' OR 'denka-seiken' OR 'denka-seiken a/b' OR 'xpect flu a & b' OR 'zstatflu-ii test' OR espline OR capilia OR rapid test

#5. (influenza OR flu) AND rapid AND (test OR testing OR detection OR diagnosis OR screening)

#6. (influenza AND flu) AND (screening OR viral) AND (test OR testing)

#7. #2 OR #4 OR #5 OR #6

#8. #2 OR #4 OR #5 OR #6 AND [humans]/lim AND [embase]/lim

We searched Pub Med/Medline and the Cochrane Library (including the Cochrane Database of Systematic Reviews, Central and the Health Technology Assessment database) from 1966 using the following strategy:

#6 Search "influenza rapid test"

#12 Search (influenza OR flu) AND rapid AND (test OR testing OR detection OR diagnosis OR screening)

#14 Search (influenza[Title/Abstract] AND flu[Title/Abstract]) AND (screening[Title/Abstract] OR viral[Title/Abstract]) AND (test[Title/Abstract] OR testing[Title/Abstract])

#16 Search Binax[Title/Abstract] OR "Directigen Flu A"[Title/Abstract] OR "Directigen Flu A+B"[Title/Abstract] OR "Flu OIA"[Title/Abstract] OR "QuickVue" [Title/Abstract] OR "Denka Seiken"[Title/Abstract] OR "Xpect Flu"[Title/Abstract] OR "ZstatFlu-II test"[Title/Abstract] OR Espline[Title/Abstract] OR Capilia[Title/Abstract] OR RapidTesta[Title/Abstract]

#17 Search #6 OR #12 OR #14 OR #16

We searched all identifiable websites of manufacturers, affiliates and marketing companies of influenza rapid tests as well as public health bodies to identify further background or unpublished evidence.


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Appendix 4

Data extraction form for Systematic Reviews

General description

NB Do not leave blank spaces. If there is no answer to the question write NR (not reported)
Non lasciare risposte in bianco. Se non vi è risposta alla domanda segna NR (non riportato)

Study ID:
(ad es Smith 2000)

Date of publication:
(Data di pubblicazione)

Published Y/N
(Pubblicato (S/N)

Form of publication:
abstract/full paper/protocol
CDSR/electronic elsewhere/paper
(Tipo di pubblicazione: abstract/articolo intero/protocollo ED INOLTRE:
Cochrane Database of Systematic Reviews/elettronica altrove/cartacea)

Biblio ref:

Type of funder: Government, mixed, private, industry, unfunded, undeclared/unknown
(Tipo di finanziamento: governo, misto, privato, farmaceutico, non finanziato, non dichiarato/ignoto)

Pub Med abstract:
(incollare abstract)

Date of last updated search (data dell'ultima ricerca aggiornata):

Methods description

Rationale
(razionale):

Objective
(obiettivo):

Searches (list databases/sources)
(ricerche - elenca fonti):

Strategy reported
(strategia riportata) Y/N

Inclusion criteria
(criteri inclusione studi primari):

Types of studies
(tipi di studi):

Types of participants
(tipi di partercipanti):

Types of intervention
(tipi di interventi):

Types of outcome measures
(tipi di esiti):

Number of included studies
(numero studi inclusi):

Included studies list
(lista studi inclusi): Y/N/Av from author

Total population from included studies (pop. totale da studi inclusi):

Excluded studies list (lista studi esclusi): Y/N/Av from author

Reasons for exclusion given (motivi di esclusione spiegata): Y/N

Flow diagram (algoritmo di studi): Y/N

Quality of primary studies evaluated: (Qualità dei studi priimari valutate)Y/N

If Y how Score/Checklist/Other (se sì come: Punteggio/Checklist/Altro)

Number of outcomes assessed (numero esiti studiati):

Meta-analysis included (meta-analisi presente): Y/N

If Y brief statistical methods description (se sì breve descrizione metodi statistici)

Sub group analysis (analisi sottogruppi) Y/N

If Y was it mentioned in the protocol (se sì prevista nel protocollo): Y/N

Heterogeneity analysed (Eterogeneità analizzata): Y/N

Heterogeneity discussed (Eterogeneità discussa): Y/N

How quality incorporated (Stratificazione per qualità):

Weighting/Sub group analysis/Narrative/Unclear

Results description

Comparison (Confronto)	Outcome (Esito)	Estimate of effect (with 95% CI) Stima di effetto (con IC 95%)

Conclusions description (descrizione delle conclusioni)

Assessment of generalisability of results (Giudizio di trasferibilità dei risultati)

Bottom line (Indicazione finale)

Quality assessment (valutazione qualità metodologica)

Number	Item	Answer Y/N/UC/NA	Notes
1.	Is there an objective? C'è un obbiettivo?		
2.	Is the objective clear? L'obbiettivo è chiaro?		
3.	Are the searches reported? Le ricerche biblio sono descritte?		
4.	Were the searches done on at least 3 sources? Le ricerche sono state condotte perlomeno su 3 fonti?		
5.	Do the searches appear thorough? Le ricerche sembrano esaustive?		
6.	Were handsearches carried out? Sono state fatte ricerche a mano?		
7.	Are the inclusion criteria explicit? Criteri di inclusione espliciti?		
8.	Are the inclusion criteria coherent with the objective? Criteri inclusione coerenti con obbiettivo?		
9.	Do the inclusion criteria include quality of primary studies as a criterion?		
10.	Was extraction done in double? L'estrazione dati è stata fatta in doppio?		
11.	Is the description of primary studies reported? La descrizione degli studi primari c'è?		
12.	If there is a meta-analysis, are interventions homogeneous? Se vi è meta-analisi gli interventi sono omogenei?		
13.	If there is a meta-analysis, are outcomes homogeneous? Se vi è meta-analisi gli esiti sono omogenei?		
14.	If there is a meta-analysis, are the study designs homogeneous? Se vi è meta-analisi i disegni di studio sono omogenei?		
15.	Is the statistical analysis appropriate? Le analisi statistiche sono appropriate?		
16.	Do conclusions flow logically from the results? Le conclusioni derivano logicamente dai risultati?		
17.	Is there a declaration of conflicts of interest? Vi è dichiarazione di conflitti di interesse degli autori?		

Notes: Y= yes; N= No; UC= unclear; NA= not applicable.

Appendix 5

Data extraction form for Primary Studies

General description

NB Do not leave blank spaces. If there is no answer to the question write NR (not reported)
Non lasciare risposte in bianco. Se non vi è risposta alla domanda segna NR (non riportato)

Study ID:
(ad es Smith 2000)

Date of publication:
(Data di pubblicazione)

Published Y/N
(Pubblicato (S/N)

Form of publication:
abstract/full paper/protocol
CDSR/electronic elsewhere/paper
(Tipo di pubblicazione: abstract/articolo intero/protocollo ED INOLTRE:
Cochrane Database of Systematic Reviews/elettronica altrove/cartacea)

Biblio ref:

Type of funder: Government, mixed, private, industry, unfunded, undeclared/unknown
(Tipo di finanziamento: governo, misto, privato, farmaceutico, non finanziato, non dichiarato/ignoto)

Pub Med abstract:
(incollare abstract)

Date of last updated search (data dell'ultima ricerca aggiornata):

Methods description

Rationale
(razionale):

Objective
(obiettivo):

Type of study (disegno di studio):

Types of participants (tipo di partercipanti):

Age (mean+SD) [years/months]:
(Età in mesi/anni media e DS)

Age (range) [years/months]:
(Età in mesi/anni distribuzione/range)

Gender:
(Sesso)

Setting:
(Contesto)

Description of incidence or prevalence of the target disease in the test and reference population:
(Descrizione della incidenza o prevalenza della condizione in questione nella popolazione oggetto del test e nella popolazione di riferimento)

Inclusion criteria:
(Criteri di inclusione)

Index test:
(test diagnostico indice)

Test duration (time units):
(Durata del test con unità di misura del tempo in minuti e specifica dell'ambiente di esecuzione)

Gold standard:
(test diagnostico di riferimento)

- Viral isolation (or shell vials): Y/N

If Y:

Type/n° samples:

Culture recommended by WHO and CDC:

[ ] Embryonated chicken eggs

[ ] MDCK

[ ] Primary rhesus monkey cell

Culture not recommended by WHO and CDC:

o Which……..

- RT PCR: Y/N

If Y:

Type/n° samples:

Sensitivity indicated Y/N: If Y:

Specificity indicated Y/N: If Y:

Notes (Real Time or end point; Commercial or in-house, target gene……..)

- Mixed systems (Viral isolation, RT-PCR and/or serology): Y/N

If Y:

Viral isolation (or shell vials): Y/N

If Y:

Type/n° samples:

Culture recommended by WHO and CDC:

[ ] Embryonated chicken eggs

[ ] MDCK

[ ] Primary rhesus monkey cell

Culture not recommended by WHO and CDC:

o Which.....

- RT PCR: Y/N

If Y:

Type/n° samples:

Sensitivity indicated Y/N: If Y:

Specificity indicated Y/N: If Y:

Notes (Real Time or end point; Commercial or in-house, target gene.....)

- Serology: Y/N

If Y:

Type/n° samples:

Notes (FC or HAI or Neutralization) .....

- Other:.....

Type/n° samples:.....

Results description

Index test	Comparator	Virus Type	Specimen type	PPV% [95% CI] (authors)	NPV% [95% CI] (authors)	Sensitivity % [95% CI] (authors)	Specitivity % [95% CI] (authors)	Other outcome misure (authors) (LRP; LRN)

a= true positive

		Condition (RS)	Total	
		+	-	
Test result	+	a	b	a+b
	-	c	d	c+d
Total	a+c	b+d	a+b+c+d	

b= false positive
c= false negative
d= true negative

Conclusions description (descrizione delle conclusioni)

Assessment of generalisability of results (Giudizio di trasferibilità dei risultati)

Bottom line (Indicazione finale)

Quality assessment (QA)

(General quality assessment tool)

Number	Item (Elemento)	Answer Y/N/UC	Notes
1.	Is there an objective? C'è un obiettivo?		
2.	Is the objective clear? L'obiettivo è chiaro?		
3.	Are the inclusion criteria coherent with the objective? Criteri inclusione coerenti con obiettivo?		
4.	Is the statistical analysis appropriate? Le analisi statistiche sono appropriate?		
5.	Do conclusion flow logically from the results? Le conclusioni derivano logicamente dai risultati?		
6.	Is there a declaration of conflicts of interest? Vi è dichiarazione di conflitti di interesse degli autori?		
7.	Is there a declaration of funding? Vi è dichiarazione di provenienza dei fondi?		

QUADAS*

Quality Assessment of Diagnostic Accuracy Studies

Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14.	Were withdrawals from the study explained?	( )	( )	( )

* Whiting P, Rutjes AWS, Reitsma JB, Bossuyt PMM, Kleijnen J. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Medical Research Methodology 2003, 3:25doi:10.1186/1471-2288-3-25 http://www.biomedcentral.com/1471-2288/3/25


