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Comparison of the Immulok Cultureset Kit and Virus Isolation for Detection of Herpes Simplex Virus in Clinical Specimens

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The Immulok test performed at 24 h after inoculation had a 73% sensitivity and 97.5% specificity for detecting herpes simplex virus in clinical specimens, as compared with viral culture in human embryonic lung fibroblasts. The test increased the proportion of positive specimens reportable at 24 h by 48% ($P < 0.01$) but failed to detect 4 of 31 (13%) specimens positive for cytopathic effect at 24 h and 13 of 32 (41%) specimens positive at ≥48 h after inoculation.

Rapid diagnosis of herpes simplex virus (HSV) infections has important implications for initiating antiviral therapy, counseling patients, and undertaking specific control measures, such as caesarean section women infected with herpes simplex virus at the time of delivery. Although virus isolation is regarded as the most sensitive test for detecting active HSV infections, its lack of rapidity and general availability have led to the development of various cytological and immunological methods for HSV diagnosis (2, 4, 5). Recently, a commercially available kit that combines virus culture in Vero cell monolayers with an indirect immunoperoxidase assay for HSV antigen detection (Immulok Cultureset; Immulok, Inc., Carpinteria, Calif.) has been promoted for use in clinical microbiology laboratories. In the present study, we compared this kit with a standard cell culture isolation system for accuracy, rapidity, and cost of detecting HSV in clinical specimens.

During a 3-month period from November 1982 to January 1983, 188 specimens submitted to the University of Virginia Clinical Virology Laboratory for HSV isolation were processed by standard isolation techniques in cell culture and by inoculation and identification in the Immulok Cultureset kit. Cotton-tipped swab specimens or vesicle aspirates were transported in 2.5-ml volumes of viral transport media (veal infusion broth containing 0.5% gelatin and 50 μg of vancomycin, 50 μg of gentamicin, and 1 μg of amphotericin B per ml, pH 7.2 to 7.4) and held at 4°C until inoculation. Specimens were vortexed for 5 to 10 s before removal of the swabs, and visibly cloudy specimens were clarified by centrifugation at 1,500 rpm for 10 min.

The standard method was to inoculate 0.2 ml of specimen onto two MRC-5 human embryonic lung fibroblast monolayers, each purchased in screw-capped tubes (16 by 125 mm) from one of two commercial sources (M. A. Bioproducts, Walkersville, Md.; Flow Laboratories, Inc., McLean, Va.). After adsorption for 60 min at 36°C in stationary racks, the inoculum was removed and the monolayers were washed once with Hanks balanced salt solution and fed with Eagle minimal essential medium containing Earles salt solution, 2% fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.), and 0.3 mg of glutamine, 50 μg of vancomycin, 50 μg of gentamicin, and 1 μg of amphotericin B per ml, pH 7.2 to 7.4. The monolayers were observed daily for 1 week for cytopathic effect (CPE) and considered positive when CPE typical for HSV was detected. On this day, a verbal report was telephoned to the requesting clinicians. Earlier work with 150 consecutive HSV-positive specimens found complete concordance between CPE positivity and the presence of HSV antigens detected by direct immunofluorescence staining (bivalent antiherpes fluorescein-conjugated rabbit antisera; M. A. Bioproducts).

The methods used for the Immulok Cultureset kits were those specified by the manufacturer. Tissue culture tubes containing Vero cell monolayers maintained in minimal essential medium with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and antibiotics were purchased weekly and held in horizontal racks at 36°C. A 0.6-ml volume of each specimen was inoculated into one culture tube, which was then incubated at 36°C for 24 h. Monolayers were fixed with 10% buffered Formalin (pH 6 to 7), washed with phosphate-buffered saline (pH 7.6), stored at 4°C for 1 to 4 days, and then stained for HSV antigens by the peroxidase-antiperoxidase technique, using the method and reagents provided by the manufac-

TABLE 1. Correlation between commercial immunoperoxidase test for herpes simplex virus performed at 24 h after inoculation of specimens and virus isolation

Immulok test	No. of specimens	HSV isolation	
		Positive	Negative
Positive HSV	49	46	3
Negative	113	10	103
Equivocal	20	7	13
Unsatisfactory	6	3	3

turer. Control slides containing HSV-infected Vero cells were included in each staining procedure. All slides were read under blind conditions by one of the investigators (F.G.H.) who was not involved in the processing of the clinical specimens. The degree of positivity was rated according to a visual scale, provided by the manufacturer, which ranged from equivocal to 3+ staining for HSV antigen. In contrast to the recommendation of the manufacturer that a minimum of three foci be considered significant, we considered the presence of one or more densely stained cells to indicate positivity (see below).

