Comparison of a commercial ELISA system with restriction endonuclease analysis for typing herpes simplex virus

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SUMMARY Isolates of herpes simplex virus which had previously been typed by restriction enzyme analysis were typed again with a commercial ELISA system using polyclonal antibodies. There was complete correlation between the two techniques. Although restriction is more precise and definitive, when typing only is required the simplicity of ELISA makes it the preferred technique.

For a long time the existence of two variants of herpes simplex virus (HSV), differentiated on clinical grounds, has been apparent. More recently, methods of typing such as plaque morphology,¹ differential neutralisation,² immunoperoxidase staining,³ DNA density analysis,⁴ guanine plus cytosine content,⁵ and the formation of intranuclear tubular structures⁶ have been described. These methods are generally accurate and satisfactory, but are tedious, time consuming, or costly.

The types of HSV are more definitively differentiated by analysis of the viral polypeptides⁷ and by restriction enzyme analysis of the viral DNA.^{8,9} The latter technique especially is highly precise; it can not only differentiate the types but also the subtypes of HSV and thus is useful in studying the epidemiology of these infections.¹⁰⁻¹² Nevertheless, although it has been miniaturised and simplified¹³ to permit the typing of many isolates at low cost, it is not sufficiently simple for use in a routine virus laboratory with limited facilities. Therefore, when only typing rather than subtyping is required, other methods may be more suitable.

The cross reactivity between the two types of HSV, which confuses conventional serotyping, may be eliminated by the use of antisera extensively absorbed against heterotypic virus or by the development of monoclonal antibodies, which have now been raised against epitopes which are largely both type specific and type universal.¹⁴ However,

use of such defined reagents may not be necessary. We have evaluated a commercial enzyme linked immunosorbent assay (ELISA) kit based on the differential reactivity of homotypic and heterotypic HSV isolates of immunoglobulin purified from polyclonal antibodies raised against HSV 1 and 2 and have compared it with restriction endonuclease typing.

Material and methods

VIRUSES

HSV standard type strains 17 syn⁺ SV (type 1) and HG 52 (type 2) were kindly provided by Mr J Lang of the Institute of Virology, Glasgow. The Bristol standard strains of SC 16 (type 1) and AR 15 (type 2)¹⁵ had been isolated and typed in this laboratory. These four strains had also been typed by restriction endonuclease analysis.¹³

Isolates of virus from single clinical specimens from 39 patients taking part in a trial of topical acyclovir for primary or recurrent genital herpes were made in human embryo fibroblast cells or Vero cells. In all cases virus for typing by either method was harvested from cells showing complete cytopathic effect, and usually both tests were carried out on the same sample.

RESTRICTION ENDONUCLEASE TYPING

All HSV isolates were typed by the method of Darville,¹³ which is outlined below.

Virus was grown and labelled with ³²P (Amersham) in 1 × 10⁵ Vero cells in one well of a 96 well micro cell culture plate (Linbro). On the develop-

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Table 1 Typing by ELISA of two standard strains of herpes simplex virus type 1 (SC 16 and 17 syn⁺ SV) and two of type 2 (AR 15 and HG 52)

Virus	Optical densities on coating immunoglobulin							
	HSV 1		HSV 2		Control		HSV 1	Type
	Measured	Corrected	Measured	Corrected	Measured	Corrected	HSV 2	
SC 16	0.39	0.37	0.3	0.19	0.0	-0.03	1.95	1
17 syn ⁺ SV	1.0	0.98	0.87	0.76	0.07	0.04	1.29	1
AR 15	0.1	0.08	0.43	0.22	0.0	-0.03	0.36	2
HG 52	0.11	0.09	0.47	0.36	0.0	-0.03	0.25	2
Control*	0.02		0.11		0.03			

*Average of uninfected cell supernatants on each coating immunoglobulin.

ment of 100% cytopathic effect the viral DNA was extracted with sodium dodecyl sulphate and phenol and precipitated with ethanol. After digestion of the DNA with the enzymes Bst I (Cambridge Biotechnology Laboratories), Pvu II, and Sst I (Bethesda Research Laboratories) the cleavage fragments were resolved by agarose gel electrophoresis and visualised by autoradiography.

ELISA TYPING

The ELISA typing was carried out without knowledge of the results of the restriction typing. A Dakopatts ELISA HSV typing kit based on the technique described by Vestergaard and Jensen¹⁶ was kindly provided for evaluation by Mercia Brocades. This kit contained normal rabbit immunoglobulin and rabbit anti-HSV type 1 (MacIntyre) and rabbit anti-HSV type 2 (MS) antibodies in the form of immunoglobulin fractions obtained by salting out and by ion exchange chromatography. They were absorbed with immobilised fetal calf serum and were supplied unconjugated and conjugated with horseradish peroxidase.

