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Field performance and new uses of rapid influenza testing in Thailand

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Summary
Objectives: Rapid influenza tests are increasingly used in surveillance systems and for clinical care in Southeast Asia. However, the performance and utility of rapid influenza tests under field conditions in rural Southeast Asia has not been evaluated.
Methods: In the context of a larger study on the causes of respiratory illness in rural Thailand, we used a rapid test to collect data on influenza burden, seasonality, and cost of illness. We compared the performance of the QuickVue® Influenza Test to tissue cell viral culture and reverse transcriptase-polymerase chain reaction (RT-PCR) among 1092 Thai patients meeting the World Health Organization case definition for influenza-like illness over a 12-month period.
Results: The sensitivity and specificity of the QuickVue test compared to viral culture were 77% and 96%, respectively. Rapid influenza tests were useful to describe the seasonality of influenza, estimate the cost of illness, increase the sensitivity of surveillance, conduct outbreak responses, and guide evaluation of suspected avian influenza virus infections.
Conclusions: Despite their high cost, rapid influenza diagnostic tests are useful tools for influenza research, surveillance, and outbreak investigations in Southeast Asia.
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Introduction

In developed countries in temperate climates, influenza is an important cause of morbidity among healthy children and adults and of mortality among the very young, the elderly, and those with chronic illnesses during seasonal influenza

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epidemics.[1–3] In developed economies, influenza may cause 10–12% of all sickness absence resulting in significant direct medical costs and lost wages.[4,5] Much less is known about the disease burden, seasonality, and cost of influenza in tropical countries. Improving the sensitivity of influenza surveillance and documenting the burden of disease in Southeast Asia is a World Health Organization (WHO) priority.[6] Virological surveillance is conducted by performing cell culture on respiratory specimens from patients with influenza-like illness (ILI). While viral culture yields isolates needed for surveillance and vaccine strain selection, results are not timely, the process is labor-intensive, and requires specialized laboratory skills.[7]

In recent years commercially available rapid influenza diagnostic tests have become available in developed countries. Rapid tests are primarily used in clinical settings where prompt testing can influence treatment decisions.[8–10] In addition, France, Switzerland, and the USA States of Hawaii and Colorado have experimented with integrating rapid tests into their influenza surveillance systems.[11–13] The high specificity and the improved speed in reporting with rapid tests can improve the early warning capacity of some influenza surveillance systems.[14] In controlled laboratory trials and in medical practices, rapid influenza tests have generally demonstrated moderate sensitivity (45–90%) and good specificity (86–100%) to detect influenza virus infection.[8,15] There has been considerably less experience with rapid influenza tests in Southeast Asia. Use of rapid tests has been limited by factors including high cost (US $7–12/test), limited availability, few available treatment options for influenza, and concern about the performance of rapid tests in the hot and humid field conditions found in rural tropical medical clinics.

To evaluate the utility and performance of rapid tests for influenza research, surveillance, and outbreak response, we employed a commercially available rapid test during a one-year period in a field setting as one component of an influenza research study in Thailand.

Materials

We used the QuickVue® Influenza Test, which detects influenza antigens in clinical specimens and provides results in 10 min. This test is a lateral-flow immunoassay that uses monoclonal antibodies specific for influenza viral nucleoprotein antigens and detects both influenza A and B, but does not distinguish between them. The test uses an extraction reagent to disrupt virus particles in the clinical specimen. Viral nucleoproteins are subsequently exposed and react with an antibody-coated strip. If influenza virus is present, a pink-to-red test line along with a blue procedural control line will appear on the test strip.

Methods

This research was carried out with the approval of, and in compliance with the standards of, the ethical review committees of the United States Centers for Disease Control and Prevention, the Thailand Ministry of Public Health, and the Tulane University School of Public Health.

