

## Lateral-flow immunoassay as a diagnostic test for influenza type A and B in children

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### ABSTRACT

**Background** The diagnosis of influenza remains difficult to establish because of its similar symptoms to those of respiratory infection caused by other viruses. The “gold standard” for the diagnosis of influenza is viral culture, which takes time to gain the result and is expensive as well. A simple, rapid, and easily used tool for detection of influenza virus type A and B is needed. **Objective** To assess the accuracy of *lateral-flow immunoassay* with Quick Vue Influenza A+B® in detecting influenza virus of type A and B.

**Methods** This was an observational study designed for diagnostic test. The subjects were children aged 0-14 years old presenting with acute respiratory infection in primary Health Care Jetis, Godean I, Godean II and Prof. Dr. Sardjito Hospital Yogyakarta, from October 2005 to May 2007. Specimens were collected from both the anterior nares and the throat by physicians for *lateral-flow immunoassay* with Quick Vue Influenza A+B® and viral culture as gold standard. *Lateral-flow immunoassay* was done in each study centre, nasal specimen was placed in an extraction reagent tube and sent to NAMRU II laboratory.

**Results** There were 255 children enrolled in this study. *Lateral-flow immunoassay* by Quick Vue Influenza A+B® has sensitivity 70% (CI95% 6;83%), specificity 93% (CI95% 90;97%), positive predictive value 68% (CI95% 54;82%), negative predictive value 94% (CI95% 91;97%), positive likelihood ratio 10,56 (CI95% 6,14;18,19) and negative likelihood ratio 0,32 (CI95% 0,21; 0,51).

**Conclusion** *Lateral-flow immunoassay* (Quick Vue Influenza A+B®), nasal swab specimen is not accurate to detect influenza virus A and B in children. [Paediatr Indones 2008;48:104-109].

**Keywords:** Influenza A and B virus, *lateral-flow immunoassay*, diagnostic test.

The diagnosis of influenza remains difficult to establish because of its similar symptoms to those of respiratory infections caused by other viruses. A study conducted by Monto *et al*<sup>1</sup> suggested that sensitivity and specificity of influenza clinical symptoms known as fever and cough were 63-78% and 55-71% respectively compared with those of viral culture. Study performed in Canada in children less than five years old obtained the percentage of sensitivity and specificity by 63% and 54% respectively compared to those of viral culture.<sup>2</sup>

The “gold standard” for influenza is viral culture takes time to gain results and is expensive as well; therefore, obtaining the prevalence of influenza is difficult. During peak season of influenza, approximately 70% of flu-like disease is caused by influenza virus,<sup>3</sup> and it is evident that around 30-50% of children are infected serologically by this virus.<sup>4</sup> Distinguishing influenza virus from other respiratory viruses is crucial because respiratory infection caused by influenza virus is closely related to the high morbidity and mortality

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which can only be treated by a specific antivirus.<sup>3</sup> The culture of influenza virus needs two to 14 days to make.<sup>4</sup>

With considerations mentioned above, a rapid, affordable, and simple diagnostic tool is needed. A rapid diagnostic tool for the detection of influenza virus can help to make the decision for the best antivirus therapy given, controlling the spreading of nosocomial infection, and it is therefore proven cost effective prior to the previous study in pediatric hospital.<sup>2,5</sup> Until recently, some rapid tests with various testing methods are optical immunoassay (FLU OIA<sup>®</sup>), lateral-flow immunoassay (Quick Vue Influenza A+B<sup>®</sup>), enzyme immunoassay (directigen FLU A+B<sup>®</sup>, Directagen FLU A<sup>®</sup>) and Viral-encoded enzyme assay (ZstatFLU<sup>®</sup>). According to study performed in Canada for children < 5 years of age, Rapid test with lateral- flow immunoassay using Quick Vue Influenza A+B<sup>®</sup> gains sensitivity and specificity of 95% and 71% respectively, positive predictive value of 64%, negative predictive value of 96%, positive likelihood ratio of 3.3 (95%CI 1.9;5.6) and negative likelihood ratio of 0,07 (95%CI 0.01;0.48) compared with those of viral culture.<sup>2</sup> In contrast, a study done by Poehling and Cazacu,<sup>6,7</sup> lateral-flow immunoassay with Quick Vue Influenza has low sensitivity of 97 -98% and high specificity of 70-74 %. This study assessed the accuracy of Lateral-flow immunoassay using Quick Vue Influenza A+B<sup>®</sup> for children in Yogyakarta, in comparison to that of viral culture as the 'gold standard'.

