http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (19): 3306-3313, 2007
ISSN 1028-8880

Designation of an In-House ELISA for Detection of VZV IgG and Determination of Antibody Avidity by Use of Diethylamine

[1]A.H. Mohagheghi Fard and [2]J. Vallely Pam
[1]Department of Virology, School of Medicine, Zahedan Medical University, Zahedan, Iran
[2]Department of Virology, School of Medicine, Manchester University, Manchester, UK

Abstract: Herpesviruses are ubiquitous in nature and both humans and animals harbour different species of this diverse family. However, only 8 species have been so far recovered from human beings. Different techniques (molecular and non-molecular) have been employed for rapid and efficient diagnosis of herpesviruses especially in case of immunocompromised hosts who are considered as high risk individuals. An indirect in-house ELISA was standardized to measure IgG antibody against Varicella-Zoster Virus (VZV) in sera from different groups of individuals. In the process of optimization of in-house ELISA, optimal dilutions of antigen, serum, conjugate and monoclonal IgG antibody along with selection of efficient blocking buffer and washing steps of microtitre plates were studied by the help of checkerboarding. The results were calculated according to Specific Binding Ratio (SBR) and cut-off procedures. The efficiency of newly developed indirect in-house ELISA was attempted and results were compared with data previously obtained by the agency of commercial kits or other serological techniques. The latter helped to investigate different technical aspects of in-house ELISA. When accuracy of ELISA was confirmed the protocol was applied in screening of the sera from immunocompetent and immunocompromised hosts that facilitated examination of the clinical aspects of the hosts. Diethylamine (DEA) in 20, 35 and 70 mM concentrations were used in order to assay IgG antibody avidity. The IgG avidity index was calculated by dividing OD of each sera obtained with denaturant to OD of the same specimen without application of denaturants. Avidity indices proved to be an important tool to differentiate between primary and recurrent infections and between seropositivity and seronegativity.

Key words: In-house ELISA, Herpes viruses, Varicella-Zoster, IgG avidity

INTRODUCTION

Varicalla-Zoster Virus (VZV) is a member of *Herpesviridae* family (α-herpesvirinae subfamily). VZV causes two common diseases chickenpox (varicella), which is considered as one of the exanthemic childhood fevers and shingles (zoster). Shingles results from recurrence of VZV infection. This latency may last for several decades (Kangro and Harper, 1995). The virus is 180 to 200 nm in diameter and identical in both varicella and zoster (Gelb, 1993). The genome, a linear double-stranded DNA molecule (80×10^6 Daltons, approximately 125 kbp) (Davison and Scott, 1986).

Pathogenesis of VZV is similar to other viral exanthems and clinical disease is quite benign and few experimental models are available (Gelb, 1993). The route of virus entry is through mucosa of upper respiratory tract and pharynx or possibly via infection of conjunctivae. VZV initially replicates at the site of entry and via the blood stream and lymphatics disseminate causing primary viraemia. Secondary viraemia takes place when the virus is taken up by cells of reticuloendothelial system, liver, spleen and other organs where it undergoes multiple cycles of replication during the rest of incubation period (Cleveland and Richman, 1987; Gelb, 1993; Kangro and Harper, 1995). The incubation period from virus entry until the appearance of mucosal lesions is approximately 14 days, rises in titers of VZV-specific IgG, IgM and IgA antibodies are detectable within 5 days of the onset of clinical disease (Arvin *et al*., 1986). CNS complications (meningoencephalitis, myelitis, Guillain-Barr syndrome) are observed in approximately 1 varicella case per 1000 mostly among children aged 5 to 14 years (Preblud *et al*., 1984). However, the risk amongst immunocompromised individuals (especially AIDS victims) rises to 35% where VZV is an important cause of mortality and morbidity (Moulignier *et al*., 1995). VZV occurs worldwide and it is disease of children aged 4-10 years and almost 10% of adults may also contract varicella. In contrast to varicella, zoster occurs

Corresponding Author: A.H. Mohagheghi Fard, Department of Virology, School of Medicine, Zahedan Medical University, P.O. Box 98135-795, Zahedan, Iran Tel: +915-341-1964

sporadically throughout the year and constitutes about 10-20% of adults during their lifetime. Herpes zoster occurs as a result of primary varicella infection, the virus remaining latent in one or more posterior root ganglion (Kangro and Harper, 1995). The virus is reactivated in the ganglion and progresses peripherally down the sensory nerve to produce the typical lesions of VZV infection. Most cases of zoster occur spontaneously but trauma and stress are supposed to cause reactivation. The most common complication of herpes zoster is post herpetic neuralgia which occurs in 50% of patients over 60 years of age rarely in those under 40s (Gelb, 1993).

