Epidem. Inf. (1988), **101**, 187–195
Printed in Great Britain

Detection of specific IgM in varicella and herpes zoster by antibody-capture radioimmunoassay

By H. O. KANGRO, A. WARD, S. ARGENT, R. B. HEATH
Department of Virology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE

J. E. CRADOCK-WATSON and MARGARET K. S. RIDEHALGH
Public Health Laboratory, Withington Hospital, Manchester M20 8LR

(*Accepted 2 March 1988*)

SUMMARY

A simple and sensitive M antibody-capture radioimmunoassay (MACRIA) is described which utilizes crude commercial VZV antigen and a single monoclonal anti-VZV antibody. This was compared to the immunofluorescence (IF) test for IgM antibody and was used to study IgM responses in sera from 261 patients with varicella and 220 patients with herpes zoster.

With MACRIA, IgM antibodies were detected in all patients with varicella. The IgM antibodies appeared shortly after onset of rash, reached peak levels between 1 and 4 weeks after onset and then declined to low or undetectable levels in most, though not all, patients after 3 months. IgM antibodies were also detected in 98·2% of patients with herpes zoster, but the levels of IgM were significantly lower than after varicella and there was wider individual variation both in magnitude and duration of the IgM responses, in some cases only lasting 2–3 weeks.

Comparison between MACRIA and IF showed good agreement in the detection of IgM antibodies following varicella. Discordant results were obtained with 13% of sera, of which 81% were taken either early or late after onset of rash and contained very low IgM levels. In contrast, 62 (28%) of the 220 sera from patients with zoster gave discordant results in the two tests, all except five being MACRIA-positive but IF-negative. The largest proportion of discordant results were obtained with sera taken more than 3 months after onset of rash, but 18 (29%) contained high IgM levels and were taken during the period of peak IgM responses. The diagnostic applications of the VZV MACRIA are discussed.

INTRODUCTION

Interest in varicella-zoster virus (VZV) infection has been stimulated by the increasing number of immunocompromised patients, in whom disease can be severe or even fatal, and by the possibilities of prevention by vaccination and of treatment with antiviral drugs. The serological investigation of VZV infections now demands methods which are more sensitive and specific than complement fixation, and which do not depend on detecting a rise in the titre of antibody.

Testing for specific IgM antibody has become an accepted method for the early serological diagnosis of many virus infections, but its value in the investigation of VZV infections has not yet been clearly established.

Most previous reports agree that IgM antibody can be detected in all cases of chickenpox, but there is disagreement about the response in patients with herpes zoster. Schmidt & Lennette (1975), who tested sucrose density-gradient fractions for neutralising activity, detected IgM in only 1 out of 22 cases (5%). Other workers have demonstrated IgM in 50–78% of cases by immunofluorescence (Ross & McDaid, 1972; Cradock-Watson, Ridehalgh & Bourne, 1979; Gershon *et al.* 1982) and in 69% by solid-phase radioimmunoassay (RIA) (Arvin & Koropchak, 1980). The antibody-capture technique, which has become the method of choice for detecting IgM antibody to many other viruses, was adapted for the detection of VZV IgM by Tedder, Mortimer & Lord (1981), who used a system in which wells coated with anti-IgM were reacted successively with the test serum, then with unpurified antigen, and finally with human immunoglobulin (from zoster convalescent serum) labelled with ^{125}I. They detected IgM antibody in 82% of cases of zoster, but unfortunately antigens from different sources have been found to vary in their suitability for use in this test. Forghani *et al.* (1984), using a pool of five monoclonal anti-VZV antibodies in an antibody-capture enzyme immunoassay, detected IgM in only 50% of cases and reported problems with non-specific binding which necessitated the use of a control antigen. It has been shown that non-specific binding in solid-phase RIA to detect IgG antibody to VZV can be reduced by using an antigen which has been purified by centrifugation on a sucrose density gradient (Clarke *et al.* 1984). However, the purification process is laborious and we have therefore developed an IgM antibody-capture radioimmunoassay (MACRIA) based on a selected monoclonal antibody to VZV which not only improves the detection of IgM but also allows the use of unpurified commercial complement-fixing antigen. We have compared this MACRIA with the immunofluorescence (IF) test for the detection of specific IgM in patients with varicella and herpes zoster.