The method used was that described in the kit. Replicate wells of immunological quality microtitre plates (Nunc) were coated at room temperature for 1 h with normal rabbit immunoglobulin or with antibody to HSV 1 or HSV 2. Eighty microlitres of each immunoglobulin was diluted in 4 ml coating buffer (0.015M Na₂CO₃, 0.035M NaHCO₃, pH 9.6) and 100 µl of diluted immunoglobulin was used in each well. The plates were then washed five times in wash buffer (0.5M NaCl, 0.0015M KH₂PO₄, 0.0065M Na₂HPO₄.2H₂O, 1% Triton X-100; pH 7.2).

After washing, 100 µl volumes of 1/100 dilutions in dilution buffer (wash buffer containing 1% bovine serum albumin) of HSV culture supernatant or uninfected cell supernatant were added to one each of the coated wells and incubated at room temperature for 2 h. The plates were washed five

times. It was essential to include the uninfected cell control since non-specific binding to anti-HSV 2 was significantly higher than to either anti-HSV 1 or to normal immunoglobulin (Table 1).

After washing, 100 µl peroxidase conjugated normal immunoglobulin or anti-HSV 1 or 2 immunoglobulin (80 µl stock diluted in 4 ml dilution buffer) was added and incubated for 1 h at room temperature. The plates were again washed five times.

The plates were then washed once for 1 min with 100 µl substrate buffer (0.0347M citric acid, 0.0667M NaH₂PO₄.2H₂O; pH 5). The substrate, orthophenylenediamine (8 mg OPD and 5 µl 30% H₂O₂ in 15 ml substrate buffer), was added at 100 µl per well and incubated for 15 min at room temperature. The reaction was stopped with 150 µl M H₂SO₄, and the absorbances were read.

A negative reaction gives no colour, a heterotypic reaction gives a light brown colour, and a homotypic reaction gives a dark brown colour. A difference in optical density of about 0.2 can easily be detected by eye at the lower range of values (<1.0), whereas differences of this magnitude between readings at or above 1.0 can not. In this comparison, however, all reactions were read by an automatic ELISA reader (Dynatech model MR580) interfaced with a Superbrain Microcomputer (Intertec Data Systems) programmed to make the following calculations necessary to ascribe types to the isolates.

For each of the three immunoglobulin specificities average absorbance values with uninfected cell supernatants were calculated and subtracted from the corresponding test absorbances. The test result is positive for the detection of HSV in a given sample if (a) the absorbance of the test well showing the higher absorbance less the absorbance of the normal immunoglobulin control well is greater than 0.1 and (b) the absorbance of that test well is at least twice that of the average control absorbance.

When virus is detected its type is determined by

dividing the HSV 1 absorbance value by the HSV 2 absorbance value. The isolate is type 1 if the calculated value is greater than 1, and type 2 if it is less than 1. Where absorbance values for the two viruses are so close that the result is equivocal, which occurs in about 10% of tests, a definitive result can be obtained by repeating the test on a more dilute sample of the specimen.

Replicates were not done routinely. Included in each run, however, were type standards, which were always identified correctly and the absorbances of which varied little.

Initially, the four standard strains of HSV were typed by ELISA and then the 39 clinical isolates were tested in the same way.

Results

The four standard strains of HSV (two each of type 1 and type 2) were correctly and clearly identified by ELISA (Table 1).

The Figure shows the striking and unambiguous differentiation by analysis with the restriction enzyme Bst I of types 1 and 2 in 12 genital isolates of HSV. These and all but one of the remaining clinical isolates were identified correctly by ELISA: of the 39 isolates 10 were type 1 and 28 were type 2 (Table 2). One isolate, however, the profile of which showed specific bands of both HSV 1 and 2, typed by ELISA as HSV 1 or 2 on different occasions. Restriction enzyme analysis of this virus labelled during growth after reisolation or passage showed only a type 1 profile, but analysis of virus labelled during reisolation from the specimen again yielded the mixed profile. This evidence and plaque purification studies (unpublished results) suggest that little type 2 was present and that it was lost on passage.

Discussion

The results of the ELISA method were unambiguous, and in all but one case correlated completely with restriction enzyme analysis. The single isolate which failed to type satisfactorily by ELISA was clearly a mixture of HSV 1 and 2 and not an intermediate strain, since in addition to yielding mixed profiles its replicate isolates yielded type 1 profiles.