With few exceptions, the avidity of specific IgG increases with the time from the onset of a primary infection (Thomas, 1995). The control of avidity maturation depends on an interplay between T-cells, B-cells, antigen presenting cells and lymphokines in the germinal centers of the lymphoid organs (Berek and Milstein, 1987). Antibody avidity plays an important role in the development of an effective immune response to infection especially to secondary infection. Detection of low avidity specific IgG antibody generally indicates that primary infection has occurred within the last one or two months. Avidity assay have been widely used to differentiate between primary and recurrent infections in case of different viral infections (Thomas, 1995; Ory *et al*., 1993; Gray, 1995; Soderlund *et al*., 1995; Gut *et al*., 1995).

MATERIALS AND METHODS

Antigen production: In this study firstly we designed an in-house avidine-biotin indirect ELISA for this we grew marsden strain on HEL cells for antigen isolation. To assay the infectivity of the virus $TCID_{50}$ quantitation was carried out and subsequent steps of the ELISA were standardized and optimized by checkerboarding. In case of modification with respect to antigen and antibody concentrations, blocking buffer, the optimal dilution of monoclonal antibody (anti-human IgG), conjugate and finally washing of the plates after coating with antigen and after antibody binding and so on were all adjusted and chosen accordingly.

Standard ELISA protocol: The wells of a microtitre plate (Falcon 3912 Micro Test III) were coated with 140 μL optimally diluted antigen in carbonate bicarbonate buffer (pH: 9.6) and then incubated overnight at 4°C in a moist chamber. The next day the plate was washed with wash buffer 5 times with the same volume i.e., 140 μL (PBS-A with 0.05% tween 20 (Sigma No. 5362) and each well was incubated with 140 μL of blocking buffer (1 g skimmed milk powder dissolved in 100 mL of PBS-A) in moist chamber for an hour and later washed 5 times with wash buffer.

In the next step 140 μL of optimally diluted serum in serum diluent (blocking buffer plus 0.5% W/V tween 20) was added to each well. Of course two wells for each serum dilution and on every plate was used as blank (no serum only diluent). The plate was then incubated over night in moist chamber at 37°C. The next day plate was washed according to optimized number of washes i.e., x15. Afterward 140 μL of optimally diluted anti-human IgG mouse monoclonal antibody (biotin labeled; Sigma product No. B-3773) in diluent, which was exactly as blocking buffer, was added to appropriate wells. Incubation period was then half an hour at 37°C in moist chamber. The plate was again washed 5 times and incubated for 5 min at 37°C before addition of 140 μL of optimally diluted avidine peroxidase (Sigma product No. A-3151) in suitable diluent (100 mL PBS-A plus 4.0 g bovine serum albumin and 4.0 g goat serum supplied by Sara Lab Ltd., Sussex, U.K). After incubation and washing of plate 3 times 140 μL of o-phenylenediamine (OPD) dihydrochloride (Sigma product No. P-8287) 10 mg mL^{-1} dissolved in citrate buffer (pH: 5.0) was added to the wells. The plate was then incubated at 37°C away from light in moist chamber for half an hour. In final step 50 μL stop solution (1N, H_2SO_4) was added to the wells and the absorbance was read at 492 nm wave length against mean of column blank.

IgG avidity assay: As explained earlier, IgG avidity assays plays an important role in distinguishing primary from other categories of viral infections, so as one of the objectives in this project was measurement of IgG avidity (also used as a measure of denaturant-induced dissociation of binding) the approach was as following.

Diethylamine (DEA) (Sigma product No. D-31) in different concentrations (20, 35 and 70 mM) were chosen as IgG denaturants. Standard ELISA was carried out for this purpose except that after antigen-antibody binding, the plate was washed 15 times with wash buffer containing one of the denaturant instead of wash buffer alone. In order to measure IgG avidity it is essential to determine O.D of the sample both in standard ELISA and in the avidity assay procedure. Therefore for every batch of samples different plates had to be prepared as:

- Plate coated with viral antigen
- Plate coated with control antigen (uninfected cells)
- Plates coated with viral antigen but subjected to avidity assay by adding required denaturants.

