JOURNAL OF CLINICAL MICROBIOLOGY, June 1994, p. 1610–1613
0095-1137/94/$04.00+0

Vol. 32, No. 6

Comparison of Techniques and Evaluation of Three Commercial Monoclonal Antibodies for Laboratory Diagnosis of Varicella-Zoster Virus in Mucocutaneous Specimens

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Received 22 November 1993/Returned for modification 18 January 1994/Accepted 22 March 1994

A comparison of direct antigen detection in cell scrapings with culture techniques (tube culture and shell vial method) for diagnosis of varicella-zoster virus (VZV) mucocutaneous infections was done in parallel in two groups of specimens. A total of 100 specimens were from patients with clinical diagnosis of VZV infection (group 1), and 69 were from patients with no suspicion of VZV infection (group 2) but mainly with herpes simplex virus infections. In addition, three commercially available monoclonal antibodies (Whittaker, Biosoft Clone 2013, and Ortho 3B3) directed against VZV antigens were evaluated in parallel in the last 87 group 1 specimens. Overall, 80% of the group 1 specimens were confirmed positive by direct detection, in comparison with 56% positive by tube culture and/or shell vial. None of the group 2 specimens were positive for VZV by any of the methods, and none of the monoclonal antibodies assayed reacted with any herpes simplex virus stock strains. Antiviral therapy and the length of evolution time of lesions affected negatively the performance of all laboratory methods, but to a lesser extent in direct detection techniques than in culture techniques. The Whittaker and Biosoft reagents (indirect immunofluorescence assay) showed statistically significant differences in sensitivity with respect to the Ortho antibody ($P = 0.002$ and $P = 0.039$, respectively; two-tailed binomial test). Direct antigen detection is a rapid, easy-to-perform, sensitive, and specific technique and appears to be the method of choice for laboratory confirmation of VZV mucocutaneous infections.

Varicella-zoster virus (VZV) causes significant morbidity and even mortality in the immunocompromised host (1–3, 5). Diagnosis of mucocutaneous VZV infections in immunocompetent patients is normally based on clinical grounds because of its typical presentation and benign clinical course. However, in immunosuppressed patients, such as those infected with human immunodeficiency virus and transplant recipients, differential diagnosis between herpes simplex virus (HSV) and VZV infections should be made for several reasons. First, the clinical presentations may be indistinguishable. Second, in human immunodeficiency virus-infected patients, chronic mucocutaneous HSV infection is a criterion for AIDS. Third, VZV and HSV show different susceptibilities to antiviral agents (namely, acyclovir), so different dosages can be used for treating infections caused by these viruses. With the advent of effective and safe antiviral therapy against VZV, there is a need for early, rapid, and accurate laboratory diagnostic tests to start specific treatment in this group of patients (1, 2).

Conventional cell culture has been considered the method of reference for laboratory diagnosis of VZV infections, but it lacks sensitivity, gives delayed results, and needs cell culture facilities (4, 10, 12, 14). Several alternative methods have been proposed. The centrifugation-culture variant (shell vial [SV] technique) is more rapid (48 h instead of 5 to 7 days), but in our experience, it has similar sensitivity (9). The Tzanck test and electron microscopy are very rapid methods, but they do not differentiate between HSV and VZV, a crucial factor for this group of patients.

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Studies performed with direct detection (DD) of viral antigens in cell scrapings by using polyclonal and monoclonal antibodies (MAbs) have given good results in terms of sensitivity and specificity, so DD has been proposed as the method of choice for diagnosis of VZV mucocutaneous infections (4, 12). However, the effect of the antiviral agents and the length of the evolution time of disease on the performance of the technique have not been evaluated. The aim of this study is to compare prospectively and in parallel the direct antigen detection method using three different commercially available MAbs with culture techniques and to determine the efficacy of the methods when antiviral therapy has been started or after different evolution times of the lesions.