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Appendix 6

List of excluded studies in phase II of the inclusion criteria

Article	Reason for exclusion
Bai, G. R, Sakoda, Y, Mweene, A. S, Fujii, N, Minakawa, H., and Kida, H. Improvement of a rapid diagnosis kit to detect either influenza A or B virus infections, eng. J Vet Med Sci. 2006 Jan; 68(1):35-40.	Animal study
Bonner, A. B, Monroe, K. W, Talley, L. I, Klasner, A. E., and Kimberlin, D. W. 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, eng. Pediatrics. 2003 Aug; 112(2):363-7.	Not-comparative
Boon, A. C, French, A. M, Fleming, D. M., and Zambon, M. C. Detection of influenza a subtypes in community-based surveillance, eng. J Med Virol. 2001 Sep; 65(1):163-70.	Not rapid test
Cohen, R, Thollot, F, Lecuyer, A, Koskas, M, Touitou, R, Boucherat, M, d'Athis, P, Corrard, F, Pecking, M., and de La Rocque, F. [Impact of the rapid diagnosis downtown in the assumption of responsibility of the children in period of influenza.] [Impact des tests de diagnostic rapide en ville dans la prise en charge des enfants en periode de grippe.], fre. Arch Pediatr. 2007 Jul; 14(7):926-31.	No original economic data
Diomande, D, Bellavoine, V, Gilles, I, Gehanno, B, Checoury, A., and Pascal, C. [Benefit of seasonal use of a rapid diagnosis of influenza virus in a paediatric emergency unit] [Benefice de l'utilisation saisonniere d'un test de diagnostic rapide (TDR) de la grippe aux urgences pediatriques.], fre. Arch Pediatr. 2006 Nov; 13(11):1463-5.	No original economic data
Gavin, P. J. and Thomson Jr., R. B. Review of rapid diagnostic tests for influenza. Clin. Appl. Immunol. Rev. 2003; 4(3):151-172.	Not a systematic review
Henley, E. Prevention and treatment of influenza. J. Fam. Pract. 2003; 52(11):883-886.	Not relevant
Kaiser, L, Briones, M. S., and Hayden, F. G. Performance of virus isolation and Directigen Flu A to detect influenza A virus in experimental human infection, eng. J Clin Virol. 1999 Dec; 14(3):191-7.	Starting point not naturally occurring influenza
Noyola, D. E. and Demmler, G. J. Effect of rapid diagnosis on management of influenza A infections, eng. Pediatr Infect Dis J. 2000 Apr; 19(4):303-7.	Effectiveness data results of IT at 1hr
Noyola, D. E, Paredes, A. J, Clark, B., and Demmler, G. J. Evaluation of a neuraminidase detection assay for the rapid detection of influenza A and B virus in children, eng. Pediatr Dev Pathol. 2000 Mar-2000 Apr 30; 3(2):162-7.	Prototype of IT results not at 30 mins
Poehling, K. A, Zhu, Y, Tang, Y. W., and Edwards, K. Accuracy and impact of a point-of-care rapid influenza test in young children with respiratory illnesses, eng. Arch Pediatr Adolesc Med. 2006 Jul; 160(7):713-8	CRTC assessing the impact of use/not use RT on resource consumption
Rawlinson, W. D, Waliuzzaman, Z. M, Fennell, M, Appleman, J. R, Shimasaki, C. D., and Carter, I. W. New point of care test is highly specific but less sensitive for influenza virus A and B in children and adults, eng. J Med Virol. 2004 Sep; 74(1):127-31	The IT needs to be modified
Rebelo-de-Andrade, H. and Zambon, M. C. Different diagnostic methods for detection of influenza epidemics, eng. Epidemiol Infect. 2000 Jun; 124(3):515-22	Does not identify manufacturer, Does not identify test Does not report disaggregate data by index and comparator tests.
Rothberg, M. B, Fisher, D, Kelly, B., and Rose, D. N. Management of influenza symptoms in healthy children: cost-effectiveness of rapid testing and antiviral therapy, eng. Arch Pediatr Adolesc Med. 2005 Nov; 159(11):1055-62.	No original efficacy and economic data
Saito, R, Li, D, Shimomura, C, Masaki, H, Le, M. Q, Nguyen, H. L, Nguyen, H. T, Phan, T. V, Nguyen, T. T, Sato, M, Suzuki, Y., and Suzuki, H. An off-seasonal amantadine-resistant H3N2 influenza outbreak in Japan, eng. Tohoku J Exp Med. 2006 Sep; 210(1):21-7	Not relevant
Sharma, P. P, Friesen, T., and Waites, K. B. Influenza testing in the diagnostic laboratory. Lab. Med. 2006; 37(6):366-370	Not a systematic review
Storch, G. A. Rapid diagnostic tests for influenza, eng. Curr Opin Pediatr. 2003 Feb; 15(1):77-84	Narratvie review
Tucker, S. P, Cox, C., and Steaffens, J. A flu optical immunoassay (ThermoBioStar's FLU OIA): a diagnostic tool for improved influenza management, eng. Philos Trans R Soc Lond B Biol Sci. 2001 Dec 29; 356(1416):1915-24	Not a systematic review
Uyeki, T. M. Influenza diagnosis and treatment in children: a review of studies on clinically useful tests and antiviral treatment for influenza, eng. Pediatr Infect Dis J. 2003 Feb; 22(2):164-77	Not a systematic review
Woolcock, P. R. and Cardona, C. J. Commercial immunoassay kits for the detection of influenza virus type A: evaluation of their use with poultry, eng. Avian Dis. 2005 Dec; 49(4):477-81	On avian influenza
Wunderli, W, Thomas, Y, Muller, D. A, Dick, M., and Kaiser, L. Rapid antigen testing for the surveillance of influenza epidemics, eng. Clin Microbiol Infect. 2003 Apr; 9(4):295-300	Not rapid test
Yan, X, Schielke, E. G, Grace, K. M, Hassell, C, Marrone, B. L., and Nolan, J. P. Microsphere-based duplexed immunoassay for influenza virus typing by flow cytometry, eng. J Immunol Methods. 2004 Jan; 284(1-2):27-38	Not relevant, not rapid test


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Appendix 7

List of Greek and Japanese studies

Greek article

Gioula, G, Exindari, M, Chatzidimitriou, D, Melidou, A., and Kyriazopoulou, V. Rapid tests in the identification of influenza A and B in clinical cases. Acta Microbiol. Hell. 2006; 51(5):374-378; ISSN: 0438-9573.

Japanese articles

Hara, M, Sadamasu, K, Takao, S, Shinkai, T, Kai, A, Fukuda, S, Shimazu, Y, Kuwayama, M., and Miyazaki, K. [Evaluation of immunochromatography test for rapid detection of influenza A and B viruses using real-time PCR], jpn. Kansenshogaku Zasshi. 2006; 80(5):522-6.

Hara, M, Takao, S, Fukuda, S, Shimazu, Y, Kuwayama, M., and Miyazaki, K. [Comparison of four rapid diagnostic kits using immunochromatography to detect influenza B viruses], jpn. Kansenshogaku Zasshi. 2005; 79(10):803-11.

Hara, M, Takao, S, Fukuda, S, Shimazu, Y., and Miyazaki, K. [Comparison of three rapid diagnostic kits using immunochromatography for detection of influenza A virsuses], jpn. Kansenshogaku Zasshi. 2004; 78(11):935-42.

Ikematsu, H, Nabeshima, A, Nabeshima, S, Kakuda, K, Maeda, N, Chong, Y, Li, W, Hayashi, J, Hara, H., and Kashiwagi, S. [Evaluation of a rapid enzyme immunoassay for detection of influenza A virus among adult and elderly patients], jpn. Kansenshogaku Zasshi. 1999; 73(11):1153-8.

Kaji, M, Kuno, H., and Oizumi, K. [Costs of influenza therapy], jpn. Kansenshogaku Zasshi. 2001; 75(6):460-3.

Kawai, N, Iwaki, N, Kawashima, T, Satoh, I, Shigematsu, T, Kondoh, K, Maeda, T, Kanazawa, H, Hirotsu, N, Miyachi, K, Kunishima, O, Ikematsu, H., and Kashiwagi, S. [Clinical symptoms of influenza infection in the 2002-2003 season], jpn. Kansenshogaku Zasshi. 2004; 78(8):681-9.

Kawakami, C, Shimizu, H, Watanabe, S, Saikusa, M, Munemura, T, Mitamura, K, Sugaya, N., and Imai, M. [Evaluation of immunochromatography method for rapid detection of influenza A and B viruses], jpn. Kansenshogaku Zasshi. 2001; 75(9):792-9.

Kubo, N, Ikematsu, H, Nabeshima, S, Yamaji, K, Nabeshima, A, Kondou, H, Chong, Y, Kashiwagi, S., and Hayashi, J. [Evaluation of an immunochromatography test kit for rapid diagnosis of influenza], jpn. Kansenshogaku Zasshi. 2003; 77(12):1007-14.

Mitamura, K. [Rapid diagnosis of influenza by EIA], jpn. Nippon Rinsho. 2000; 58(11):2229-33.

Mitamura, K. and Kawakami, C. [Rapid diagnostic tests for influenza], jpn. Nippon Rinsho. 2003; 61(11):1914-20; ISSN: 0047-1852

Mitamura, K, Sugaya, N, Nirasawa, M, Takahashi, K, Shimizu, H., and Hirai, Y. [Impact of influenza A virus infection as a cause of pediatric hospitalization and use of rapid antigen test of influenza A virus], jpn. Kansenshogaku Zasshi. 1998; 72(9):883-9.

Mitamura, K, Sugaya, N, Shimizu, H, Nirasawa, M, Takahashi, K, Hirai, Y., and Takeuchi, Y. [Optical immunoassay test for rapid detection of influenza A and B viruses: an evaluation], jpn. Kansenshogaku Zasshi. 1999; 73(10):1069-73.

Mitamura, K, Yamazaki, M, Ichikawa, M, Kimura, K, Kawakami, C, Shimizu, H, Watanabe, S, Imai, M, Shinjo, M, Takeuchi, Y., and Sugaya, N. [Evaluation of an immunochromatography test using enzyme immunoassay for rapid detection of influenza A and B viruses], jpn. Kansenshogaku Zasshi. 2004; 78(7):597-603.

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Appendix 8

List of included studies

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Appendix 9

Description of studies included in the systematic review

A description of the studies subdivided by type of rapid test follows. A single index test is described for each study, see table 1 for data relating to remaining ITs.

QuickVue Influenza A+B (Quidel corp.)

Agoritsas[19] – During an influenza season (no information on viral circulation is reported), the authors enrolled 122 eligible children with influenza-like illness (ILI). Selection criteria were unclear. The outcomes reported were not subdivided by virus type. The IT (Index Test) is compared with viral cultures type A2, with sensitivity varying from 69 to 85%, depending on typology of specimen, (other indicators were not reported for an accurate diagnosis). The IT is compared with RS (Reference Standard) mixed type A2+B1 with a sensitivity of 69-85%, a specificity of 97-98%, PPV and NPV (Positive Predictive Value and Negative Predictive Value) of 96-98% and 78-87% respectively, depending on typology of specimen. The IT was carried out in laboratory and the study was conducted only partly in a correct manner. Even though both RS had replicable characteristics, only type B1 resulted in a correct classification for the conditions, but it was performed on only one part of the sample.

Bellei[48] - During an influenza season (no information on viral circulation is reported), the authors enrolled 33 eligible patients with ILI (adults before starting antiviral treatment). Selection criteria were unclear. The outcome measures were not subdivided by virus type, and used only one specimen type (NPS). The IT was compared with viral cultures type A1 with a sensitivity and specificity, respectively of 85.5% and 75.0% (other indicators for accurate diagnosis were not reported). The IT was conducted in laboratory. Laboratory comparisons were well conducted and the authors provide sufficient details for RS replication.

Mehlmann[27]- During an influenza season (no information on viral circulation is reported), the authors enrolled an unclear number of eligible patients with ILI on the basis of unclear selection criteria. The outcome measures were not subdivided by virus type or by specimen typology. The IT is compared with viral cultures type A2 with a sensitivity, specificity, PPV and NPV of 93% [83-97%], 100% [92-100%], 100% [93-100%], 92% [81-97%] respectively. The second comparator type B1 showed a sensitivity, specificity, PPV and NPV of IT respectively of 85% [74-92%], 97% [87-100%], 98% [90-100%], 82% [69-90%]. The length of time taken to complete the IT is not clear. The study was correctly conducted only in part, even though the RS has replicable characteristics, only the RS type B1 was appropriate for the correct classification of the conditions.

Poehling[29] - During an influenza season (no information on viral circulation is reported), the authors enrolled 303 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by age group but not subdivided by virus type and used only one type of sample (NS). The IT is compared with RS type mixed A2 and BN1 with a sensitivity, specificity, PPV and NPV respectively reported as 74%, 98%, 74%, 98%. The study was conducted in a laboratory. Data for replicability are not sufficient as replicability was supported only by RS type B1. The study was not correctly designed as the comparator and the IT were conducted using two specimens from the same patient.

Harden[33] - During an influenza season (no information on viral circulation is reported), the authors enrolled 157 eligible patients. Selection criteria were unclear. The outcome measures were not subdivided by virus type and used only one specimen type (NPA). The IT with comparator RT-PCR type B4 had a sensitivity, specificity, PPV and NPV reported as 44% [32-58%], 97% [91-99%], (not reported any other indicators for accurate diagnosis). The IT was conducted by a physician. The IT and RS were performed on two different samples taken from the same patient. RS was appropriate, but the authors do not provide sufficient details for replication of their methods.

Quach[49] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcome measures where not subdivided by virus type and used only one specimen type (NPA). The IT compared with mixed viral cultures type A1+A2 with a sensitivity, specificity, PPV and NPV respectively reported as 79.2% [68.2-90.2%], 82.6% [77.9-87.3%], 49.4%, 94.9%. The IT was conducted in a laboratory. The reference standard is appropriate but no indications are provided for the replication of the IT and RT.

Rashid[37] – During one influenza season (no clear information on viral circulation is reported) 567 pilgrims attending the Hajii were enrolled. Participants presented with ILI symptom within 1 week of onset. The outcome measures where not subdivided by virus type and used only one specimen type (NS). The IT with comparator RT-PCR type B3 has sensitivity, specificity, PPV and NPV, LRP and LRN of 22%, 99%, 72%, 92%, 22% e 0.79%. It reported the sensitivity of 22% for virus A and 23% for virus B. The IT was conducted in the laboratory. The RS is appropriate and the authors provide sufficient details for replication of their methods. but IT and RS were performed on two different specimens.

Pregliasco[36] - During two influenza seasons (no information on viral circulation is reported), the authors enrolled 928 eligible patients with ILI. Selection criteria were unclear. The outcome measures where not subdivided by virus type or by influenza season and used only one specimen type (NS for the first season; TS for the second season). The comparator for the first influenza season had mixed virus culture type A1 + A2 with a sensitivity, specificity, PPV and NPV respectively reported as 36.5% [25-49.6%], 82.1% [78.2-85.5%], 22.6% and 90.1%. In the second influenza season the IT was compared with RS mixed type A1+A2 with a sensitivity, specificity, PPV and NPV respectively reported as 54.5% [24.6-81.9%], 98.5% [96.3-99.4%], 54.5%, 98.5%. The second comparator was type B3 with a sensitivity, specificity, PPV and NPV respectively reported as 58.3% [28.6-83.5%], 98.8% [96.7-99.6%], 66.3%, 98.5%. The IT was conducted in paediatricians' surgery during the first season and in a laboratory for the second season. The RS are appropriate: In particular, the viral culture is appropriate (only A1). The authors provide sufficient data for replication for both. In the first season the reference standard and the IT where performed from two different specimens from the same patient.

