Evaluation of Five Monoclonal Antibody-Based Kits or Reagents for the Identification and Culture Confirmation of Herpes Simplex Virus

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Received 28 June 1990/Accepted 7 December 1990

Five immunofluorescence (IF) kits or reagents (Bartels [Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.], Imagen [CellTech Diagnostics, Ltd., distributed by Analytab Products, Plainview, N.Y.], Ortho [Ortho Diagnostics Systems, Inc., Raritan, N.J.], Syva [Syva Co., Palo Alto, Calif.], Whittaker [Whittaker Bioproduts, Walkersville, Md.]) were evaluated for typing and laboratory confirmation of herpes simplex virus (HSV). Of 101 clinical isolates tested by each kit or reagent, results for 97 of them were in agreement. Identification of the four isolates with discordant results was performed by restriction endonuclease analysis of the viral DNA. The sensitivity and specificity of the Imagen and Bartels kits were 100%. For the Ortho, Syva, and Whittaker kits or reagents, the HSV type 1 (HSV-1) and HSV type 2 (HSV-2) sensitivities were 96.9% and 100%, 100% and 100%, and 97.4 and 100%, respectively, and the specificities were 100 and 97.4%, 100 and 92.4%, and 100 and 97.4%, respectively. There was one false-positive HSV-2 isolate identified by each of the Ortho and Whittaker kits or reagents. Three false-positive HSV-2 isolates occurred by staining with Syva, giving the erroneous indication of dual isolates. Several isolates stained with Imagen and Whittaker reagents displayed dull IF patterns. A dull green background occurred in some type-2 isolates tested with the Ortho kit. The intensities of IF staining by the Bartels and Syva kits were satisfactory; however, the latter displayed a specificity of 92.7%. A total of 38 and 63 specimens were finally designated as HSV-1 and HSV-2, respectively. Identification of each isolate with the Bartels kit was consistently interpretable and is recommended as the typing and confirmatory assay of choice.

The typing of herpes simplex virus (HSV) is important in (i) patient counseling (for prognostic purposes), (ii) epidemiological studies, (iii) the measurement of vaccine or drug efficacy, and (iv) educational settings (3-5, 11, 13, 16). Additionally, the utilization of HSV typing reagents may serve as a confirmatory culture assay and assist in the identification of viral antigen in the spin-amplified shell vial assay system (8).

The widespread impetus by most laboratory technicians and physicians to proceed with the typing of HSV isolates has resulted in the development of numerous commercial typing kits and reagents. One of the most commonly used typing system consists of fluorescein (e.g., fluorescein isothiocyanate [FITC])-labeled type-specific or type-common monoclonal antibodies. A need exists to periodically evaluate the newer kits and reagents and to compare these with earlier related products. Accordingly, such an undertaking was the purpose of this study.

MATERIALS AND METHODS

Specimens. HSV isolates were amassed during the latter part of 1989 and early part of 1990 from among thousands of genital (vulva, vagina, cervix, labia, penis, scrotum) and nongenital (face, throat, nasopharynx, eye, tongue, sputum, bronchoalveolar lavage, rectum, buttock, torso lesions, perianal area) specimens submitted to the diagnostic virology laboratory. Positive HSV isolates were frozen at -78°C until reisolation and parallel testing by each of the antigen detection kits or reagents in this study. Collection and transport of specimens to the laboratory have been described previously (12).

Virus isolation. All specimens received for testing were inoculated into human lung carcinoma (A549), primary rhesus monkey kidney, and rabbit kidney cell tube cultures. Toxic cultures or cultures displaying a questionable cytopathic effect were filtered and/or passed into human neonatal kidney and human foreskin fibroblast (MRC-5) cell cultures. The cell cultures were obtained from Whittaker Bioproduts (Walkersville, Md.) and ViroMed Laboratories, Inc. (Minneapolis, Minn.).

Detection of HSV antigen in infected cell cultures. Infected A549 or rabbit kidney cell cultures displaying a +2 to a +3 cytopathic effect (scale of +1 through +4) were agitated well on a vortex mixer and subjected to removal by using a cell scraper. The cells were washed in phosphate-buffered saline supplemented with 2% fetal bovine serum, placed on glass slides, air dried, and then fixed in cold acetone (methanol required for the Ortho [ORT] HSV typing kit). Infected cells were prepared in quintuplicate for parallel staining with the ORT HSV 1 & 2 dichromatic typing reagent (lot no. 89030; Ortho Diagnostics Systems, Inc., Raritan, N.J.), the Bartels HSV fluorescent monoclonal antibody test for the identification and differentiation of HSV type 1 (HSV-1) and HSV type 2 (HSV-2) in inoculated cell cultures (BAR; lot no. 1026; Bartels Immunodiagnostic Supplies, Inc., Bellevue, Wash.), the Syva MicroTrak HSV-1/HSV-2 culture confirmation and typing test (lot no. 2H219; Syva Co., Palo Alto, Calif.), the Imagen HSV typing test (lot no. C880420021A; CellTech Diagnostics, Ltd., distributed by Analytab Products, Plainview, N.Y.), and the FITC murine monoclonal bivalent immunoglobulin G (common) and FITC murine...
TABLE 1. Identification of discordant HSV isolates by RE analysis of viral DNA