Specimens. A total of 169 cell scrapings from cutaneous lesions were processed. In most instances, sampling was performed by laboratory personnel or by a well-trained physician by absorbing the vesicle fluid followed by vigorous swabbing of the lesion base. All were sent to the laboratory in 2 ml of viral transport medium (Celltechs; Difco, Detroit, Mich.) and were processed within 3 h of sampling, except for 19 specimens referred to us from other hospitals, which were processed within 24 h of receiving them. The specimens were divided into two groups: 100 were from 51 patients with definitive clinical VZV infection (group 1), whereas the remaining 69 were included in group 2 (no suspected VZV infection; 41 of them from patients with clinical diagnosis consistent with HSV infections). The definitive clinical diagnosis was always made by a dermatologist or an infectious disease physician. Of 100 group 1 specimens, 58 were from patients treated with specific antiviral therapy (acyclovir or foscarnet). Of the 51 group 1 patients (40 zoster and 11 varicella patients), 33 were normal hosts, 11 were human immunodeficiency virus-infected patients, 5 suffered from hematological malignancies, and 2 were

renal transplant recipients. To analyze the effects of the evolution time of lesions and antiviral therapy, sequential sampling was done in 17 group 1 patients when feasible. A brief protocol with clinical and laboratory variables was filled in for each specimen and entered in a computerized data base.

Conventional tube culture (CC). Culture tubes containing MRC-5 fibroblasts (Biomérieux, Marcy l'Etoile, France) were inoculated with 300 μl of the specimen, incubated at 37°C for 15 days, and read on 5 days/week for typical cytopathic effect on cultures. Positive tubes were confirmed by immunofluorescent staining using MAbs directed against VZV.

SV. An aliquot (200 μl) of the specimen was inoculated into shell vials with a round coverslip containing a MRC-5 monolayer and processed as previously described for other viruses (7). Briefly, vials were centrifuged at 700 × *g* for 45 min and adsorbed at 37°C for 1 h, the medium was replaced, and then the vials were incubated for 48 h at 37°C. The coverslips were fixed in methanol (10 min) and stained by an indirect immunofluorescence assay (IFA) using a MAb against VZV (Clone 2013; Biosoft, Paris, France). Coverslips were screened with an epifluorescence microscope at a magnification of ×160, and results were confirmed at a magnification of ×400. An image showing nuclear fluorescence that spread to the cell membrane and to contiguous cells was observed in the case of a positive result.

DD. An aliquot (1.5 ml) of the specimen was centrifuged in an Eppendorf-type conical tube at 5,000 × *g* for 10 min. The supernatant was discarded, and the sediment was resuspended in the remaining liquid. One drop (ca. 30 μl) of the suspension was placed onto each of three wells of microscopic slides and left to dry. The slides were fixed in cold acetone (−20°C) for 10 min, dried, and finally stained.

Three different commercially available MAbs were used. Two of them reacted with a VZV membrane antigen, one in an IFA (Whittaker Murine anti-VZV; Whittaker, Walkersville, Md.) and the other in a direct fluorescence assay (DFA) (Ortho VZV 3B3; Ortho, Raritan, N.J.). The third MAb (Clone 2013) was directed against a nuclear antigen and used in an IFA. In group 1 specimens, only the last 87 were studied with the Ortho reagent.

Slides were observed in an epifluorescence microscope at a magnification of ×160, and results were confirmed at ×400, searching for cells showing membrane fluorescence (Whittaker and Ortho) or nuclear fluorescence (Biosoft). The specimen was considered positive when at least three typical fluorescent cells were observed in each preparation.

Cross-reactivity of MAbs. A total of 30 cell cultures (MRC-5 fibroblasts) infected with clinical HSV strains (18 with HSV type 1 [HSV-1]; 12 with HSV-2) were stained with Whittaker and Biosoft reagents. Thirty-one different HSV cultures (23 of HSV-1; 8 of HSV-2) were tested with the Ortho MAb.

Statistical analysis. We used a two-tailed binomial test for double comparisons of MAbs. The chi-square test for trends was performed to analyze the effect of the treatment. A *P* value of <0.05 was accepted as statistically significant.

Table 1 shows the results obtained with group 1 specimens. Overall, 80% of them were positive by DD, in comparison with the lower positivity rate (56%) of CC and/or SV techniques. Differences between culture methods and direct staining were especially notable in specimens obtained during antiviral therapy. A total of 33 positive specimens were diagnosed only by DD, in comparison with 1 positive specimen diagnosed by culture methods alone. When focusing on patients, 38 of 51 VZV-infected patients were diagnosed by culture techniques, in contrast with 45 such patients diagnosed by at least one of the DD methods. In one patient, a VZV-positive diagnosis was only made by culture; conversely, in eight of them only DD was positive.