Calculation of ELISA results: The results were calculated by cut-off and Standard Binding Ratio (SBR). In the cut-off method of results calculation a serum was considered positive for IgG antibodies, if the mean of the test serum OD exceeded by more than three standard deviations the mean OD given by 12 replicate test of a negative control serum. Whereas in the SBR method of results calculation a serum was regarded positive if the SBR (mean test serum OD with viral antigen/ mean test serum OD with cell control antigen) was greater than 2 (Klapper *et al*., 1990).

Calculation of IgG avidity: Once the O.D of the samples from different plates were known as mentioned above IgG avidity was measured by following equation.

$$\text{IgG avidity (\%)} = \frac{\text{(average OD of wells with denaturant)}}{\text{(Average OD of wells without denaturant)}} \times 100$$

In order to assess the reproducibility of the ELISA interassay and intrassay were attempted by testing a set of sera already tested by commercial kits (FAMA and biokit ELISA). Furthermore, 556 samples from normal children, 213 samples from leukaemic children were assayed by in-house ELISA. In the next step VZV IgG avidity indices were too calculated by 20, 35 and 70 mM Diethylamine (DEA). The latter was more applicable in case of primary and recurrent infections of VZV.

RESULTS

Reproducibility and comparison of in-house VZV IgG ELISA with other tests: A set of sera (n = 53) that had been tested by commercial ELISA (Northumbria Biologicals, Cramlington, UK) and in-house FAMA in the diagnostic laboratory were subjected to newly developed VZV IgG ELISA. This helped compare the in-house ELISA in terms of accuracy and reproducibility with the other tests. Virtually all sera except one gave concordant results in the in-house ELISA by SBR and cut-off [52/53 (98%)]. Figure 1 indicates overall distribution of VZV IgG avidities by scatter diagram.

Screening and IgG avidity measurement of sera from normal children: A total of 556 sera collected from normal children within 3 years were subjected to VZV IgG ELISA and avidity assay. These samples were supplied by Dr. J. Thomas, Preston Public Health Laboratory. The prevalence of doubly positive sera increased with age ($p<0.001$) with largest increase between 3<5 and 5<7 but a continuing rise until 11<13 years as shown in Table 1.

Furthermore as the results indicated the proportion of serum specimens positive only by SBR decreased marginally with increasing age ($p>0.1$) and prevalence of sera giving uniformly negative readings declined throughout the age groups ($p<0.001$) with the largest falls being between 3<5 and 5<7 years and between 9<11 and 13<15 years. Figure 2 also shows trend of VZV IgG antibody distribution amongst normal children.

Distribution of avidity indices in sera from normal children : As stated earlier, one of the objectives of this project was use of denaturants in order to establish avidity assay of VZV IgG, since there were many reports indicating certain differences in avidity indices in recent and remote infection. For this reason the same set of sera were subjected to avidity assay by application of 20, 35 and 70 mM DEA. The overall findings are shown in Table 2.

Doubly negative samples revealed avidities which were similar for all DEA concentrations and virtually low. However, with all DEA concentrations sera positive by both methods of results calculation had higher avidity

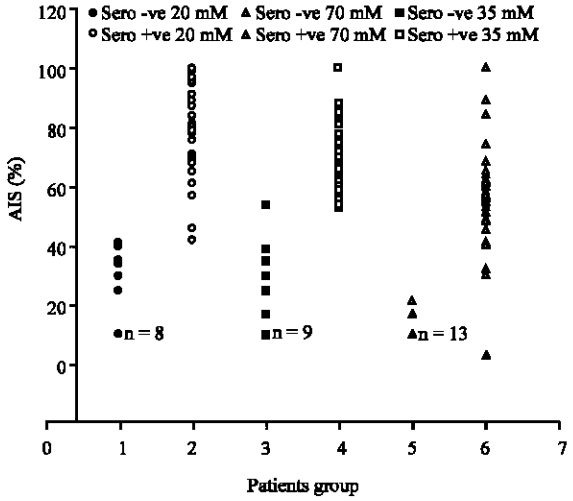

Fig. 1: Comparison of VZV IgG avidites for serum samples

Table 1: VZV IgG ELISA results for sera from normal children grouped by age

	Age range in years (% of total)						
Result	1<3	3<5	5<7	7<9	9<11	11<13	13<15
+ +	29 (41%)	41 (44%)	57 (65%)	69 (76%)	47 (73%)	63 (84%)	64 (86%)
+ -	0	0	2 (2%)	0	0	0	0
- +	8 (11%)	12 (13%)	5 (6%)	6 (7%)	2 (3%)	3 (4%)	5 (7%)
- -	34 (48%)	40 (43%)	24 (27%)	16 (17%)	15 (24%)	9 (12%)	5 (7%)
Total	n = 71	n = 93	n = 88	n = 91	n = 64	n = 75	n = 74

