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Evaluation of Two Immunofluorescence Assays with Monoclonal Antibodies for Typing of Herpes Simplex Virus

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An indirect immunofluorescence assay and a direct immunofluorescence assay were evaluated for typing clinical isolates of herpes simplex virus (HSV). The indirect immunofluorescence assay (Electro-Nucleonics, Inc.) correctly identified 16 HSV type 2 (HSV-2) isolates, but failed to identify 4 of 14 HSV-1 isolates because of background fluorescence and instability of reagents. Forty-nine HSV-1 isolates were correctly typed by direct immunofluorescence assay (Kallestad Laboratories, Inc.), but 1 of 39 HSV-2 isolates did not react with the HSV-2 type-specific antibody conjugate.

Typing of herpes simplex virus (HSV) by immunofluorescence with conventionally produced animal antisera is generally unreliable because of the extensive antigenic cross-reactivity of HSV type 1 (HSV-1) and HSV-2 (2, 5). Type-specific monoclonal antibodies have the potential for unambiguous differentiation of HSV-1 and HSV-2. In this study we compared restriction endonuclease analysis with two immunofluorescence assays that utilize monoclonal antibodies for typing clinical isolates of HSV. Monoclonal antibodies are available from other commercial sources, but were not evaluated.

Clinical isolates were grown in either Vero cells (American Type Culture Collection, Rockville, Md.) or WI-38 cells (Flow Laboratories, McLean, Va.). Vero cells were grown and maintained in medium 199 (K. C. Biologicals, Lenexa, Kans.) supplemented with 10% heat-inactivated fetal bovine serum and 25 μg of gentamicin sulfate per ml. WI-38 cells were maintained in modified Eagle medium with 10% heat-inactivated fetal bovine serum, 0.4% sodium bicarbonate, 100 U of potassium penicillin G per ml, and 25 μg of gentamicin sulfate per ml.

HSV clinical isolates were obtained from the Diagnostic Virology Laboratory, Cardinal Glennon Memorial Hospital for Children, St. Louis, Mo.; M. Menegus, University of Rochester Medical Center, Rochester, N.Y.; T. Smith, Mayo Clinic, Rochester, Minn.; and Binghamton General Hospital, Binghamton, N.Y. HSV-1 (F) and HSV-2 (G) prototype strains were obtained from B. Roizman, University of Chicago, Chicago, Ill.

Restriction endonuclease analysis of NaI gradient-purified viral DNA was performed as previously described (1), except that electrophoresis was performed in submerged agarose gels at 30 V for 16 h. All viral DNAs were typed by digestion with at least one enzyme (*Hpa*I, *Bgl*II, and *Hin*dIII from Boehringer Mannheim, Indianapolis, Ind.; *Kpn*I and *Sst*I from Bethesda Research Laboratories, Gaithersburg, Md.). Restriction enzyme analysis of HSV-1 (F) and HSV-2 (G) DNAs was performed simultaneously with each analysis of clinical isolates. Designation of type was made by comparison with the restriction fragment patterns of the prototype strains.

Thirty isolates were typed by indirect immunofluorescence with kits of two separate lots provided by Electro-Nucleonics, Inc. (ENI), Columbia, Md. This test utilizes HSV type-common and HSV-2 type-specific monoclonal antibodies, which are used in conjunction with fluorescein isothiocyanate-conjugated goat anti-mouse antibodies in the indirect test. HSV-1 isolates react with only the type-common antibody, whereas HSV-2 strains react with both the type-common and HSV-2 type-specific antibody.

Eighty-eight isolates were typed by direct immunofluorescence with reagents provided by Kallestad Laboratories, Inc., Chaska, Minn. This test employs type-specific fluorescein isothiocyanate-conjugated monoclonal antibodies that are directed against epitopes on either HSV-1 or HSV-2.

Infected cells, harvested when 75 to 100% of the cell culture displayed a typical cytopathic effect, were pelleted by centrifugation and suspended in phosphate-buffered saline (pH 7.2) before application to the slide. Air-dried slides were fixed in acetone (−20°C) and stored at −70°C before testing. Staining was performed according to the manufacturer's instructions. Slides were examined at ×400 with a Nikon Optiphot microscope with a 50-W mercury arc bulb.

Intensity of fluorescence was graded as follows: 4+, glaring-green fluorescence; 3+, bright-green fluorescence; 2+, dull-green fluorescence; 1+, very-dim-green fluorescence.

