



# Evaluation of a Direct Immunofluorescence Assay, Dot-Blot Enzyme Immunoassay, and Shell Vial Culture in the Diagnosis of Lower Respiratory Tract Infections Caused by Influenza A Virus

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*We prospectively evaluated the efficacy of two commercial rapid methods for antigenic detection, a dot-blot enzyme immunoassay (EIA-DB) (Directigen FluA, Becton-Dickinson, USA) and a direct immunofluorescence assay (DIF) (Monofluokit Influenza A, Diagnostics Pasteur, France), compared with the shell-vial culture in the MDCK line, incubated 2 to 3 days and stained with the monoclonal antibody clone IA-52, the diagnosis of lower respiratory tract caused by Influenza A virus (IA). In the study period the presence of IA virus was detected in 59 of the 377 samples analyzed (15.7%). Only the SVC method detected all positive samples (100% sensitivity), being used as a reference method for comparison with the other techniques). The EIA-DB technique detected 50 cases (84.7%)*

*and the DIF only 35 (59.3%). In nine (15.2%) cases the diagnosis was obtained only with the SVC method. The results of the comparison of the EIA-DB technique with SVC were: sensitivity 84.7%, specificity 100%, positive predictive value 100%, and negative predictive value 97.2%. The DIF technique gave values of 59.3%, 100%, 100%, and 92.9%, respectively. A statistically significant difference was observed between the sensitivity of the EIA-DB and the DIF method ( $p = 0.0001$ ). In view of the results we recommended the use of the EIA-DB as a screening method when infection by the IA is suspected. But to obtain the maximum diagnostic yield all samples would be inoculated in a shell vial culture with the MDCK cell line. © 1996 Elsevier Science Inc.*

## INTRODUCTION

The influenza A virus (IA) is the main etiologic agent in the epidemic outbreaks of flu that occur every winter throughout the world. These respiratory infections affect patients of all ages but present with increased morbidity and mortality in both the very young and the very old [Murphy and Webster, 1990]. Until recently the isolation of the IA virus could only be achieved by inoculation in the allantoic and am-

niotic cavities of embryonated eggs or in primary cellular cultures of monkey kidney [Harmon and Kendal, 1989; Murphy and Webster, 1990]. The majority of strains fail to produce a visible cytopathic effect in the cell cultures so that it is necessary to use indirect methods (hemadsorption) to detect the presence of the virus. In addition, positive results in conventional cultures are only obtained after incubation periods of 3 to 5 days [Harmon and Kendal, 1989; Murphy and Webster, 1990].

In view of the clinical significance of infections caused by the IA virus on very young children, it has become necessary to develop alternative, rapid diagnostic methods for this entity. Rapid methods at present available include the direct immunofluorescence (DIF) and the dot-blot enzyme immunoassay (EIA-

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DB) antigen detection system that enable us to reach a diagnosis in less than an hour [Daisy et al., 1979; Shalit et al., 1985; Grandien et al., 1985]. The shell vial culture (SVC) system applied to the IA virus displays a sensitivity very close to that of conventional culture with the advantage of reduction at the time of incubation of the samples [Mills et al., 1989; Seno et al., 1990].

A prospective study of the efficacy of two rapid antigen detection methods, and EIA-DB (Directigen FluA, Becton & Dickinson) and a DIF (clone IA-52, Monofluokit Influenza A, Pasteur Diagnostics) compared with isolation in SVC with the MDCK cell line (Viricell, Spain) in the detection of the IA virus was carried out in 377 nasopharyngeal aspirates from children with lower tract respiratory infections, which were negative for other viruses in previous tests.