++: Positive by SBR and cut-off, + - : Positive by SBR and negative by cut-off, - +: Negative by SBR and positive by cut-off, - -: Negative by SBR and cut-off

Table 2: Distribution of VZV IgG avidity indices in sera from normal children

	20 mM DEA			35 mM DEA			70 mM DEA		
Result	≤30%	31-50%	≥51%	≤30%	31-50%	≥51%	≤30%	31-50%	≥51%
+ +	27	37	306	28	73	269	35	101	234
n = 370	(7.3%)	(10%)	(83%)	(7.6%)	(20%)	(73%)	(9.5%)	(27%)	(63%)
+ -	2	0	0	2	0	0	2	0	0
n = 2	(100%)			(100%)			(100%)		
- +	18	1	22	15	5	21	21	6	14
n = 41	(44%)	(2.4%)	(54%)	(37%)	(12%)	(51%)	(51%)	(15%)	(34%)
- -	139	4	2	139	4	0	141	2	0
n =143	(97%)	(2.8%)		(97%)	(2.8%)		(99%)	(1.4%)	

Each value is number of sera having the given ELISA ratio and cut-off results and an IgG avidity index in the specified range. Results in order ratio then cut-off

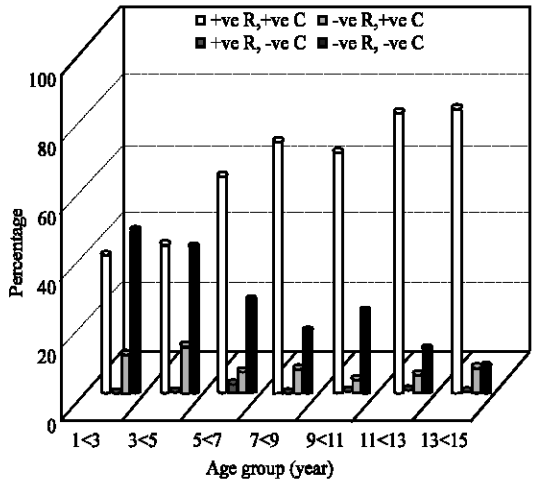

Fig. 2: Age-related distribution of VZV IgG antibodies amongst normal children footnote: R represents SBR and C denotes to cut-off

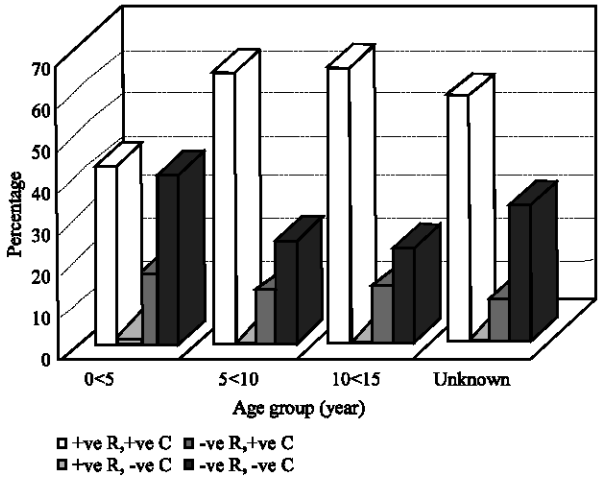

Fig. 3: Age-related distribution of VZV IgG antibodies within leukaemic children footnote: R represents SBR and C denotes to cut-off

Table 3: Age distribution of VZV IgG ELISA results for children with leukaemia

Result	Up to 5 years	5<10 years	10<15 years	Unknown age
+ +	38 (42%)	27 (64%)	15 (65%)	17 (58%)
+ -	1 (1.5%)	0	0	0
- +	15 (16.5%)	5 (12%)	3 (13%)	3 (10%)
- -	36 (40%)	10 (24%)	5 (22%)	9 (32%)
Total	90	42	23	29

+ +: Positive by SBR and cut-off, + - : Positive by SBR and negative by cut-off, - + : Negative by SBR and positive by cut-off, - - : Negative by SBR and cut-off

indices than those solely by ratio or doubly negative ($p<0.001$) and a similar difference was seen between the latter two categories of sample ($p<0.001$). Interestingly, for doubly positive serum specimens, the avidity indices increased with age, with almost the entire increase between 1<3 and 3<5 years at 70 mM DEA ($p<0.001$) and the main increase between 1<3 and 7<9 years at 20 mM DEA ($p<0.001$).