MATERIALS AND METHODS

Viral antigens

Partially purified VZV, which was used as an antigen for the production of monoclonal antibodies, was prepared as described previously (Clarke *et al.* 1984). Briefly, VZV (Marsden strain) was harvested from infected cultures of human embryo lung (HEL) cells by treatment with trypsin/versene, concentrated, and then centrifuged on 10–50% (w/w) sucrose gradients at 50000 ***g*** for 1 h at 4 °C. The virus-containing fractions were identified by RIA (Campbell-Benzie, Kangro & Heath, 1981), mixed, and then dialysed against phosphate-buffered saline (PBS, pH 7·3). The virus was concentrated by centrifugation and the pellets were finally resuspended in PBS to $\frac{1}{10}$ of the original volume, sonicated at 20 kHz for 60 s and then stored in aliquots at −70 °C. Unpurified commercial antigen (Hoechst UK Ltd) was used in RIA tests for screening hybridoma cultures, in assays for measuring VZV IgG antibody and in the MACRIA for VZV IgM.

Serum specimens

The specimens, comprising 310 sera from 261 patients with varicella and 233 sera from 220 patients with herpes zoster, were selected from samples collected at the two collaborating laboratories. The clinical diagnosis was confirmed by detecting a rise in the titre of complement-fixing antibody, and/or by virus isolation.

Production of monoclonal antibody

Balb/C mice were inoculated intraperitoneally (i.p.) with 250 μl of purified VZV antigen in half-strength Freund's complete adjuvant, with booster doses given at 4 and 8 weeks. Spleen cells were harvested 3 days after the final dose. These were mixed with myeloma cells (JkAg8653) at a ratio of 5–7 : 1 and fused with 1 ml of 50% polyethylene glycol (PEG). After washing with serum-free medium the cells were resuspended in selective medium, containing hypoxanthine/aminopterine/thymidine (HAT), and seeded at a concentration of $3–5 \times 10^5$ viable cells per well into 96-well microplates containing feeder layers of macrophages (2×10^5 per well). The plates were incubated at 37 °C in 5% CO_2 and at days, 4, 8 and 12 the wells were re-fed with 0·1 ml HAT medium. At 14 days the medium was changed to medium plus hypoxanthine/thymidine (HT) and subsequent changes were with medium only. From about the third week the wells were assayed for antibody and the cells in positive wells were transferred to 24-well plates containing feeder layers. Hybridomas which produced antibody were cloned twice and then cultivated in 25 cm^2 bottles.

Ascitic fluid was produced in female Balb/C mice aged 12–14 weeks, primed with 'Pristane' 1 week prior to i.p. inoculation with $0·5–2 \times 10^6$ cloned hybridoma cells. The ascitic fluid was harvested 2–3 weeks later, clarified and stored at −20 °C. The antibody titres of ascitic fluids, measured by RIA, were in the range $10^6–10^7$.

Purification and radiolabelling of monoclonal antibody

The monoclonal antibody (2C2.6B) chosen for use in the MACRIA is directed against a viral structural protein of molecular weight 150–180 kDa, which is abundant in the nuclei of infected cells. This antibody showed no detectable cross-reaction with cellular proteins or with other herpesviruses, and blocking tests showed that strong antibody responses develop to the recognized epitope following infection with VZV (to be published). Mouse IgG was purified from ascitic fluid by ion-exchange chromatography on DE-52 (Whatman). The major antibody-containing fraction was concentrated (Minicon) to contain approximately 1·5 mg/ml protein and then radiolabelled with ^{125}I using 'Iodogen' (Salacinski *et al.* 1979). Labelled antibody was purified by gel filtration through a $0·9 \times 60$ cm column of Sephacryl S-300 (Pharmacia UK Ltd).