Simmerman[40] - During an influenza season (without unclear information on virus circulation), the authors enrolled 1,092 ILI eligible Thai patients. Selection criteria were unclear. The outcome measures where not subdivided by virus or sample type, but were subdivided by period of circulation. The comparator for the first influenza season had mixed virus culture type A1 + A2 with a sensitivity, specificity, PPV and NPV respectively reported as 77%, 96%, 82%, 95%. The second comparator was of type B1 but no accuracy outcomes are reported. The IT was conducted in an outpatients department. The RS is appropriate and the authors provide sufficient details for replication. The IT and the RS were performed on two different samples.

Hurt[44] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus or specimen type. The IT is compared with mixed viral cultures type A1 and A2 with a sensitivity, specificity, PPV and NPV respectively reported as 30-67%, 100%, 100% and 89-96%. The IT was conducted in a laboratory. The study partly used the correct comparator for the IT/RS. Only the inoculation in MDCK cells is adequate for influenza virus culture according to WHO and CDC recommendations (see Appendix 9). The authors provide sufficient details for RS replication. The study used another five IT (Binax Now Influenza A&B; Denka Seiken Quick Ex-Flu; Espline Influenza A&B-N; Rockeby Influenza A Antigen Test; BD Directigen EZ Flu A+B).

Cazacu[15] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of eligible children patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus type and used only one type of sample (NS). The IT is compared with viral cultures type A2 with a sensitivity, specificity, PPV and NPV respectively reported as 70.4%, 97.7%, 84.4%, 94.9%. The IT was conducted in a laboratory. The RS is inappropriate and the authors do not provide sufficient details for RS replication. The study also used another IT (Directigen Flu A+B - Becton Dickinson).

Ruest[46] - During an influenza season (no information on viral circulation is reported), the authors enrolled 192 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus type and used only one sample type (NPA). The IT comparator had viral cultures type A1 with a sensitivity, specificity, PPV, NPV, LRP and LRN respectively reported as 91%, 86%, 78%, 95%, 6.5 [5.3-8] and 0.10 [0.05-0.22]. The study used a second comparator (RT-PCR type B4) with a sensitivity, specificity, PPV, NPV, LRP and LRN and IT respectively reported as 86%, 90%, 87%, 90%, 8.6 [4.9-15.2] e 0.16 [0.09-0.74]. The IT was conducted in a laboratory. The RS was appropriate (viral isolation and RT-PCR); sufficient details are provided for the replication of RT-PCR (sensitivity not reported), but not for viral isolation. The study used another index test (Directigen Flu A+B EIA).

Rodriguez[13] – During an influenza season (no information on viral circulation is reported), the authors enrolled 1,521 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus or specimen type (PS,NW, NS). The IT comparator had viral cultures type A3 with a sensitivity, specificity, PPV and NPV respectively reported as 95%, 76%, 81% and 93%. The IT was conducted in a laboratory. The study was not well described and the number of samples submitted for the IT or RS are unclear. The cell type used for the viral cultures was not specified and it was not possible to express an opinion on the appropriateness of the standard. The authors do not provide sufficient details for RS replication. The study reports three more IT (Flu OIA, ZStat Flu, Directigen fluA).

Directigen Flu A+B EIA (Becton Dickinson)

Landry[23] - During the peak influenza season the authors enrolled 152 eligible patients with ILI (no information on viral circulation is reported). Selection criteria were unclear. The outcome measures were subdivided by virus type (only virus A was detected) and used one specimen type NP. The IT is compared with antigen detection (C), but data for diagnostic accuracy were not reported. The IT was conducted in laboratory. The RS was not appropriate for the classification and the authors provide sufficient details for RT replication.

Drinka[19] – During four influenza seasons (no information on viral circulation is reported), the authors enrolled 327 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus type and used one specimen type (NPS). The IT is compared with viral cultures type A3 with a sensitivity, specificity, PPV and NPV respectively reported as 64%, 99% and 94% (other indicators of diagnostic accuracy were not reported). The IT was conducted in a laboratory. The cell type used for the viral cultures were not specified and it was not possible to express an opinion on the appropriateness of the standard and data were not sufficient to be replicable.

Alexander[41] - During an influenza season (no information on viral circulation is reported), the authors enrolled 193 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type (only virus A was detected) and used more than one specimen type (NPA, NS, PS, BW). The IT is compared with mixed viral cultures type A1 and A2 with a sensitivity, specificity, PPV and NPV respectively reported as 80.8%, 100%, 100%, 83.2%. The same value of diagnostic accuracy which verified the second comparator represented the antigen detection (C). The study used a third comparator representing the RT-PCR type B4 with a sensitivity, specificity, PPV and NPV the IT respectively reported as 83%, 97.9%, 97.5%, 85.6%. Where the RT took place is unclear. The authors used RETCIF (rapid enhanced tissue culture immunofluorescence) for viral culture only MDCK are cells recommended for influenza virus culture by the WHO. RT-PCR is an appropriate reference standard. The authors provide sufficient details only for viral culture.

Chan[47] - During an influenza season (no information on viral circulation is reported), the authors enrolled 250 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type (only for absolute data) and used one specimen type only (NPA). The IT was compared with viral cultures type A1 with a sensitivity, specificity, PPV and NPV respectively reported as 92.59%, 94.89%, 83.3% and 97.89%. The IT was conducted in a laboratory. The RS was appropriate and there were sufficient data to be replicable.

Grondal[32] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of elligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type and used one specimen type (NPA). The IT is compared with RT-PCR of B3 with a sensitivity, specificity, PPV and NPV for virus A respectively reported as 29.3%, 99.2%, 85.7% and 89.8%. For virus B with a sensitivity, specificity, PPV and NPV respectably reported as 10%, 99.6%, 66.7% and 93.9%. The IT was conducted in a laboratory. The RS was appropriate and the authors provide sufficient details for reference test replication.

Rahman[30] - During an influenza season (no information on viral circulation is reported), the authors enrolled 818 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type and used one specimen type (NPS). The IT is compared with viral cultures type A1 and with sensitivity and specificity for the virus A respectively reported as 41% and 98% (other indicators of diagnosis accuracy were not reported). The IT for virus B sensitivity and specificity of 50% and specified 99% (other indicators of diagnostic accuracy were not reported). The study also reported aggregate data by virus with a sensitivity, specificity, PPV and NPV respectively reported as 42% 28-57%], 96% [89-99%], 86% [65-95%], 74% [65-82%]. The IT was conducted in a laboratory. The RS is appropriate and sufficient data are provided for replication but no indications are provided for the replication of the index test.

Reina[38] - During a one year study (no information on viral circulation is reported), the authors enrolled 93 paediatrics and 67 adults. Selection criteria were unclear. The outcome measures were subdivided by virus type and used two types of specimen (NPA for pediatrics, TS for adults). The IT is compared with viral cultures type A1. In adult the sensitivity, specificity, PPV, NPV, for virus A were respectively reported as 72.7%, 100%, 100% and 95.1%, for virus B sensitivity, specificity, PPV, NPV were respectively reported as 41.1%, 100%, 100% and 79.5%. For the pediatric patients the sensitivity, specificity, PPV, NPV, for virus A respectively reported as 86.6%, 100%, 100% and 92.1%, for virus B sensitivity, specificity, PPV, NPV respectively reported as 62.5%, 100%, 100% and 88.6%. The IT was conducted in a laboratory. The reference standard is appropriate but the authors do not report sufficient details for replication.

Hamilton[21] - During an influenza season (no information on viral circulation is reported), the authors enrolled 300 eligible patients with ILI according to unclear selection criteria. The outcome measures were not subdivided by virus type and used one specimen type (NA). The IT is compared with viral cultures type A2 with a sensitivity, specificity, PPV and NPV respectively reported as 75%, 93%, 74%, 93%. The IT was conducted in a laboratory. The reference standard was inappropriate and the authors does not provide sufficient details for reference test replication. The study also used another IT (ZstatFlu-II).

Landry[25] - The authors enrolled 89 eligible patients with ILI, but the period of the study is unclear. Selection criteria were unclear. The outcome measures were subdivided by virus type and used one specimen type NPS/NPA. The IT is compared with viral cultures type A1 with a sensitivity, specificity, respectively reported as 55.9%, 100% (no other indicators of diagnostic accuracy were reported). The IT was conducted in a laboratory. The RS was appropriate and presented sufficient data to be replicable. The study also used another IT (Now Flu A and B - Binax).

Smit[47] - During an influenza season (no information on viral circulation is reported), the authors enrolled 448 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type and used more than one specimen type (NPS, TS, NW). The IT is compared with mixed viral cultures type A1 and with a sensitivity, specificity, PPV and NPV for virus respectively reported as 53%, 99.7%, 97% and 83%. For virus B with a sensitivity, specificity, PPV and NPV respectively reported as 33% and 100%. The IT was conducted in a laboratory. The RS is appropriate (viral isolation with two different cell types, one of them recommended by WHO and CDC). The authors provide sufficient details for RT replication. The study used another two ITs (Binax Now FluA&B; Binax Now Flu A - Binax Now Flu B).

Dunn[43] - During a not defined period of the study (no information on viral circulation is reported), the authors enrolled an unspecified number of patients on the basis of not clearly defined selection criteria. The outcome measures were subdivided by virus type and the specimen type used was not reported. The IT was compared with mixed viral cultures type A1+A2 with a range of a sensitivity, specificity, for the virus respectively reported as 57.5-60% and 99.6-100% (no other indicators of diagnostic accuracy were reported). The IT was compared with antigen detection (C) with sensitivity, specificity respectively reported as 57.1-75% and 96.9-99.6% (no other indicators of diagnostic accuracy were reported). The third comparator was a mixed system A1 + A2 + C with a range of sensitivity, specificity respectively reported as 70-82.4% and 99.3-100% (no other indicators of diagnostic accuracy were reported). The length of time taken to complete the IT is not reported. Only the RS viral culture is appropriate, and sufficient details are available for replication. The study used another IT (Quick S-influ A/B -Denka Seiken).

Weinberg[32] - During an influenza season (no information on viral circulation is reported), the authors enrolled 178 eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type but not by specimen type. The IT was compared with a mixed system of A2+B3+Directigen Flu (A+B) + DirectigenEZ Flu (A+B)+Now Flu A Now Flu B (the comparator was taken from the IT), with a sensitivity, specificity by virus 29-66% and 99-100% (no other indicators of diagnostic accuracy were reported). The IT was conducted in a laboratory. The reference standard is a mixed system. It is considered inapprorpriate, as the index test is included in the RS (i.e. the true positive is identified also on the basis of 2 positive results in the rapid test). The study used another two ITs (Directigen EZ Flu (A+B); Binax Now Flu A Now Flu B).

Directigen Flu A (Becton Dickinson)

Noyola[28] - During an influenza season (no information on viral circulation is reported), the authors enrolled 497 eligible patients with ILI. Selection criteria were unclear. The subdivision of the outcome measures by virus type is unclear and they used one specimen type (NPW). The IT is compared with viral cultures type A2. With a sensitivity, specificity, PPV and NPV, virus A respectively reported as 89.7%, 98.1%, 93.5% and 96.9%. The study reports the outcomes for virus A/B with a sensitivity, specificity, PPV and NPV respectively reported as 74.3%, 98%, 93.5% and 90.7%. The IT was conducted in a laboratory. The study was not well described, and the RS used was not appropriate according to WHO and CDC recommendations. However there are sufficient data reported for replication. The study used another IT (Zstat Flu -ZxmeTx).

BD Directigen EZ Flu A+B (Becton Dickinson)

Hurt[44] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unspecified number of eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by virus type, but not by specimen type. The IT is compared with mixed viral cultures type A1 + A2 with a sensitivity, specificity, PPV and NPV by virus respectively reported as 30-69%, 100%, 100% and 90-96%. The IT was conducted in a laboratory. The study in part followed the correct comparator for the IT/RS. Only the inoculation in MDCK cells is adequate to culture the influenza virus. All the samples followed WHO and CDC guidelines. The authors provide sufficient details for RS replication. The study used another five IT (Binax Now Influenza A&B; Denka Seiken Quick Ex-Flu; Espline Influenza A&B-N; Rockeby Influenza A Antigen Test; Quick Vue Influenza A+B).

FLU OIA (BioStar, Inc., Boulder, Colorado)

Covalciuc[17] - During an influenza season (no information on viral circulation is reported), the authors enrolled 148 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus. The IT is compared with cultural virus type A1 with a sensitivity, specificity, by virus specimen respectively reported as 62.1-88.4% e 51.5-79.5% (no other indicators of diagnostic accuracy are reported). The time taken to complete the IT is unclear. The RS is appropriate and well described and the study can be replicated.