Screening and IgG avidity measurement of sera from leukaemic children: As described earlier, sera from paediatric leukaemia patients were the next set of samples subjected to screening and avidity assay by VZV ELISA as already done for normal children.

The proportion of sera giving doubly positive or doubly negative results respectively increased or decreased between ages <5 and >5 years ($p<0.01$ or $p<0.05$) but there was no parallel change with age for sera positive only by cut-off (Table 3). Figure 3 shows distribution of VZV IgG antibodies amongst leukaemic children.

Distribution of avidity indices in sera from leukaemic children: As distribution of avidity indices were measured for normal children it was also done for leukaemic children as shown in Table 4. Serum specimen positive by SBR and cut-off had avidity indices which were mostly ≥51% and higher with 20 mM rather than 70 mM DEA ($p<0.02$). The antibody avidity with 35 mM DEA was intermediate between that for the other two denaturant concentrations but not significantly different

Table 4: Distribution of VZV IgG avidity indices in sera from leukaemic children

	20 mM DEA			35 mM DEA			70 mM DEA		
Result	≤30%	31-50%	≥51%	≤30%	31-50%	≥51%	≤30%	31-50%	≥51%
++ 8	19	70	14	22	61	19	24	54	
n =97	(8%)	(20%)	(72%)	(14%)	(23%)	(63%)	(20%)	(25%)	(56%)
+ - 0	0	1	0	0	1	1	0	0	
n = 1			(100%)			(100%)	(100%)		
- + 11	1	14	14	5	7	6	7	13	
n = 26	(42%)	(4%)	(54%)	(54%)	(19%)	(27%)	(23%)	(27%)	(50%)
- - 57	2	1	57	1	2	57	1	2	
n =60	(95%)	(3%)	(2%)	(95%)	(2%)	(3%)	(95%)	(2%)	(3%)

Footnote: Each value is number of sera having the given ELISA ratio and cut-off results and an IgG avidity index in the specified range. Results in order ratio then cut-off

Table 5: VZV ELISA and avidity results on single sera from patients with VZV associated disease

	ELISA results		Avidity index by DEA (%)			
Patient's No.	Ratio	Cut-off	20 mM	35 mM	70 mM	Diagnosed disease
1	130.00	+ ve	57	65	33	Chickenpox
2	10.30	+ ve	40	51	21	Chickenpox
3	1.20	+ ve	40	70	34	Chickenpox
4	7.80	+ ve	72	54	63	Chickenpox
5	16.50	+ ve	73	52	38	Chickenpox
6	1.10	+ ve	73	69	46	Chickenpox
7	3.40	+ ve	67	72	60	Chickenpox
8	14.50	+ ve	19	34	10	Chickenpox
9	20.40	+ ve	64	30	26	Chickenpox
10	9.40	+ ve	27	28	24	Chickenpox
11	41.00	+ ve	66	63	51	Chickenpox
12	244.00	+ ve	79	71	63	Chickenpox
13	20.50	+ ve	40	28	17	Chickenpox
14	14.00	+ ve	24	<10	<10	Chickenpox
15	27.20	+ ve	77	63	57	Zoster
16	19.50	+ ve	61	64	49	Zoster
17	59.00	+ ve	74	53	34	Zoster
18	6.30	+ ve	72	70	55	Zoster
19	49.00	+ ve	72	67	58	Zoster
20	62.00	+ ve	62	60	53	Zoster
21	79.00	+ ve	63	59	48	Zoster
22	9.03	+ ve	70	53	55	Zoster
23	14.00	+ ve	68	61	57	Zoster
24	38.00	+ ve	71	75	59	Perineal rash
25	30.50	+ ve	74	61	62	Perineal rash
26	27.60	+ ve	70	65	68	Steven's Johnson
27	25.00	+ ve	65	69	53	Steven's Johnson
28	11.60	+ ve	68	53	51	Encephalitis
29	8.50	+ ve	69	51	46	Encephalitis
30	91.80	+ ve	73	67	75	Palatal palsy
31	137.00	+ ve	68	64	57	Palatal palsy
32	101.00	+ ve	77	62	58	Palatal palsy

from either. For sera positive by cut-off alone the avidities decreased between 20 and 35 mM DEA ($p<0.05$) but increased between 35 mM and 70 mM DEA ($p<0.05$), so that overall there was no change in avidity from 20 to 70 mM DEA ($p>0.1$).