For the EIA-DB method 125 µl of the sample was inoculated onto the surface of the nitrocellulose membrane of the testing device and manufacturer's instructions were followed. Samples giving a purple triangle on the membrane (antigen presence) were considered positive regardless of the intensity. For the DIF technique 200 µl of the sample were cyto-centrifuged (Cytospin 3, Shandon) at 700 rpm for 10 minutes. After drying they were fixed with acetone at -20°C for 10 minutes. The slides were stained with a monoclonal antibody directed against the IA virus nucleoprotein. Samples presenting fluorescent intracytoplasmic inclusions were considered positive. For the SVC method 200 µl of the sample were added to two vials of the MDCK cell line and centrifuged at 700 × g for 45 minutes. After an absorption period of 60 minutes at 36°C the sample was decanted and maintenance medium with 2 µg/ml of trypsin was added. The cultures were incubated for two days if the EIA-DB or DIF were positive or three days if both were negative, at 36°C, and subsequently stained with the same monoclonal antibody used for the DIF technique.

In the study period (January 1995 to February 1996) the presence of IA virus was detected in 59 of the 377 samples analyzed (15.7%). Only the SVC method detected all positive samples (100% sensitivity), being used as a reference method for comparison with the other techniques). The EIA-DB technique detected 50 cases (84.7%) and the DIF only 35 (59.3%) (Table 1). The results of the comparison of the EIA-DB technique with SVC were: sensitivity 84.7%, specificity 100%, positive predictive value 100%, and negative predictive value 97.2%. The DIF technique gave values of 59.3%, 100%, 100%, and 92.9%, respectively. A statistically significant difference (Chi-square test) was observed between the sensitivity of the EIA-DB and the DIF method ( $p = 0.0001$ ).

TABLE 1 Comparison of EIA-DB, Direct Immunofluorescence (DIF), and Shell Vial Culture (SVC) for Detection of Influenza A Virus in 377 Nasopharyngeal Samples

	EIA-DB	DIF	SVC	No. (%) with given result
	+	+	+	35 (59.3)
	+	-	+	15 (25.4)
	-	-	+	9 (15.2)
	+	-	-	0
	-	+	-	0
	+	+	-	0
	-	+	+	0
	-	-	-	318
Total	50 (84.7)	35 (59.3)	59 (100)	377

The various studies carried out for the comparison of conventional culture with the SVC technique in the isolation of the IA virus have given widely varying sensitivity values. The first studies carried out by Espy et al. [1986] and Guenther and Linnemann [1988], gave values of 56 and 62%, whereas more recent studies provide values of 88% [Bartholoma and Forbes, 1989]. To achieve the maximum sensitivity in SVC certain requisites, such as the use of the MDCK cell line, the addition of trypsin (2 µg/mL) to the maintenance medium, and the use of highly specific monoclonal antibodies, must be complied with [Bartholoma and Forbes, 1989; Murphy and Webster, 1990].

In our study we have observed a sensitivity of 84.7% for the EIA-DB system compared with SVC, with 9 (15.2%) cases in which this system was negative and the culture positive (false negatives). The only existing comparative study evaluating this antigenic system compares it with conventional cell culture and obtains a sensitivity of 100% with 8.4% of false positives [Warner et al., 1991]. In our study we obtained no false positives and the majority of true positives were clear and without difficulties in reading or interpretation. When comparing the DIF technique with the SVC we obtained a sensitivity lower (59.3%) than that of the EIA-DB, which is, in our opinion, inadequate, so that this technique cannot be recommended for rapid diagnosis. In addition, in 15 cases (25.4%) the sample was positive in the EIA-DB method but negative in the DIF. In all cases the number of epithelial cells in the sample was very low, in spite of which the IA virus grew in cell culture.

In view of these results we consider it advisable to use a rapid antigen technique as a screening method when infection by the IA virus is suspected. Of the two methods evaluated, the EIA-DB displayed the greater sensitivity and negative predictive value. This, together with its rapidity (15 minutes) and the

objectivity of the results, suggests this as the method of choice. The DIF is a much more laborious and subjective technique that frequently presents with the problem of scarcity of epithelial cells in the sample. In view of the failure to detect any DIF positive results where the EIA-DB is negative it seems unnecessary to perform the fluorescence technique in negative EIA-DB samples.

In spite of the use of an antigen screening method, it is essential to carry out the inoculation of all

samples in cell cultures to obtain the maximum diagnostic yield. Nowadays it seems that we may use the SVC method in the isolation of the IA virus with highly satisfactory results and leave conventional culture or chicken embryos to the reference centers.

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