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# Assessing sensitivity and specificity of rapid diagnostic test: the importance and challenges of Influenza surveillance in Indonesia



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## ABSTRACT

**Background:** The rapid detection of influenza viruses from clinical samples is important for providing information to assist public health and clinical decision-making, including infection prevention and control measures. The SD Bioline rapid influenza test is a point-of-care (POC) diagnostic test that is based on influenza-specific monoclonal antibodies. The purpose of this study is to assess the performance of this assay against the reference diagnostic standards of RT-PCR in influenza-like illness (ILI) surveillance activity.

**Methods:** A total of 4,262 nasal and throat swab specimens were collected from ILI patients at sentinel hospitals and public health centers across 10 provinces in Indonesia. Specimens were tested with the SD Bioline rapid influenza test in the sentinels, and rRT-PCR test was conducted in referral laboratory in Jakarta. Data analysis was

performed by comparing the results of the rapid test and the rRT-PCR test.

**Results:** The performance evaluation on the SD Bioline rapid influenza demonstrated the specificity of test 97% for both influenza A and B. However, the overall sensitivity of this rapid influenza test for these samples was low at 28% for influenza A and 21% for influenza B.

**Conclusions:** Overall, the results indicated that the SD Bioline rapid influenza test has a high specificity but has only low sensitivity for the detection of influenza from the throat and nasal swabs. Therefore, the integration of rapid test in the influenza surveillance needs further assessment to obtain optimal benefits for both clinical and public health sectors.

**Keywords:** Influenza, rapid diagnostic test, surveillance

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## INTRODUCTION

Influenza is a respiratory tract infection caused by influenza virus and has drawn national and international attention due to the considerable morbidity and mortality rates associated with the infection. Globally, it is estimated that there are 3–5 million severe influenza cases resulting in annual fatalities ranging from 500,000 to 1 million.<sup>1</sup> The emergence of novel influenza virus strains and its pandemic potential has led to active global surveillance on the spread of influenza which provides data on the influenza strain circulated annually to facilitate vaccine composition and early warning response.<sup>2,3</sup> The surveillance also showed that seasonal variations are present in temperate regions with peaks in the winter; however, there are no clear or marked variations in tropical areas.<sup>4,5</sup>

Indonesia has established influenza surveillance mechanisms since 1999 with limited sentinels.<sup>6</sup> The emergence of Avian Influenza (A/H5N1) human cases in 2005 increased the awareness of the importance of surveillance of influenza.<sup>7,8</sup> Therefore, Indonesia has strengthened influenza surveillance by establishing a laboratory-based influenza-like illness (ILI) surveillance in several provinces across the country in 2007.<sup>9</sup> The ILI surveillance

conducted in minimum-resource settings of sentinels also incorporate laboratory tests, including a rapid diagnostic test at the sentinels and molecular test at a reference laboratory in Jakarta.

Rapid diagnostic test (RDT) has been used in several influenza surveillance tests as a point-of-care test. This test provides assistance to diagnose influenza for doctors and paramedics as influenza has an array of clinical symptoms which are difficult to differentiate from other respiratory tract infections. RDTs are one of laboratory methods that could overcome the problems, especially in Indonesia as it is a vast archipelago country with limited transportation access between islands and laboratory facilities. However, RDTs also have some drawbacks, including the sensitivity and specificity. Therefore, this study aimed to assess the performance of this assay against the reference diagnostic standards of RT-PCR in influenza-like illness (ILI) surveillance activity.

## MATERIAL AND METHODS

### Surveillance study sites and patient enrollment

The Indonesia influenza surveillance between April 2007 and March 2009 was an outpatient sentinel

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system involving public health centers, hospitals, and National Institute of Health Research and Development (NIHRD) Laboratory affiliated to the Ministry of Health. Surveillance was conducted in 10 public health centers and 10 hospitals located in 10 out of 33 provinces (Figure 1). The provinces were West Java, DKI Jakarta, Central Java and East Java in Java island, Nanggroe Aceh Darussalam, Riau, and Lampung in Sumatera and one province at each of the islands of Kalimantan, Sulawesi, and Papua. The sentinel selection criteria were the number of ILI patients per month; the public health centers should have a minimum of 30 ILI patients per month, and the sentinel hospitals must have 10 ILIs per month.