Three HSV-1 isolates and six HSV-2 isolates were correctly typed with reagents from ENI lot 8715 (Table 1). HSV-1-infected cells fluoresced with an intensity of 3+ to 4+ with the type-common antibody. HSV-2-infected cells fluoresced with 3+ to 4+ intensity with the type-common antibody, but only 1+ to 2+ with the HSV-2 type-specific antibody. Diffuse, dim cytoplasmic fluorescence was observed with cells infected with HSV-1 clinical isolates stained

TABLE 1. Comparison of ENI immunofluorescence (IF) and restriction endonuclease analysis of viral DNA for typing HSV isolates

Type by restriction endonuclease analysis	Type by IF[a]		Indeterminate
	HSV-1	HSV-2	
HSV-1	10	0	4[b]
HSV-2	0	16	0

[a] Results of two separate ENI lots.
[b] Lot 8802.

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TABLE 2. Comparison of Kallestad immunofluorescence (IF) endonuclease analysis for typing HSV isolates

Type by restriction endonuclease analysis	Type by IF		Not reactive
	HSV-1	HSV-2	
HSV-1	49	0	0
HSV-2	0	38	1

with either type-common or HSV-2-specific antibodies. Background fluorescence was also observed with HSV-1-infected cells reacted with only phosphate-buffered saline and the conjugate. No background fluorescence was seen with the HSV-1 control slides provided with the kit, with uninfected cells, or with HSV-2-infected cells. This background fluorescence somewhat obscured the HSV-specific granular fluorescence. Binding of conjugate via HSV-1 induced Fc receptors may have been responsible for this background fluorescence (3).

ENI lot 8802 was used to type the remaining 21 isolates (Table 1). Isolates were typed in four batches over a 6-week period. Antibodies and conjugate were stored at −20°C according to kit instructions. Again, diffuse background fluorescence was seen with the HSV-1-infected cells, which obscured the HSV-specific fluorescence in four cases. The intensity of fluorescence of HSV-1-infected cells diminished from 2+ to 4+ to 1+ to 2+ after reagents had been stored a few weeks. The instability of the reagents combined with the diffuse background fluorescence probably explain the failure to type four of the HSV-1 strains. With the second ENI lot, equal fluorescence intensity (2+ to 4+) was observed for HSV-2-infected cells stained with both the type-common and the HSV-2 type-specific antibody even after storage of reagents.

With Kallestad direct fluorescein isothiocyanate conjugates we correctly typed 87 of 88 clinical isolates (Table 2). These reagents gave virtually no background with either of the monoclonal antibody preparations in homologous or heterologous infected cells. The HSV-1–specific reagent showed diffuse cytoplasmic fluorescence. Sometimes small granules were visible in addition to the diffuse fluorescence, but these were also exclusively cytoplasmic. The HSV-2 monoclonal antibody reacted with an antigen that was exclusively nuclear. The usual fluorescence pattern was relatively large granules sometimes with stained nucleoli appearing as very large granules. The average staining intensity with both the HSV-1 and HSV-2 specific monoclonals was 3+ to 4+.

ENI has chosen to use a type-common monoclonal antibody and a HSV-2 type-specific antibody. The type-common antibody could be used in cases where one wished to simply detect HSV without actually typing a specimen or isolate. It has not yet been shown that the epitope against which this antibody is directed is more highly conserved than other viral epitopes, so it is possible that some HSV strains may not react. The background fluorescence observed with HSV-1-infected cells as well as diminished intensity of fluorescence upon storage were major undesirable factors with these reagents. Because of these problems, the ENI products were not tested further.

The direct conjugates from Kallestad Laboratories were tested more extensively. The most desirable aspects of these reagents were the virtual absence of background fluorescence and the clearly distinguishable HSV-1 and HSV-2 fluorescence patterns. These characteristics contributed to more easily read slides and enhanced the reliability and accuracy by diminishing the possibility of technical errors in staining. These reagents were also more stable and yielded essentially unchanged fluorescence after 8 weeks of storage.

The failure of one HSV-2 strain to react with the HSV-2-specific conjugate underscores the risk of using a single monoclonal antibody for typing HSV. The problem of nonreactivity of certain isolates with monoclonal antibodies has been investigated and discussed in detail by others (4, 5). Some epitopes are more highly conserved than others, but the genetic variability of HSV is such that one could not expect any single epitope to be absolutely conserved in all clinical isolates. Thus an overall sensitivity of 100% is perhaps an unreachable goal with reagents employing single monoclonal antibodies.

The use of monoclonal antibodies for typing HSV isolates promises reliability comparable to that of restriction endonuclease analysis. However, problems such as background fluorescence, weak intensity of fluorescence, instability of reagents, and narrow antigenic specificity must first be addressed.

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