Detection of Influenza Antigen with Rapid Antibody-Based Tests after Intranasal Influenza Vaccination (FluMist)

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Rapid tests for influenza antigen detection are frequently used, but it is not known how receipt of intranasal influenza vaccine affects results of these tests. We tested healthy adults who received either intranasal or intramuscular influenza vaccine. Of the 14 intranasal vaccine recipients, 7 (50%) had a direct fluorescent antibody test (DFA) result and 2 (14%) had an enzyme immunoassay (EIA) result that was positive for influenza antigen within 7 days after vaccination. No subjects had positive EIA results on day 12 or 13 after vaccination. For some intranasal vaccine recipients, rapid influenza-antigen detection tests yield positive results within 1 week after vaccination.

A live, attenuated, trivalent influenza vaccine for intranasal administration (FluMist; MedImmune Vaccines) was introduced in the 2003–2004 influenza season for vaccination of healthy individuals aged 5–49 years. Shedding of the live attenuated vaccine has been studied by culture-based methods. In one study of children aged 8–36 months attending day care facilities, viral shedding after intranasal vaccine administration was evaluated by culture of nasal swab specimens obtained 3 times per week for 21 days [1]. Approximately 80% of children shed virus detectable by culture for a median period of 7.6 days. Another, much older study of 2 cold-adapted live influenza vaccine recipients of this vaccine had a viral culture positive for influenza B virus 5 days after vaccination. Rapid immunodiagnostic tests were not used in any of these studies.

Currently, influenza is rarely diagnosed by slow, expensive culture-based methods. It is usually diagnosed clinically and suspected cases are confirmed by results of rapid, antibody-based tests that detect viral antigens in respiratory secretions. These include either EIAs or direct fluorescent antibody staining (DFA) of secretions to detect cells with influenza antigens. Rapid tests are useful in confirming influenza and may decrease attention to other important diagnostic considerations. Today, other diagnostic possibilities include severe acute respiratory syndrome (SARS) and perhaps also early presentations of illnesses linked to bioterrorism. Detection of influenza antigens in an intranasal vaccine recipient with these illnesses could result in mismanagement from a clinical or infection-control perspective. This confusion might be minimized if a positive test result were reasonably attributed to vaccination (although this would not eliminate the unlikely but possible concurrence of bona fide influenza infection and another emerging infectious disease). In addition, given the early and severe influenza season this year (2003–2004), a shortage of parenteral vaccine, and recent publicity over deaths due to influenza, it is possible that many persons will receive intranasal vaccine. We could not find any published studies evaluating whether subjects who receive intranasal vaccine test positive for viral antigens, as measured by rapid, antibody-based diagnostic tests for influenza. Therefore, we conducted a descriptive study to determine the frequency of viral antigen detection in nasopharyngeal secretions after intranasal vaccine vaccination.

Study design. The study was reviewed and approved by the institutional review board of Massachusetts General Hospital (Boston, Massachusetts). The study was conducted at this hospital in early November 2003, before detection of significant numbers of influenza cases in the community. Seventeen healthy adult volunteers aged 18–49 years were enrolled. Exclusion criteria included direct occupational contact with patients, pregnancy, history of asthma, severe allergy to chicken eggs, and having a household member known to be immunosuppressed. Subjects were randomly assigned, on the basis...
of their order of attendance, to receive intranasal vaccine spray (0.5 mL per dose [0.25 mL in each nostril]; total dose, $10^{6.5}–10^{7.5}$ median tissue culture infectious dose [TCID$_{50}$] units) ($n = 14$) or intramuscular inactivated influenza vaccine (0.5 mL per dose; Fluvirin, Evans Vaccines) ($n = 3$). Nasopharyngeal swab specimens were obtained before vaccination (day 0) and at 3 time points after vaccination: day 2 or 3, day 6 or 7, and day 12 or 13 (2-day windows were allowed, to maximize the chance of subjects completing all follow-up visits).

Two sterile Dacron (i.e., polyester) swabs were used to collect nasal and throat secretions separately. Swabs were then combined by vortexing together in 1 mL of sterile saline to create 1 sample per subject per time point, and samples were stored at 4°C until testing. Study participants recorded oral temperatures daily and recorded symptoms such as runny nose, malaise, sore throat, cough, body aches, headache, and sinus pain for 7 days after vaccination. Vortexed swab samples were tested by laboratory staff not aware of the subjects’ vaccine assignments. Three tests were used: two EIAs (Directigen Flu A and Directigen Flu A+B; Becton-Dickinson) and a microscopic DFA (SimulFluor FluA/FluB Immunofluorescence Assay; Light Diagnostics) for rapid, qualitative detection of influenza virus types A and B.

