JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2001, p. 730–732 Vol. 39, No. 2
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.2.730–732.2001

Evaluation of a Rapid Optical Immunoassay for Influenza Viruses (FLU OIA Test) in Comparison with Cell Culture and Reverse Transcription-PCR

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Received 13 July 2000/Returned for modification 8 September 2000/Accepted 8 November 2000

The FLU OIA test was evaluated with 146 throat swab specimens from subjects with a flu-like illness in six Canadian clinics during the 1999–2000 flu season. The rate of positivity of the FLU OIA test (41.5%) was significantly lower than that of cell culture (55.2%) or reverse transcription-PCR (55.9%) during a season in which only influenza A virus was detected.

Rapid diagnosis of influenza virus infections is required for outbreak control measures in health care facilities and for outpatient management of subjects with flu-like illnesses. In view of the short window of opportunity for administering antiviral therapy (7, 11, 12), conventional detection methods for influenza virus (cell culture, immunofluorescent assays, and serology) are of little benefit in most private clinics due to the long delay before results are obtained or the need for laboratory expertise. Thus, point-of-care (POC) tests that could reliably and rapidly identify all types of influenza viruses are greatly needed. In the study described here, we performed an independent multicenter evaluation of one of the new POC tests for influenza, the FLU OIA test (Biostar Inc., Boulder, Colo.), using freshly collected throat swab specimens from subjects with a well-defined flu-like illness.

Participants with a flu-like illness of ≤72 h in duration were enrolled by general practitioners at six outpatient clinics in the following Canadian cities: Riverview (New Brunswick), Québec City and Montréal (Québec), Toronto (Ontario), Edmonton (Alberta), and Vancouver (British Columbia). The clinical case definition for influenza-like illness consisted of a temperature of ≥37.8°C or feverishness (in the case of antipyretic use in the last 6 h) with two of the four following clinical symptoms: cough, myalgia, sore throat, and headache. Enrollment was initiated on 23 November 1999, after the first influenza cases were reported in western Canada, and continued until 14 March 2000.

Two pharyngeal swab specimens were collected from each patient. The first swab was kept at 4°C for a maximum of 24 h before testing by the FLU OIA test, which is an optical immunoassay for the rapid detection of influenza A and B viral antigens (nucleoprotein) from various upper respiratory tract samples. The test was performed according to the recommendations of the manufacturer by physicians or nurses who had received a standardized 45-min phone training. The other swab was immediately placed in 2 ml of a viral transportation medium (VTM) (M4-RT; Micro-Test, Inc., Lillburn, Ga.) and sent at 4°C to a central laboratory in Québec City. Within 72 h of sample collection, an aliquot of 200 μl of VTM was inoculated in a vial containing Madin-Darby canine kidney (MDCK) cells. The presence of a cytopathic effect or a positive hemadsorption test result was confirmed by viral typing with monoclonal antibodies against influenza A and B viruses (Whittaker, Walkersville, Md.) (2). The nested multiplex reverse transcription (RT)-PCR for influenza viruses AH1, AH3, and B was performed as described previously (6). The rates of positivity of the detection assays were compared by the use of McNemar's exact test. The influence of dichotomized variables on the rate of positivity of each individual assay was analyzed by the chi-square test.

A total of 146 pharyngeal swab specimens were collected from subjects who fulfilled the clinical case definition (Table 1). The mean and the median ages of the subjects were 37.8 and 36.0 years, respectively (age range, 0.6 to 83.0 years). The mean and the median oral temperatures at the time of the visit were 37.9 and 38.0°C, respectively (temperature range, 37.0 to 40.0°C). Finally, the mean and median times between the onset of symptoms and sample collection were 37.2 and 36.0 h, respectively (range, 5.0 to 72.0 h). All isolates were identified as influenza virus type A by culture and were further classified as AH3 (76/80, 95%) and AH1 (4/80, 5%) by multiplex RT-PCR.

The rate of positivity of the FLU OIA test was significantly lower than that of RT-PCR ($P = 0.004$) or cell culture ($P = 0.009$), as shown in Table 2. However, RT-PCR and cell culture identified similar numbers of positive samples ($P = 1.000$). The sensitivity, specificity, and positive and negative predictive values of the FLU OIA test by using cell culture and RT-PCR as "gold standards" were 54.0, 74.1, 72.7, and 55.8% and 56.0, 77.2, 76.3, and 57.1%, respectively. The influence of demographic variables and clinical symptoms on the rate of positivity of each detection test was analyzed (Table 1). A positive culture was significantly associated with the presence of cough ($P = 0.005$) and headache ($P = 0.05$). The same two variables were also associated with a positive RT-PCR result ($P = 0.004$ and 0.012 for cough and headache, respectively), whereas only cough was significantly associated with a positive FLU OIA test result ($P = 0.001$) (data not shown). Of note, there was no

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TABLE 1. Descriptive data and culture positivity rate for 146 subjects with a flu-like illness[a]