Hindiyeh[22] - During an unspecified period within an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided by specimen type. The IT was compared with viral cultures type A1 with a sensitivity, specificity, PPV and NPV on the total of the specimen respectively reported as 48%, 88%, 64% and 79%. The IT is compared with antigen detection (C), with a sensitivity, specificity, PPV and NPV on the total of the specimens respectively reported as 81%, 96%, 88% and 94%. Finally the IT is compared with a mixed system (A1 + C) with a sensitivity, specificity, PPV and NPV on the total of the specimen respectively reported as 54%, 97%, 91% and 77%. The IT was conducted in a laboratory. Only the RS A1 (isolated virus in PRMK) is adequate to identify the virus and therefore appropriate and replicable.

Hermann[35] - During an unclear period of the study (no information on viral circulation is reported), the authors enrolled 268 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus type, but were subdivided by specimen type. The IT is compared with viral cultures type A1 with a sensitivity, specificity, for the NPA specimen respectively reported as 46.3% and 90.8% (no other indicators of diagnostic accuracy are reported). The IT is compared with antigen detection (C), with a sensitivity, specificity, for specimen type respectively reported as 40.4-77.5% and 82-89.1% (no other indicators of diagnostic accuracy are reported). However the IT is compared with RT-PCR type B3 with a sensitivity, specificity, for specimen type respectively reported as 48.8-86.6% and 75.5-93.9% (no other indicators of diagnostic accuracy are reported). Finally the IT is compared with a mixed system A1 + C + B3 with a sensitivity, specificity, for specimen type respectively reported as 39.3-78.6% and 84.2-95.9% (no other indicators of diagnostic accuracy are reported). The IT was conducted in a laboratory. The RS viral culture A1 and RT-PCR are appropriate and replicable. The viral isolation was performed just on nasopharyngeal aspirates using cells recommended by the WHO and CDC.

Schultze[39] - During an unclear period of an influenza outbreak in 1998-1999 (no information on viral circulation is reported), the authors enrolled 378 eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided by virus or by specimen type. The IT is compared with a mixed system of A1 + C with a sensitivity, specificity, NPV on the total of specimens respectively reported as 64.4%, 94.9% e 73% (no other indicators of diagnostic accuracy are reported). The IT was conducted in a laboratory. The reference standard is not appropriate (the use of viral culture is appropriate, but is part of a mix system which was not appropriate). The data for replicability were sufficient.

Boivin[41] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcome measures were subdivided only for virus type and used only one specimen type (PS). The IT is compared with viral cultures type A1 with a sensitivity, specificity, PPV and NPV respectively reported as 54%, 74.1%, 72.7% and 55.8%. The IT is compared with RT-PCR type B3 with a sensitivity, specificity, PPV and NPV respectively reported as 56%, 77.2%, 76.3% and 57.1%. The IT was conducted in an outpatients clinic. The study was not conducted correctly: the RS and IT were taken from two different samples from the same patient. The RS is appropriate, but there were only sufficient data to replicate the RT-PCR.

Binax Now Flu A & Flu B Test (Binax Inc),

Cruz[18] – During a period of seven months with unclear data on virus circulation, the authors enrolled 3561 Eligible patients with ILI. Selection criteria were unclear. The outcome measures were not subdivided for virus or specimen type. The IT is compared with viral cultures type A2 with a sensitivity, specificity, respectively reported as 61.6% [60.3-63.2%] and 95.7% [95.1-96.3%]. For virus A a LR of 15.3% [13.0-18.2%] was reported (other indicators for diagnostic accuracy are not reported). The IT was conducted in a laboratory. The study was not conducted correctly: the RS was not described sufficiently to allow a replication, and was not appropriate (the cells used were not those recommended for influenza viral isolation defined by the WHO and CDC).

Rahman[31] - During a part of the influenza season (no information on viral circulation is reported) the authors enrolled 143 of 932 eligible patients with ILI. Selection criteria were unclear. Outcomes are not reported separately by virus type and the authors used one specimen type only (NPS). The IT is compared with A1 viral culture with a sensitivity and specificity respectively reported as 65% and 100%. No other measures of diagnostic accuracy are reported. The authors also compare the IT with B2 RT-PCR, with a sensitivity and specificity respectively reported as 61% and 100%. No other measures of diagnostic accuracy are reported. Lastly the authors compared the index test with a mixed A1+B2 RS with sensitivity and specificity respectively reported as 61%, 100%, 100% and 89%. The IT was carried out in a clinic. The viral culture as RS is appropriate (cell type recommended by WHO and CDC) and well described. No information about RT-PCR is reported. Only for viral culture A1 were sufficient data reported to ensure replicability.

Magauran[26] – The authors report enrolling 348 patients during two influenza seasons. Selection criteria were unclear. No information on virus circulation is reported. Outcomes and specimen type are not reported separately by virus type. The authors compared the IT (carried out in a laboratory) with A2 type viral culture and a 81% NPV. No other measures of diagnostic accuracy are reported. The RS is not appropriate to assess the IT and the data are insufficient to assess reproducibility.

Fader[20] - During an influenza season (no information on viral circulation is reported) the authors enrolled an unspecified number of eligible patients with ILI. Selection criteria were unclear. Outcomes are reported only by virus type A and not subdivided by specimen type. The IT is compared with A2 viral culture with a sensitivity and specificity PPV and NPV respectively reported as 64.9%, 98.4%, 89.3% and 93.2%. The IT was carried out in a laboratory. The study was not conducted correctly: The RS is not appropriate (the cells used were not those recommended for the influenza viral isolation defined by the WHO and CDC). There was sufficient data reported to ensure replicability.

Booth[45] - During an influenza season (no information on viral circulation is reported), the authors enrolled an unclear number of adults and children. Selection criteria were unclear. The outcome measures were subdivided by virus type, but not for specimen. The IT is compared with viral cultures type A1 with a sensitivity, specificity, PPV and NPV for virus respectively reported as 47-80%, 99-100%, 88-97% and 96%. The IT was compared with antigen detection (C) with a sensitivity, specificity, PPV and NPV respectively reported as 60-83%, 95-99%, 66-75% and 98%. Finally the IT is compared with a mixed system A1 + C with a sensitivity, specificity, PPV and NPV for virus respectively reported as 50-79%, 98-100%, 85-88% and 97%. The IT was conducted in a laboratory. Only the RS viral culture A1 is appropriate. The authors report that only the speci-

mens which were positive for FLU A or B were tested with the IT but Table1 reports that the IT was conducted on all samples, so the text is contradictory. The authors do not provide sufficient details for reference test replication. The study used another IT (Immunocard Stat! Flu A&B plus).

Binax Now Flu A (Binax Inc) - Binax Now Flu B (Binax Inc)

Smit[47] - During an influenza season (no information on viral circulation is reported) the authors enrolled 448 ILI eligible children. Selection criteria were unclear. Outcomes are reported by subgroup for virus type, and was used more than one specimen type (NPS, TS, NW). The IT is compared with a mixed viral culture A1+A2 with a sensitivity and specificity, PPV and NPV respectively reported as 58%, 99%, 94% and 89%. For B virus the sensitivity and specificity are 33% and 100% (No other measures of diagnostic accuracy are reported). The index test was carried out in a laboratory. RS is appropriate: (viral isolation with two different cell type, one of them recommended by WHO and CDC). The authors provide sufficient details for reference test replication. The study used two other ITs (Directigen Flu A+B; DirectigenEZ Flu A+B).

ImmunoCard STAT! Flu A and B (Meridian Bioscience INC)

Weitzel[51] - During a period of nearly two years (no information on viral circulation is reported) the authors enrolled 203 eligible patients with ILI (travellers). Selection criteria were unclear. Outcomes are reported by subgroup for virus type, and was used one specimen type. The IT is compared with a mixed system A1+B2 with a sensitivity and specificity for virus respectively reported as 64-67%, 99-100%. The PPV and the NPV for the total virus is 95% [85-100%]. The IT was carried out in a laboratory. The reference is appropriate but the study should be considered inaccurate. The authors report that two specimens were collected from each patient, but one was tested with the IT, the other with the RS. As a consequence the results are not comparable or replicable.

Xpect Flu A/B (Remel Inc.)

Cazacu[16] - During an influenza season (no information on viral circulation is reported) the authors enrolled 400 eligible patients with ILI. Selection criteria were unclear. Outcomes are reported by subgroup for virus type, by test duration (15 or 30 minutes), but not by specimen type. The IT is compared with a viral culture type A2 with a sensitivity and specificity, PPV and NPV for virus at 30 minutes respectively reported as 92.4-97.8%, 100%, 100% and 98.2-99.7%. The IT at 30 minutes has a sensitivity and specificity, PPV and NPV respectively reported as 93.7-97.8%, 100%, 100% and 97.8-98.5%. The study was not conducted correctly: the RS was not appropriate (the cells used were not recommended for the isolation of influenza virus by WHO or CDC). There were insufficient data for RT replication.

ZstatFlu (Zymetx Corp.)

Hulson[23] - During two influenza seasons (no information on viral circulation is reported) the authors enrolled 268 eligible patients with ILI. The outcomes reported are not sub grouped for virus, they used one specimen type (OPS). The IT is compared with a viral culture type A3 with a

sensitivity and specificity for PPV, NPV, LRP and LRN respectively reported as 65%, 83%, 79%, 70%, 3.82 and 0.42. The IT was carried out in a laboratory. The study is not well described and the number of samples submitted for the IT or RS (viral isolation) is not clear. The cell type used for the viral cultures was not specified and therefore it is not possible to express a judgment on the appropriateness of the standard.

Quick Ex-Flu (Denka Seiken)

Hurt[44] – During an influenza season (no information on viral circulation is reported) the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcomes reported are sub grouped for virus, but not by specimen type. The IT is compared with mixed viral culture type A1 + A2 with a sensitivity and specificity for PPV and NPV respectively reported as 30-71%, 100%, 100% and 90-96%. The study partly used the correct comparator for the IT/RS. Only the inoculation in MDCK cells is appropriate for influenza virus culture. The authors provide sufficient details for RS replication. The study used another five IT (Binax Now Influenza A&B; BD Directigen EZ Flu A+B; Espline Influenza A&B-N; Rockeby Influenza A Antigen Test; Quick Vue Influenza A+B).

Quick S-influ A/B (Denka Seiken)

Dunn[43] - During an unspecified period (no information on viral circulation is reported) the authors enrolled an unclear number of eligible patients with ILI. Selection criteria were unclear. The outcomes reported are sub grouped by virus but the specimen types used are not reported. The IT is compared with a mixed viral culture type A1 + A2 with a sensitivity and specificity respectively reported as 57.5-66.7% and 99.6-100% (no other indicators of diagnostic accuracy are reported). The IT is compared with an antigen detection (C) with a mixed viral culture type A1 + A2 + C with a sensitivity and specificity respectively reported as 70.6-80% and 100% (no other indicators of diagnostic accuracy are reported). The study used another IT (Directigen Flu A+B). The length time taken for the IT is not reported. Only the RS type viral culture is appropriate with sufficient data for replication.

Espline Influenza A&B-N (Fujirebio Corp)

Hurt[44] – During an influenza season (no information on viral circulation is reported) the authors enrolled an unspecified number of eligible patients with ILI. Selection criteria were unclear. The outcomes reported are sub grouped by virus, but not by specimen type. The IT is compared with a mixed viral culture type A1 + A2 with a sensitivity and specificity PPV and NPV respectively reported as 30-67%, 100%, 100% and 96-89%. The study partly used the correct comparator for the IT/RS. Only the inoculation in MDCK cells is adequate to culture influenza viruses. With regards to the WHO and CC recommendations, all the samples submitted to such methodology. The authors provide sufficient details for RS replication. The study used another five IT (Binax Now Influenza A&B; Denka Seiken Quick Ex-Flu; BD Directigen EZ Flu A+B; Rockeby Influenza A Antigen Test; Quick Vue Influenza A+B).

Rockeby Influenza A antigen test (Rockeby)

Hurt[44] - During an influenza season (no information on viral circulation is reported) the authors enrolled an unknown number of eligible patients with ILI. Selection criteria were unclear. The outcomes reported are only for virus A. The IT is compared with a mixed viral culture type A1 + A2 with a sensitivity and specificity PPV and NPV respectively reported as 10%, 100%, 100% and 74%. The study was conducted in a laboratory. The study partly followed the correct procedures for the IT/RS. Only the inoculation in MDCK cells is adequate for culture of influenza viruses,d this was used for all samples as recommended by WHO and CDC. The authors provide sufficient details for RS replication. The study used another five IT (Binax Now Influenza A&B; Denka Seiken Quick Ex-Flu; Espline Influenza A&B-N; BD Directigen EZ Flu A+B; Quick Vue Influenza A+B).

