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Evaluation of a New Dot Blot Enzyme Immunoassay (Directigen Flu A+B) for Simultaneous and Differential Detection of Influenza A and B Virus Antigens from Respiratory Samples

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We report a prospective evaluation of a new dot blot enzyme immunoassay (EIA) method for the direct, rapid, qualitative, simultaneous, and differential detection of the influenza A (IA) and B (IB) virus antigen in different respiratory samples. The EIA method was compared with the shell vial culture system (MDCK cell line) used with the same samples. We studied 160 samples from 93 (58.1%) pediatric patients (hospital emergency room) and from 67 (41.9%) adult patients (sentinel network). Seventy-four(46.2%) samples were considered positive; of them, 46 (62.2%) were from pediatric patients and 28 (37.8%) were from an adult group ($P < 0.05$), with overall positive values of 49.9% and 41.7%, respectively. All 74 (100%) of the positive samples were isolated in cell culture versus the 68.9% that were detected as positive by the new EIA method ($P < 0.05$). Of the 41 samples positive for the IA virus, the EIA detected 34 (82.9%) positive samples; of the 33 samples positive for the IB virus, the EIA detected 17 (51.5%) positive samples ($P < 0.05$). No false-positive reaction was detected with the EIA method (specificity and positive predictive value of 100%). The overall results obtained in the comparison between the new EIA and the shell vial culture had a sensibility of 82.9% and predictive negative values of 92.4% for the IA virus and 51.5% and 84.3%, respectively, for the IB virus. This evaluation shows sensitivity and specificity percentages for the new EIA method that is acceptable for routine use in IA virus detection. The results obtained were worse for IB virus detection, but this new EIA method is actually the only one with the capacity to differentiate between the two influenza viruses.

Influenza is an infection caused by the influenza A and B (IA and IB) viruses, which present as epidemic outbreaks in the winter months. This epidemiological fact is of use in the clinical diagnosis of this infection. Usually, this infection may be considered self-limiting in healthy populations. Nevertheless, in the very young and in immunodepressed patients it may lead to an increase in morbidity and mortality (5, 13).

However, it is necessary to carry out a definitive etiological diagnosis at the beginning of, and during, each epidemiological period in order to establish the prevalence and appearance of new strains or subtypes not included in the recommended vaccine (1, 15). At the same time, the appearance and the availability of neuraminidase inhibitors requires a rapid (these new antivirals are most effective when given within 48 h of symptoms) and specific diagnosis of influenza virus infection (7, 9).

The diagnosis of influenza infection is largely clinical, but this method has been demonstrated to be both insensitive and nonspecific (15, 22). The reference method (gold standard) for laboratory diagnosis of influenza is the isolation of the virus. This may be carried out by inoculation in embryonated hens' eggs, in laboratory reference only, or by means of cell culture (classical or shell vial type) (8, 17, 21). These methods, however, have serious drawbacks in that they are slow and laborious and require from 2 to 7 days to reach the final result. Consequently, rapid techniques based on the detection of viral antigens or of physiological viral activity (neuraminidase) have been developed (2, 6, 14, 16). Immunofluorescence is a highly sensitive and specific technique, but it requires a minimum number of cells in the sample and an expert technician for good interpretation (6, 16). The enzyme immunoassay systems (EIA) have provided high sensitivity, high specificity (nucleoprotein as the antigen), rapid diagnosis (less than 15 min), and technical simplicity. The majority of these systems are performed on a solid membrane and are based on an enzymatic reaction with the development of a visual color (2, 11, 16, 19).

The aim of this study was to carry out a prospective evaluation of a new EIA method in the direct, qualitative, simultaneous, and differential detection of the IA and IB virus antigens in different clinical samples of symptomatic patients.

From January to December 2001 we evaluated the efficacy of a new commercial rapid EIA method for the differential detection of the IA and IB viruses in clinical samples of two different population groups. Samples were taken from adult patients attended to in the sentinel network (community-based study) by using a throat swab vigorously rubbed on both tonsillar surfaces and the posterior pharynx. In the case of patients attended to in the pediatric emergency room of our hospital (pediatric group), a nasopharyngeal aspirate was taken. Both types of samples were inoculated in compatible liquid transport medium (Earle's minimum essential medium with 0.5% bovine serum albumin) for viruses and sent as soon as possible to the virology laboratory.

Each of the samples was subjected to antigen detection

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TABLE 1. Results obtained from the comparison between shell vial culture and the rapid EIA test

Sample and virus	Results (%)			
	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Sentinel				
IA	72.7	100	100	95.1
IB	41.1	100	100	79.5
Pediatric				
IA	86.6	100	100	92.1
IB	62.5	100	100	88.6
Overall				
IA	82.9	100	100	92.4
IB	51.5	100	100	84.3

against IA and IB viruses with the new rapid differential EIA membrane test (Directigen Flu A+B; Becton & Dickinson Co., Sparks, Md.) following the manufacturer's recommendations. At the same time, each sample was inoculated in two shell vials of the MDCK cell line (Vircell, Granada, Spain), which were incubated for 72 h at 36°C, after which the monolayers were stained with a specific monoclonal antibody against the IA (clone IA52/9) and IB (clone IB82/2) viruses (Monofluokit Influenza; Sanofi Diagnostics Pasteur, Marnes la Coquette, France) by an indirect immunofluorescence assay.

Statistical analysis was carried out on results of different comparisons by performing the Student's t test on paired data. All P values are two-tailed and considered significant if they are less than 0.05.