The use of polyclonal antibodies for typing herpes simplex virus gives incorrect results in direct immunofluorescence¹⁷ and immunoperoxidase¹⁸ tests, whereas the use of type specific monoclonal antibodies in direct¹⁹ and in indirect¹⁸ immunofluorescence correlates completely with restriction enzyme analysis. This suggests that monoclonal antibodies might also be more accurate

Bst I restriction endonuclease profiles of genital isolates of herpes simplex virus (HSV). Tracks 5, 6, 8, 11, 12 are HSV 1, tracks 1-4, 7, 9, 10 are HSV 2.

than polyclonal antibodies in typing HSV by ELISA. Gerna *et al.*,²⁰ however, reported complete correlation of the ELISA technique with typing by the inhibition of indirect haemagglutination. Our results confirm this finding in comparison with restriction enzyme analysis, which is the most definitive typing method. It is clearly an advantage to measure the differential absorbances with an automatic ELISA reader interfaced with a computer, which can ascribe type without observer subjectivity. The value of monoclonal reagents may lie in their use for

Table 2 Typing by ELISA of 39 isolates of HSV from genital herpes previously typed by restriction endonuclease analysis

Patient	Optical densities*			HSV 1	Type	
	HSV 1	HSV 2	Control	HSV 2	ELISA	Restriction
1	0.09	0.3	-0.02	0.3	2	2
2	0.35	0.62	-0.01	0.57	2	2
3	0.02	0.18	-0.02	0.1	2	2
4	>1.5	0.24	0.35	>6.25	1	1
5	0.16	0.62	-0.02	0.26	2	2
6	0.32	0.69	-0.02	0.46	2	2
7	0.25	0.7	-0.02	0.36	2	2
8	0.9	>1.5	0.03	<0.6	2	2
9	0.95	>1.5	0.1	<0.63	2	2
10	0.42	0.1	0.16	4.2	1	1
11	0.56	0.11	0.09	5.1	1	1
12	0.8	0.32	0.2	2.5	1	1
13	0.08	0.9	0.12	0.1	2	2
14	0.25	0.6	0.03	0.42	2	2
15	0.4	1.23	0.06	0.33	2	2
16	0.29	0.73	0.0	0.4	2	2
17	0.29	0.83	0.01	0.35	2	2
18	0.07	0.39	0.01	0.18	2	2
19	0.13	0.05	-0.04	2.6	1	1
20	0.25	0.02	-0.02	12.5	1	1
21	0.03	0.9	0.03	0.03	2	2
22	0.3	0.18	-0.01	1.67	1	1
23	0.48	0.3	-0.01	1.6	1	1
24	0.67	0.59	0.0	1.14	1	1, 2
24†	1.08	1.14	0.04	0.95	2	1, 2
25	0.48	0.27	0.0	1.78	1	1
26	0.65	0.5	-0.04	1.3	1	1
27	0.14	0.32	-0.02	0.44	2	2
28	0.46	0.7	0.0	0.66	2	2
29	0.1	0.27	0.0	0.37	2	2
30	0.38	0.63	-0.01	0.6	2	2
31	0.47	0.74	0.01	0.64	2	2
32	0.46	0.89	0.0	0.52	2	2
33	0.31	0.52	0.0	0.6	2	2
34	0.39	0.6	-0.02	0.65	2	2
35	0.23	0.8	0.0	0.29	2	2
36	0.29	0.56	0.0	0.52	2	2
37	0.35	0.54	0.0	0.65	2	2
38	0.57	1.02	0.0	0.56	2	2
39	0.56	1.02	0.0	0.55	2	2

*Corrected for control readings with uninfected cell supernatants.

†Patient number 24 was shown by restriction endonuclease analysis to be infected with both type 1 and 2.

measurement by eye. A disadvantage of any serological method of typing is that mixed infections or infections with intermediate strains might not be detected as such. Only one such infection was seen in this series of 39, however, and none has been detected in any of the other isolates of HSV analysed by restriction endonuclease analysis in this laboratory. Furthermore, mixed infection has not been reported frequently by other workers—thus its incidence is presumably slight.

Further comparison between the two methods must be based on the considerations of simplicity, convenience, cost, and safety. Requiring fewer manipulations, the ELISA technique is easier and more convenient to perform than restriction enzyme analysis; it is also less time consuming and yields results more quickly. Although equivalent in virological safety with restriction enzyme analysis, ELISA does not carry the radiological risks, albeit

slight, of the former. A high concentration of detergent is used in dilution and other buffers in order to "solubilise" the antigen; an advantage of this is that viral infectivity is destroyed, making the method virologically safer. The costs of the two methods are about the same. Although restriction enzymes are not cheap, such small quantities of each and of other reagents are used that the cost per test is low.

In conclusion, the ELISA system tested has proved to be as good as restriction endonuclease analysis for the differentiation of the types of herpes simplex virus. It is a safe and easy technique to perform and only if subtyping is required is the latter technique to be preferred.

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