IgM antibody-capture radioimmunoassay (MACRIA)

Polystyrene beads were coated by overnight incubation at 4 °C with anti-human IgM (Dako Ltd) diluted 1 in 400 in 1 mM-HCl. After washing with PBS the coated beads were re-incubated overnight at 4 °C with PBS containing 1% bovine

serum albumin in order to block any unoccupied binding sites. Serum specimens, diluted 1 in 100 in PBS containing 0·1 % Tween 20 and 10 % normal rabbit serum (NRS), were dispensed in duplicate 200 μl volumes in 20-well reaction trays (Abbott Laboratories). Each test included positive serum standards containing 40, 10, 3·3 and 1 arbitrary units of VZV-specific IgM antibody, as well as a negative control serum without VZV antibody. The coated beads were added to the wells and the trays were incubated for 4 h at 37 °C. The beads were then washed twice with PBS-Tween. Volumes of 200 μl of VZV antigen, diluted 1/10 in PBS-Tween plus 10 % NRS, were added to each well and the plates were incubated overnight at room temperature. The beads were then washed again with PBS-Tween. Labelled monoclonal antibody was diluted in PBS containing 0·2 % Tween, 15 % NRS and 15 % human serum (lacking antibody to VZV and herpes simplex virus) to give approximately 50000 counts per min in a volume of 200 μl. This dilution was added to the wells in 200 μl volumes and the test was incubated at 37 °C for 4 h. The beads were then washed as before and the radioactivity was counted for 10 min. The mean number of counts minus the background was calculated for each serum and the IgM unitage was determined from a calibration curve constructed from the standards as described previously (Tedder, Mortimer & Lord, 1981).

Other serological tests

The immunofluorescence test for VZV IgM antibody was performed as described previously (Cradock-Watson, Ridehalgh & Bourne, 1979). Briefly, fixed cover-slip cultures of MRC-5 cells infected with VZV were treated first with dilutions of serum and then with a working dilution of fluorescein-labelled globulins prepared against human IgM (Wellcome Diagnostics).

The RIA for assaying VZV IgG antibody in human serum (Campbell-Benzie, Kangro & Heath, 1981) was adapted for screening mouse hybridoma cultures by using ^{125}I-labelled anti-mouse IgG and IgM antibodies (Tago Inc., Tissue Culture Services Ltd).

RESULTS

We initially tried to establish a VZV MACRIA based on the work of Tedder, Mortimer & Lord (1981), using unpurified antigen and labelled human immunoglobulin from zoster convalescent serum, but our results were frequently unsatisfactory because of non-specific binding. This problem was largely eliminated by using antigen purified on a sucrose density gradient (Clarke *et al.* 1984). A comparison of the modified test with IF (not reported here) showed that it detected IgM in sera taken at optimum times from patients with varicella or zoster, but did not always detect lower levels of antibody in specimens taken soon after the onset or beyond the first month – particularly from cases of zoster. The use of a monoclonal antibody to VZV in place of the polyclonal human antibody not only increased the sensitivity but also permitted the use of crude antigen preparations without the problem of high background binding. The results presented below were obtained in the MACRIA with radiolabelled anti-VZV monoclonal antibody and unpurified commercial VZV antigen.

