

Validation of Self-swab for Virologic Confirmation of Influenza Virus Infections in a Community Setting

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Few studies have investigated the validity of self-collected nose and throat swabs for influenza confirmation in community settings. We followed outpatients with confirmed influenza with sequential measurement of viral loads and applied log-linear regression models to the viral shedding patterns. Among 176 outpatients with confirmed influenza, the detection of virus and quantitative viral loads obtained from self-swabs was consistent with statistical predictions based on earlier and later measurements, suggesting that self-collected nose and throat swabs can be a valid alternative for virologic confirmation of influenza A or B infection in a community setting.

In community-based studies of acute respiratory illnesses, clinical specimens from the upper respiratory tract may be collected from patients at different stages of disease for virological testing. Although those clinical specimens are typically collected by trained healthcare professionals (HCPs) in a clinic setting, self-collection by the patient at home may be a more acceptable, economical, and logistically feasible alternative. We investigated whether self-collected nose and throat swabs (NTSs) from patients in a community setting could provide a valid alternative for virologic confirmation of influenza A or B virus infection. Based on 2 similarly designed community-based studies, we modeled the viral shedding patterns from illness onset, adjusting

for delays between clinical symptom onset and specimen collection, and compared the quantitative viral load measurements in self-swabs with model-based predictions.

METHODS

Sources of Data

We conducted 2 separate community-based studies of influenza virus infection in Hong Kong with broadly similar protocols for recruitment and follow-up. In both studies, outpatients with recent-onset acute respiratory illness who presented within 48 hours of symptom onset were recruited; of individuals who provided informed consent, those with a positive result on a QuickVue Influenza A+B rapid diagnostic test (Quidel Corp) were invited to continue with follow-up. In one prospective, multicenter study (Influenza Resistance Information Study [IRIS]), patients recruited between 20 January 2010 and 24 November 2010 were followed up to examine natural prevalence and/or emergence of resistance to antivirals among circulating influenza virus strains. In a separate household transmission study (HTS), eligible patients were recruited between 9 January 2008 and 29 September 2008 and followed up as part of a study investigating the effectiveness of nonpharmacological interventions [1].

Specimen Collection

In the IRIS, NTSs were collected by a trained HCP on days 1 and 6 after recruitment in the outpatient clinic and self-collected by subjects at home on day 3 after receiving detailed instruction on swab technique from the HCP at baseline. During the clinic visits, the nasal swab was collected by inserting and rotating a separate flocked sterile swab (Copan) through each nostril into the posterior nares, and throat swabs were collected by swabbing a sterile flocked swab on both the tonsillar fossae and posterior pharynx. The flocked end of the 2 nasal swabs and the throat swab were then transferred to a vial containing Copan Universal Transport Medium by breaking the prescored breaking point of the plastic swab shaft. Specimens collected in the clinic on day 1 and day 6 were stored directly in a clinic refrigerator at 4°C–8°C after collection.

Face-to-face instruction on how to perform a nasal and throat swab on oneself or one's child was given to the patient or to the parents of children aged <8 years by the HCP during the baseline visit on day 1. Patients also received a patient instruction leaflet and a kit containing the 3 swabs, an individually wrapped tongue depressor, a transport medium vial, and a sealable plastic bag. Patients kept the day 3 swabs in a refrigerator at

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home after collection and returned them to the clinic on the day 6 visit. All specimens were sent by courier in insulated transport container to the central laboratory at Erasmus Medical Centre within 7 days of collection.

In the HTS, all NTSs were collected by a trained HCP at home visits on days 1, 4, and 7 after recruitment. Nasal swabs were collected by inserting and rotating a sterile plain swab (viscose-tipped collection swab with a snappable plastic stick; EURO-TUBO) into the anterior nares, and throat swabs were collected by rubbing a second sterile swab against the tonsillar fossa. Both swabs were then snapped off into a tube containing viral transport medium (0.5% bovine serum albumin in Earle's balanced salt solution with antibiotic). Specimens were stored in an insulated transport container with at least 2 ice packs immediately after collection. Specimens were then either delivered directly or first stored overnight in a study outpatient clinic in a 2°C–8°C refrigerator and then delivered the next day to the central testing laboratory at Queen Mary Hospital by courier in ice boxes.

Laboratory Methods

Slightly different laboratory procedures were used in the 2 studies. For the IRIS, influenza A and influenza B matrix gene-specific reverse-transcription polymerase chain reactions (RT-PCRs) were performed as described elsewhere [2]. Dilutions of an electron microscopic-counted influenza virus A/PR/8/34 stock (Advanced Biosciences) and B/Lee/40 (Advanced Biotechnologies) were run in parallel for conversion of RT-PCR threshold cycle (Ct) values into a quantitative measurement of viral particles per milliliter (vp/mL) [2].

For the HTS, samples were eluted and cryopreserved at –70°C immediately after receipt in the laboratory. Specimens were then tested by a quantitative RT-PCR assay to detect the presence of influenza A or B virus and determine molecular viral loads in RNA copies per milliliter (copies/mL) using standard methods as described elsewhere [1, 3–5].