We enrolled patients of all ages during a 12-month period from September 1, 2003 to August 31, 2004. Patients that met the WHO ILI clinical case definition of a fever greater than 38 °C with either cough or sore throat and no alternative diagnosis were enrolled during two clinic days per week at five of eight hospital outpatient clinics in Sa Kaeo province of rural eastern Thailand.[16] The province shares a border with Cambodia, a 2003 population of 438 557, and an average annual household income of US $248.[17] The province has seven public hospitals, a single military hospital, and no private hospitals. Sa Kaeo is the site of an active, population-based pneumonia surveillance system carried out through collaboration between the Thailand Ministry of Public Health (MOPH) and the US Centers for Disease Control and Prevention (CDC).[18]

Patients with ILI who presented to the outpatient department were asked to enroll in the study. After obtaining signed consent, the swab provided in the test kit was used to collect a nasal specimen and the QuickVue rapid test was performed according to the manufacturer's instructions by one of three research nurses trained by the Thailand National Institute of Health (NIH) influenza laboratory. The research nurses were asked to evaluate the ease of use and durability of the rapid test during the one-year study period. The results of the test were promptly reported to both the patient and the physician. Summary reports of the number and proportion of positive rapid influenza tests were submitted weekly to strengthen the national influenza surveillance system.

Nasopharyngeal samples were also collected from all patients using a Dacron swab, inserted into 3% nutrient broth viral transport media and placed on wet ice. Specimens were then refrigerated for less than 48 h at 2–8 °C until transported to the provincial hospital for aliquoting and storage at −70 °C. Once weekly, specimens were transported on dry ice to the Thai NIH laboratory in Bangkok for viral culture in Madin–Darby canine kidney cells (MDCK) and human epithelial (HEp-2) cells using established procedures. Specimens were centrifuged at 4500 rpm for 20 min and 0.1 mL of each supernatant was inoculated onto confluent monolayers of MDCK and HEp-2 cells. MDCK cells were maintained in minimal essential medium (MEM) supplemented with 0.2% BSA and 0.2 μg/mL TPCK–trypsin, and HEp-2 cells were maintained in MEM with 2% FBS. Both cells were incubated at 35 °C for 7–10 days and observed daily for cytopathic effect (CPE). If a sample showed CPE, it was further tested by immunofluorescence assay (IFA) using a commercial respiratory virus monoclonal antibody panel to detect influenza A and B, parainfluenza viruses types 1, 2, and 3, respiratory syncytial virus, and adenovirus (Chemicon Cat. No. 3105). Cultures not showing CPE were passaged once using harvested scraped cells and fluid as inoculum and observed for CPE. Both CPE negative and positive samples were confirmed by IFA.[19]

For reverse transcriptase-polymerase chain reaction (RT-PCR) testing, specimens were added to lysis buffer AL (QIAGEN Inc., Valencia, CA, USA) and held at −70 °C until shipping to CDC on dry ice. Total nucleic acid was extracted using the QIAamp® Virus BioRobot MDx kit (QIAGEN) following manufacturer's instructions. An RT-PCR assay panel consisting of respiratory syncytial virus, human parainfluenza viruses 1, 2, and 3, human metapneumovirus, influenza viruses A and B, adenovirus, and picornavirus was performed as previously described,[20] but with the following modifications: the sense-strand primers of each set were 5′-end-labeled with the fluorescent dye, Cy5, and amplicons were analyzed using

the CEQ™ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). All assays were run with standardized viral nucleic acid extracts and nuclease-free water for positive and negative controls, respectively, and all samples were tested by RT-PCR for the human GAPDH enzyme to ensure adequate recovery of sample RNA and absence of RT-PCR inhibitors.

Demographic and risk factor data were collected at the time of enrollment. Patients who were positive for influenza with the rapid test were then given a cost diary to facilitate the recording of healthcare utilization and household costs associated with the illness. Three weeks after enrollment, each patient with a positive rapid influenza test was interviewed by a research nurse to collect cost of illness data based on the cost diary.

In 2003, to prepare for use of the rapid test during outbreak investigations, Thai and US epidemiologists received training to conduct the rapid test according to the manufacturer's guidelines. During early 2004 when cases of human H5N1 infection were reported in Thailand, several hundred laboratory staff and nursing personnel in MOPH hospitals also received training from scientists at the Thailand National Influenza Center. Rapid tests were then distributed to MOPH facilities across Thailand for use with patients suspected of having avian influenza H5N1 infection.