## Methods

This study aimed to confirm the diagnostic value of lateral-flow immunoassay using Quick Vue Influenza A+B to diagnose influenza A and B in children. The gold standard employed was viral culture. Subjects were recruited by consecutive sampling.

The subjects were children aged 0 to 14 years old living in Yogyakarta receiving treatment in Dr. Sardjito Hospital and Primary Health Cares of Jetis, Godean I, and Godean II presenting acute respiratory infection symptoms. Patients who had antivirus before were excluded from the study. This study was approved by the ethical commission of Medical Faculty of Gadjah Mada University.

Each subject underwent history, physical examination, Quick Vue Influenza A+B test and viral culture. History taking, clinical examination and Quick Vue Influenza A+B test were done by skilled health professionals while viral culture was conducted in NAMRU II virology laboratory, Jakarta. Quick Vue test and viral culture were done independently and both examiners were blinded.

Quick Vue Influenza A+B test was done within 24 hours after the specimen is taken from nasal swab with sterile cotton bud. The procedure of taking nasal swab done by inserting the cotton bud to the nostril, upper and lateral cartilage, and by spinning the tool gently. The specimen is placed in a tube containing an extraction reagent by dipping and stirring the cotton bud three times, its tip touches the base and the edge of the tube; it is then taken out from the tube by stirring it three times as well. Put Quick Vue test paper inside the tube and assess the result after 10 minutes, if a pink line appears above the blue control line, the result of influenza A is confirmed. On the contrary, if the pink line appears under the blue control line, the Influenza B is positive. The result is invalid if the Quick Vue test paper does not show any appearance of a blue control line after 10 minutes.

The isolation and viral culture test used specimen from nasal swab and throat swab was obtained by inserting a cotton bud to the anterior of the nose, upper and lateral cartilage, and stirring it gently, put it in a tube and then broke its bud and closed the tube tightly. Specimen of throat swab was obtained by inserting a cotton bud and stroking it gently to the right and left sides of peritonsillar and faring and putting it in a tube. Break its bud and close the tube tightly. Each tube was labeled and stored in a refrigerator with temperature of 2-8°C before it is sent to NAMRU II virology laboratory, Jakarta. Specimen tubes to be sent for viral culture were placed in ziplock, and it was then put in biobottle containing absorbent. Biobottle was put in fiberboard box with 4-5 bars of ice in it.

The procedure of isolation and viral culture was as follows: samples from nose and throat were placed in 1 cc media of Hanks' Balanced Salt Solution (HBSS), that is, media consisting of gelatin, 100 U/ml penicillin, 100 mg/ml streptomycin, and 25 U/ml mycostatin. Samples were stored in the temperature of -70°C. By

using 24 sterile wells, influenza virus is then planted in the cell of Madin-Darby canine kidney that also contains suspension of penicillin, streptomycin, L-glutamine, 1 mmol/l HEPES, and 10% serum of fetal calf. After monolayer confluent is formed in the wells (more and less after two days of viral planting), the medium is aspirated and 0.2 ml of specimen is inoculated. Well plates containing specimen are centrifuged and incubated for seven days with trypsin and serum-free medium. The plates are then used for the identification of virus. The identification of influenza virus used immunofluorescence assay test with a specific monoclonal antibody and hemagglutination inhibitor test to determine serotype of influenza virus. Immunofluorescence assay test was done after the incubation for seven days; the plates were washed and centrifuged. One drop of cell precipitate was put in 12 Teflon-slides wells (cell-lines) and was reacted to a specific monoclonal antibody. Influenza virus was then observed through staining of fluorescent isothiocyanate-conjugated goat antimouse IgG with epifluorescence microscope. Hemagglutination inhibitor test used turkey hemoglobin to identify influenza serotype. Virus isolate was reacted to antiserum which is in accordance with WHO standard and Centre for reference and research on Influenza, Melbourne, Australia. If agglutination occurred, influenza was positive. The final diagnose was established based on the result of viral culture.