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Fluorescence</th>
<th>Endonuclease analysis</th>
<th>Specimen site (age [y] [sex])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imagen</td>
<td>BAR</td>
<td>ORT</td>
</tr>
<tr>
<td>801-2</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>1396</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2193-2</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3839</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

* F, Female; M, male.
* False positive.
* False negative.

The monoclonal type 1 antibody (WHT; lot nos. 8V0230 and 8V0233; Whittaker Bioproducts, Walkersville, Md.) was used in this study. The BAR, Syva, and Imagen kits consisted of individual type-specific monoclonal antibodies to which FITC was directly conjugated. The same FITC indicator system exists with the WHT type 1 and common antibodies. The ORT kit consisted of type 1- and type 2-specific monoclonal antibodies in a single reagent that were tagged with phycoerythrin and FITC, respectively. The excitation and emission of phycoerythrin were compatible with those of a conventional fluorescence microscope equipped for FITC-conjugated antibodies. Such a dual-component system precluded the use of separate reagents, because under the appropriate excitation, a yellow-orange (viz., phycoerythrin) or an apple-green (viz., FITC) signal is produced. Staining according to the specifications of the manufacturers. Fluorescence was detected with an epifluorescence microscope (Nikon) equipped with a tungsten-halogen Koehler illuminator and an immunofluorescence 460- to 490-nm excitation filter.

The Syva reagents consisted of a single HSV-1 type-specific monoclonal antibody produced against the glycoprotein C complex (molecular weight [MW], 80,000 to 120,000). The HSV-2 type-specific reagent consisted of two monoclonal antibodies. The first monoclonal antibody was reactive against the HSV-2-specific induced protein ribonucleotide reductase (MW, 140,000). The other monoclonal antibody recognized an HSV-2-specific epitope of a glycoprotein B doublet (MW, 120,000 to 130,000) (18). The WHT HSV bivalent reagent was reactive against three proteins in the 75,000- to 98,000-MW range for HSV-1 and seven proteins in the 44,000- to 110,000-MW range for HSV-2. The clone of the hybridoma was derived from a fusion of SP-2/0 mouse myeloma cells and splenocytes from BALB/c mice which were immunized with the MS strain of HSV antigen. The clone of the hybridoma (HSV-1 antibody) was derived from a fusion of SP-2/20 AG 14 mouse myeloma cells and BALB/c mouse splenocytes immunized with the MacIntire strain of HSV-1 antigen (19). A single monoclonal antibody was present in each reagent supplied by ORT (15). A cocktail of two or more monoclonal antibodies made up each of the Imagen and Bartels HSV-1 and HSV-2 typing reagents (2, 2a).

In order to identify whether any of the kits or reagents tested in this study cross-reacted with other viruses routinely isolated in the clinical setting, the following experiment was performed. Slides containing cells infected with adenovirus types 2 and 7, echovirus type 9, poliovirus type 2, coxsackievirus type B2, respiratory syncytial virus, and cytomegalovirus (the viruses were obtained from the Nassau County Medical Center Virology Laboratory culture collection) were prepared as described above. All slides were stained in parallel with each kit or reagent used in this study.

Confirmatory HSV identification using RE analysis of the viral DNA. Specimens with discordant results were subpassed two times and again tested by each of the typing kits or reagents. Those specimens whose staining reactions remained discrepant were identified by restriction endonuclease (RE) analysis of the HSV DNA. Briefly, the specimens in question were grown in A549 cells from frozen original isolates. Reference strains HSV-1 (strain F) and HSV-2 (strain G), which were obtained from the American Type Culture Collection (Rockville, Md.), were grown in African green monkey kidney cells (CV-1). The isolation, purification, and analysis of HSV DNA have been described previously (12). After treatment of DNA with HindIII (Bethesda Research Laboratories, Gaithersburg, Md.), DNA fragments were separated by overnight electrophoresis in 0.5% agarose gels and stained with ethidium bromide. An EcoRI enzyme digest of bacteriophage lambda (Sigma Chemical Co., St. Louis, Mo.) was used as a marker of molecular weights. In an effort to determine the ability of the DNA assay to detect the presence of a dual herpesvirus infection, the following experiment was performed. Equal titers of HSV-1 (strain F) and HSV-2 (strain G) (measured by plaque assay) were co-cultured in CV-1 cells. Additionally, the intensities and numbers of immunofluorescent cells in individually infected cultures were determined and found to be approximately equal prior to mixing. The plaque and immunofluorescence staining assays were performed by routine procedures (12, 17). Analysis of the HSV DNA in coculture and individually infected cultures was performed as described above.