Each sentinel collected admission data of at least 10 patients in a week. Patients were enrolled in the surveillance if presenting with ILI symptoms, including axillary temperature  $> 37.8^{\circ}\text{C}$  or had a history of fever with a sore throat or a cough and had not been diagnosed for other diseases.<sup>10</sup> Trained medical personnel collected demographic and clinical symptoms of patients using a standardized questionnaire at the time of enrollment. Informed consent was obtained from each participant who was willing to enroll in this study.

### Specimen collection and laboratory testing

The nasal and throat swab specimens were taken from the enrolled ILI patients. The hospital and health center's staff then conducted laboratory test on the specimens using rapid diagnostic kit. The rapid diagnostic kit used in the surveillance was rapid immunochromatography SD Bioline rapid influenza test (Standard Diagnostics, Inc., Korea). Briefly, we prepared a tube containing 300- $\mu\text{l}$

diluent buffer and put the collected swab into the tube. The swab was swirled well to absorb the buffer. After the swab had been disposed, a test strip kit was placed in the tube and allowed to react with influenza virus-specific monoclonal antibodies for 10–15 minutes. The purple line in the influenza A or influenza B sections was considered a positive result.

Each swab was placed in another vial containing 1.5-ml sterile viral transport medium (VTM). The vials were then stored at  $4^{\circ}\text{C}$  for 3–4 days on average in each sentinel before they were shipped off for further molecular testing at the NIHRD Laboratory, Jakarta, on a weekly basis. Next, Realtime RT-PCR (rRT-PCR) technique based on WHO guidelines were performed by the NIHRD laboratory.<sup>10</sup> First, RNA was extracted from the specimens using the QIAamp Viral RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Next, the RNA were stored at  $-70^{\circ}\text{C}$  until the molecular test was performed. Each specimen was first tested for Flu A and Flu B, followed by subtyping H5N1, H1N1, and H3N2 on Flu A-positive specimens. The typing and subtyping procedure utilized primers and probes designed and provided by US-CDC, Atlanta.<sup>9</sup> One-step rRT-PCR was performed on a final volume of 25  $\mu\text{l}$  containing 5- $\mu\text{l}$  RNA, 12.5- $\mu\text{l}$  buffer mix, and 0.5- $\mu\text{l}$  Superscript III/Platinum Taq-Enzyme mix (Invitrogen, Carlsbad, CA, USA) in IQ5 real-time thermocycler (BIORAD, USA). A positive control provided by CDC Atlanta was included in each rRT-PCR examination to ensure the validity of the results.

### Statistical analysis

Data collected from the surveillance system were entered using Microsoft Office Excel 2007 and



**Figure 1** Map of hospitals (red dots) and public health centers (blue dots) influenza like illness surveillance sentinels in 2007–2009.

analyzed using Stata software version 09 (Stata Corporation, College Station, TX, USA). Data were then analyzed to determine the sensitivity, specificity, and the positive and negative predictive values of the rapid test by comparing to rRT-PCR.

## RESULTS

Specimens from 4262 patients were collected, and 56% of the samples collected were from men. The age group with the highest number of ILI cases was >14 years (36%), with the mean patient age of 16 years (median 9 years; range 1–86 years). ILI patients came mostly on the second day of illness (35%). The median time of the first visit to the sentinels was day 3.

Table 2 shows that the monthly number of ILI cases enrolled since April 2007 until August 2008 ranged from 155 to 303 cases with a mean of 243 cases. The number of cases decreased significantly since September 2008 and continued to decline to the lowest number until March 2009. However, the influenza positivity rate was high (23.4%) during this period.

We analyzed the sensitivity and the specificity of rapid test used in the surveillance by comparing the rapid test results with the rRT-PCR results. Overall, the sensitivity of this rapid test was low for the detection of both influenza A and B. Table 3 shows that the highest sensitivity of the rapid test was from the specimens collected on Day 1, which was only 30%.

