

## Development of an immunofluorescence test for the serodiagnosis of herpes zoster ophthalmicus

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**SUMMARY** An indirect immunofluorescence test has been developed and evaluated for the serodiagnosis of herpes zoster ophthalmicus (HZO) by the detection of antivariella zoster virus (VZV) antibody. The results show that, in patients with HZO, anti-VZV IgG antibody titre usually rises rapidly after onset. One hundred and seven of the 134 sera (80%) from patients with a clinical diagnosis of HZO had an anti-VZV IgG titre of  $\geq 256$ , and IgM antibody at a level of 1 in 8 was present in six of them. In comparison only two of the 216 sera (1%) from patients with a clinical diagnosis of ocular infections other than those caused by VZV had such IgG titres. It was concluded that, on the basis of results of a single sample of serum, it is possible to make a provisional diagnosis of HZO with a high degree of confidence.

Varicella-zoster virus (VZV) can affect any part of the eye with varying degrees of severity, producing rather a pleomorphic clinical picture. The clinical diagnosis of HZO, when associated with typical skin lesions presents no problems. However, sometimes there are minimal or no skin lesions or there is an

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atypical rash which may mimic that of herpes simplex virus (HSV).<sup>1</sup> Hence a rapid and sensitive laboratory diagnosis is important in the differential diagnosis of these atypical cases.

Several laboratory tests are available for the diagnosis of VZV infections. Demonstration of viral antigens in clinical specimens is rapid, but it is useful only during the first few days of infection when

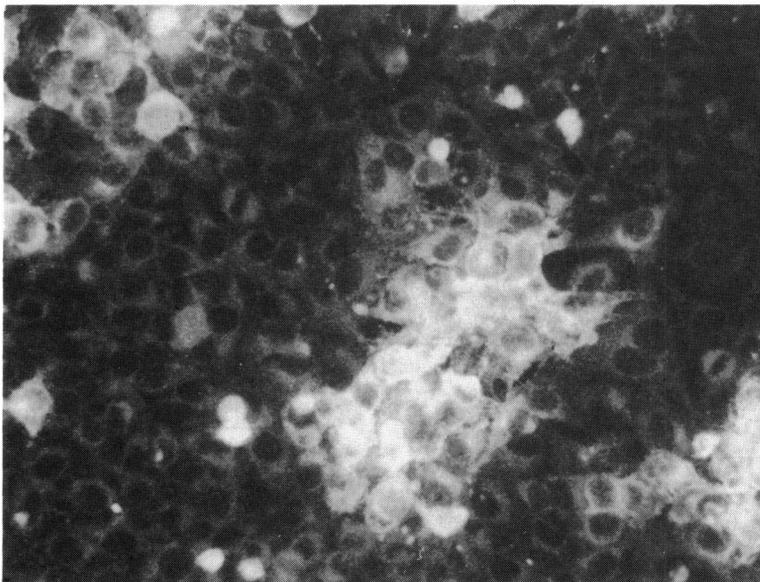


Fig. 1 Brightly fluorescing VZV infected cells growing as plaques in HeLa cell monolayer. ( $\times 540$ ).

vesicles are present.<sup>2</sup> Virus isolation in cell culture is a definitive means of diagnosis, but this is possible in only a few cell lines and only during the first few days of infection. Several serological tests are available, including immunofluorescence tests for the diagnosis of VZV infections,<sup>3,4</sup> but none have been evaluated for the differential diagnosis of HZO.

We report the development of an immunofluorescence test for the serodiagnosis of HZO using a single sample.

### Patients, Materials, and methods

#### PATIENTS

Sera were collected from patients attending the Zoster Clinic and Keratoconjunctivitis Clinic at Moorfields Eye Hospital in London and stored at  $-20^{\circ}\text{C}$  until examined. A total of 350 sera were collected from patients with HZO, ocular HSV, and other ocular viral infections. All the sera were examined for anti-VZV IgG antibody. IgM was determined only in the sera from HZO patients. In addition, anti-HSV 1 IgG titre were determined in a subsample of sera from the HZO patients by means of a microimmunofluorescence test.<sup>5</sup>

#### Materials and methods

**Antigen preparation.** A strain of VZV isolated from a case of chicken-pox (kindly supplied by the Virology Department, Institute of Child Health, London) was serially passaged in HeLa cells for antigen preparation. The infected monolayers prepared in flasks were trypsinised and resuspended in growth medium. The total number of HeLa cells per ml of growth medium was adjusted to approximately 60 000 per ml of growth medium. 0.2 ml of this suspension, which contained sufficient numbers of VZV infected cells to give 50–100 VZV plaques per monolayer (Fig. 1), was placed in each of 10 wells of the Belco Micro-Slide culture chamber (A R Horwell Ltd) and the chambers incubated for 48 hours at  $35^{\circ}\text{C}$ . The monolayers were fixed with acetone for 10 minutes at

room temperature. The fixed slides were stored at  $-20^{\circ}\text{C}$  until used. The stability of the antigen under these conditions of storage was satisfactory for up to three months.