Test parameters. Test parameters were performed by using those calculations described previously (14).

RESULTS

Among the 101 HSV-positive specimens tested, 38 were identified as HSV-1 and 63 were identified as HSV-2. Four discordant specimens were revealed that were confirmed to be HSV-1 by RE analysis of the viral DNA. Comparison of the DNA digests with controls coinfected with HSV-1 and HSV-2 failed to suggest the existence of any dual infections. Testing of infected cells by using the Syva type-specific monoclonal antibodies resulted in three false-positive type 2 signals, giving the erroneous indication of dual (i.e., HSV-1 and HSV-2) infections. The ORT and WHT reagents each incorrectly typed two different HSV isolates (from the vulva and eye, respectively). The Imagen and BAR HSV type-specific monoclonal antibodies properly identified all herpesvirus isolates in the study (Table 1). The sensitivities of the ORT, Syva, and WHT kits or reagents for HSV-1 and HSV-2
were 97.4 and 100%, 100 and 100%, and 97.4 and 100%, respectively; and the specificities were 100 and 97.4%, 100 and 92.7%, and 100 and 97.4%, respectively.

The individual BAR and Syva type-specific monoclonal antibodies consistently produced a clear +4 (on a scale of +1 through +4) apple green fluorescence signal. The staining of individual cells was apparent, with virtually no obfuscating green or dull green background in the positive or negative wells. The intensities of the Imagen and WHT monoclonal antibodies did not, in general, approach that of the fluorescein observed with the BAR and Syva products. Background staining was not a problem with most specimens tested with Imagen or WHT kits. However, several positive specimens tested with these reagents produced a dull green signal, resulting in an interpretive effort. Identification of HSV-1 isolates by the ORT dichromatic reagent was not problematic. However, dull green cytoplasmic cellular staining persisted in approximately one-third of the HSV-2 isolates tested. Retesting of these poorly staining isolates yielded no change in the intensity of the signal. No clearly discernible differences in staining intensities were observed following the retesting of the HSV-2-infected cells in question, regardless of whether the cells were fixed in methanol or acetone. The inability of the FITC-conjugated antibody in the ORT reagent to affect the characteristic apple green immunofluorescence signal made our interpretation (and identification) of the HSV-2 isolates difficult.

None of the kits or reagents cross-reacted with cells infected with viruses other than HSV.

DISCUSSION

The decision to use one and not another of the monoclonal antibody-based typing kits or reagents evaluated in this study may be properly addressed after several important criteria are considered. Such factors would include, at the very minimum, the sensitivity and the specificity of the assay, the intensity of the signal, and to a lesser extent, whether the reagents in question are supplied as type specific (individual typing reagents) or type common (combined reagents prepared against HSV-1 and HSV-2). In the current study, all of the kits tested except one were composed of individual type-specific monoclonal antibodies. The reagents available from Whittaker Bioproducts, however, consisted of a type-common and an HSV-1 type-specific monoclonal antibody. The overt limitation with the incorporation of a type-common reagent into a typing assay would be the inability to discern a dual, albeit rare, isolate (6). A similar situation was reported in the Virgo (ELECTRO NUCLEOINS, Inc.) HSV typing kit (12). Although the Virgo kit was shown to be a sensitive and specific assay, the type-common antibody and the additional use of a primary antibody and an FITC-conjugated secondary antibody probably had a negative effect on the system’s incorporation into the clinical laboratory setting. Direct fluorescein-tagged primary monoclonal antibodies are looked upon favorably by many laboratory technicians, because such systems minimize the number of manipulative steps.