Statistical Analysis

Previous studies have suggested that following influenza virus infection, viral load rises to a peak around the time of illness onset and then, for influenza A, declines approximately log-linearly over the subsequent 5–10 days to undetectable levels and, for influenza B, plateaus with a more gradual decline [6, 7]. We specified multivariable linear regression models for the log viral load on the first and third measurement (typically 0 and 7 days, respectively, after recruitment), with the same slope but separate intercepts for each individual to allow for between-person variability in peak viral loads. We fitted separate models for each study and for influenza A and B and adjusted for age and oseltamivir treatment. Interaction terms with time were included to allow the slope of the regression line to vary by age and oseltamivir treatment. Viral loads for specimens with measured load below the lower limit of quantification (LLOQ) were

imputed as half the LLOQ. This random-effects regression model constructed using the first and third measurement was used to predict viral loads expected on the second measurement (typically 3 days after recruitment), which were then compared with the observed viral loads on the second swabs, which were collected by the patients in IRIS and by an HCP in the HTS. We calculated mean differences with 95% confidence intervals (CIs) based on the *t* distribution.

RESULTS

One hundred thirty-eight subjects with confirmed influenza A and 58 with confirmed influenza B were recruited in the IRIS in 2010, including 43% aged <15 years (range, 2–85 years); 53% were female, and 53% were prescribed oseltamivir treatment. One hundred eighty-eight subjects with confirmed influenza A and 118 with confirmed influenza B were recruited into the HTS in 2008, of whom 73% were aged <15 years (range, 0–79 years); 54% were female, and 25% were prescribed oseltamivir treatment. The demographic characteristics of subjects with influenza A versus B were similar. Oseltamivir treatment was more common during the period of peak pandemic A (H1N1) activity (data not shown).

Among subjects with a positive RT-PCR result for influenza A at the first measurement and a self-swab available, 109 of 121 (90%) subjects in the IRIS had detectable virus in the self-collected swab 2–5 days after illness onset. In the HTS with an HCP-collected swab, 132 of 183 (72%) subjects had detectable virus in the swab 2–7 days after illness onset. For influenza B, the corresponding statistics were 49 of 55 (89%) for the IRIS (2–5 days after onset) and 74 of 117 (63%) for the HTS (2–7 days after onset).

Trends in influenza A viral load are shown in Figure 1A for 138 subjects from the IRIS and in Figure 1B for 188 subjects from the HTS. In the IRIS, the influenza A viral loads determined from swabs taken at the second measurement were slightly lower on average than the expected values based on the random-effects regression model (Figure 1E). The mean difference between observed and predicted viral load on the second measurement was –0.50 (95% CI, –.69 to –.31) log₁₀ vp/mL. In the HTS, viral loads determined from swabs taken at the second measurement were slightly higher on average than the expected values based on the random-effects regression model (Figure 1F), with a mean difference of 0.31 (95% CI, .08–.54) log₁₀ copies/mL.

Trends in influenza B viral loads are shown in Figure 1C for 58 subjects from the IRIS and in Figure 1D for 118 subjects from the HTS. In both studies, the differences between observed and predicted viral loads on the second measurement were small and statistically insignificant (Figure 1G and 1H). In the IRIS, the mean difference was 0.16 (95% CI, –.18–.51) log₁₀ vp/mL, and in the HTS, it was 0.14 (95% CI, –.16–.43) log₁₀ copies/mL.