Table 1 Evidence table

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Agoritsas 2006	III-b	Cross sectional study	USA influ season: 03/04 and 04/05 Children Hospital	122 ILI children mean age: 5Y age range: 2W-18Y Gender: NR	1) QuickVue (Quidel corp.) Test duration: 15' Carried out in: laboratory	virus detected: A/B disaggregate result: NR	366 total specimens - 122 NS - 122 NPS - 122 NPW Not acceptable by manufacturer: NPW Fresh specimen	a) A2 b) a) + B1	1) vs a) Sens NS: 78% [NR] NPS: 85% [NR] NPW: 69% [NR] PPV, NPV and Spec: NR 1) vs b) PPV NS: 96% [NR] NPS: 98% [NR] NPW: 98% [NR] NPV NS: 82% [NR] NPS: 87% [NR] NPW: 78% [NR] Sens NS: 78% [NR] NPS: 85% [NR] NPW: 69% [NR] Spec NS: 97% [NR] NPS: 98% [NR] NPW: 98% [NR]	QA: Y= 3; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: UC; - virus circulation: NR (months of influenza season: NR); - disaggregate results for specimen: Y; - disaggregate result for virus: NR. - appropriate RS: N - replication of RS: Yes
Alexander 2005	I-b (only part of the study)	Cross sectional study	AUS Aug-Oct 03 Pediatric Hospital	193 ILI children median age: 2 Y Gender: NR	2) Directigen flu A+B (Beckton Dickinson) Test duration: 10'-15' Carried out in: NR	virus detected: A Disaggregate result; Y (only virus A isolated)	- 183 NPA - 4 NS - 5 PS - 3 BW Not acceptable by manufacturer: NS, PS, BW Fresh specimen	a) A1+A2 b) C c) B4	2) vs a) or b) PPV 100% [NR] NPV 83.2% [NR] Sens 80.8% [NR] Spec 100% [NR] 2) vs c) PPV 97.5% [NR] NPV 85.6% [NR] Sens 83.0% [NR] Spec 97.9% [NR]	QA: Y=2 ; N/UC=5 QUADAS: Y=5; N/UC=9 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: only virus A isolated - disaggregate results for specimen: NR - appropriate RS: a) and c) - replication of RS: data sufficient for a)

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Bellei 2003	I-b	Cohort prosp.	BRAZ May-Oct 00 Place: NR	33 patient treated with antiviral drug Age range 18-56 Y Gender: NR	1) QuickVue (Quidel corp.) Test duration: 10' Carried out in: laboratory	virus detected: A/B disaggregate results: NR	99 NPS total specimen - 33 NPS - 24 h symptoms (before starting antiviral treatment) - 33 NPS after 24 h antiviral treatment - 33 NPS after 72 h antiviral treatment Not acceptable by manufacturer: NPS Thawed specimen	a) A1	33 NPS - 24 h symptoms (before starting antiviral treatment) 1) vs a) PPV NR [NR] NPV NR [NR] Sens 85.5 [NR] Spec 75.0 [NR] Other data are available (after 24 h of symptoms)	QA: Y=3 ; N/UC=4 QUADAS: Y=10; N/UC=4 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR - disaggregate results for specimen: only 1 type of specimens - appropriate RS: Y - replication of RS: data sufficient Laboratory comparison well conducted
Boivin 2001	I-b (only part of the study) see notes	Multicenter cross sectional	CANADA 23 Nov 99 -14 Mar 00 General practitioners at 6 outpatient clinics	ILI patients (number of patients: NR) (median age: 36 Y; age range: 6M-83Y) (48% female and 52% male)	3) FLU OIA A/B (Biostar) Test duration: < 20' Carried out in: outpatients clinics by physicians or nurses	virus detected: A/B Disaggregate result: NR	146 PS total specimen (2 from each patient) Not acceptable by manufacturer: PS Thawed specimen	a) A1 b) B3	3) vs a) PPV 72.7% [NR] NPV 55.8% [NR] Sens 54.0% [NR] Spec 74.1% [NR] 3) vs b) PPV 76.3% [NR] NPV 57.1% [NR] Sens 56.0% [NR] Spec 77.2% [NR]	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: sufficent; - virus circulation: NR; - disaggregate result for virus: only virus A detected by RS - disaggregate results for specimen: only 1 type of specimens - appropriate RS: Y - replication of RS: Y, only b) RS and IT are performend on two different specimens from the same patient. The results aren't comparable
Booth 2006	II-b (only part of the study)	Multicenter cross sectional	AUS May-Nov 04 Emergency Dep. Sidney children Hospital	Adults and children (number of patients: NR) Age adults range: NR Age children range: 1-16 Y Gender: NR	4) Immunocard Stat! Flu A&B Plus (Meridian Biosciences, INC) Test duration: NR 5) Binax NowFlu A&NowFlu B (Binax INC) Test duration: NR Carried out in: laboratory	Virus detected A+B Disaggregate results: Y	224 total specimen TS+NS=59 (from adults) NPA =165 (from child) Not acceptable by manufacturer: IT 4): TS; IT 5): TS, NPA Fresh specimen	a) A1 b) C c) a) + b)	Virus A 4) vs a) PPV 88% [NR] NPV 96%[NR] Sens 80%[NR] Spec 98% [NR] 4) vs b) PPV 61% [NR] NPV 98%[NR] Sens 83% [NR] Spec 94%[NR] 4) vs c) PPV 81%[NR] NPV 97%[NR] Sens 81% [NR] Spec 97%[NR] 5) vs a)	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: Y - disaggregate results for specimen: NR - appropriate RS: only a) - replication of RS:data NOT sufficient The test index's implementation is provided only for ImmunoCard STAT (not for Binax)

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
									PPV97% [NR] NPV 96% [NR] Sens 80% [NR] Spec 99%[NR] 5) vs b) PPV 66% [NR] NPV 98% [NR] Sens 83% [NR] Spec 95% [NR] 5) vs c) PPV 85% [NR] NPV 97% [NR] Sens 79% [NR] Spec 98% [NR] Virus B 4) vs a) PPV 100% [NR] NPV 96%[NR] Sens 47%[NR] Spec 100% [NR] 4) vs b) PPV 86% [NR] NPV 98%[NR] Sens 60% [NR] Spec 100%[NR] 4) vs c) PPV 100%[NR] NPV 97%[NR] Sens 50% [NR] Spec 100%[NR] 5) vs a) PPV 88% [NR] NPV 96% [NR] Sens 47% [NR] Spec 100%[NR] 5) vs b) PPV 75% [NR] NPV 98% [NR] Sens 60% [NR] Spec 99% [NR] 5) vs c) PPV 88% [NR] NPV 97% [NR] Sens 50% [NR] Spec 100% [NR]	

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Cazacu 2003	IV-c	Cross sectional	USA Jan-Apr 02 Children Hospital (for admission or emergency department)	ILI children (number of patients: NR) Mean age: 5 Y Age range: 3 M - 28 Y Gender: NR	1) Quick Vue (Quidel, San Diego,) 2)Directigen Flu A+B (Becton Dickinson) Test duration: >=15' Carried out in: laboratory	IT 1) Virus detected A/B Disaggregate results: NR IT 2) Virus detected A + B Disaggregate results: Y	356 NS total specimen IT 1) Not acceptable by manufacturer: NS Thawed specimen	a) A2	1) vs a) PPV 84.4% [NR] NPV 94.9% [NR] Sens 70.4% [NR] Spec 97.7% [NR] 2) vs a) PPV 88.6% [NR] NPV 95.2% [NR] Sens 72.2% [NR] Spec 98.3% [NR] Other disgregate data (for virus) are available for IT 2).	QA: Y=3 ; N/UC=4 QUADAS: Y=3; N/UC=11 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: Y (only for IT 2) - disaggregate results for specimen: Y - appropriate RS: N - replication of RS:data NOT sufficient
Cazacu 2004	IV-c	Multicenter cross sectional	USA Jan-Apr 03 3 hospitals (Texas; Florida; NY)	400 ILI patients (adults and children) Age: NR Gender: NR	6) Xpect Flu A/B (Remel Inc.) Test duration: 15'-30' Carried out in: laboratory	Virus detected A+B Disaggregate results: Y	400 total specimens - 239 NS - 122 NPS - 30 PS - 4 TA - 3 S - 4 BW Not acceptable by manufacturer: NS, PS, TA Thawed specimen	a) A2	6) vs a) - (A+B) at 15 min PPV 100.0% [98,8-100] NPV 97,5% [95,9-99] Sens 94,4% [95,1-96,6] Spec 100.0% [98,8-100] 6) vs a) - (A+B) at 30 min PPV 100.0% [98,8-100] NPV 97,9% [96,4-99,3] Sens 95,2% [93,1-97,3] Spec 100.0% [98,8-100] Other disgregate data (for virus) are available.	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: Y - disaggregate results for specimen: NR - appropriate RS: N - replication of RS:data NOT sufficient Laboratory comparison not well conducted. Number of specimens and results for centre: NR
Chan 2002	I-b	Cross sectional	CHINA Mar-Apr 00 Queen Mary Hospital (Department of microbiology)	250 patients Median age class: 2-11 Y Modal age class: < 2 Y Gender: NR	2) Directigen flu A+B (Beckton Dickinson) Test duration: NR Carried out in: laboratory	Virus detected A/B Disaggregate results: NR Virus detected A+B Disaggregate results: Y only absolute data	250 NPA total specimen Not acceptable by manufacturer: OK Thawed specimen	a) A1	2) vs a) PPV 83.3% [NR] NPV 97.89% [NR] Sens 92.59% [NR] Spec 94.89% [NR] 2) vs b): data not reported+J37	QA: Y=2 ; N/UC=5 QUADAS: Y=9; N/UC=5 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR (only absolute data disaggregate for virus) - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS:data sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Covalciuc 1999	I-b	Multicenter cross sectional	USA Jan-Apr 98 Emergency rooms, physician offices, employee clinics, and urgent-care facilities (3 cities - Midwest, Southwest, Roky Mountain).	184 ILI patient Age range: 2 M - 76 Y Median age class= 17-54 Y Gender: NR	3) FLU OIA (BioStar, Inc., Boulder, Colorado) Test duration: 15' Carried out in: NR explicitly (laboratory)	Virus detected A/B Disaggregate results: NR	404 total specimens (but 1 missing) - 79 NA - 142 NPS - 112 TS - 70 S Not acceptable by manufacturer: OK Fresh specimen	a) A1	3) vs a) PPV NA: NR% [NR] NPS: NR% [NR] TS: NR% [NR] S: NR% [NR] Total specimen: NR% [NR] NPV NA: NR% [NR] NPS: NR% [NR] TS: NR% [NR] S: NR% [NR] Total specimen: NR% [NR] Sens NA: 88.4% [74.9-96.1%] NPS: 83.3% [68.6-93.0%] TS: 62.1% [42.3-79.3%] S: 81.1% [64.8-92.0%] Total specimen: 80.1% [72.9-86.2%] Spec NA: 69.4% [51.9-83.7%] NPS: 76.2% [66.7-84.1%] TS: 79.5% [69.2-87.6%] S: 51.5% [33.5-69.2%] Total specimen: 73.1% [67.2-78.5%]	QA: Y=3 ; N/UC=4 QUADAS: Y=7; N/UC=7 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS:data sufficient Number of specimens and results for centre: NR

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Cruz 2006	IV-b	Cross sectional	USA Aug 03 - Mar 04 Children Hospital (bed tertiary care)	3561 patients Median age: 1,4 Y Age range: 1 D - 41 Y Gender: NR	5) Binax Now Flu A & Flu B Test (Binax Inc) Test duration: NR Carried out in: laboratory	Virus detected A/B Disaggregate results: NR	4383 total consecutive specimens - 4302 NW - 59 TA - 10 NS - 8 BL - 2 S - 2 SF Not acceptable by manufacturer: TA, BL,S, SF Fresh specimen	a) A2	5) vs a) PPV NR [NR] NPV NR [NR] Sens 61.6 % [60.3-63.2%] Spec 95.7% [95.1-96.3%] Virus + A LR 15.3 % [13.0-18.2%] Other disgregate data (by period of influenza season, ED/not ED , age) are available	QA: Y=5 ; N/UC=2 QUADAS: Y=4; N/UC=10 - Selection criteria: UC; - virus circulation: UC (influenza B prevalence was low); - disaggregate result for virus: NR (influenza B prevalence was low); - disaggregate results for specimen: NR - appropriate RS: inappropriate (cell are not recommended by WHO or CDC) - replication of RS:data NOT sufficient
Drinka 2006	IV-c	Cross sectional	USA Veterans nursing home 01-05 influenza seasons (NO cases were identified in 02-03)	327patients Mean age: 74 (± DS 10) Gender: NR	2) Directigen AB (Beckton Dickinson) Test duration: NR Carried out in: laboratory	Virus detected A/B Disaggregate results: NR	327 NPS total specimen Not acceptable by manufacturer: OK Fresh specimen	a) A3	2) vs a) PPV 94% [NR] NPV NR [NR] Sens 64%[NR] Spec 99% [NR]	QA: Y=1 ; N/UC=6 QUADAS: Y=3; N/UC=11 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: Y - appropriate RS: inappropriate (cell type is not reported) - replication of RS:data NOT sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Dunn 2003	I-b (only part of the study)	Cross sectional	Context: NR Setting: NR 02 respiratory virus season	Number of patients: UC Age data: NR Gender: NR	7) Quick S-influ A/B (Denka-Seiken) 2) Directigen Flu A+B (Becton Dickinson) Test duration: NR Carried out in: NR	IT 7) and 2) Virus detected A+B Disaggregate results: Y	255 total specimens Specimen type: NR Thawed specimen	a) A1 +A2 b) C c) a + b	Vir A: (val %) 7) vs a) sen 57.5 ;spec 100 7) vs b) sen 57.1; spec 96.9 7) vs c) sen 70.6; spec 100 7) vs d)sen 44; spec 100 2) vs a) sen 57.5; spec 100 2) vs b) sen 57.1; spec 96.9 2) vs c) sen 82.4; spec 100 2) vs d) sen 36; spec 100 Vir B (val %) 7) vs a) sen 66.7 ;spec 99.6 7) vs b) sen 83.3; spec 99.6 7) vs c) sen 80; spec 100 7) vs d)sen 33.3; spec 98.9 2) vs a) sen 60; spec 99.6 2) vs b) sen 75; spec 99.6 2) vs c) sen 70; spec 99.3 2) vs d) sen 33.3; spec 100	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: Y; - disaggregate results for specimen: NR - appropriate RS: appropriate only a) - replication of RS:data sufficient Context and setting: NR