Data were analyzed using SPSS 12.0 (SPSS Inc, Chicago, Illinois, USA) and EpiInfo 6.0 (US CDC, January 2001) statistical software. The Chi-square test was used to compare proportions and a p value of <0.05 was considered significant.

Results

We enrolled 1092 patients (age range 1 month—86 years; median 35 years) of whom 587 (54%) were less than six years of age. Five hundred and fifty-seven (51%) enrolled patients were male. Four hundred and nineteen patients (38%) and 988 (90%) of specimens were collected within two and four days of the onset of symptoms, respectively.

One hundred and ninety-two of 1092 (18%) rapid tests were positive using the QuickVue test while viral culture indicated that 205 of 1092 (19%) were positive for influenza. Of these isolates 178 (87%) and 27 (13%) were influenza type A and type B viruses, respectively. No cases of avian influenza A (H5N1) infection were identified.

When compared to cell culture, the QuickVue influenza test produced 34 false positive and 47 false negative results with a sensitivity and specificity of 77% and 96%, respectively. Of the 34 specimens that were positive by rapid test but negative by viral culture, 28 (82%) were RT-PCR positive. Of the 47 results that were negative by rapid test but positive by cell culture, RT-PCR was positive in only four (9%) cases (Table 1). There was substantial agreement between the rapid test and both cell culture and RT-PCR (Kappa 0.751 and 0.758, respectively) and almost perfect agreement between cell culture and RT-PCR methods (Kappa 0.810).[21]

Table 1 Comparison of results by test method ($n = 1092$)

Rapid test	Cell culture	RT-PCR	Number of specimens
Pos	Pos	Pos	151
Neg	Neg	Neg	823
Pos	Neg	Pos	28
Pos	Pos	Neg	7
Pos	Neg	Neg	6
Neg	Pos	Neg	4
Neg	Pos	Pos	43
Neg	Neg	Pos	30

The proportion of rapid tests positive for influenza was highest during the months of June—August (Figure 1). Over the 12-month study period, the QuickVue test had a positive predictive value (PPV) of 82% and a negative predictive value (NPV) of 95%. Three hundred and eighty-two patients were enrolled during the months of June through August and 127 (33%) of these patients tested influenza positive by cell culture. During this three-month period of highest influenza activity, the PPV of the rapid test increased to 88% while the NPV decreased to 90% (Table 2).

All nasopharyngeal specimens were tested using RT-PCR methods and 252 (23%) of these specimens were influenza positive. Tissue cell culture identified influenza in 205 (81%) of these specimens and was significantly less sensitive than RT-PCR ($p < 0.0001$). When the rapid test results were compared to RT-PCR testing on these specimens, the QuickVue test had a lower sensitivity of 71% ($p = 0.144$) and an increased specificity (98%) ($p = 0.019$) than when the test was compared to tissue cell culture. In children less than six years of age, the QuickVue test agreed with the cell culture result in 57 of 70 (81%) cases while the test identified 101 of 135 (75%) of culture positive patients aged 18 years or older ($p = 0.28$). The QuickVue test was not more likely to indicate a positive result when viral culture was positive for influenza

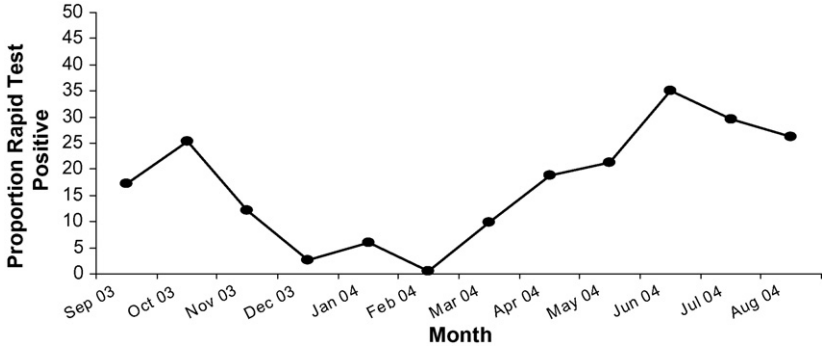

Figure 1 Proportion of rapid test influenza positive outpatient specimens by month ($n = 1092$).