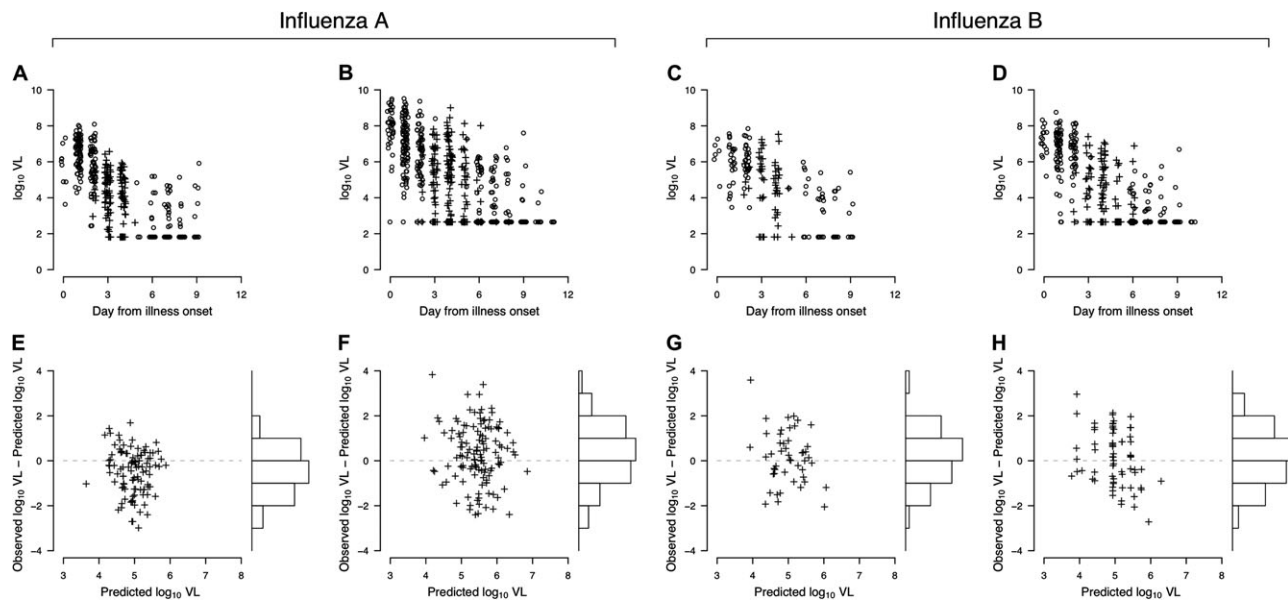


Figure 1. A–D, Molecular viral loads on first and third measurements (*circles*) and second measurement (*crosses*) for influenza A from the Influenza Resistance Information Study (IRIS) (A) and the household transmission study (HTS) (B) and for influenza B from the IRIS (C) and the HTS (D). E–H, Difference between observed and expected molecular viral load (VL) at the second measurement, with a histogram summarizing the differences, for influenza A from the IRIS (E) and the HTS (F) and for influenza B from the IRIS (G) and the HTS (H). The second measurement (*crosses*) was collected by self-swab in the IRIS (A, C, E, G) and by a healthcare professional in the HTS (B, D, F, H). For the IRIS, the lower limit of detection (LLOD) of the influenza A assay was 54 viral particles per milliliter (vp/mL), and the lower limit of quantification (LLOQ) was 131 vp/mL; the LLOD of the influenza B assay was 168 vp/mL, and the LLOQ was 194 vp/mL. For the HTS, the LLOD of the influenza A and B assays was 550 copies/mL, and the LLOQ was 900 copies/mL.

DISCUSSION

Results from the HTS, in which all 3 swabs were collected by trained HCPs, showed that the viral load on the second measurement could accurately and reliably be predicted from a log-linear model based on the first and third measurements. Applying the same approach to the IRIS data, we found that viral loads from self-swabs on the second measurement were very similar to the viral loads that we would have expected if the second swab had been collected by a trained HCP. Our results therefore support the feasibility and validity of using self-swabs as an alternative approach to permit laboratory confirmation of influenza-associated illnesses in a community setting.

Previous studies have demonstrated the feasibility of using parent-collected NTSs from children, in either a hospital setting or community setting, for laboratory confirmation of influenza and other respiratory virus infections without any significant loss in sensitivity [8–10]. Our results further extend this to self-collected swabs by patients in the community setting, both for qualitative disease confirmation and quantitative viral load estimation. In the IRIS, 90% of self-collected swabs contained detectable influenza virus approximately 4–6 days after illness onset, indicating no substantial loss in sensitivity for qualitative virus detection through this approach. In the HTS, the lower proportion of specimens with detectable virus in the second

swab can be attributed to the slightly longer average delay from illness onset.

Our results also suggest that self-swabs work generally well for quantitative measurement of viral loads. For influenza B, there was no significant difference between those obtained from self-swabs and the values predicted from the other 2 swabs by HCPs (Figure 1G and 1H). For influenza A, overall trends in viral loads between the 2 studies also appear similar (Figure 1A and 1B). Although self-swabs from the IRIS were associated with a lower viral load than the predicted value (Figure 1E and 1F), these results should be interpreted with some caution as differences in the type of swabs and transport media used, collection site and technique, delays between collection and transport to the laboratory, and laboratory procedures between studies may have led to artifactual differences. We therefore only compared the predicted and observed viral loads within each study because results from the 2 studies were not directly comparable. Although the log-linear model fit the data well and provided reasonable predictions of viral loads in the HTS (Figure 1), more complex models might better represent the decline in viral loads over time. Although we did not explicitly model the shedding patterns of different influenza A subtypes, we have not previously identified substantial differences [2, 5].

No reports in the literature exist on the validity of self-swab for longitudinal studies of influenza virus infection and illness in

a community setting. Because of the need for multiple sequential respiratory specimens over the course of illness, such studies typically require multiple clinic visits by the patients or multiple home visits by the HCPs and are thus costly and complicated, which may also affect study compliance. Self-swab would thus be an attractive alternative, and further validation of this approach would benefit the design of future community-based studies. Further studies employing collection of NTSs by both the patients and HCPs in a parallel or randomized fashion could help to enable finer calibration of the measurements obtained by self-swabs.

Notes

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R. T. D. is an employee of Hoffmann-La Roche. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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