Comparison of Immunofluorescence with Commercial Monoclonal Antibodies to Biochemical and Biological Techniques for Typing Clinical Herpes Simplex Virus Isolates†

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Immunofluorescence with monoclonal antibody reagents from two commercial sources for differentiating herpes simplex viruses types 1 and 2 demonstrated 100% agreement with cell culture selectivity (chicken embryo and guinea pig embryo cells) and (E)-5-(2-bromovinyl)-2'-deoxyuridine sensitivity for typing a total of 94 clinical herpes simplex virus isolates.

Herpes simplex viruses (HSV) remain the most common viral isolates encountered in clinical laboratories (9, 13). Although HSV can be differentiated into types 1 and 2, many laboratories do not routinely type their HSV isolates. Some of the reasons in the past for not typing HSV isolates included the lack of technical time and necessary equipment or the unavailability of commercial reagents. Proper typing of clinical HSV isolates will become more important with the increased development and use of antiviral compounds, since HSV types 1 and 2 (HSV-1 and HSV-2, respectively) have been shown to vary in their susceptibilities to many antiviral compounds studied previously (3).

Numerous laboratory methods have been used to distinguish between HSV-1 and HSV-2. These techniques include in vitro virus neutralization (15), immunofluorescent-antibody staining (4, 11), radioimmunoassay (14), enzyme-linked immunosorbent assay (10), restriction endonuclease analysis (6), nucleic acid hybridization (2), selective growth in different cell culture systems (12), and selective sensitivity to (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU) (7). Previous studies in our laboratory have demonstrated that cell culture selectivity with chicken embryo and guinea pig embryo (GPE) cells and BVDU sensitivity with GPE cells provide two methods of HSV typing that are highly specific, easily interpretable, and do not require special equipment (18). However, both of these methods use cell culture techniques which require approximately 1 week from the initial preparation of cell cultures until final typing results are obtained.

Although immunofluorescent-antibody staining can provide rapid results, polyclonal antisera have been shown to be unreliable for typing clinical HSV isolates because of the extensive cross-reactivity between HSV-1 and HSV-2 (18). The advent of monoclonal antibody (MAb) technology has allowed the commercial production of antibody preparations directed against specific determinants on HSV-1 and HSV-2 (1, 5). In the present study, 94 clinical HSV isolates were analyzed by immunofluorescence (IF) with MAb HSV typing reagents from two commercial sources (Ortho Diagnostics, Inc., Raritan, N.J., and Syva Co., Palo Alto, Calif.), and the IF typing results were compared with HSV type identification by cell culture selectivity and BVDU sensitivity. Low-passage HSV isolates from clinical specimens previously submitted for virus isolation or identification and stored at −70°C were used throughout the study.

Cell culture selectivity assays were performed as described previously (12, 18). Isolates demonstrating comparable plaque formation in chicken embryo and GPE cells were designated as HSV-2, whereas isolates that only produced plaques in GPE cells were designated as HSV-1. BVDU sensitivity assays were also performed as previously described (7, 18). BVDU was kindly provided by E. De Clercq, Rega Institute for Medical Research, Leuven, Belgium. In the presence of BVDU, a plaque reduction of ≥2 log10 indicated that the isolate was HSV-1, and no effect on plaque-forming ability indicated HSV-2.

Slides for IF staining were prepared from infected GPE cell cultures showing a 2 to 3+ HSV-induced cytopathic effect. Cell culture medium was removed, and infected cells were removed by scraping and then suspended in approximately 0.2 ml of phosphate-buffered saline, pH 7.2. Single drops of infected cell suspensions were added to individual wells of toxoplasmosis slides (Belleco Glass, Inc., Vineland, N.J.). After being air-dried, the slides were fixed for 10 min in cold acetone and either tested immediately or stored at −70°C. All slides were prepared and tested under code. IF procedures with commercial MAb kits designed to differentiate HSV-1 and HSV-2 were performed in accordance with the instructions of the manufacturers. The MicroTrak kit (Syva Co.) contained HSV-1- and HSV-2-specific murine MAb preparations labeled with fluorescein isothiocyanate and Evans blue counterstain; this kit used a direct IF staining procedure. The Cultureset kit (Ortho Diagnostics) contained HSV-1- and HSV-2-specific murine MAb preparations and also a preparation of antimouse immunoglobulins labeled with fluorescein isothiocyanate; this kit used an indirect IF staining procedure. All slides were read by a single individual with a fluorescence microscope at a magnification of 100 to 400×.