## DISCUSSION

Influenza is one of the most common diseases that has symptoms similar to those of respiratory diseases caused by other viruses. It is difficult for clinicians to diagnose influenza virus infection on the basis of physical diagnostics alone. Several laboratory methods have been utilized to identify influenza virus from patient specimens, including virus culture and molecular techniques (PCR). These laboratory confirmations assist the influenza surveillance program and vaccine development by providing vital data annually on the circulation of influenza strains circulation data. The recent advancement of molecular technology (rRT-PCR) has made the influenza diagnostics faster and more robust than conventional methods. However, the molecular method requires specific infrastructures, equipment, and trained laboratory technicians, which are impractical to deploy given the prohibitive costs of setting up and running such facilities custom-designed to provide diagnoses for influenza infection. Therefore, several manufacturers have been attempting to develop rapid diagnostic tests which will allow diagnosing influenza at the point-of-care for better patient management.

Influenza rapid diagnostic test is mostly based on antigen-based systems which focus on the conserved region of influenza genes such as NP. The studies on the specificity and sensitivity of several influenza RDT kits showed that mostly RDTs have low sensitivity and high specificity. These results indicate that when RDTs show negative results, there is a possibility for influenza virus in the specimens to go undetected. Therefore, further testing should be performed to ensure accurate results. The present study has been conducted recently; thus, the sensitivity in relation to influenza A detection was 28%, much higher than the influenza B detection at 21% as found through other studies.<sup>11,12</sup> The difference in sensitivity levels between influenza A and B detection is suggested to be related to the level of virus load needed for the rapid test to provide accurate results. The rapid test requires a higher viral load of Influenza B than it does in the case of influenza A virus.<sup>13</sup> The sensitivity of the rapid test also increases when the test is performed at the peak of influenza season. When the circulation of influenza virus is low, positive results of rapid test might turn out to be in fact false-positives; this, therefore, requires further testing using techniques with higher sensitivity, such as rRT-PCR.<sup>14,15</sup>

Specimens that provide results with better sensitivity and specificity with rapid diagnostic tests are those taken on the first day of the ILI onset.<sup>13</sup> According to a study conducted by Tanei M et al., the sensitivity and specificity of the rapid test increased if the length

**Table 1** Demographic characteristics of the ILI patients

Characteristics	N (%)
Number of respondents	4262
Sex	
Male	2402 (56)
Female	1860 (44)
Age group	
<1 years	248 (6)
1–4 years	1176 (28)
5–14 years	1302 (30)
>14 years	1536 (36)
Illness onset till specimen collection	
Day 1	408 (10)
Day 2	1493 (35)
Day 3	1133 (27)
Day 4	605 (14)
Day 5	234 (5)
> Day 5	389 (9)

**Table 2** Number of influenza-positive and ILI cases identified with rRT-PCR tests month-wise

	Month	Flu A	Flu B	Negative	ILI cases	
2007 ( <i>n</i> = 1992)	April	7	4	212	223	
	May	9	13	230	252	
	June	11	7	230	252	
	July	10	10	270	290	
	August	3	12	261	276	
	Sept	10	8	200	218	
	Oct	8	18	153	179	
	Nov	10	7	212	229	
	Des	19	8	128	155	
	2008 ( <i>n</i> = 2212)	Jan	67	31	155	253
		Feb	55	21	175	251
		March	61	56	186	303
April		32	53	191	276	
May		14	31	205	250	
June		22	10	232	264	
July		9	4	190	202	
August		8	5	244	257	
Sept		9	1	80	90	
Oct		2	0	2	4	
Nov		23	0	21	44	
Des		5	1	12	18	
2009 ( <i>n</i> = 58)	Jan	2	0	12	14	
	Feb	2	0	13	15	
	March	1	4	24	29	
		399	304	3560	4262	