In this study we analyzed 160 clinical samples from 93 (58.1%) pediatric patients and 67 (41.9%) patients of the sentinel network (adults). Of these samples, 74 (46.2%) were considered positive (detection and/or viral isolation). Of the positive samples, 46 (62.2%) were from the pediatric group and 28 (37.8%) were from the sentinel group ($P < 0.05$), giving an overall positivity of 49.4% for the pediatric samples and 41.7% for the sentinel group.

All 74 (100%) of the positive samples were isolated in cell culture, 68.9% of which were detected as positive by the EIA method ($P < 0.05$). The IA virus was isolated in 41 samples (25.6%), and the IB virus was isolated in 33 (20.6%) samples. Of the 41 samples positive for the IA virus the EIA method detected 34 (82.9%) positive samples, and of the 33 samples positive for the IB virus the EIA method detected 17 (51.5%) ($P < 0.05$). Of the 46 positive pediatric samples the IA virus was isolated in 30 and was detected by the EIA method in 26 (86.6%). The IB virus was isolated in 16 samples in this group and was detected by the EIA method in 10 (62.5%). Of the 28 positive samples from the sentinel group, the IA virus was isolated in 11 samples and was detected by the EIA method in 8 (72.7%). The IB virus was isolated in 17 samples in this group and was detected by the EIA method in 7 (41.1%) samples.

No false-positive reaction was detected with the EIA method studied, giving us a specificity and positive predictive value of 100%. Table 1 shows the overall results and the results obtained for each group in the comparison between isolation in cell culture and antigen detection (new EIA method).

The new EIA method examined in this study is rapid and simple, and it permits the simultaneous and differential detection of IA and IB virus antigens. The comparison between the cell culture (shell vial) and this new EIA method showed an overall sensitivity of 68.9%, somewhat lower than expected. However, if we separate the two viruses detected we find that the sensitivity for IA was 82.9%, while for the IB virus it was 51.5% ($P < 0.05$).

In a previous study carried out with the same antigen detection EIA method against only the IA virus (Directigen Flu A; Becton & Dickinson), a sensitivity of 84.7% was observed (16). Therefore, the present method displays a practically identical behavior against the IA virus, maintaining very similar sensitivity values, similar to results of other studies (6, 11, 23).

One of the advantages of the new EIA method is the ability to specifically detect the IB virus. Until now no other EIA method with this capacity had been commercialized, so there are no previous studies with which we can compare our results. Reina et al. (18) have previously reported a sensitivity of 66.6% for an indirect immunofluorescence technique against the shell vial culture in the detection of the IB. This value is slightly higher than that detected by the EIA method evaluated in this study, confirming the idea that, in general, immunofluorescence techniques are usually somewhat more sensitive than the EIA methods for the influenza viruses (6, 16, 23).

One of the main problems when evaluating different methods for the antigenic detection of respiratory viruses is the type of sample studied. For this reason we divided the patients into two different groups, both for reasons of age (children and adults) and for the type of clinical sample analyzed (nasopharyngeal aspirate and pharyngeal swab). The majority of studies concerning other EIA methods with the ability to detect simultaneously, although not differentially, the IA and IB viruses have shown important variations in sensitivity according to the type of sample studied (2, 3, 20). Thus, in the study of Schultze et al. (20) we find that the optical immunoassay method (Flu OIA) displays an overall sensitivity of 71.8% in pediatric samples and of 51.4% in adult samples. In the same way, Covalciuc et al. (2) reported that, with this same method, the highest sensitivity is obtained with nasal aspirate (88.4%), and the lowest sensitivity is obtained with the throat swab (62.1%).

In our study the new EIA method displayed a sensitivity of 86.6% for pediatric samples and 72.7% for adult samples (sentinel network) in the detection of the IA virus. The difference between the two groups and/or types of sample was greater in the case of IB virus (62.5% versus 41.1%; $P < 0.05$). It seems obvious, once more, that the type of sample and, therefore, the viral load present is what probably determines the sensitivity of the different antigen detection methods against the majority of respiratory viruses (4, 10, 20). This phenomenon does not affect the cell culture, which scarcely displays differences in sensitivity according to the type of clinical sample (2, 3,16, 23).

Over the study period we found no false-positive antigen detection with the new EIA method, establishing a specificity and positive predictive value of 100%. Therefore, a positive result with this method provides, with a high degree of certainty, the diagnosis of infection by the influenza viruses. It may be used as a rapid screening method for patients with symptoms of infection by the influenza viruses. A negative result in the test does not exclude the existence of viral infec-

tion, especially for those caused by the IB virus. In general, the antigenic detection methods for the IB virus have displayed lower sensitivity percentages, and alternative methods, such as cell culture or reverse transcription-PCR, should be used (12, 18, 23).

The availability of antiviral drugs effective against the IA and IB viruses justifies the need for the rapid and specific detection of infection caused by these viruses (7, 9). In addition, the efficacy of these drugs is maximum when they were used within the first 48 h of the appearance of the disease. Therefore, physicians require rapid and simple diagnostic methods which they themselves can use in their consulting rooms. However, since sometimes the reading of results obtained by EIA methods can be difficult (14, 19), a sample should be always sent to the laboratory for confirmation, culture, isolation, and typing the virus for epidemiological studies (10, 22).

In summary, this study shows sensitivity and specificity percentages for the new EIA method which is acceptable for routine use in antigen detection of IA virus, and it is comparable with other no-differential methods. The results obtained are worse for IB antigenic detection, but this new EIA is actually the only one with the capacity to differentiate between the two influenza viruses. At the same time, it was possible to confirm the variations in the behavior of this antigenic method depending on the sample used. It has been shown to be highly effective for nasopharyngeal aspirates from children attended to in hospital emergency rooms and has been shown to be somewhat less effective for the detection of these viruses in adult patients from the sentinel network.

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