No change in avidity with any DEA concentration was detected for samples negative by both methods of results calculation. The avidity indices for these sera were mostly low (<30%) and lower than when the results were doubly positive ($p<0.001$) or positive by cut-off alone ($p<0.001$). Sera positive by cut-off but either negative or positive by ratio showed similar avidities with 70 mM DEA, but the former had the lower avidities at 35 mM ($p<0.001$) and 20 mM DEA ($p<0.001$).

Role of IgG avidity in distinguishing varicella from zoster: As mentioned above avidity assay is a suitable method of differentiation between recent and remote viral infections, this is also applicable in case VZV IgG avidity. For this reason, a set of sera belonging to different patients all suffering from VZV associated diseases including varicella, zoster and encephalitis and so on were collected and subjected to screening and avidity measurement (Table 5). Unfortunately, no dates of onset were available for chickenpox patients.

The SBRs were marginally higher with zoster [6/9 (67%) >16] than with varicella [6/14 (43%) >16, $p>0.1$]. Furthermore, avidities were higher with zoster than varicella however, no set of avidity indices were

absolutely characteristic of either disease. For example with 20 mM DEA an index of >50% had a sensitivity of 100% for zoster but a positive predictive value for the same disease of only 9 (53%) of 17. At 70 mM DEA an avidity of <30% had sensitivity of 43% (6/14) and a positive predictive value of 100% (6/6) for varicella. If all three avidities were >50% the sensitivity and positive predictive value for zoster were 67% (6/9) and 60% (6/10). Two or three rather than one avidity index of <30% provided no more sensitive or discriminatory marker for varicella.

DISCUSSION

Optimization of in-house ELISA: In the optimization process of the ELISA for VZV IgG antibodies every step of the protocol had to be standardized. The best way of standardization of an ELISA was checkerboarding. For this purpose three sets of sera were chosen for every step of optimization process; namely strong positive, weak positive and negative. Confirmation of the reactions of these sera was obtained from diagnostic laboratory for VZV. Therefore, checkerboarding was set up for every step including: antigen, serum, anti-IgG monoclonal antibody and enzyme conjugate dilutions. Besides, washing times both after coating the plates and after antibody binding were investigated however, the optimization of ELISA might have been further improved by using smaller dilution steps in checkerboards. The optimal protocol was chosen after careful consideration of the results of every checkerboarding and in particular where the best discrimination was observed between strong positive, weak positive and negative sera.

Choice of ELISA protocol: In previous studies, indirect ELISA has been proved efficient for the detection of antibodies to human herpesviruses (De Mattia *et al*., 1991; Shen *et al*., 1992; Yamanaka *et al*., 1992). The ELISA in this project was therefore indirect and avidine-biotin amplification increased its sensitivity. After adding biotinylated monoclonal antibody the plates were washed and avidine peroxidase was then added to the appropriate wells. By doing this unbound antigen and antibody were washed away before conjugate and subsequently substrate addition. The latter reduced the chances of false positivity and increased specificity and sensitivity.

Interpretation of results: As described earlier two methods of interpretation were employed for VZV IgG ELISA cut-off and SBR (Klapper *et al*., 1990) therefore both modes for the calculation of results were already mentioned. However, these two methods occasionally generated discrepant readings on the same serum or serial sera from the same patient. One cannot easily claim that one method is more acceptable than the other. The cut-off method had the theoretical advantage that the range for positive results theoretically excludes all but 0.05% of negative sera (Klapper *et al*., 1990). The choice of negative control sera was clearly crucial, a large number of sera (say 100) from persons never infected with the virus in question would have been ideal. These samples could have been tested repeatedly to establish the negative range of the test.

IgG avidity and choice of denaturant: Urea at 7 and 8 M has been widely used for avidity determination (Blackburn *et al*., 1991; Thomas *et al*., 1992; Ward *et al*., 1993). In this project 8 M urea was also assessed but unsatisfactory results were obtained besides making 8 M urea in the large quantities needed for this project was somewhat difficult and costly. Hence, DEA which was already employed by other workers (Thomas and Morgan-Capner, 1991; Thomas *et al*., 1993) was also evaluated at 20, 35 and 70 mM concentrations. DEA not only produced better results but also was more easily prepared and handled than 8 M urea. The avidity indices for sera from patients with recent primary or recurrent infection declined with increasing DEA concentration because of the effects of more powerful denaturation on antigen-antibody bonds of a given avidity. The greater decline in avidity with increasing DEA concentrations for sera collected after primary rather than recurrent VZV infection presumably reflected the lower avidities seen after primary infection. It was clear that testing with more than one DEA concentration was helpful for detection of differences in antibody avidity after primary or recurrent VZV infection and the maturation of that avidity after primary infection. For example, low avidity index (<30%) was always predictive of recent infection except when the denaturant was 70 mM DEA, but the association between recent recurrent infection and a high antibody avidity index (>50%) was strongest with the latter denaturant concentration.