A total of 94 clinical HSV isolates were typed by cell culture selectivity and BVDU sensitivity. Their sources and type identification are shown in Table 1. Approximately 60% of the isolates were HSV-1, and the remaining 40% were identified as HSV-2.

A total of 84 of these HSV isolates were tested with the
MicroTrak kit, and IF typing demonstrated 100% agreement with both cell culture selectivity and BVdU sensitivity (Table 2). IF typing results were easily interpretable, with one of the two MAb reagents demonstrating bright-green cytoplasmic fluorescence with the infected cells for each HSV isolate and the other MAb reagent demonstrating no reaction (only red counterstained cells). Typing results were obtained in approximately 1.5 h from the time that the infected cells were harvested.

The Cultureset kit was used to test 76 of the 94 HSV isolates, and IF typing again demonstrated 100% agreement with the biochemical and biological techniques (Table 2). A positive reaction with the HSV-1 MAb preparation was demonstrated by an intense green fluorescence in both the nucleus and cytoplasm of the HSV-1-infected cells. The HSV-2 MAb preparation showed fluorescence primarily in the nucleus of the HSV-2-infected cells. IF typing results were again easy to interpret; however, the lack of a counterstain did necessitate a closer examination of the wells with the nonreactive infected cells. Since the Cultureset kit used an indirect IF procedure, typing results were obtained in approximately 2.5 h after the harvesting of the infected cells.

Of the 94 HSV isolates, 67 were tested with both the direct and indirect IF typing kits, and comparable results were obtained in all cases. Also, uninfected GPE cells did not bind any of the MAb reagents. Although only GPE cell cultures were used in this study, additional testing in our laboratory has shown that both HSV typing kits are suitable for differentiating HSV-1 and HSV-2 in other cell culture systems. These cell types include A549 cells, human embryonic kidney cells, human newborn foreskin fibroblasts, MRC-5 cells, and primary rabbit kidney cells (data not shown).

This study demonstrates that IF with commercial MAb reagents is comparable to cell culture selectivity and BVdU sensitivity for typing clinical HSV isolates. In addition, IF typing results for 10 of the HSV isolates showed agreement with the results of a previous restriction endonuclease analysis (8). Both direct and indirect IF methods were equally sensitive and specific. IF typing results could also be obtained within hours rather than in days, as is required for cell culture methods.

### Table 2. Comparison of methods for typing clinical HSV isolates

<table>
<thead>
<tr>
<th>Cell culture selectivity and BVdU sensitivity</th>
<th>Immunofluorescence</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MicroTrak</td>
<td>Cultureset</td>
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<tr>
<td></td>
<td>HSV-1</td>
<td>HSV-2</td>
</tr>
<tr>
<td>HSV-1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>HSV-2</td>
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<td>0</td>
</tr>
<tr>
<td>Respiratory tract</td>
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<td>5</td>
</tr>
<tr>
<td>Extremities</td>
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<td>2</td>
</tr>
<tr>
<td>Not specified</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*As determined by cell culture selectivity and BVdU sensitivity.*

With the increased use of antiviral chemotherapy, IF staining with MAb reagents may offer an advantage over other methods for HSV typing. For example, a recent study has shown that HSV-1 strains which are resistant to acyclovir (thymidine kinase-negative mutants) can yield false typing results in the BVdU typing assay (16). However, problems may arise from the use of preparations containing MAb to only a single viral epitope, since mutant viruses that are no longer reactive with the particular MAb may appear (17). The appearance of nonreactive HSV should produce fewer problems with typing than with direct examination of clinical specimens, since the presence of a characteristic HSV-induced cytopathic effect in cell cultures not reactive with the MAb reagents would warrant testing by alternative typing methods. Therefore, pools of MAb with different specificities may be more applicable to the direct examination of clinical specimens.

In summary, IF with commercial MAb reagents provides an easy, rapid, and specific method for typing HSV isolates in clinical laboratories.

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### LITERATURE CITED