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Fader 2005	III-b	Cross sectional	USA ED, pediatric outpatient clinic, impatient setting ILI season 03 - 04	Number of patients: NR Median age class: 0-5 Y Gender: NR	5) Binax NOW Flu A&B (Binax, Inc.) Test duration= 15' Carried out in: virology laboratory	Virus detected A Disaggregate results: N (only virus A)	455 total specimens - NS (number NR) - NA (number NR) Not acceptable by manufacturer: OK Fresh specimen	a) A2	5) vs a) Vir A PPV 89.3% [NR] NPV 93.2% [NR] Sens 64.9% [NR] Spec 98.4% [NR] Other disgregate data (by age group) are available	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: Y (only virus A); - disaggregate results for specimen: NR - appropriate RS: N - replication of RS:data sufficient Context and setting: NR
Grondal 2005	I-b	Cross sectional	Germany Jan-Jun 03 (ILI season) Department of Paediatrics in University	Number of patients: NR Age range: 0-16 Y Median age: 2,25 Y Gender: NR	2) Directigen flu A+B (Beckton Dickinson) Test duration: NR Carried out in: laboratory	Virus detected A+B Disaggregate results: Y	299 NPA total specimen Not acceptable by manufacturer: OK Fresh specimen	a) B3	2) vs a) Vir A PPV 85,7% [NR] NPV 89,8% [NR] Sens29,3% [NR] Spec 99,2%[NR] Vir B PPV 66,7%[NR] NPV 93,9%[NR] Sens 10% [NR] Spec 99,6%[NR]	QA: Y=3 ; N/UC=4 QUADAS: Y=4; N/UC=10 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: Y; - disaggregate results for specimen: Y (only 1 type) - appropriate RS: appropriate - replication of RS:data sufficient Population: NR Difference between total of specimens collected (635) in the study and specimens tested with rapid test (299).
Hamilton 2002	IV-b	Cross sectional	USA Paediatric Hospital Jan-Mar 01 (00 - 01 ILI season)	300 patients Mean age: 43 M Median age : 20 M Mode age: 2 M Age range: 12 D - 19 Y Female:136; male:164	1)Directigen flu A+B (Beckton Dickinson) Test duration: NR 8)ZstatFlu - II (ZymexTx) Test duration: <30' Carried out in: laboratory	Virus detected A/B Disaggregate results: NR for IT 2)	300 NA total specimens Not acceptable by manufacturer: NA Thawed specimen	a) A2	2) vs a) PPV 74% [NR] NPV 93% [NR] Sens75% [NR] Spec 93% [NR] 8) vs a) PPV 75% [NR] NPV 96% [NR] Sens 88% [NR] Spec 92% [NR]	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: NR (for IT 2); - disaggregate results for specimen: Y (only 1 type) - appropriate RS: N - replication of RS: data NOT sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Harden 2003	II b	Cross sectional	UK General Practiotioner Jan - Mar 2001 Oct - Mar 2002	157 ILI children median age: 3 Y age range: 6 M - 12 Y gender: 100 boys	1) QuickVue (Quidel corp.) Test duration: 12' Carried out in: studio medico	virus detected: NR disaggregate result: NR	157 NS Not accettable by manufacturer: acceptable Fresh specimen	a) B4	1) vs a) PPV NR [NR] NPV NR [NR] Sens 44% [32-58] Spec 97% [91-99]	QA: Y=5 ; N/UC=2 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: Y (IT and RS have been permormed on two different specimens from each pt) - appropriate RS: Y - replication of RS: N
Herrmann 2001	I-c (only part of the study)	Cross sectional	Sweden Period: NR Setting: NR	268/289 patient with suspect influenza virus infection Age range: 2 M - 83 Y Gender: NR	3) FLU OIA (BioStar, Inc., Boulder, Colorado) Test duration:15-20' Made in laboratory	Virus detected A/B Disaggregate results: NR	184/347 total specimen - NPA 105/268 - NPS 79/79 Not acceptable by manufacturer: NPA Fresh specimen	a) A1 b) C c) B3 d) a + b + c	3) vs b) Total: Sens 56.5% [NR]; Spec 89.1% [NR] NPA: Sens 40.4% [NR]; Spec 94.3% [NR] NPS: Sens 77.5% [NR]; Spec 82.0% [NR] 3) vs c) Total: Sens 64.4%[NR]; Spec 86.1% [NR] NPA: Sens 48.8% [NR]; Spec 93.9% [NR] NPS: Sens 86.6% [NR]; Spec 75.5% [NR] 3) vs a) NPA: Sens 46.3%[NR]; Spec 90.8%[NR] 3) vs d) Total: Sens 55.7% [NR]; Spec 90.8% [NR] NPA: Sens 39.3% [NR]; Spec 95.9% [NR] NPS: Sens 78.6% [NR]; Spec 84.2% [NR]	QA: Y=0 ; N/UC=7 QUADAS: Y=6; N/UC=8 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: Y - appropriate RS: only a) and c) appropriate - replication of RS: data sufficient Context: NR. Difference between totalspecimens collected and specimens tested with IT.

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Hindiyeh 2000	I-c (only part of the study)	Cross sectional	USA 98-99 ILI respirtory virus season ARUP Laboratory	Number of patients: NR Age data: NR Gender: NR	3) FLU OIA (BioStar, Inc., Boulder, Colorado) Test duration: 20' Carried out in: laboratory	Virus detected A/B Disaggregate results: NR	145 total specimen - 15 S - 79 NS + NPW - 30 TS - 5 BAL - 16 NR Not acceptable by manufacturer: NS, NPW, BAL Fresh specimen	a) A1 b) C c) a+b	3) vs c) Total specimen: PPV 91% [NR] Total specimen: NPV 77% [NR] Total specimen: Sens 54% [NR] Total specimen: Spec 97% [NR] 3) vs a) Total specimen: PPV 64% [NR] Total specimen: NPV 79%[NR] Total specimen: Sens 48%[NR] Total specimen: Spec 88%[NR] 3) vs b) Total specimen: PPV 88%[NR] Total specimen: NPV 94%[NR] Total specimen: Sens 81%[NR] Total specimen: Spec 96% [NR] 3) vs c) S: Sens 90% [NR] Spec 100% [NR]; NW+NPW: Sens 46% [NR] Spec 96% [NR]; TS: Sens 25% [NR]; Spec 94% [NR]	QA: Y=2 ; N/UC=5 QUADAS: Y=4; N/UC=10 - Selection criteria: UC; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: Y - appropriate RS: Y only a) A1 - replication of RS:data sufficient Context: NR. Biostar was a co financer

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Hulson 2001	NA	Cross sectional	USA I) Jan-Mar 99 and II) nov 99 - Jan 00 Private family practice clinic	I) 268/382 consecutive ILI patient Age: around 30Y Gender: NR II) 90/225 consecutive ILI patient Age: around 32Y Gender: NR 358 total patients	8) ZstatFlu (Zymetx Corp.) Test duration: NR Carried out in: laboratory	Virus detected A/B Disaggregate results: NR	OPS total specimen: NR Not acceptable by manufacturer: OPS Thawed specimen	a) A3	8) vs a) PPV 79% [NR] NPV 70%[NR] Sens 65%[NR] Spec 83%[NR] LR+ 3.82 LR- 0.42	QA: Y=1 ; N/UC=7 QUADAS: Y=2; N/UC=12 - Selection criteria: Y; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: Y - appropriate RS: unclear (cell type not specified) - replication of RS:data insufficient The study is not well described: it is unclear how many samples are analyzed with index test and reference standard.

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Hurt 2007	I-b	Cross sectional	AUSTRALIA Jun-Oct 06 Royal Children Hospital	Number of patients: NR Age range: 4-64 Y (percentile 78%: <=5 Y) 41% female; 59% male.	5) Binax Now Influenza A&B (Binax; Portland, USA) 10) BD Directigen EZ Flu A+B (Beckinton D,USA) 7) Denka Seiken Quick Ex-Flu (Denka; Japan) 9) Espline Influenza A&B-N (Fujirebio, Japan) 11) Rockeby Influenza A Antigen Test (Rockeby, Singapore) 1) QuickVue Influenza A + B Test (Quidel, San Diego. USA) Test duration: NR Carried out in: laboratory	Virus detected A+B Disaggregate results: Y	177 total specimens - 150 NPA - 8 NS - 8 BAL - 6 S - 5 TS Not acceptable by manufacturer: IT 5) - NPA, BAL, S, TS IT 10) - …… IT 7) - NPA, BAL, S, TS IT 9) - NPA, BAL, S IT 11) - …… IT 1) - NPA, BAL, TS Fresh specimen	a) A1 + A2	VIR A - VIR B 5)vs a) PPV97% PPV 100% NPV 91% NPV 96% Sens 73% Sens 30% Spec 99% Spec 100% 10)vs a) PPV 100% PPV 100% NPV 90% NPV 96% Sens 69% Sens 30% Spec 100% Spec 100% 7)vs a) PPV 100% PPV 100% NPV 90% NPV 96% Sens 71% Sens 30% Spec 100% Spec100% 9)vs a) PPV 100% PPV 100% NPV 89% NPV 96% Sens 67% Sens 30% Spec 100% Spec 100% 11)vs a) [5)detect only vir A] PPV 100% NPV 74% Sens 10% Spec 100% 1)vs a) PPV 100% PPV 100% NPV 89% NPV 96% Sens 67% Sens 30% Spec 100% Spec 100%	QA: Y=3 ; N/UC=4 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: Y; - disaggregate results for specimen: NR - appropriate RS: Y - replication of RS:data sufficient Context: NR.

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Landry, 2003	III-b	Cross sectional	USA Period: peak influenza season Emergency department (after midnight)	Number of patient: NR Adults Gender: NR	2) Directigen Flu A+B EIA (Becton Dickinson) Test duration: 15' Made in laboratory	Virus detected: A Disaggregate results: Y Only virus A was detected	152 NPS total specimens Fresh specimen	a) C	Vir A 2) vs a) PPV NR% [NR] NPV NR% [NR] Sens NR% [NR] Spec NR% [NR] (n. IT 2+)/(n. a)+)=16/31 (n. IT 2-)/(n. a)-)=121/121 other data about number of DFA-positive cells and EIA result are aviable.	QA: Y=3 ; N/UC=4 QUADAS: Y=4; N/UC=10 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: Y; - disaggregate results for specimen: Y - appropriate RS: N - replication of RS:data sufficient The study reported only descriptive results
Landry, 2004	I-c	Cross sectional	USA period: NR Clinical Virology Laboratory	89 total patient - 50 children - 39 adult gender: NR	2) Directigen Flu A+B EIA (Becton Dickinson) 5) Now Flu A and B tests (Binax Inc., Portland, ME) Test duration: NR Made in laboratory	Virus detected: A+B Disaggregate results: Y	59/89 total specimen (+ for RS) - 77 NPS - 12 NPA Fresh specimen	a) A1	2) vs a) Sens 55.9%[NR] Spec 100.0% [NR] 5) vs a) Sens 52.5% [NR] Spec 93.3% [NR] Other disaggregate absolute data (for virus and age group) are aviable	QA: Y=0 ; N/UC=7 QUADAS: Y=6; N/UC=8 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: Y; - disaggregate results for specimen: NR - appropriate RS: Y - replication of RS:data sufficient
Magauran, 2007 (SP)	IV-c	Cross sectional	USA 2 period study 1. 2004-2005 influenza season 2. 2005-2006 influenza season (the objective of this period is incompatible with our) Clinic Clinical Virology Laboratory	348 patients Modal age class: > 50 Y Gender: NR	5) Binax NOW flu A and Flu B (BINAX Inc, Scarborough, Maine) Test duration: 15'-30' Carried out: laboratory	Virus detected: A/B Disaggregate results: NR	696 total mixed specimens Fresh specimen	a) A2	5) vs a) PPV NA% [NA] NPV 81% [NR] Sens NA% [NA] Spec NA% [NA]	QA: Y=0 ; N/UC=7 QUADAS: Y=1; N/UC=13 - Selection criteria: NR; - virus circulation: NR; - disaggregate result for virus: NR; - disaggregate results for specimen: NR - appropriate RS: N - replication of RS: data not sufficient The study reported only descriptive results