## Results

During the period of October 2005 to May 2007, there were 255 subjects with acute respiratory infection in Dr. Sardjito Hospital and Primary Health Cares of Jetis, Godean I and Godean II, who met inclusion criteria. Most of the subjects were 6-14 years old, that is, as many as 139 (54.5%). Male (52.9%) was greater in number than female (47.1%).

On evaluation the clinical examination, fever, cough, and rhinorrhea (69.4%) were the most clinical symptoms of respiratory infection in this study, followed by fever, cough, rhinorrhea, and dyspnea; fever and cough; fever and rhinorrhea; and fever, cough, and dyspnea (Table 1).

Out of 255 subjects, 765 specimens were obtained. Subjects that showed positive influenza

culture were 43 (16.8%). Influenza virus type A was the major cause of influenza in this study by 76.7% compared to influenza virus type B (Table 2). Out of 43 subjects infected by influenza, 30 subjects (70%) showed result of positive Quick Vue Influenza.

Influenza affected more in male group (54.7%) and in age of 1-5 years old (47.6%). Fever, cough, and rhinorrhea were the most clinical symptoms of influenza-confirmed acute respiratory infection, that is, as many as 32 children (76%) (Table 3).

In this study, the diagnostic value of lateral-flow immunoassay test with Quick Vue Influenza A+B® was tested. Compared to viral culture test as the gold standard. The sensitivity of influenza test with lateral-flow immunoassay using Quick Vue Influenza A+B was 70%, meaning that the tool has a 70% ability to detect virus in Influenza group and the specificity of this tool was 93%, meaning that this tool has a 93%

**Table 1.** Subjects' characteristics

Characteristics	Number, n (%) n= 255
Sex	
Male	135 (52.9)
Female	120 (47.1)
Age	
< 1 years old	10 (3.9)
1 – 5 years old	106 (41.5)
6 – 14 years old	139 (54.5)
Time of specimen taking	
Fever less than 3 days	238 (93.3)
Clinical symptoms	
Fever and cough	20 (7.8)
Fever and rhinorrhea	19 (7.5)
Fever, cough, and rhinorrhea	177 (69.4)
Fever, cough and dyspnea	6 (2.4)
Fever, cough, rhinorrhea, and dyspnea	33 (12.9)

**Table 2.** Distribution on result of positive influenza viral culture

Type of Influenza virus	Number, n (%) n= 43
Type A	34 (79)
Type B	9 (21)

**Table 3.** Distribution on clinical symptoms of influenza-confirmed acute respiratory infection

Clinical symptoms	Number, n (%) n= 43
Fever and cough	0 (0)
Fever and rhinorrhea	7 (16)
Fever, cough, and rhinorrhea	32 (76)
Fever, cough, and dyspnea	1 (2)
Fever, cough, rhinorrhea, and dyspnea	3 (7)

ability to detect virus in non-influenza group. Pretest probability or prevalence of disease in this study was 17% (95%CI 12;21 %). The positive predictive value was 68%, meaning that if the result of the diagnostic test was positive, there were only 68% of probability that subject suffered from influenza. A 94% negative predictive value means that if the result of the diagnostic test was negative, the subject would have probability of 94% not to suffer from influenza.

Lateral-flow immunoassay using Quick Vue Influenza had a positive likelihood ratio of 10.56, meaning that the result of positive diagnostic test was 10.56 times greater occurring in subjects suffering from influenza than those without influenza. The negative likelihood ratio of this tool was 0.32, meaning that the result of negative diagnostic test was 0.32 times greater occurring in subjects suffering from influenza than those without influenza.

**Table 4.** Sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio of Quick Vue Influenza

Diagnostic value	Result	95%CI
Sensitivity	70%	56;83
Specificity	93%	90;97
Positive predictive value	68%	54;82
Negative predictive value	94%	91;97
Positive likelihood ratio	10.56	6.14;18.19
Negative likelihood ratio	0.32	0.21;0.51

## Discussion

The result shows that influenza is more common in the age of 1 – 5 years old (41.5%) and in male (52.9%). The highest incidence of influenza occurs in the age of 6-23 months old. Children being exposed to influenza virus had a higher viral concentration and the virus lived longer than adults.<sup>8,9</sup>

The prevalence of influenza in this study was 17%, which was almost the same as Beckett study<sup>10</sup> in six cities in Indonesia, which found the prevalence of influenza by 11%. A study by Cazacu performed in Texas also indicated similar influenza prevalence by 15%, while study in Canada, showed prevalence of influenza at the peak season as high as 51%.<sup>2</sup>

Fever with cough and rhinorrhea (76%) was the most common clinical symptom found in this study.