Table 2 Performance of the QuickVue® Influenza Test compared to viral cell culture

Time period	Specimens (*n*, % positive)	Sensitivity (%)	Specificity (%)	PPV (%)	*p* value[a]	NPV (%)	*p* value[a]
12 months	1092 (192, 18)	77	96	82		95	
Lowest prevalence (September–May)	710 (78, 11)	77	96	73		97	
Highest prevalence (June–August)	382 (114, 30)	77	96	88	(0.009)	90	(0.00001)

[a] Comparison of PPV and NPV during highest and lowest influenza prevalence periods. PPV, positive predictive value; NPV, negative predictive value.

type B virus 22/27 (82%) than when compared to influenza type A 136/178 (76%) ($p = 0.51$).

In July 2003, an outbreak of febrile respiratory illness was reported in a remote boarding school for hill tribe children in the mountains of northern Thailand. For the first time in Thailand, a team equipped with rapid influenza tests was dispatched to conduct an epidemiological investigation. Nine of 19 (47%) of nasal swabs collected from acutely ill children tested positive for influenza virus, later confirmed by tissue cell culture as influenza A/Fujian/411/2002 H3N2.[22]

Discussion

We chose to evaluate the QuickVue test because prior experience in the USA suggested it was a simple and durable test that might be useful in the more rigorous environments found in rural Thai clinics. We hypothesized that the extremes of heat and humidity and the variability in specimen collection, handling, and testing common to field settings might negatively impact the performance of the rapid test when compared to laboratory evaluations conducted in developed countries. However, we found that under field conditions in rural Thai clinics, the QuickVue Influenza Test had a sensitivity and specificity compared to viral culture of 77% and 96%, respectively. This compares favorably with findings from laboratory evaluation studies in Canada, the USA, and Japan, where the test yielded a median sensitivity of 79.2% (range 74–95%) and a median specificity of 91.9% (range 76–98%).[25–27] During our study year, influenza infection demonstrated marked seasonal variation. Positive rapid test results were significantly more likely to be confirmed by cell culture (true positive) during the seasonal peak of influenza activity while false positives were more likely to occur in low prevalence months. Overall, the positive and negative predictive values of the test were 82% and 95%, respectively.

Consistent with other studies, we found that RT-PCR was a more sensitive method to identify influenza virus infection than tissue cell culture.[23,24] The rapid test yielded 34 false positive specimens and 47 false negative results when compared to cell culture, but in the majority of these cases RT-PCR testing agreed with the rapid test. This is probably due to the significant reduction in the recovery of influenza viruses caused by transport delays in the inoculation of samples into cell culture and the deleterious effects of the freeze–thaw cycle. These data suggest that RT-PCR could be considered the new gold standard, especially for specimens that must be frozen and shipped.

Children with influenza infections may experience higher viral loads and shed virus longer than adults. Thus, some rapid tests have demonstrated increased sensitivity in younger age groups.[15,23,28] In this study, the rapid test was not more sensitive in children under six years of age when compared to adults aged 18 years and older. Some studies indicate that rapid tests may be less sensitive for influenza type B virus than for influenza type A.[29,30] This may be due to lower viral loads during infection with influenza virus type B than with influenza type A.[31,32] The QuickVue test was equally sensitive in detecting infection with both influenza type A and type B viruses. Our experience in this study afforded insight into several potential new uses for rapid influenza testing in developing countries.

Uncomplicated viral respiratory infections can represent a significant economic burden to poor families as a result of lost work, costs of transportation to medical clinics, and out-of pocket treatment costs.[33] To minimize recall and misclassification bias, patients must be correctly diagnosed and interviewed early in the course of illness. However, clinical diagnosis of influenza is unreliable and obtaining viral cell culture results can take several weeks.[34,35] Use of the QuickVue test allowed us to effectively identify symptomatic influenza patients in the outpatient department who were later interviewed to collect cost of illness data (reported elsewhere).