In the presentation of avidity index results, they were mostly divided into 3 groups: ≤ 30% (band 1), 31-50% (band 2) and ≥ 51% (band 3). Samples with an avidity index of ≤ 30% were considered to indicate low avidity, samples with indices of 31-50% were regarded as showing intermediate avidity and samples with high avidity indices were those with indices ≥ 51% (Thomas and Morgan-Capner, 1991). In addition avidity of <10% or >70% were considered to represent very low or very high antibody avidity.

Sensitivity of VZV IgG ELISA: In order to confirm the accuracy of the in-house VZV IgG ELISA 55 samples that had been already tested by commercial ELISA and in-house FAMA for VZV IgG antibodies were examined using the in-house ELISA. Most samples [40/55 (73%)] gave identical results (positive = 32 and negative = 8) in all three tests. The in-house ELISA proved to be marginally more sensitive than the in-house FAMA, but there was only one possibly false positive FAMA result confirming the high specificity of the latter assay. The commercial ELISA appeared to generate both false positive and false negative results, perhaps because it included no cell control antigen and lacked avidin-biotin amplification step. All the antibody avidity evidence confirmed that the in-house ELISA was more sensitive than both the other assays and that it was of higher specificity than the commercial ELISA.

Age-related changes in VZV IgG seropositivity and avidity in normal and leukaemic children: The peak period of primary infection has been reported as either 2-6 years (Jawetz *et al*., 1991) or 4-10 years (Kangro and Harper, 1995) with approximately 10% of adults remaining susceptible. The seroprevalence data reported here indicated that most primary VZV infections occurred in children aged 3-7 years, but infection continued to occur until 11<13 years. This applied irrespective of whether seropositivity was defined as reactivity by both SBR and cut-off or by either approach alone in the VZV IgG ELISA. In children aged 13>15 years the seropositivity rate was approximately 90%. Again, the VZV avidity changes with age were those expected if most primary infections occurred in children aged 3-7 years and these changes were similar in sera giving ELISA positive readings by SBR and cut-off or by both methods of results calculation. This supported the view that VZV seropositivity could be defined as reactivity in the IgG ELISA detectable by either means of results calculation.

Comparison of the prevalence of antibodies in normal and leukaemic children was made difficult by the different groupings of the ages of the populations. Nonetheless, it was clear that there was little effect of the cancer on the prevalence of VZV IgG antibodies. For instance, the prevalences of SBR and cut-off positive VZV ELISA results virtually the same among normal and leukaemic children in age groups 1<5 or 0<5 years [120/164 (43%) versus 38/90 (42%)] and 5<9 or 5<10 years [126/179 (70%) versus 27/42 (64%)]. In the age group 11<15 years or 10<15 years for the two groups of children, a difference in VZV seroprevalence documented by doubly positive ELISA readings was noted [127/149 (85%) versus 17/29 (59%), $p<0.001$]. This might have merely reflected the increase in VZV seroprevalence with age and the greater age of the first of the latter two groups.

CONCLUSION

The accuracy of in-house ELISA was evident after assessment of the assay by different modes so these proved in-house ELISA reproducible. Specific IgG avidity assay can distinguish between primary and recurrent VZV infection. As expected almost all sera from patients with zoster and other VZV complications showed high avidity indices (>50%). Low avidity was predictive of recent varicella so the latter finding plays an important role in differentiation of recent from recurrent infection.

REFERENCES

Arvin, A.M., C.M. Koropchak, B.R.G. Williams, F.C. Grumet and S.K.H. Foung, 1986. Early immune response in healthy and immunocompromised subjects with primary VZV infection. J. Infect. Dis., 154: 422-429.

Berek, C. and C. Milstein, 1987. Mutation drift and repertoire shift in the maturation of the immune response. Immunol. Rev., 96: 23-41.

Blackburn, N.K., T.C. Besselaar, B.D. Schoub and K.F. O'Connell, 1991. Differentiation of primary cytomegalovirus infection from reactivation using the urea denaturation test for measuring antibody avidity. J. Med. Virol., 33: 6-9.