This result was not different from the previous study that influenza diagnosis could be established by 77–87% based on the clinical symptoms, that is, fever and cough.<sup>1,3,11</sup>

In this study, 43 patients (17%) were found to have positive culture. Influenza virus type A (79%) was the major cause in children than influenza virus Type B (21%). The influenza viral strain obtained in this study consisted of influenza virus A/H1N1, A/H3N2, A/H5N1, B/Shanghai, B/Hong Kong, B/Sichuan. These results were in accordance with the report of WHO that since 2005 influenza virus A H3N2 has been the predominant subtype in most countries.<sup>8,12</sup> Different from study by Cazacu<sup>7</sup> in Texas, influenza Virus type B was the major cause of influenza (23.4 %) compared to influenza virus type A.

Quick Vue influenza is immunodiagnostic influenza A and B test with lateral-flow immunoassay, using a monoclonal antibody specific to influenza antigen. The result of diagnostic test showed that lateral-flow immunoassay using Quick Vue Influenza A+B had the sensitivity by only 70%, meaning that this tool had a 70% ability to detect influenza and 30% of influenza cases were not detected because of false negative result. However, this tool had a high specificity of 93%, meaning that this tool had a 93% ability to detect non influenza cases and 7% for misdiagnosed cases because of false positive result. This study results were almost the same as a study by Cazacu<sup>7,18</sup> that, from 356 children with acute respiratory infection symptoms, sensitivity of Quick Vue Influenza was 70.4% and specificity was 97.7% and as a study by Poehling<sup>6</sup> from 625 children with acute respiratory infection symptoms, sensitivity of Quick Vue Influenza was 74% and specificity was 98%.

However, the sensitivity of this study was lower than that of the study by Ruest *et al*<sup>2</sup> in Canada. The study by Ruest *et al*<sup>2</sup> showed that the sensitivity of Quick Vue Influenza A+B<sup>®</sup> at age of = 5 years old was 95%, this was probably related to different specimens used. This study used nasal swab while Ruest used nasopharyngeal aspirate as the material of Quick Vue Influenza test.<sup>2</sup> The material test or specimen of Quick Vue Influenza can be one of these three options: nasal aspirate/nasopharyngeal, nasal wash, or nasal swab. The effect of specimen choosing



as the test material of immunodiagnostic Influenza is not clear yet. Only specimen of nasopharyngeal aspirate has the biggest sensitivity, but, for children, it remains unknown. Therefore, nasal swab is easier and quicker to do.<sup>13,14</sup>

The best time to take influenza specimen is immediately after the symptom appears because virus is commonly in the maximum titer when the symptom develops and virus replicates for 10-14 days. Fever caused by influenza infection happens since the first day of infection.<sup>4,9,15</sup> Virus titer is optimum on the first day of symptom and specimen had better be taken within 4–5 days after the onset of influenza. For children, positive influenza culture tends to be more frequent to be found in the first three days of influenza symptom.<sup>16</sup> In this study, 93.3% of the specimens were examined within the first three days from the onset and 6.7% of specimens were examined after three days from the onset.

False negative result in this study was related to viral concentration in the specimen. Lateral-flow immunoassay using Quick Vue Influenza has limitation, that is the ability of detecting virus depends on viral concentration in the specimen. If the concentration is below the minimal concentration that is able to be detected by Quick Vue Influenza, this tool, therefore, cannot detect influenza virus. Each strain has its different minimum level.

Interpretation of diagnostic test result varies from one situation to another, depending on the estimation of the prevalence of disease in a given situation. In this study, positive and negative predictive values were almost similar to the previous study, which is between 64% and 74% for positive value and between 96% and 98% for negative value.

The limitation of this study was that sample size was estimated not based on prevalence of influenza in Indonesia because the data of influenza prevalence in Indonesia was not available. It is then probable that the sample size did not represent the population in Indonesia.

In conclusion, Lateral-flow immunoassay using Quick Vue Influenza A+B, nasal swab specimen has a low sensitivity and a high specificity to detect influenza virus type A and B. Therefore it is not accurate to detect influenza virus type A and B in children.

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