**Immunofluorescence test.** Doubling dilutions of the sera ranging from 1 in 8 to 1 in 4096 were prepared in phosphate buffered saline (PBS) at pH 7.3. The dilutions initially examined for the presence of IgG were from 1 in 16 to 1 in 512. The presence of IgM was screened at a dilution of 1 in 8. Further higher dilutions were tested when required.

Approximately 10  $\mu\text{l}$  of each of the dilutions was spread on appropriate monolayers on the slide and incubated at  $37^{\circ}\text{C}$  in a moist chamber for 30 minutes. The slides were washed in PBS for 15 minutes in a magnetic stirrer. The monolayers were air dried and covered with appropriately diluted antihuman IgG or IgM conjugated with fluorescein isothiocyanate (Hyland Laboratories Ltd, Lorne Laboratories Ltd). Incubation and washing were repeated as before except for an extra final wash of the slides in distilled water for 5 minutes. The slides were air dried and mounted in a glycerol mountant<sup>6</sup> and examined with a Zeiss Standard 18UV microscope fitted with a Zeiss filter set 10. The tests were read under a  $\times 16$  Neofluar objective and  $\times 10$  eyepieces.

The end point of the titration was taken as the last dilution in which cells with specific fluorescence could be clearly seen.

**Reproducibility of the test.** The reproducibility of the test was determined by testing aliquots of two positive sera stored at  $-20^{\circ}\text{C}$  on five separate occasions.

### Results

**Reproducibility of the test.** The results showed complete agreement in titre in four of the five aliquots for each of the two sera tested. The remaining aliquot showed only a one-tube difference in titre (Table 1).

**Levels of distribution of anti-VZV IgG.** The distribution and levels of anti-VZV IgG in the sera of all the categories of patients are shown in Table 2. The

Table 1 Reproducibility of the immunofluorescence test: five aliquots of two positive sera tested on five separate occasions

Aliquots of sera	Anti-VZV IgG titres	
	Serum 1	Serum 2
1	16	512
2	16	512
3	16	1024
4	32	512
5	16	512

Table 2 Distribution of levels of anti-VZV IgG in the different patient groups

Clinical diagnosis	Number of sera							Total
	Anti-VZV IgG titres							
	$\leq 16$	32	64	128	256	512	$\geq 1024$	
HZO	9	4	4	10	26	46	35	134
HSV ocular	15	4	2	0	0	0	0	21
Adenovirus ocular	84	18	15	7	0	0	0	124
Other 'viral' ocular	46	9	12	2	0	2	0	71

results show that 107 of the 134 (80%) of the sera from patients with HZO had a titre of  $\geq 256$ . In comparison, only two out of a total of 216 patients (1%) with ocular problems other than those of HZO had a titre of  $\geq 256$  (Table 2).

**Level of anti-VZV IgM in patients with HZO.** IgM at a level of 1 in 8 was found in 6 of the 134 sera from patients with HZO.

**Cross reactivity with HSV 1.** Sera of 27 patients with HZO were tested against HSV-1 antigen in addition to the VZV antigen. Twenty-one had an anti-HSV1 IgG titre of 64 or less, four had a titre of 128, and the remaining two had a titre of 256. In all but two samples anti-VZV IgG titre was at least fourfold greater than the anti-HSV1 titre.

**Anti VZV IgG titres and skin lesions.** The results are presented in Fig. 2. Anti-VZV IgG titres increased within about four to six days of onset of skin lesions of HZO and appeared to fall to baseline levels after the second month.

## Discussion

In this study a simple, practical, and reproducible serological test has been developed for the diagnosis of HZO using a single serum sample. It has been possible to make a provisional diagnosis of HZO with a high degree of confidence.

Previous studies evaluating immunofluorescence (IF) serological tests for the detection of antibody against VZV have used human diploid cells with a

limited life span for the preparation of antigen.<sup>17</sup> In this study we used HeLa cells, which have the advantage of being a continuous cell line. HeLa are known to be satisfactory for maintenance of VZV in serial passage and for the preparation of complement fixing antigen.<sup>8</sup>

The use of fixed monolayers as antigen makes the morphology of VZV plaques well preserved: specific fluorescence was confined to the plaques or foci of VZV-infected cells which result from the cell to cell spread and was not seen in the surrounding uninfected HeLa cells (Fig. 1). This makes the end point easier to read and would explain the good reproducibility of the test (Table 1). Previous workers have used infected cell monolayers as antigen, but the methods of preparation have been cumbersome.<sup>17</sup> The fixed antigen slides stored at  $-20^{\circ}\text{C}$  could be used for at least three months and thus are practical for routine use. The test is economical, since the Belco chambers make it possible to have 10 infected monolayers on the same slide.