**Results.** All prevaccination nasopharyngeal swab specimens obtained on day 0 (i.e., before vaccination) were negative for influenza A and B by all 3 rapid influenza tests. For 3 control subjects who received parenteral vaccine, all samples yielded negative rapid test results, although 1 of these 3 individuals did not return for the final study visit. Results of EIA and DFA tests for intranasal vaccine recipients are shown in table 1. On day 2 or 3 after vaccination, 1 (7%) of 14 intranasal vaccine recipients was positive for influenza antigens by EIA, and 6 (42%) of 14 were positive by DFA. On day 6 or 7 after vaccination, 1 (7%) of 14 subjects was positive by EIA and 2 (14%) of 14 were positive by DFA. Each subject who had a sample with a positive EIA result also had a positive DFA result for the same influenza strain (A or B). Five subjects who were negative by EIA were positive by the more sensitive DFA, which requires interpretation by an expert microscopist. Of 14 subjects who received the intranasal vaccine, 1 (subject 5, who had a positive test result on day 2), did not complete the final follow-up visit. No subjects had specimens positive by EIA on day 12 or 13. Neither of the 2 subjects with positive test results on day 6 or 7 had a positive DFA results on day 12 or 13; therefore, this more labor-intensive test was not performed on other specimens. Day 2 or 3 after vaccination was the time point with the highest proportion of influenza-positive specimens. On day 2 or 3 after vaccination, 6 of 14 intranasal vaccine recipients had mild symptoms, such as runny nose, sore throat, cough, and malaise; no subject required treatment or limited their daily activities. No subject was febrile.

**Discussion.** Rapid antigen testing of respiratory secretions is widely used to confirm the diagnosis of influenza with high sensitivity and specificity. Published studies comparing the EIA Directigen Flu A with viral culture reported sensitivity values ranging from 39% to 100% in various sample types and patient populations [4–8]. The sensitivity of the Directigen Flu A+B EIA, compared with shell vial culture, was 82.9% for detection of influenza virus type A, and it was 51.5% for detection of influenza virus type B [9]. The Directigen EIAs are frequently used in clinical practice.

In this study, 7 (50%) of 14 intranasal vaccine recipients were positive for influenza virus by either EIA or DFA (or both) at some point during a period 2–7 days after vaccination. DFA

<table>
<thead>
<tr>
<th>Subject</th>
<th>Test result, by day after vaccination and type of test</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
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<tr>
<td></td>
<td>EIA</td>
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<tr>
<td>P3</td>
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<td>P16</td>
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<td>Total no. (%) of subjects</td>
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**NOTE.** ND, not done.
a Influenza A.
b Influenza B.
was more sensitive than was EIA; results were positive for 8 samples, but only 2 of these also had positive EIA results. Most positive results were obtained on day 2 or 3 after vaccination.

These results are temporally comparable to those reported for use of EIA to detect virulent influenza virus after experimental inoculation of human volunteers. Kaiser et al. [10] experimentally infected 14 adult volunteers with influenza A virus (inoculum, 10^5 TCID₅₀), and viral shedding was carefully evaluated for 8 days. Quantitative viral culture and the Directigen Flu-A EIA were used to detect influenza virus in a variety of specimens, including nasopharyngeal wash, nasopharyngeal swab, throat swab, and throat gargle-fluid specimens. The largest numbers of samples were positive on day 2 or 3 after inoculation. Nasopharyngeal wash samples were the most virus-rich specimens, by quantitative culture. Nasopharyngeal wash samples were analyzed, and 86% of all subjects inoculated with virulent influenza had a positive Directigen Flu A test result at some point during the 8-day study period, although sensitivity varied if culture results were used as the standard. These authors found that 79% of subjects had positive EIA results at some point during the 8 days if either a nasopharyngeal or throat swab sample was analyzed. On day 2 or 3 after intranasal inoculation with virulent influenza A, 7 (50%) of 14 adult volunteers had nasopharyngeal or throat swab specimens that were positive for influenza virus antigen by EIA, compared with 1 (7%) of 14 volunteers who had nasopharyngeal swab specimens positive by EIA at the same time point.

These results and those of another study of elderly subjects with naturally acquired influenza [7] suggest that a combination sample of nasopharyngeal and throat swabs was a good clinical specimen for our study; nasopharyngeal wash specimens are not routinely obtained from adults. We did not culture our samples for influenza virus, so it cannot be stated with certainty that these subjects shed vaccine virus, although this is highly likely. It is difficult to estimate what percentage of our subjects may have been culture positive, given the conflicting data for toddlers and adults inoculated with the intranasal vaccine FluMist or its investigational precursors [1–3]. Our findings show that respiratory secretions obtained from subjects who received FluMist (in the formulation approved by the US Food and Drug Administration) may test positive for influenza antigens as a result of vaccination. The greatest number of positive test results were obtained on day 2 or 3 after vaccination. This seemed to correlate with the timing of mild upper-respiratory symptoms related to the intranasal vaccine, and is temporally similar to shedding patterns seen in human challenge studies of influenza [10]. Physicians should use caution in interpreting rapid influenza antigen detection tests for recipients of intranasal vaccine, especially within 1 week after receipt of the vaccine. Detection of antigen >1 week after vaccination seems unlikely. Additional studies of adults that further characterize shedding of this live intranasal vaccine virus by both culture and rapid diagnostic methods may help inform future use of this vaccine, especially among health care workers.

Acknowledgment

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References