The Syva typing kit resulted in three false-positive HSV-2 signals, giving the erroneous indication of dual HSV-1 and HSV-2 infections. Previous studies in our laboratory with the Syva kit incorrectly identified 2 of 101 positive specimens as dual infections (12). In reports of recent studies, Gleaves and coworkers (9, 10) reported a total of three dual isolates obtained with the Syva kit. However, the Seattle researchers did not confirm their dual isolates obtained from 57 (10) and 75 (9) positive specimens, nor did they corroborate the identification of the dual isolates by testing with additional individual type-specific monoclonal antibodies. In a related study, an absence of dual isolates among 81 positive specimens tested was noted (1). One might conclude that this absence was real, or perhaps a subjective interpretation of some fluorescent signals among the positive specimens tested might have occurred. Upon retesting of our dual isolates with the Syva kit, we observed the same results, in which there was a difference in the number of stained cells and, to a lesser extent, in the intensity of signal between each of the two HSV-infected cell preparations. The more intensely stained and the larger number of stained cells indeed proved to be the correct type. However, nonspecific staining of cells with the heterotypic monoclonal antibody was of sufficient intensity and number (ca. 15 cells per field) to allow a positive result to be reported. Confirmatory testing of discordant specimens by RE analysis of the viral DNA indicated the presence of only serotype 1 HSV. The detection of mixed DNA patterns following coinfection of strains F and G in approximately the same ratio seen by immunofluorescence confirmed the sensitivity of the assay system. In dual infections, however, it is theoretically possible for a strain of lower titer to be selected against during culture amplification. As a result, analysis of the nucleic acid might reveal only the predominant strain. Such a scenario was unlikely in this study, because repeated culture amplifications of the specimens in question failed to efface or change the immunofluorescence intensities of the HSV-2 false-positive signals. The latter data strongly suggest that if HSV-2 had actually been present in the specimens with discordant results, type 2 banding patterns would have been identified. Furthermore, RE analyses were performed on specimens with discordant results which were passed only two to three times. The absence of bands characteristic of HSV-2, as well as the reported rarity of dual infections (6), lends credibility to our conclusions.

The BAR kit consistently produced a clear apple green fluorescence signal. Background staining did not occur with either of the individual type-specific reagents, resulting in the distinct visual separation between HSV types. The Syva kit approached the level of fluorescence intensity and absence of background staining as those described with the BAR kit. However, the inaccurate appearance of several dual isolates in this and an earlier study (12) denotes some limitation in the kit’s specificity.

Several specimens tested with the Imagen and WHT kits produced insufficient fluorescence signals (i.e., dull green stain approaching apple green) such that repeated testing was warranted. Repeated testing did not resolve the difficulty of interpreting the results for the few specimens in question.

The ORT dichromatic typing reagent is unique among other monoclonal antibody-based culture confirmation and identification kits, in that the test has the theoretical ability to differentiate HSV-1 from HSV-2 within a single cell (slide) population. Among the 101 positive specimens tested by ORT, however, ca. 33% of the HSV-2 culture confirmation slide preparations displayed a relatively dull green signal. Additionally, the signal intensity from nonspecific staining of background (i.e., apparently uninfected) cells in the HSV-2-positive cell preparations approached that of the infected cells. Compared with the BAR and Syva kits, interpretation of HSV-2 isolates by use of the ORT kit centered more toward the subjective than the discreet. It should be pointed out that the ORT reagent was developed for use in the
spin-amplified or shell vial assay, because the component antibodies “recognize antigens expressed early in the virus replication cycle” (15). Although the precise natures (e.g., molecular weights) of the component monoclonal antibodies were ill defined, one might speculate that the number of poorly stained specimens observed in this study was due to a paucity of early antigen. If such a factor were the case, however, interpretive difficulty would have occurred for all of the HSV-2 isolates tested; this effect was not observed. Single-cycle replication studies, using a high multiplicity of infection, were not performed (7). Consequently, a heterogeneity of early and late viral antigens would be expected to occur in all of the infected cell populations tested with the ORT kit.

An explanation for the decreased sensitivity and specificity identified among some of the kits or reagents tested in this study may only be addressed in general at present. Accordingly, monoclonal antibodies prepared against a single (or more than one) epitope are not necessarily expected to react with all strains. Conversely, one may not rule out the possibility that the antibodies react with cellular components resembling viral antigens.

A pragmatic approach must be taken prior to the decision to incorporate into the clinical setting any one of the typing kits or reagents evaluated in this study. First, many laboratories consider the factor of cost. Manufacturers and distributors realize this point and price their products competitively. The kits and reagents for detection of HSV tested in this study varied in cost only minimally. Additionally, no significant differences in manipulative procedures were found. In conclusion and based on the criteria of signal intensity and background level and the sensitivity and specificity, the most appropriate choice for the typing and identification (and culture confirmation) of HSV isolates is the BAR HSV fluorescence monoclonal antibody test.

ACKNOWLEDGMENTS

We greatly appreciate the excellent technical assistance of Pat Costello and Clara Smith.

REFERENCES