An improved understanding of influenza seasonality in Thailand and early identification of seasonal epidemics will allow for more effective timing of vaccination and for improved clinical management. Our daily use of rapid influenza tests in outpatient clinics functioned as a sensitive, active surveillance system. Real-time knowledge of the proportion of ILI patients who were influenza positive provided unprecedented insight into the level of circulating human influenza viruses during 2003–4 and contributed valuable new information on the seasonality of influenza in Thailand.

The July 2003 outbreak of febrile respiratory illness in a boarding school in the mountains of northern Thailand was the first use of rapid influenza tests for an epidemiologic investigation in Thailand. Rapid influenza tests enabled investigators to promptly identify influenza as the cause of the outbreak. Immediate identification of the etiology of the outbreak facilitated treatment decisions by local clinicians, guided control measures such as cohorting, quarantine, and school closure. Thailand MOPH epidemiological response teams are now routinely supplied with rapid influenza tests when investigating respiratory illness outbreaks.

During 2004, East and Southeast Asia experienced an outbreak of avian influenza H5N1 that resulted in more than 100 million dead or culled poultry, and 44 human cases with 32 deaths in Thailand and Vietnam.[36] Reassortment of the H5N1 avian virus with circulating human influenza A viruses

Table 3 Potential impact of rapid testing on virological surveillance efficiency[a]

	ILI patients	Rapid tests performed	Specimens cultured	Influenza isolates	Missed isolates	Efficiency % (*n* isolates/*n* cultured)
Culture all specimens	1092	0	1092	205	0	19 (205/1092)
Culture only rapid test positive specimens	1092	1092	192	158	47	82 (158/192)

[a] Assumes rapid test sensitivity of 77%, specificity of 96%, and 19% culture positive. ILI, influenza-like illness.

could generate a novel strain that would cause a global influenza pandemic.[37,38] The Thailand MOPH incorporated rapid influenza testing into its diagnosis and treatment algorithm and distributed 59 200 rapid influenza tests to 904 hospitals and public health offices across the country. Briefly, if the rapid test result was negative in patients with clinical features incompatible with H5N1 infection and without a clear history of poultry exposure, specimens were generally not forwarded to central laboratories for viral culture. If the rapid test returned positive, additional expedited testing was conducted to distinguish between infection with H5N1 and human influenza A virus. The results of these rapid tests were not systematically recorded and although they were considered to be a useful tool in the outbreak response, there are no published studies on the accuracy of rapid tests to detect human avian influenza infection. Therefore, clinical management decisions should be guided by specific H5N1 testing algorithms with close attention to exposure history and clinical presentation.[39] The pandemic threat of H5N1 avian influenza virus underscores the importance of maintaining a sensitive human influenza surveillance system.

The WHO Influenza Surveillance Network consists of 112 National Influenza Centers (NIC) in 83 countries and four collaborating centers worldwide.[16] The network functions as a global virologic surveillance system that collects influenza isolates for strain surveillance, pandemic preparedness, and annual vaccine composition decisions. For NICs in developing countries, equipping and staffing complex laboratories needed to characterize influenza viruses is challenging. Considering the high costs and limited resources, approaches to maximizing efficiency and increasing the number of isolates submitted to the WHO network merits attention. For physicians, access to rapid influenza tests is appealing to improve clinical care and may serve as an incentive to submit specimens.[9,12] The application of rapid testing could allow influenza laboratories to focus efforts on clinical specimens that are most likely to yield influenza isolates, thereby increasing efficiency and possibly reducing costs. While conducting viral cell culture on all specimens is ideal, using rapid tests as a screening tool for influenza surveillance could strengthen overall virological surveillance (Table 3).

In summary, the rapid influenza test demonstrated moderate sensitivity and high specificity under field conditions in rural Thailand. The research nurses reported that the test was easy to perform, simple to interpret, and the test kit materials were durable in field conditions. The rapid test demonstrated utility in a variety of roles including collecting cost-of-illness data, conducting investigations of human influenza outbreaks, and responding to the avian influenza A/H5N1 outbreak. While their high cost per test currently limits the large-scale application of rapid influenza tests in resource-poor countries, growing market competition among manufacturers may decrease costs and increase availability in the future. Further evaluation is needed to determine if integrating rapid influenza tests can improve the performance of national influenza surveillance systems in Southeast Asia.

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