**Table 3** Sensitivity and specificity of rapid test for detection of influenza virus compared to rRT-PCR test

	N = 4262	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
<b>By results</b>					
Influenza A positive	399	28	97	51	92
Influenza B positive	304	21	97	37	93
<b>Time from illness onset till specimen collection</b>					
Day 1	408	30	99	74	92
Day 2	1493	25	97	65	97
Day 3	1133	29	96	62	86
Day 4	605	19	96	17	86
Day 5	234	11	97	44	86

of time from the onset of symptoms until the specimen's collection was approximately 12 hours. If it was less than 12 hours, it would give a false-negative

result.<sup>13</sup> This phenomenon is correlated with the time influenza virus starts to appear in respiratory secretions after their proliferation in the epithelial cells of the respiratory tract (24 hours before the onset of symptoms). Peak viral shedding occurs 24 hours after the onset of symptoms; after that point the amount of virus starts to decrease rapidly after that time.<sup>16</sup> In this study, the majority of cases came to the health centers on the second or third day of the onset of illness, and this can be assumed as the cause of the low sensitivity of RDTs evaluated in this study. Moreover, poor specimen collection and storage will also interfere with test results as specimen storage (>3 days at 4°C) may negatively affect results.<sup>17,18</sup>

The incorporation of the rapid test as part of the influenza surveillance might increase clinicians' participation as has been demonstrated in Hawaii.<sup>19</sup> RDT requires minimum training, does not require special expertise or specific tools or technologies such as rRT-PCR examination. Furthermore, results can be obtained immediately; say within a span 15–30 minutes. This in turn may improve case management, including administration of antibiotics. Also noteworthy here are the challenges and difficulties involved in the transportation of specimens to the nearest regional laboratory or to different regions in the of Indonesian archipelago for molecular testing. Therefore, there is always going to be delays in providing information in a timely fashion concerning receipt of results of laboratory tests performed for diagnosis. As the rapid test reduces costs of transporting specimens, cost of surveillance may be less expensive. However, as the rapid test only gives better performance in the influenza epidemic situation,<sup>20</sup> the decision to integrate RDT into public health surveillance should be carefully made for the regions that had not shown a clear peak in the spread of influenza seasonally, such as Indonesia.

## CONCLUSION

In conclusion, the rapid test (SD Bioline) used in this surveillance demonstrated a high specificity but only low sensitivity in relation to the detection of influenza. Although we have evaluated only one of the commercially available influenza rapid test kits, our findings still highlight the need to further assess the rapid diagnostic tests that will be used for influenza surveillance. Further analysis on cost-effectiveness and the impact of RDT on clinical management should be conducted in the future.

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## DISCLOSURE

No competing interest.