TABLE 1. Comparison of direct antigen detection methods with CC and SV in specimens from group 1 patients (clinical diagnosis of VZV infections)

Commercial antibody (technique of DD) and result	No. of all specimens studied (n = 100[a])		No. of specimens (n = 58[b]) from treated patients	
	Total	Positive by CC and/or SV	Total	Positive by CC and/or SV
Whittaker (IFA)				
Positive	86	55	49	23
Negative	14	2	9	1
Biosoft (IFA)				
Positive	82	55	46	24
Negative	18	2	12	0
Ortho (DFA)				
Positive	64	48	35	22
Negative	23	1	16	0

[a] Only the last 87 specimens were studied with the Ortho reagent.
[b] Only the last 51 specimens were studied with the Ortho reagent.

None of the 69 group 2 specimens (no suspicion of VZV infection) were positive by SV or DD with any of the MAbs tested, including the 42 samples in which HSV was isolated in cell culture (23 with HSV-1; 19 with HSV-2), thus showing a complete specificity of these methods. Moreover, none of the cell cultures infected with HSV stock strains reacted with any of the commercial MAbs.

With respect to the efficiency of the different MAbs tested in our study, the IFAs showed better results than did the DFA, as reflected in Table 2. The Whittaker reagent led us to the confirmation of the highest number of diagnoses. Differences were statistically significant (binomial test) both between Whittaker and Ortho reagents (P = 0.002) and between Biosoft and Ortho MAbs (P = 0.039) but not when Whittaker and Biosoft products were compared (P = 0.125).

Table 3 shows the effect of the evolution of lesions on the laboratory techniques in the group of patients without antiviral treatment. It is noteworthy that results for nearly all specimens obtained within the first 5 days of disease were confirmed by DD methods. Finally, Table 4 reflects the analysis of data from specimens from treated patients, showing statistically significant differences (P < 0.0001; chi-square test for trends) between culture methods and direct staining. More than 85% of specimens taken after 1 to 3 days of treatment were

TABLE 2. Comparison of three commercial MAbs for DD of VZV in the 87 specimens studied in parallel

Reagent(s) giving positive results	Positive specimens	
	No.	%
Whittaker	76	87.4
Biosoft	71	81.6
Ortho	64	73.6
All three	63	72.4
Only Whittaker	6	6.9
Only Biosoft	1	1.1
Only Ortho	1	1.1
Whittaker and Biosoft	7	8.1
Whittaker and Ortho	0	0
Biosoft and Ortho	0	0

TABLE 3. Effect of the days of evolution of lesions on positivity of laboratory tests in 42 specimens from patients without antiviral treatment

Method(s)	No. of positive specimens/no. tested (% positive) with evolution of:	
	<5 days	≥5 days
CC and/or SV	23/26 (88.5)	10/16 (62.5)
DD		
Whittaker IFA	25/26 (96.2)	12/16 (75.0)
Biosoft IFA	26/26 (100.0)	10/16 (62.5)
Ortho DFA	23/24 (95.8)	6/12 (50.0)

confirmed by either the Whittaker or Biosoft MAb, in contrast with the lowest positivity obtained with CC or SV.

VZV can cause severe and life-threatening disease in immunocompromised patients. A rapid and accurate laboratory method could facilitate early diagnosis and, when necessary, the earlier administration of a specific therapy, which is an important factor in the efficiency of the antiviral treatment (1–3).

Direct antigen detection in clinical specimens accomplishes many of the characteristics desirable for these purposes. It is rapid (turnaround time: 1 to 2 h) and easy to perform, making this method readily available to most laboratories without sophisticated instruments or technologies. In addition, DD has shown excellent sensitivity. Calculations from Table 1 give sensitivities ranging from 73.6 to 86.0% for DD methods, which are superior to those obtained for CC and SV together (56.0%). When clinical diagnosis was used as the standard, reported sensitivities from the literature ranged from 52.9 to 100% for DD techniques, from 5.3 to 61.5% for CC, and from 25.2 to 65.2% for SV (4, 6, 8–15). It is obvious from these data that the stringency of clinical diagnosis greatly affected the performance of the different techniques. Because of the difficulties inherent in the nature of our specimens (about 60% from treated patients; 37% with a time of evolution of ≥5 days), our results confirm the excellent sensitivity of antigen detection. The high proportion of either specimen or patient results confirmed by DD techniques also confirm the wide reactivity of the three MAbs.