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Mehlmann 2007	I-b (only part of the study)	Prospective cross-sectional	USA 29 Dec 05 - 2 Feb 06 Scott & White Hospital and Clinic	N° of patients: NR Median age: 7.5Y Age range: 3M - 86Y Gender: NR	1) QuickVue Influenza A + B (Quidel corp.) Test duration: <30' Carried out in: UC	Virus detected A (only influenza A was detected) Disagregate results: Y	102 total specimens - NS: NR - NPS: NR - NPA: NR Fresh specimen	a) A2 b) B1	1) vs a) PPV 100% [93-100] NPV 92% [81-97] Sens 93% [83-97] Spec 100% [92-100] 1) vs b) PPV 98% [90-100] NPV 82% [69-90] Sens 85% [74-92] Spec 97% [87-100]	QA: Y=5; N/UC=2 QUADAS: Y=8; N/UC=6 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: N - appropriate RS: Y only b) - replication of RS: Data sufficient
Noyola 1999	III-c	Comparative cross sectional	USA 30 Dec 98 - 20 Mar 99 Children Hospital	479 ILI patients Mean Age: 3.8 Y Age range: 7 D - 27 Y 293 male - 186 female	8) Zstat Flu; ZymeTx) Test duration: 30' 12) Directigen FluA (Becton-Dickinson) Test duration: 30' Carried out in: laboratory	Virus detected: A/B - A Disaggregate results: UC	479 total NW specimens 479 NW for IT 8) 417 NW for IT 12) Fresh specimen	a) A2	8) vs a) PPV=76.3 NPV=89.9 Sens = 70.1Spec=92.4 12) vs a) VIRUS A PPV=93.5 NPV=96.9 Sens = 89.7 Spec=98.1 12) vs a) VIRUS A/B PPV=93.5 NPV=90.7 Sens = 74.3 Spec=98	QA: Y=3; N/UC=4 QUADAS: Y=3; N/UC=11 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: UC - disaggregate results for specimen: Y - appropriate RS: N (cell types not recommended by WHO and CDC) - replication of RS: Data sufficient
Poehling 2002	III-b	prospective cross-sectional (comparative study)	USA 10 Jan 00 - 15 Feb 00 Children Hospital	303 ILI patients 2 pt group classified by age and symptoms Age range: 6 months - 19Y Gender: predominantly male	1) QuickVue (Quidel corp.) Test duration: 10', Carried out in: laboratory	Virus detected A + B Disagregate results: N	233 NS (from 233 pt/303 pt) Fresh specimen	a) A2 + B1	A+B 1) vs a) PPV 74.00% [NR] NPV 98.00% [NR] Sens 74.00% [NR] Spec 98.00% [NR] Other disaggregate data for pt group are available.	QA: Y=5; N/UC=2 QUADAS: Y=6; N/UC=8 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: Y - appropriate RS: N - replication of RS: Y

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Pregliasco 2004	I-b	Prospective cross-sectional	ITALY 2 influenza season: Dec 00 - Mar 01 / Dec 01 - Mar 02 Paediatricians of the Italian surveillance network	First season: 586 ILI pt Age range: 0Y - 6 Y Gender: NR Second season: 342 ILI pt Age range: 0Y - 14Y Gender: NR	1) QuickVue influenza test (Quidel corp.) Test duration: 10' Carried out in: first season: pediatrician office second season: laboratory	Virus detected A + B Disagregate results: N	First season: 505 NS Second season: 339 TS Not acceptable by manufacturer: TS Fresh specimen	Season 00-01: a) A1 + A2 Season 01-02: a) A1 + A2 b) B3	Season 00-01 1) vs a) PPV 22.6% [NR] NPV 90.1% [NR] Sens 36.5% [25.0-49.6] Spec 82.1% [78.2-85.5] Season 01-02 1) vs a) PPV 54.5% [NR] NPV 98.5% [NR] Sens 54.5% [24.6-81.9] Spec 98.5% [96.3-99.4] 1) vs b) PPV 63.6% [NR] NPV 98.5% [NR] Sens 58.3% [28.6-83.5] Spec 98.8% [96.7-99.6] Other data are available about curve of influenza virus detected.	QA: Y=5; N/UC=2 QUADAS: Y=6; N/UC=8 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS:Y
Quach 2002	II-b	Cross sectional (comparative study)	CAN Feb - Mar 2001 (7 weeks) Montreal Children's Hospital	N° of patients: NR Age: NR Gender: NR	1) QuickVue influenza test (Quidel corp.) Test duration: 10' Carried out in: laboratory	Virus detected A + B Disagregate results: N	300 NPA Not acceptable by manufacturer: NPA Fresh specimen	a) A1 + A2	1) vs a) PPV 49.4% [NR] NPV 94.9% [NR] Sens 79.2% (68.2 – 90.2) Spec 82.6% (77.9 – 87.3)	QA: Y=2; N/UC=5 QUADAS: Y=8; N/UC=6 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS: data NO sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Rahman 2007 (May)	I-b (only part of the study)	Retrospective comparative cross-sectional (from a population-based cohort study of influenza vaccine)	USA From 22 Jan 07 for 10 weeks Physicians	143/932 ILI patients/elegible patient for influenza vaccine 73/143 pt had RT 63% age>= 17Y Age range: NR (Age>= 6M) Gender: NR	5) Binax NOW flu A and Flu B (BINAX Inc, Scarborough, Maine) Test duration: 15' Carried out: Clinic center during evening and weekend	Virus detected A/B Disaggregate results: NR	73/118 NPS total specimen Not acceptable by manufacturer: NPS acceptable Fresh specimen	a) A1 b) B2 c) a)+b)	5) vs a) Sens 65% [NR] Spec 100% [NR] 5) vs b) Sens 61% [NR] Spec 100% [NR] 5) vs c) PPV 100% [NR] NPV 89% [NR] Sens 61% [NR] Spec 100% [NR] Other disaggregate result, for group age, are available	QA: Y=5; N/UC=2 QUADAS: Y=9; N/UC=5 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: NR - disaggregate results for specimen: Y - appropriate RS: Yes - replication of RS: data sufficient only for a)
Rahman 2007 (Nov)	I-b	Retrospective comparative cross-sectional (from a population-based cohort study of influenza vaccine)	USA From 3 Jan 05 for12 weeks Physicians and Regional clinic centre	818 total ILI patients (who had culture) 118/818 pt had RT Median age: 44Y 50% male	2)Directigen Flu A+B (Directigen; Becton-Dickinson, Sparks, Md.) Test duration:10' Carried out in: laboratory (specimens for RT take by Physicians)	Virus detected A + B Disaggregate results: N	118 NPS Not acceptable by manufacturer: NPS acceptable Fresh specimen	a) A1	2) vs a) Virus A PPV NR [NR] NPV NR [NR] Sens 41.00% [NR] Spec 98.00% [NR] Virus B PPV NR [NR] NPV NR [NR] Sens 50.00% [NR] Spec 99.00% [NR] Virus A + B PPV 86% [65-95] NPV 74% [65-82] Sens 42% [28-57] Spec 96% [89-99] Other disagregate data (for age group) are available	QA: Y=5; N/UC=2 QUADAS: Y=8; N/UC=6 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: Y - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS: data sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Rashid 2007	I-b see notes	cross-sectional	UK 05-06 Virology Centre and Health Protection Agency	567 pilgrims attending Hajj, who presented within 1 week of onset of ILI symptom Median age: 41Y Age range: 1-85Y 89& male	1) QuickVue influenza test (Quidel corp.) Test duration: 10' Carried out in: laboratory	Virus detected A+B Disaggregate results: Y	555 pairs NS Not acceptable by manufacturer: OK Fresh specimen	a) B3	1) vs a) PPV 72% [NR] NPV 92% [NR] Sens 22% [NR] Spec 99% [NR] LRP 22 [NR] LRN 0.79 [NR] Vir A: Sen 22% Vir B: Sen 23% Other disaggregate results (for age, group vaccination) are available	QA: Y=5; N/UC=2 QUADAS: Y=6; N/UC=8 - Selection criteria: Y - virus circulation: UC - disaggregate result for virus: Y - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS: Y. Index test and RS performed on two different samples.
Reina 2002	II-b	Prospective cross-sectional	SPAIN Jan - Dec 01 sentinel network and pediatric emergency room	160 total ILI patients: 93 pediatric 67 adults Age: NR Gender: NR	2)Directigen Flu A+B (Directigen; Becton-Dickinson, Sparks, Md.) Test duration:NR Carried out in: laboratory	Virus detected A + B Disaggregate results: Y	160 total specimens - 93 NPA - 67 TS Not acceptable by manufacturer: all acceptable Fresh specimen	a) A1	Vir A: adults 2) vs a) PPV 100% [NR] NPV 95.1% [NR] sens 72.7% [NR] spec 100% [NR] pediatrics 2) vs a) PPV 100% [NR] NPV 92.1% [NR] sens 86.6% [NR] spec 100% [NR] Vir B: adults 2) vs a) PPV100% [NR] NPV 79.5% [NR] sens 41.1% [NR] spec 100% [NR] pediatrics 2) vs a) PPV 100% [NR] NPV 88.6% [NR] sens 62.5% [NR] spec 100% [NR]	QA: Y=4; N/UC=3 QUADAS: Y=6; N/UC=8 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: Y - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS: data NO sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Rodriguez, 2002	NA	Cross-sectional (comparative study)	USA 13 Dec 99 - 13 Jan 00 Private practice	152 ILI patients Median class 3Y-10Y Modal class < 3 years Age range: < 3Y: 64 pt 3Y - 10Y:42 pt 10 Y - 15Y:21 pt 15 Y- 20Y:14 pt >20Y:11 pt Gender: NR	3) Flu OIA (Biostar) Test duration: 15' 1) Quickvue (Quidel) Test duration:15' 8) Z Stat Flu (Zymetx) Test duration: 30' 12) Directigen Flu A (Beckton Dickinson) Test duration: NR Carried out in: laboratory	Virus detected A + B Disaggregate results: N [Yes for IT 12)]	N° specimens: NR PS NW NS Not acceptable by manufacturer: IT 3): PS, NW, NS IT 1): PS IT 8): PS, NW, NS IT 12):PS, NW, NS Fresh specimen	a) A3	3) vs a) PPV 84.00% [NR] NPV 92.00% [NR] Sens 93.00% [NR] Spec 82 [NR] 1) vs a) PPV 81.00% [NR] NPV 93.00% [NR] Sens 95.00% [NR] Spec 76.00% [NR] 8) vs a) PPV 80.00% [NR] NPV 72.00% [NR] Sens 72.00% [NR] Spec 95.00 %[NR] 12) vs a) PPV 86.00% [NR] NPV 94.00% [NR] Sens 95.00% [NR] Spec 84.00% [NR]	QA: Y=2 ; N/UC=5 QUADAS: Y=5; N/UC=9 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: N - appropriate RS: UC - replication of RS: data NOT sufficient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Ruest 2003	I-b (only part of the study)	Prospective cross-sectional (comparative study)	CANADA 14 Jen to 13 Feb - 01 Hospital Microbiology Laboratory	192 ILI patients - 70 children - 122 adults Median age: 39Y Age range: 1D - 98Y Gender: NR	2)Directigen Flu A+B (Directigen; Becton-Dickinson, Sparks, Md.) Test duration:15' 1) QuickVue Influenza Test (Quidel corp.) Test duration: 10' Carried out in: laboratory	Virus detected A + B Disaggregate results: Y	200 total NPA Specimens tested: 2) vs culture: 183/200 NPA 2) vs RT-PCR: 184/200 NPA 1) vs culture:198/200 NPA 1) vs RT-PCR: 199/200 NPA Not acceptable by manufacturer: NPA for IT 1) Fresh specimen	a) A1 b) B4	All patients 2) vs a) PPV 89.00% [NR] NPV 92.00% [NR] Sens 86.00% [NR] Spec 94.00% [NR] LRP 14.3 [6.9 - 29.6] LRN 0.15 [0.08 - 0.28] 1) vs a) PPV=78.00% [NR] NPV=95.00% [NR] Sens = 91.00% [NR] Spec=86.00% [NR] LRP 6.5 [5.3 - 8.00] LRN 0.10 [0.05 - 0.22] 2) vs b) PPV=97.00% [NR] NPV=87.00% [NR] Sens = 80.00% [NR] Spec=98.00% [NR] LRP 40.00 [10.1 - 158.4] LRN 0.22 [0.14 - 0.34] 1) vs b) PPV=87.00% [NR] NPV=90.00 % [NR] Sens = 86.00% [NR] Spec=90.00% [NR] LRP 8.6 [4.9 - 15.2] LRN 0.16 [0.09 - 0.74] Other disgregate data for patients group are available	QA: Y=3 ; N/UC=4 QUADAS: Y=5; N/UC=9 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: Y (different numerousness tested) - appropriate RS: Y - replication of RS: data sufficient only for b)