Cleveland, P.H. and D.D. Richman, 1987. Enzyme immunofiltration staining assay for immediate diagnosis of HSV and VZV directly from clinical specimens. J. Clin. Microbiol., 25: 416-420.

Davison, A.J. and J.E. Scott, 1986. The complete DNA sequence of VZV. J. Gen. Virol., 67: 1759-1816.

De Mattia, D. and T. Stroffolini *et al*., 1991. Prevalence of cytomegalovirus infection in Italy. Epidemiol. Infect., 107: 421-27.

Gelb, L.D., 1993. Varicella-zoster Virus. In: The Human Herpesviruses. Roizman B., R.J. Whitley and C. Lopez (Eds.), 1st Edn., Raven Press, New York, pp: 257-308.

Gray, J.J., 1995. Avidity of EBV VCA-specific antibodies; distinction with recent primary infection, past infection and reactivation. J. Virol. Methods, 52: 95-104.

Gut, J.P., C. Lablanche, S. Behr and A. Kirn, 1995. Symptomatic mumps virus reinfections. J. Med. Virol., 45: 17-23.

Jawetz, E., J.L. Melnick and E.A. Adelberg, 1991. Herpesviruses. In: Medical Microbiology. Brooks, G.F. and J.S. Bute *et al.* (Eds.), 19th Edn., Appleton and Lange, pp: 418-440.

Kangro, H.O. and D.R. Harper, 1995. Varicella-Zoster. In: Principles and Practice of Clinical Virology. Zuckerman, A.J., J.E. Bantavala and J.R. Pattison (Eds.), 3rd Edn., John Wiley and Sons Ltd., pp: 37-67.

Klapper, P.E., G.M. Cleator, D. Prinja-Wilks, D.J. Morris, and G. Morrel, 1990. Immunoradiometric assay for cytomegalovirus specific IgG antibodies; Assay development and evaluation in blood transfusion practice. J. Virol. Methods, 27: 327-340.

Moulignier, A., G. Pialoux, H. Dega, B. Dupont, M. Huerre and M. Baudrimout, 1995. Brain stem encephalitis due to VZV in a patient with AIDS. Clin. Infect. Dis., 20: 1378-1380.

Ory, F.D., J. Antonaya, M.V. Fernandez and J.M. Echevarria, 1993. Application of low-avidity IgG studies to diagnosis of EBV infectious mononucleosis. J. Clin. Microbiol., 31: 1669-1671.

Preblud, S.R., W.A. Orenstein and K.J. Bart, 1984. Varicella; clinical manifestation, epidemiology and health impact in children. Pediatr. Infect. Dis., 3: 505-509.

Shen, C.Y., W.W. Chang, S.F. Chang, M.F. Chao, E.S. Huang and C.W. Wu, 1992. Seroepidemiology of CMV infection among children between the ages of 4 and 12 years in Taiwan. J. Med. Virol., 37: 72-75.

Soderlund, M., C.S. Brown, B.J. Cohen and K. Hedman, 1995. Accurate diagnosis of B19 infection by measurement of IgG avidity. J. Infect. Dis., 171: 710-713.

Thomas, H.I.J. and P. Morgan-Capner, 1991. Rubella-specific IgG1 avidity: A comparison of methods. J. Virol. Methods, 31: 219-128.

Thomas, H.I.J., P. Morgan-Capner, A. Roberts and L. Hesketh, 1992. Persistent rubella-specific IgM reactivity in the absence of recent primary rubella and rubella reinfection. J. Med. Virol., 36: 188-192.

Thomas, H.I.J., P. Morgan-Capner and N.S. Connor, 1993. Adaptation of a commercial rubella-specific IgG kit to assess specific IgG avidity. Serodiagn Immuno. Ther. Infect. Dis., 1: 13-16.

Thomas, H.I.J., 1995. Specific antibody avidity studies in clinical microbiology: Past, present and future. PHLS Microbiol. Digest, 12: 97-102.

Ward, K.N., J.J. Gray, M.W. Fotheringham and M.J. Sheldon, 1993. IgG antibodies to HHV-6 in young children: Change in avidity of antibody correlate with time after infection. J. Med. Virol., 39: 131-138.

Yamanaka, T. and K. Kiuoyani *et al.*, 1992. Detection of cytomegalovirus in urine samples by an enzyme linked immunosorbent assay using a monoclonal antibody against the viral 150 kilo Dalton protein. J. Clin. Microbiol., 30: 685-690.