HSV was isolated from 66 of 188 (35%) specimens during the study (Table 1). In six instances (3% of specimens), the Immulok test result was considered unsatisfactory because the monolayers were lost in processing. Although extensive infection of the monolayer could have theoretically caused detachment of the cells, the lost monolayers were equally divided between culture-positive and -negative specimens. Of the remaining 63 culture-positive specimens, the Immulok test was positive for 46 (73% sensitivity [$P < 0.001$] versus culture; chi-square test), negative for 10, and gave equivocal results for 7 (Table 1). The predictive value of a positive Immulok test was high (94%). In contrast, the positive predictive value of an equivocal result was found to be low (35%), since equivocal staining results were observed in 7 of 63 (11%) culture-positive and 13 of 119 (11%) culture-negative specimens.

In the present study, the sensitivity of the Immulok test performed at 24 h was substantially lower than the 94% sensitivity stated by the manufacturer (package insert, Immulok Cultureset, December 1982) and lower than that of other systems combining cell culture with immunological means of detection (3). An analysis by type of specimen found that the test had its lowest sensitivity (56%) in detecting HSV in female genital tract specimens (Table 2). This may relate to the wide range of viral titers in genital herpes specimens (3). Although the manufacturer recommended that a minimum of three foci of staining be considered significant, 9 of 46 (20%) Immulok-positive, culture-positive specimens had only one to three densely stained cells in the monolayer.

TABLE 2. Comparison of virus isolation and Immulok test results according to specimen type

Type of specimen	No. tested	No. (%) culture positive	No. (%) Immulok positive
Genital (female)	86[a]	18 (21)[a]	10 (12)[a]
Genital (male)	19	9 (45)	7 (37)
Orofacial	35	15 (43)	13 (37)
Vesicle, site unspecified	11	7 (64)	6 (55)
Ulcer, site unspecified	5	5 (100)	5 (100)
Other	23	9 (39)	5 (22)

[a] These figures exclude three Immulok positive, culture negative specimens. $0.05 < P < 0.1$, culture versus Immulok, chi-square test.

Of 119 culture-negative specimens, 103 had negative and 13 had equivocal Immulok test results (97.5% specificity). The predictive value of a negative test was 91% a value which decreased to 85% when both negative and equivocal test results were combined. The 2.5% false-positive rate was due to three specimens judged to show 2+ specific staining of multiple cells by two independent observers.

Of 63 culture-positive specimens for which Immulok tests were performed. 31 (49%) were positive for CPE at 24 h after inoculation, compared with 46 (73%) positive by the Immulok test ($P < 0.01$; chi-square test). However, 4 of 31 (13%) specimens positive for CPE at 24 h gave negative or equivocal Immulok results (Table 3). The sensitivity of the Immulok test decreased significantly as the time to CPE development increased (Table 3), presumably because of lower virus concentrations in the specimens. Only 2 of 8 (25%) culture-positive specimens which developed CPE at ≥3 days after inoculation were Immulok positive at 24 h, as compared with 27 of 31 (87%) specimens positive for CPE at 24 h ($P = 0.003$; Fisher exact test). The use of

TABLE 3. Relationship between time to HSV isolation detected by CPE in human diploid fibroblast monolayers and Immulok test results

Immulok test result	No. of specimens positive for CPE on post-inoculation day			
	1	2	3	≥4
Positive	27[a]	17	2	0
Negative or equivocal	4[a]	7	2	4
% Sensitivity	87	71	50	0

[a] $P < 0.001$, chi-square test; distribution of positive versus negative or equivocal Immulok test results on days 1 through 3 or greater. Total percent sensitivity, 73%.

larger inocula or longer culture periods before fixation and staining might have increased the sensitivity of the Immulok test, but these variables were not studied.

The direct costs per specimen for reagents and equipment to our laboratory were $11.44 and $4.40 for the Immulok test and the isolation method, respectively. The corresponding personnel costs, based on work units (6), were estimated to be $14.52 and $11.37, respectively. In summary, although the Immulok test increased the number of positive specimens reportable at 24 h by 48% compared with virus isolation, the test was significantly less sensitive than isolation and was more costly. The Immulok test performed at 24 h after specimen inoculation was not an adequate substitute for standard virus isolation. Its usefulness as an adjunct to isolation will need to be determined by comparative testing against other immunoassays (1, 3) that appear to offer greater sensitivity.

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