As expected, the length of the evolution time of lesions determined the efficiency of all laboratory techniques. However, from the point of view of an early and effective antiviral therapy, it should be stressed that the most relevant laboratory confirmations should be performed during the first days of evolution. Nearly all specimens from patients with less than 5 days of evolution after onset were confirmed by all three DD techniques. The only pitfalls of DD methods in this subset of specimens occurred in one low-quality sample with poor cell content (fewer than 10 cells per well [data not shown]). It is true that culture techniques also confirmed a high percentage of cases, but only after 2 days and 4 to 15 days (mean, 7.1 days) for SV and CC, respectively. In the only report from the literature we know of analyzing this aspect, Schmidt et al. (12) were not able to culture VZV in specimens collected more than 5 days after onset. These differences could be explained, in part, by the relatively high number of patients suffering from chronic VZV infections (mainly patients with AIDS) included in our study, as well as by the optimal sampling and culture procedures.

TABLE 4. Effect of antiviral treatment on positivity of laboratory tests excluding specimens from patients with chronic zoster

Method(s)	No. of specimens	No. of positive specimens/no. tested on treatment day:					
		1	2	3	4	5	6
CC and/or SV	46	7/9	5/14	1/12	2/5	0/5	0/1
DD							
Whittaker IFA	46	9/9	13/14	9/12	5/5	1/5	0/1
Biosoft IFA	46	9/9	13/14	8/12	4/5	1/5	1/1
Ortho DFA	40	7/7	8/12	6/10	4/5	1/5	0/1

An additional advantage of DD techniques is the possibility of confirming diagnoses of patients treated before specimen collection. With the advent of a safe and effective therapy, it is conceivable that this situation would occur more and more, mainly in some referral laboratories. Data from Table 4 show that results for up to about 90% of specimens taken within 1 to 3 days of starting therapy can be confirmed by some DD methods. As expected, antiviral therapy strongly affects both culture techniques (Tables 1 and 4).

Although the overall efficiency of the three MAbs assayed in our study was excellent, we were able to find several qualitative and quantitative differences between them. First, IFA techniques were statistically superior to DFA in terms of sensitivity. Second, the intensity of the fluorescence (data not shown) was higher with the two MAbs for which an indirect method was used. The best results were obtained with the Whittaker reagent and showed significant differences with respect to the Ortho MAb results. Finally, we subjectively prefer MAbs directed against membrane antigens because of the easier recognition of the positive fluorescent cells in comparison with nuclear staining. This could be of importance in critical samples with low cell content in which the fluorescent nucleus could be confused with artifacts. However, it is important to point out that the Biosoft reagent (nuclear fluorescence) did not give any false-positive results in our study.

All DD techniques proved to be 100% specific when clinical diagnosis was used as the "gold standard." None of the group 2 specimens were positive either by DD or by SV, despite the high proportion of culture-confirmed HSV specimens included in this subset. Other authors, as well as our previous experience in evaluating some of these MAbs, also confirmed specificity (6, 9–11, 14).

An important point to ensure maximal efficiency of the DD method is the need to obtain a good-quality specimen. Although we did not exclude from the analysis of results those specimens with a low cell content, it is important to remark that all failures in confirming diagnosis in specimens taken from patients without treatment and with few days of evolution were obtained in specimens with these characteristics. On the other hand, one might expect that the performance of the DD assay would be lowered in normal practice, when sampling conditions were less stringent. Consequently, we now tentatively recommend rejecting as inadequate those preparations with fewer than 20 cells per well.

In conclusion, DD using MAbs is a rapid, easy-to-perform, sensitive, and specific method to diagnose VZV infections, allowing specific antiviral treatment to start earlier. Accordingly, DD appears to be the method of choice for laboratory confirmation of VZV. Proper sampling and quality control of the specimen submitted to the laboratory are strongly recommended for optimum performance.

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