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# Short Report: Rapid-Test Based Identification of Influenza as an Etiology of Acute Febrile Illness in Cambodia

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*Abstract.* Influenza can be manifested as an acute febrile illness, with symptoms similar to many pathogens endemic to Cambodia. The objective of this study was to evaluate the Quickvue influenza A+B rapid test to identify the etiology of acute febrile illness in Cambodia. During December 2006–May 2008, patients enrolled in a study to identify the etiology of acute febrile illnesses were tested for influenza by real-time reverse transcriptase PCR (RT-PCR) and Quickvue influenza A+B rapid test. The prevalence of influenza was 19.7% by RT-PCR. Compared with RT-PCR, the sensitivity and specificity of the rapid test were 52.1% and 92.5%, respectively. The influenza rapid test identified the etiology in 10.2% of enrollees and ≥ 35% during peak times of influenza activity. This study suggests that rapid influenza tests may be useful during peak times of influenza activity in an area where several different etiologies can present as an acute febrile illness.

Several studies have highlighted the use of rapid influenza testing to improve early detection of epidemics and conduct outbreak responses.[1,2] Rapid influenza testing can be used to identify patients with influenza in a timely manner and influence clinical treatment.[3,4] Although rapid testing for influenza has been demonstrated to be less sensitive when compared with the polymerase chain reaction (PCR),[5] it could be an attractive diagnostic tool in resource-limited settings lacking laboratory capabilities to identify influenza by culture or molecular techniques. There is limited data on the use of rapid influenza testing in Southeast Asia and regions where numerous etiologies of acute febrile illness are endemic and could require prompt treatment (e.g., malaria, dengue, typhoid fever).

Hospital-based and clinic based influenza surveillance was established to ascertain the etiologies contributing to acute febrile illness in patients in Cambodia.[6] We report the performance of a rapid influenza test conducted at the sites of enrollment to identify influenza as an etiology of febrile illness during December 2006–May 2008 in Cambodia. Outpatients were initially recruited from two referral hospitals, but during the course of the study, seven additional healthcare facilities were added; two in August 2007, one in October 2007, one in December 2007, one in February 2007, one in March 2008, and one in April 2008. Study sites were located ≤ 50 km of Phnom Penh in southcentral Cambodia.

Patients were clinically evaluated by a physician or medical assistant and recruited for study participation if they met inclusion criteria: at least 24 hours of fever (a measured tympanic membrane temperature > 38.0°C), were ≥ 2 years of age, and after medical examination, had no obvious source of infection. Influenza-like illness was defined as fever (> 38.0°C) with cough and/or sore throat. Eligible subjects voluntarily enrolled in accordance with an Institutional Review Board protocol approved by U.S. Naval Medical Research Unit No. 2 in compliance with all applicable Federal regulations governing the protection of human subjects and the National Ethics Committee of the Royal Kingdom of Cambodia, Ministry of Health.

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For each enrolled patient, one throat and one nasal swab was collected and placed in a vial containing 2–3 mL of virus transport medium. All inoculated vials were kept at 4°C until received by the laboratory 24–72 hours after collection. Medical personnel were trained by study investigators by using the manufacturer's instructions on the proper collection of a nasopharyngeal swab and interpretation of the QuickVue Influenza A+B Test (Quidel Inc., San Diego, CA). Color digital photographs of all rapid test results were taken and forwarded for laboratory supervisor's confirmation. RNA was extracted from nasal and throat swabs by using QIAamp viral RNA mini kits (QIAGEN, Hilden, Germany) according to the manufacturer's instruction and stored at –70°C. Influenza virus genome was detected by using a reverse real-time PCR (Centers for Disease Control and Prevention, Atlanta, GA) developed to detect influenza A and B viruses and influenza A viruses of H1, H3, and H5 subtypes and performed as described.[6]

A total of 1,327 patients were enrolled during December 2006–May 2008. Among the participants, the median age was 10 years (interquartile range [IQR] = 5–22 years) and 50% were male. The median time of illness prior to presentation for healthcare services was 3 days (IQR = 2–3 days). During this period, 261 (19.7%) enrollees were identified as influenza positive by RT-PCR, and 147 participants were influenza positive by rapid test. Among the 147 rapid test–positive results, 136 had concordant results by RT-PCR. Overall, the sensitivity and specificity of the Quickvue influenza rapid test compared with PCR was 52.1% and 92.5%, respectively. Among the 136 concordant results, 72 (52.9%) were influenza A and 64 (47.1%) were influenza B. Sensitivity for influenza A and B was 47% and 57%, respectively. One case identified as rapid influenza A positive at the field site was later confirmed to be influenza B.

Among PCR-positive influenza patients, the median age of rapid test–positive patients was 8 years (IQR = 5–12 years) and the median age of rapid test–negative patients was 10 years (IQR = 5–17 years). There was a significant difference between the sensitivities of the rapid test between age groups, 56.9% among patients 2–18 years of age and 22.2% among patients > 18 years of age ($P = 0.0001$). The median day of presentation for health care services was 3 days for rapid test positive-patients (IQR = 2–3 days) and rapid test–negative patients

(IQR = 2.5–4 days). A comparison based on day of fever presentation identified a significant difference among patients presenting at days 0–3 (61.1%) and day 4 (22.4%) ($P < 0.0001$). There was no significant difference in rapid test results based on a clinical presentation with influenza-like illness; 74.8% of rapid test–positive patients and 79.3% of rapid test–negative patients had influenza-like illness ($P = 0.39$).

Among 1,327 patients enrolled who had an acute febrile illness, use of an influenza rapid test resulted in 136 (10.2%) patients who were diagnosed with influenza at the time of their health care visit. During the peak of influenza activity (August–December 2007),[6] influenza rapid tests identified 20–35% of the acute febrile cases as influenza infections. There was a significant difference in the sensitivity of the rapid test when used at times of peak influenza activity compared with when influenza was not circulating (55.6% versus 25.1%, respectively; $P = 0.002$).

In this study, the overall sensitivity of the influenza rapid test was similar to those of published studies.[5,7–9] The cost of influenza PCR testing (US $21–25/test) and infrastructure requirements prevent its routine clinical use in Cambodia. A laboratory-based surveillance program that monitors influenza activity in a region could determine times to implement rapid testing beyond the influenza surveillance sentinel sites to help manage and diagnose illnesses. Limiting the use of rapid tests (US $7–12/test)[10] to times determined by a laboratory-based surveillance program would be a more cost-effective alternative than year-round testing.

This study was conducted in a region endemic to several causes of acute febrile illness (e.g., dengue, malaria, typhoid) that can present with similar symptoms and can make a clinical diagnosis of disease difficult. This study suggests that rapid influenza tests may be useful during peak times of influenza activity or during outbreaks in an area where several different etiologies can present as an acute febrile illness.

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