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Schulltze 2001	III-b	Prospective cross-sectional (comparative study)	SWITZ Influenza outbreak: 98-99 Pediatric and geriatric clinics, peditrician, general practicioners and physicians in Swiss Sentinal Surveillance Network	378 ILI patients: - pediatric and adolescence Mean age: 3.8Y Age range: 1Y - 18Y 126 female - 131 male -adults Mean age: 50Y Age range: >18Y - 103Y 76 female - 45 male	3) Flu OIA (Biostar, USA) Test duration: 16' Carried out in: laboratory	Virus detected A + B Disaggregate results: N	400 total specimens Paediatrics and adolescence: - NA=264 - NPS=4 - TS=1 - S=1 Adults: - NA=119 - NPS=2 - TS=8 - S=1 Not acceptable by manufacturer: all acceptable Fresh specimen	a) A1 + C	Virus A and/or B 3) vs a) PPV NR% [NR] NPV 73% [NR] Sens 64.4% [56.3-71.7] Spec 94.9% [89.8-97.7] Other disgregate data for patients group are available	QA: Y=5 ; N/UC=2 QUADAS: Y=6; N/UC=8 - Selection criteria: UC; - virus circulation: NR - disaggregate result for virus: N - disaggregate results for specimen: N - appropriate RS: N - replication of RS: data sufficient
Simmerman, 2006	I-b see notes	Multicenter cross sectional	THAI 1 Sept 03 - 31 Aug 04 Hospital outpatient clinics	1092 ILI Thai patients Median age: 35Y Age range: 1M - 86Y 51% male	1) QuickVue Influenza Test (Quidel corp.) Test duration: 10' Carried out in:outpatients department	Virus detected A + B Disaggregate results: N	For IT: specimen type NR Indicate by author: nasal specimens For RS: NPA Fresh specimen	a) A1 + A2 b) B1	1) vs a) PPV 82.00% [NR] NPV 95.00% [NR] Sens 77.00% [NR] Spec 96.00% [NR] 1) vs b) Sens UC SpecUC. Other disaggregate data (follow and high prevalence) are available+J65	QA: Y=5 ; N/UC=2 QUADAS: Y=8; N/UC=6 - Selection criteria: UC; - virus circulation: UC - disaggregate result for virus: N - disaggregate results for specimen: N - appropriate RS: Y - replication of RS: data sufficient RS and IT are performend on two different specimens from the same patient

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Smit, 2006	I-b	Cross sectional (comparati ve study)	NEW ZEL Winter Influenza season: 04 Hospital	448 ILI children and adults Median age: 34Y Age range: 7Y - 101Y 228 female - 220 male	2)Directigen Flu A+B (Directigen; Becton-Dickinson, Sparks, Md.) Test duration:10' 5) Binax NOW Flu A & NOW Flu B (NOW; Binax, Portland, Maine) Test duration:15' 5A) Binax NOW Flu A(NOW; Binax, Portland, Maine) Test duration:15' 5B) Binax NOW Flu B (NOW; Binax, Portland, Maine) Test duration:15' Carried out in: laboratory	Virus detected A +B Disaggregate results: Y	521 total specimens - 338 NPS - 162 TS - 19 NW - 2 Swab: sites NR Not acceptable by manufacturer: IT 2): NW, IT 5) TS Fresh specimen	a) A1 + A2	Virus A: 2) vs a) PPV 97.00% [NR] NPV 83.00% [NR] sens 53.00% [NR] spec 99.7% [NR] 5) vs a) PPV 93.00% [NR] NPV 88.00% [NR] sens 59.00% [NR] spec 99.00% [NR] 5A) vs a) PPV 94.00% [NR] NPV 89.00% [NR] sens 58.00% [NR] spec 99.00% [NR] Virus B: 2) vs a) PPV NR [NR] NPV NR [NR] sens 33.00% [NR] spec 100% [NR] 5) vs a) PPV NR [NR] NPV NR [NR] sens 33.00% [NR] spec 100% [NR] 5B) vs a) PPV NR [NR] NPV NR [NR] sens 33.00% [NR] spec 100% [NR]	QA: Y=3 ; N/UC=4 QUADAS: Y=5; N/UC=9 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: Y - disaggregate results for specimen: N - appropriate RS: Y - replication of RS: Y

Study ID	Level of evidence	Design	Context/setting	Population	Index Test (IT)	Virus type	Specimen type	Reference standard (RS)	Results IT) vs RS) [IC,95%]	Quality assessement (QA) & QUADAS Notes
Weinberg 2005	III-c	Prospective cross-sectional	USA Jan - Mar 03 Hospital	178 ILI patients Mean age: NR Age range: <1Y - 92 Y Gender: NR	2)Directigen Flu A+B (Directigen; Becton-Dickinson, Sparks, Md.) Test duration: 8' 10) Directigen EZ Flu A+B (EZ; Becton-Dickinson), Test duration: 16' 5) NOW Flu A NOW Flu B (NOW; Binax, Portland, Maine) Test duration:15,5' Carried out in: laboratory	Virus detected A + B Disaggregate results: Y	178 total specimens - 31 NPS - 64 BAL - 75 NW - 8 TA/S Not acceptable by manufacturer: IT 2):NW, TA, S; IT 5): BAL, TA, S IT Thawed specimen	a) A2 + B3 + 2) +10) + 5)	2) vs a) PPV 93.00% [68-100] NPV 85.00% [74-92] Sens 56.00% [35-76] Spec 98.00% [91-100] 2a) vs a) PPV 56.00% [30-80] NPV 89.00% [83-94] Sens 39.00% [20-61] Spec 94.00% [89-98] 5) vs a) PPV 93.00% [66-100] NPV 81.00% [58-95] Sens 76.00% [50-93] Spec 94.00% [73-100] Other disaggregate data (for virus) are available	QA: Y=2 ; N/UC=5 QUADAS: Y=2; N/UC=12 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: Y - disaggregate results for specimen: N - appropriate RS: N - replication of RS: data sufficient
Weitzel 2007	II-c see notes	Prospective cross-sectional (comparative study)	GER Feb 05 - Nov 06 Institute of Tropical Medicine	203 ILI travellers Median age: 37 Y Age range: 4Y - 80 Y male: 51%	4)ImmunoCard STAT! Flu A and B (Meridian Bioscience INC) Test duration: 20' - 25' Carried out in: laboratory	Virus detected A + B Disaggregate results: Y	203 NS for IT 4) 203 NS for [a)+b)] The results are calculated on 203 NS Fresh specimen	a) A1 + B2	4) vs a) PPV 95% [85-100] NPV 95% [85-100] Sens 67% [49-84] Spec 99% [88-100] Other disaggregate data (for virus) are available	QA: Y=3 ; N/UC=4 QUADAS: Y=2; N/UC=12 - Selection criteria: UC - virus circulation: NR - disaggregate result for virus: Y - disaggregate results for specimen: Y - appropriate RS: Y - replication of RS: N Results are not comparable. Two specimens were collected from each patients.One was tested by IT and the other by RS. Origin data travellers: NR

Key:

BW = Bronchoalveolar wash
NPS = Nasopharyngeal swabs
NPW = Nasopharyngeal wash
NS = Nasal swab
NW = Nasal wash
OPS = Oropharingeal swab
PA = Nasopharyngeal Aspirate
PS = Pharyngeal swab
S = Sputum
SF = Sinus fluid
TA = Tracheal aspirate
TS = Throat swabs
TW = Tracheoalveolar wash
Y = Yes
N = No
UC = Unclear
NR = Not reported
NA = Not applicable
For the key of RS see appendix 10

Appendix 10

List of reference standards (RS) used in the studies

The RS used in the 39 included studies were classified on the basis of the type of technology (viral culture, RT-PCR and antigen detection) and of appropriateness, i.e. the correct and meaningful use of the method. In addition we commented on the reproducibility of the test on the basis of the quantity and quality of the reported data describing how the test was carried out.

The RS were classified as follows:

Viral culture

We constructed three "A" levels of approriateness:

A1: use of WHO or CDC recommended types of cells

A2:use of other specificied (but no WHO or CDC recommended) types of cells

A3: use of unspecified cells

Of these we considered as appropriate only the A1 subgroup as it was compised of MDCK or pRhM cell lines recommneded by WHO. Our assessment of reproducibility of the test was made on the basis of the quantity and quality of the reported data describing how the test was carried out (Table 1)

RT-PCR

We constructed four "B" levels of appropriateness:

B1: RT-PCR real time, with reported sensitivity in the study or its bibliographical references;

B2: RT-PCR real time, without reported sensitivity in the study or its bibliographical references;

B3: RT-PCR end point, with reported sensitivity;

B4: RT-PCR end point, without reported sensitivity.

B type were all considered as appropriate and the studies using RT-PCR and reporting its sensitivity were considered more accurate. Our replicability assessment included this factor.

We also assessed the type of amplified RT-PCR target gene (Table 2) but this variable was not included in our overall conclusions.

Antigen detection

This technique was classifed as type "C" and considered inappropriate. Our assessment of reproducibility of the test was based exclusively on the presence of a detailed description of how the test was carried out (Table 3).

The following tables synthesise the types and appropriateness of assays used as RS.

Table 1: Classification of RS used in the studies and appropriateness criteria

Virus Isolation

* Viral isolation in embryonated chicken eggs, Madin-Darby canine kidney (MDCK), primary Rhesus Monkey (pRhM) cells indicated as gold standard for influenza diagnosis by WHO and others organisms. Eggs have not been used in the consid-

Reference standard	Virus isolation system	Appropriateness
A1	cells recommended*	YES
A2	others§	NO
A3	not reported	NO

ered studies. [WHO. Manual on Animal Influenza Diagnsois and Surveillance. 2002; HHS. Pandemic influenza plan. Released Nov 2, 2005. http://www.hhs.gov/pandemicflu/plan/]

§ Cells not recommended for influenza virus isolation, but indicated for viral isolation of several respiratory viruses: ex. human foreskin fibroblast, human lung carcinoma (A549), human hepitelial (Hep2), rhesus monkey kidney, Buffalo green monkey kidney (BGM), human diploid fibroblasts (MRC-5), mink lung, Rhabdomyosarcoma cells (RD), etc.

Table 2: Classification of RS used in the studies and appropriateness criteria

RT-PCR

Key: M = matrix gene; NP = nucleoprotein gene; NS = non structural protein genes; HA = haemagglutinin gene;

Reference standard	Real time	Sensitivity^	Target gene		Appropriateness
			Influenza A	Influenza B	
B1	YES	YES	M	NP	YES
			M; HA	-	
			M	HA	
			NS	NS	
B2	YES	NO	HA	M	YES
			not reported		
B3	NO	YES	HA	HA	YES
			NS	NS	
			HA; M	HA;M	
B4	NO	NO	M	NS	YES
			M; HA; NA	M; HA; NA	
			not reported		

NA = neuraminidase gene
^ indicated in the text or in a reference

Table 3: Classification of RS used in the studies and appropriateness criteria

Antigen Detection (IFA or EIA)

Reference standard	Appropriateness
C	NO

References:

World Health Organization. Manual on Animal Influenza Diagnosis and Surveillance. 2002 http://www.who.int/vaccine_research/diseases/influenza/WHO_manual_on_animal-diagnosis_and_surveillance_2002_5.pdf

U.S. Department of Health & Human Services. HHS Pandemic Influenza Plan. November 2005

http://www.hhs.gov/pandemicflu/plan/pdf/HHSPandemicInfluenzaPlan.pdf

Centers for Disease Control and Prevention. Manual for the surveillance of vaccine preventable diseases. Brammer L, Fukuda K, Klimov A, Cox N. Chapter 5: Influenza. Centers for Disease Control and Prevention, Atlanta, GA, 2002 http://www.cdc.gov/nip/publications


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Appendix 11
Designation of level of evidence

As all included studies were of the same design (cohort), the study design was not considered as a major factor for the assignement of the level of evidence. Our assessment of the best methodological studies was based on other factors:

Appropriateness of RS was assessed using the criteria described in Appendix 10.

Replicability of RS was assessed using the criteria described in Appendix 10.

Study quality was assessed on the basis of the information in the relevant answer items of the generic QA and QUADAS tools. We considered the best studies those with the highest number of "Yes" answers. The number of Yes answers could vary from 0 (no Yes answers) to 21 (Yes answers to all the questions). We divided the studies into three levels:

a) 21 - 15: high methodological quality;

b) 14 - 7: medium methodological quality;

c) 6 - 0: low methodological quality;

Table 1: shows the quality level of included studies grouped by the two dimensions considered (appropriateness and replicability)

Table 1: Level of evidence quality

Level	Appropriateness	Replicability	QA + Quadas total positive items
I–a	Y	Y	21 - 15
I-b	Y	Y	14 -7
I-c	Y	Y	6 - 0
II–a	Y	N	21 - 15
II-b	Y	N	14 -7
II-c	Y	N	6 - 0
III–a	N	Y	21 - 15
III-b	N	Y	14 -7
III-c	N	Y	6 - 0
IV–a	N	N	21 - 15
IV-b	N	N	14 -7
IV-c	N	N	6 - 0


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