## REFERENCES

- Russell CA, Jones TC, Barr IG, Cox NJ, Garten RJ, Gregory V, Gust ID, Hampson AW, Hay AJ, Hurt AC, et al: The global circulation of seasonal influenza A (H3N2) viruses. In *Science*. Volume 320. New York, NY;2008;(5874):340-346.
- Pan American Health Organization (PAHO) (2004) Final report of the XIV Meeting on Vaccine Preventable Diseases of the Pan American Health Organization. Washington (District of Columbia): PAHO. Available at: [http://www.paho.org/English/AD/FCH/IM/TAG16\\_FinalReport\\_2004.pdf](http://www.paho.org/English/AD/FCH/IM/TAG16_FinalReport_2004.pdf). Accessed 19 November 2013.
- Saha S, Chadha M, Al Mamun A, Rahman M, Sturm-Ramirez K, Chittaganpitch M, et al. Influenza seasonality and vaccination timing in tropical and subtropical areas of southern and south-eastern Asia. *Bulletin of the World Health Organization*. 2014; 92(5):318-330.
- Vibound C, Alonso WJ, Simonsen L. Influenza in tropical region. *PloS Med*. 2006;3(4):e89. <https://doi.org/10.1371/journal.pmed.0030089>.
- Moura FE, Perdigão AC, Siqueira MM. Seasonality of influenza in the tropics: a distinct pattern in northeastern Brazil. *The American journal of tropical medicine and hygiene*. 2009; 81(1):180-183.
- Becket CG, Kosasih H, Ma'roef C, Listyaningsih E, Elyazar IRE, Wuryadi S, et al. Influenza Surveillance in Indonesia: 1999–2003. *Clin Infect Dis*. 2004;39(4): 443-449. DOI: <https://doi.org/10.1086/422314>.
- Sedyaningsih ER, Isfandari S, Setiawaty V, Rifati L, Harun S, Purba W, et al. Epidemiology of Cases of H5N1 Virus Infection in Indonesia, July 2005–June 2006. *The Journal of Infectious Diseases* 2007; 196:522–7. DOI: [10.1086/519692](https://doi.org/10.1086/519692).
- Escorcia M, Attene-Ramos MS, Estrada MJ, Nava GM. Improving global influenza surveillance: trends of A(H5N1) virus in Africa and Asia. *BMC Research Notes*2012;5:62. DOI: [10.1186/1756-0500-5-62](https://doi.org/10.1186/1756-0500-5-62).
- Kosasih H, Roselinda, Nurhayati, Klimov A, Xiyan X, Lindstrom S, et al. Surveillance of influenza in Indonesia, 2003–2007. *Influenza Other Respiratory Viruses*. 2013;7(3):312-20.
- World Health Organization Global Influenza Programme. WHO manual on animal influenza diagnosis and surveillance. (2002) [cited 30 September 2012]. Available from: [http://www.who.int/csr/resources/publications/influenza/WHO\\_CDS\\_CSR\\_NCS\\_2002\\_5/en/print.html](http://www.who.int/csr/resources/publications/influenza/WHO_CDS_CSR_NCS_2002_5/en/print.html).
- Yoo Y, Sohn JW, Park DW, Kim JY, Shin HK, Lee Y, et al. Clinical Evaluation of the SD Bioline Influenza Virus Antigen Test for Rapid Detection of Influenza Viruses A and B in Children and Adults during the Influenza Season. *Clin Vaccine Immunol*. 2007;14(8):1050–1052.
- Kenmoe S, Tchendjou P, Tetang SM, Mossus T, Ripa NM, Guillet M, et al. Evaluating the performance of a rapid antigen test for the detection of influenza virus in clinical specimens from children in Cameroon. *Influenza and Other Respiratory Viruses*. 2014;8(2): 131–134. DOI: [10.1111/irv.12210](https://doi.org/10.1111/irv.12210).
- Tanei M, Yokokawa H, Murai K, Sakamoto R, Amari Y, Boku S, et al. Factors influencing the diagnostic accuracy of the rapid influenza antigen detection test (RIADT): a cross-sectional study. *BMJ Open* 2014;4: e003885. doi:10.1136/bmjopen-2013-003885.
- Uyeki TM, Prasad R, Vukotich C, Stebbins S, Rinaldo CR, Ferng YH, et al. Low sensitivity of rapid diagnostic test for influenza. *Clin Infect Dis* 2009;48:e89– 92.
- Al Johani SM, Al Balawi M, Al Alwan B, Al Hefdhhi R, Hajeer A. Validity of two rapid point of Care Influenza tests and Direct Fluorescence assay in comparison of Real Time PCR for swine of origin Influenza virus. *J Infect Public Health*. 2011; 4(1):7-11. doi: 10.1016/j.jiph.2010.10.004.
- Treanor JJ. Influenza virus. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and practice of infectious diseases, 7th edn. Philadelphia: Churchill Livingstone. 2005:2271–2.
- Sakai-Tagawa Y, Ozawa M, Tamura D, Le MQ, Nidom CA, Sugaya N, et al. Sensitivity of influenza rapid diagnostic tests to H5N1 and 2009 pandemic H1N1 viruses. *J Clin Microbiol*.2010;48:2872–7.
- Kasper MR, Putnam SD, Sovann L, Yasuda CY, Blair PJ, Wierzba TF. Short Report: Rapid-Test Based Identification of Influenza as an Etiology of Acute Febrile Illness in Cambodia. *Am. J. Trop. Med. Hyg*. 2011;85(6):1144–1145.
- Effler PV, Ieong MC, Tom T, Nakata M. Enhancing public health surveillance for influenza virus by incorporating newly available rapid diagnostic tests. *Emerging infectious diseases*. 2002;8(1):23-8.
- Gavin PJ, Thomson RB. Review of rapid diagnostic tests for influenza. *Clinical and Applied Immunology Reviews*. 2004;4(3):151-72.



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