The test showed a high degree of sensitivity and specificity in the differential diagnosis of HZO from other ocular viral infections. The results show that 80% of the 134 sera from patients with a clinical diagnosis of HZO had anti-VZV IgG titre of  $\geq 256$ . Such titres were found in only 2 of 216 sera from patients with a clinical diagnosis of ocular 'viral' infections. In 27 (20%) of the HZO patients lower titres ( $< 256$ ) were found. Two-thirds of these were probably due to early collection of sera because they were taken within one week of onset of rash. In addition a review of clinical diagnosis in 17 cases showed that one had a probable HSV infection and in two the diagnosis of zoster was not confirmed clinically.

Anti-VZV IgG was present in all the sera of patients with HZO studied here. The titres increased rapidly, usually within a week, peaked at 10 to 15 days, and appeared to fall after the second month (Fig. 2). Thus the test has an obvious advantage in cases of chronic infection or late acute cases. The number of observations after two months was small for any valid conclusion to be drawn beyond this period.

The problem of cross reactivity between VZV and HSV infections has been noted previously.<sup>9</sup> In this study anti-VZV IgG was detected in all 21 patients with HSV ocular infections but only in low titres ranging from 16 to 64 (Table 2). Anti-HSV1 IgG was also detectable in HZO patients, but, again, the majority had titres in the range of up to 64 (Table 2). This suggests a degree of cross reactivity between these viruses. However, the degree of cross reaction detected was not sufficient to interfere with the interpretation of the result for the diagnosis of HZO.

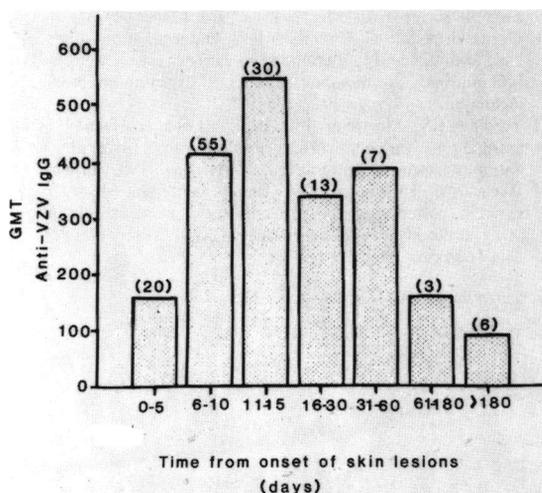


Fig. 2 Correlation between levels of anti-VZV IgG titres and time from onset of skin lesions in HZO patients. Each bar represents geometric mean anti-VZV IgG titres in HZO patients at various times after onset of skin lesions. Figures in parentheses indicate numbers of observations for each time interval.

Table 3 Levels of anti-VZV and anti-HSV1 IgG in 27 patients with HZO

Patient	Onset (days)	Anti-VZV	Anti-HSV1*
1	12	2048	32
2	90	128	0†
3	5	2048	16
4	5	512	0
5	—	2048	32
6	14	512	0
7	42	1024	64
8	21	512	0
9	—	64	16
10	—	512	16
11	6 yr	32	16
12	5	2048	32
13	14	1024	128
14	12	512	64
15	8	2048	32
16	12	1024	64
17	15	1024	0
18	10	2048	0
19	28	512	128
20	6	128	256
21	11	512	128
22	50	1024	64
23	7	512	16
24	1	16	0
25	28	1024	256
26	12	1024	128
27	10	2048	16

\* Determined by using an indirect microimmunofluorescence test.<sup>5</sup>  
 † 0 = <16.

Cradock-Watson *et al.*<sup>4</sup> found IF to be more sensitive than the complement fixation test in detecting VZV antibodies, but even in their study the range of these antibodies in HSV patients was relatively low (titres 8 to 64).

Anti-VZV IgM was found in only six of the 134 patients with HZO. Various authors have suggested that the presence of IgM is uncommon in zoster, which is usually considered a secondary VZV infection.<sup>9,10</sup> However, others have found IgM in acute varicella or zoster by the IgM capture radioimmunoassay (RIA) test,<sup>11</sup> IF,<sup>4</sup> and RIA.<sup>12</sup> The differences in IgM detection

between this study and that of Cradock-Watson *et al.*, who found IgM in 75% of 55 patients with herpes zoster, is difficult to explain. However, the difference may be related to the differences in methods and patient selection.

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