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Evaluation of a Direct Immunofluorescence Cytospin Assay for the Detection of Herpes Simplex Virus in Clinical Samples

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A comparison between a direct immunofluorescence assay (DFA) and the shell-vial culture (SVC) was conducted to evaluate their efficacies according to the quality and origin of the sample and the type of herpes simplex (HSV) responsible for the infection. The SVC detected all 58 HSV-infected samples, while the DFA detected only 49 (84.5%) positive samples. The DFA detected HSV type 1 in 22 of 89 samples (24.7%) and HSV type 2 in 27 of 96 samples (28.1%). Compared with the SVC, the DFA had a sensitivity of 75.8% for HSV type 1 and 93.1% for HSV type 2. The sensitivity of the DFA depends on the quality of the sample. Thus, while DFA is recommendable as a screening method, the SVC remains the method of choice for obtaining the maximum diagnostic yield from the sample.

Infections caused by herpes simplex virus (HSV) affect principally the genitourinary tract, the eyes, the mouth, and the skin. These infections are especially severe in the disseminated form in immunocompromised patients (1, 2).

The definitive diagnosis of infection by HSV requires isolation of the virus in conventional cell culture (tube culture) or in shell-vial culture (SVC) and positive identification of cytopathic characteristics; HSV type is determined by subsequent typing using type-specific monoclonal antibodies (1-4). The use of methods for the direct detection of HSV in samples reduces the time required to establish an etiologic diagnosis. The

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Table 1: Comparison between the number of adequate samples, the results of the direct immunofluorescence assay (DFA), and the results of shell-vial culture (SVC) in the detection of HSV type 1.

Origin of samples	Total no. of samples (n = 89)	No. (%) of adequate samples* (n = 64)	No. of positive samples	
			SVC (n = 29)	DFA (n = 22)
Orolabial	9	9 (100)	3	3
Nasal	8	3 (37.5)	3	1
Mouth	27	13 (48.1)	9	6
Conjunctival	9	6 (66.6)	3	2
Perianal	6	4 (66.6)	2	1
Skin	30	29 (96.6)	9	9

* Samples with > 25 epithelial cells were considered adequate.

rapid technique most frequently used in the direct detection of HSV antigens in cell smears is the direct immunofluorescence assay (DFA) (4–8). However, this method displays varying sensitivities (60–80%), depending on the type of sample and the quality of reagents used (5–11).

The use of the cytocentrifugation technique in the direct detection of other viral antigens in clinical samples (12) has permitted its application in the diagnosis of HSV infections (11). This prompted us to compare a DFA with cytopsin-prepared slides with the SVC, with respect to its efficacy according to the type of sample and to the type of HSV.

Materials and Methods. All clinical samples collected between January 1995 and December 1996 from patients with suspected HSV infection were studied. A total of 185 samples were included: 46 from skin lesions, 36 from penile lesions, 31 from the female genital tract, 27 from the mouth, 18 from anal or perianal lesions, nine from orolabial lesions, nine from conjunctival-corneal lesions, eight from nasal lesions, and one from a tracheal aspirate.

Samples were placed in 2 ml of viral transport medium and vortexed. For performance of the DFA, 400 µl (200 µl per slide) was cytocentrifuged (Cy-

topsin 3, Shandon Scientific, UK) on two slides at 700 rpm for 10 min. After air drying, the slides were fixed with acetone at –20°C for 10 min and then stained with fluorescein-labeled mouse monoclonal antibodies to HSV type 1 (HSV-1) and HSV type 2 (HSV-2) (Syva MicroTrack HSV1/HSV2, USA) following the manufacturer's instructions. The sample was considered adequate for testing by the DFA if the total number of epithelial cells present was >25 per slide. A sample was considered positive if at least two epithelial cells with specific fluorescence were detected. The results of the DFA were read without prior knowledge of the results obtained in the SVC and, in all cases, by a different person.

From the initial cell suspension 1 ml was inoculated into two shell-vials (0.5 ml per vial) of the Vero cell line (Vircell, Spain). The vials were then centrifuged at 700 x g for 45 min. They were allowed to rest at 36°C for 30 min and the supernatant was discarded. To each vial 1 ml of maintenance medium (MEM with 1% fetal bovine serum) was added. The vials were incubated at 36°C and subsequently stained with the same monoclonal antibodies used in the DFA at 24 h or, if the first vial was negative, at 48 h.