Variable	Category	No. (%) of observations	% Subjects with positive culture
Study site	East (Riverview, Québec City, Montréal)	67 (45.9)	61.2
	Central (Toronto)	30 (20.5)	35.7
	West (Edmonton, Vancouver)	49 (33.6)	58.3
Visit date (mo-day-yr)	11-23-93 → 12-23-99	51 (34.9)	53.1
	12-24-99 → 01-24-00	59 (40.4)	60.3
	01-25-00 → 03-14-00	36 (24.7)	50.0
Age (yr)	<40	83 (56.8)	50.0
	≥40	63 (43.2)	62.3
Gender	Female	70 (48.3)	62.9
	Male	75 (51.7)	47.2
Time (h) since symptom onset	≤36	77 (54.2)	54.8
	>36	65 (45.8)	56.7
Temp (°C)	≤38	93 (64.1)	51.1
	>38	52 (35.9)	60.8
Antipyretic use in last 6 h	No	68 (47.2)	55.2
	Yes	76 (52.8)	55.4
Flu vaccination in 1999	No	133 (91.1)	54.6
	Yes	13 (8.9)	61.5
Underlying illnesses	No	122 (83.6)	50.8
	Yes	24 (16.4)	66.7
Cough	No	17 (11.6)	23.5
	Yes	129 (88.4)	59.5[b]
Sore throat	No	33 (22.6)	65.6
	Yes	113 (77.4)	52.2
Headache	No	21 (14.4)	35.0
	Yes	125 (85.6)	58.5[b]
Myalgia	No	16 (11.0)	60.0
	Yes	129 (89.0)	55.1
Use of neuraminidase inhibitors	No	138 (94.5)	54.3
	Yes	8 (5.5)	50.0

[a] See text for case definition.
[b] Statistically significant difference ($P \leq 0.05$).

significant difference in the rate of positivity of the FLU OIA test among clinics ($P = 0.153$).

Although the growth of the virus is essential for characterization of its antigenic properties, this method is not suitable for immediate management of influenza in most clinics due to the delay before results are obtained and the technical expertise needed. A simple enzyme immunoassay, the Directigen FLU A test (Becton Dickinson, Meylan, France), which can directly detect influenza A virus in clinical samples in <15 min, has been available for >5 years. Compared to conventional cell culture, the Directigen FLU A test has sensitivity and specificity values of 62 to 87% and 94 to 100%, respectively, depending on the type of respiratory samples and the age of the subjects (1, 5, 9, 10). However, this test does not detect type B influenza viruses, which could be problematic in some flu seasons, considering that neuraminidase inhibitors effective against all types of influenza viruses are now available (7, 8, 11, 12).

The Biostar FLU OIA test is a new optical immunoassay intended for on-site detection of influenza A and B virus nucleoprotein antigens from various types of respiratory samples

TABLE 2. Rate of positivity for influenza by detection assays

Assay(s)	No. of samples with positive results/ total no. of samples	%
RT-PCR	80/143	55.9
Cell culture	79/143	55.2
RT-PCR and/or cell culture	83/143	58.0
FLU OIA test (Biostar)	56/135	41.5[a]

[a] Statistically significant difference ($P < 0.05$) compared to RT-PCR and cell culture.

in <20 min. Although the test requires minimal technical expertise, our results show that it is significantly less sensitive than culture on MDCK cells for detection of influenza A virus from throat swab specimens (Table 2). Of note, our samples were collected from subjects with a standardized flu-like illness definition, which should have increased the probability of influenza virus detection. Among clinical symptoms, cough and headache were associated with higher rates of positivity for the detection assays. As recently reported by our group with a different study population, cough is the most important clinical predictor of influenza (odds ratio = 6.68) (3). Colvalciuc et al. (4) have reported that viral culture and the FLU OIA test had equal abilities to detect influenza A or B virus antigens in samples from four different sites (nasopharyngeal swab, throat swab, nasal aspirate, and sputum samples) when a positive viral culture from any of these sample sites was considered as a reference. However, the sensitivity and specificity of the FLU OIA test were only 62.1 and 79.5%, respectively, compared to the results of viral culture when only throat swab samples were considered. The performance of the test was even lower in our study, with sensitivity and specificity values of 54.0 and 74.1%, respectively. Furthermore, contrary to the previous study, almost all FLU OIA test-positive, culture-negative samples (13 of 15 [86.7%]) were negative by RT-PCR. The discrepancy between the two studies could be explained by a higher sensitivity of our cell culture method, variable performance of the rapid test by some investigators, slightly different criteria for selection of subjects with a flu-like illness, age of the patients, different viral types or subtypes, etc.

In summary, our prospective multicentric study indicates that throat swab samples are not optimal samples for use with the rapid FLU OIA test. Different types of samples (nasal aspirate, nasopharyngeal swab, and even sputum samples) may be associated with a better test performance (4), although their collection is probably not as convenient as the collection of throat swab samples in busy outpatient clinics. Other rapid antigen detection tests for influenza A and B viruses are now available, but their performances need to be carefully evaluated with respect to viral types (types A and B), sample sites, and categories of patients (pediatric, geriatric) (13). In addition, the cost-benefit utility of these rapid tests should be compared to that of clinical diagnosis by using a valid case definition during different periods of influenza virus activity (3).

We thank the following physicians for collecting throat swab samples from subjects with a flu-like illness: Jean Maziade (Québec City), Guy Tellier (Montréal), Allan Kaplan (Toronto), Allan J. Kelly (Edmonton), Michael Myckatyn (Vancouver), and Brian Davidson (Riverview).

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