Results and Discussion. Of the 185 samples, 147 (79.4%) were considered adequate for testing by

Table 2: Comparison between the number of adequate samples, the results of the direct immunofluorescence assay (DFA), and the results of shell-vial culture (SVC) in the detection of HSV type 2.

Origin of samples	Total no. of samples (n = 96)	No. (%) of adequate samples* (n = 83)	No. of positive samples	
			SVC (n = 29)	DFA (n = 27)
Perianal	12	6 (50)	4	4
Skin	16	16 (100)	5	5
Pene	36	30 (83.3)	12	10
Female genital	31	30 (96.7)	7	7
Tracheal	1	1	1	1

* Samples with > 25 epithelial cells were considered adequate.

the DFA. The DFA detected the presence of HSV in 49 samples (26.4%), while the SVC detected HSV in 58 (31.3%). The DFA detected HSV-1 in 22 samples (22/89, 24.7%) and HSV-2 in 27 samples (27/96, 28.1%). All the HSV (100%) were isolated in the SVC, while the DFA detected only 84.5% ($p = 0.0001$). Four samples inadequate for testing by DFA (2 from the mouth, 1 from a perianal lesion, and 1 from a penile lesion) were positive by SVC. When comparing the DFA with the SVC according to the type of HSV isolated, we found a sensitivity of 75.8% for HSV-1 (Table 1) and 93.1% for HSV-2 (Table 2) ($p = 0.001$).

One of the main difficulties of the DFA occurs when the sample is of poor quality, that is, when the sample lacks a sufficient number of epithelial cells to permit the observation of the presence of viral antigens. The majority of authors consider that a sample is inadequate for testing by DFA when <20 epithelial cells per slide are present. In these cases a negative result does not exclude the possibility of an HSV infection (9, 11).

In our study, which included samples of different origins, 20.5% were considered inadequate. This percentage is somewhat higher than the 9.5% reported by Landry et al. (11), even though we also concentrated the samples by means of cytocentrifugation. The principal reason for this difference is that, in the study of Landry et al. (11), the authors centrifuged the specimens prior to preparing the slides and used a cut-off of <20 epithelial cells.

It seems clear that the quality of the sample depends on its origin, with genital and cutaneous samples providing the greatest quantity of epithelial cells (83–100%). In HSV infections the quality of the sample and the quantity of viral particles present depend on the type of infection (primary or recurrent), the site of infection, and the state of the infection site. Vesicular lesions (orolabial, cutaneous, and genital) provide greater diagnostic yield in the DFA (5–7). When the lesions proceed to ulcerative or necrotic lesions, the diagnostic yield of the DFA decreases significantly (1, 3, 10).

The different results obtained in infections by HSV-1 and HSV-2 can be explained by the DFA's dependence on the quality of the sample. The DFA detected 22 cases of HSV-1 infection, presenting a sensitivity of 75.8% when compared with SVC, and a negative predictive value of 89.5%. In these infections the majority of samples were ob-

tained from the mouth and cutaneous zones with nonvesicular lesions. On the other hand, the majority of samples studied for HSV-2 were of genital origin, male and female, and in this type of sample the quality has proved to be superior (5–7). As a result, the DFA detected 93.1% of the cases of HSV-2 infection confirmed by culture, presenting a negative predictive value of 97.1% and a statistically significant difference with respect to detection of HSV-1.

Unlike other investigators (10, 11), we found no sample that was positive in the DFA and negative in SVC. Thus, the diagnostic efficacy of the SVC was 100% and that of the DFA 84.5%, a difference which is statistically significant. This observation is not surprising because we compared 400 μ l of the sample in the DFA versus 1 ml of the sample in the SVC, so the results are biased in favor of the SVC. However, it is possible that the use of the same monoclonal antibodies for both the DFA and the SVC eliminates differences that may arise due to differences in the quality of these reagents (10, 11).

In view of our results, we believe that cell culture, especially the SVC method, is still the method of choice for establishing the etiologic diagnosis of infections by HSV. The sensitivity of the DFA is dependent not only on the origin of the sample or the HSV type causing the infection, but also on the quality of the specimen. If specimens with an adequate designation of 80% or better are used, the sensitivity of DFA for HSV-1 and HSV-2 is 100% and 92%, respectively (combined sensitivity, 95%). Moreover, the DFA can be performed in only 60–90 min. Thus, while DFA is recommendable as a rapid screening method, it cannot replace cell culture, the performance of which is necessary to confirm the detection by DFA and to have the isolate available for susceptibility and epidemiological studies.

Acknowledgement

This study was supported in part by the Spanish National Institute of Health (INSALUD, Baleares).

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