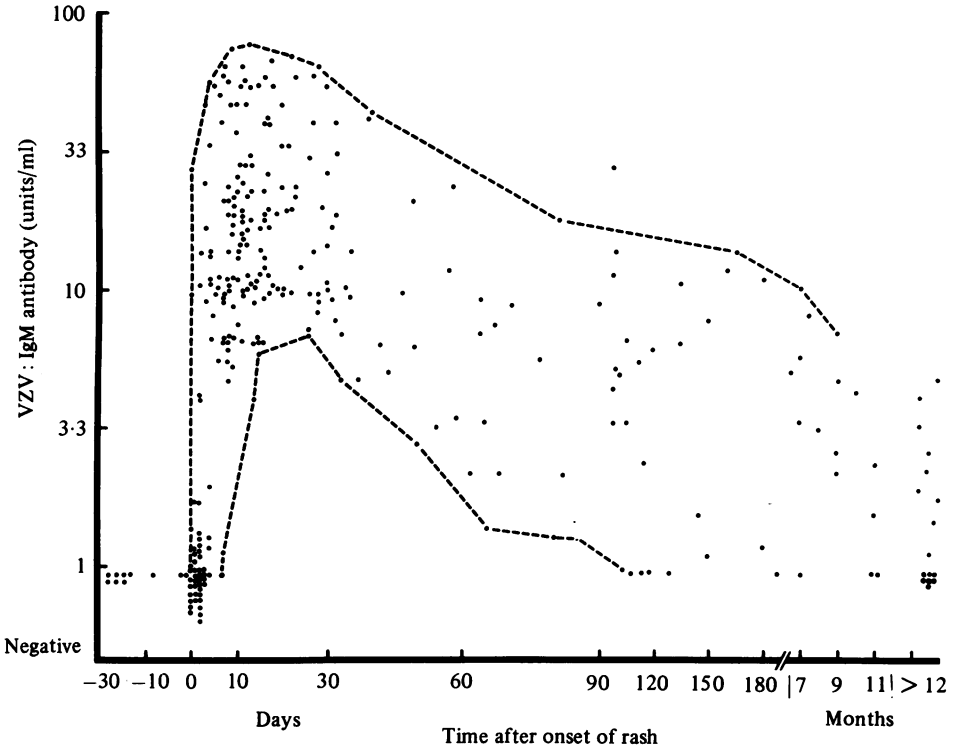

Fig. 1. VZV IgM antibody levels measured by MACRIA in sera from patients with varicella. The two dots outside the norm line represent sera from an individual with an exceptionally prolonged IgM response.

IgM response in varicella

IgM antibody was detected in all 261 patients (Fig. 1). It was not found in any of the sera taken before the onset of the rash, but was present in 3 out of 11 sera (27%) taken on the first day and in 28 of 49 sera (57%) taken 1–4 days after the onset. All 183 sera taken 5–85 days after the onset were IgM-positive. The response reached a peak 7–30 days after the rash, with IgM levels ranging from 5 to 80 units. Thereafter the levels declined, and the first negative results were obtained 100 days after the rash. However, there was wide individual variation, and even 6–12 months after the rash 9 out of 16 patients (56·3%) had between 1 and 10 units of IgM.

IgM response in herpes zoster

IgM antibody was detected in 216 out of 220 patients (98·2%). Its appearance was closely associated with the onset of the rash (Fig. 2). IgM was not detected in specimens taken before the rash, but was present in 6 out of 30 sera (20%) taken during the first 2 days and in 30 out of 52 sera (57·7%) taken 3–7 days after the onset. As in varicella, the IgM responses reached a peak in 7–30 days. During this period some patients had IgM levels as high as those seen after varicella, but the geometric mean was lower (12·3 units compared with 21) and the range was wider. IgM was undetectable in 4 of 81 (4·9%) patients tested 7–30 days after zoster, and

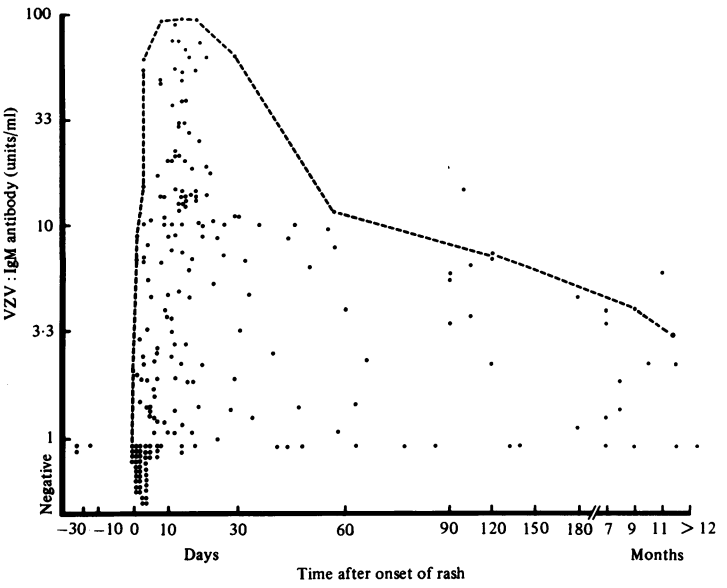

Fig. 2. VZV IgM antibody levels measured by MACRIA in sera from patients with zoster. The two dots outside the norm line represent sera from an individual with an exceptionally prolonged IgM response.

the sera from a further 9 patients (11·1 %) contained only 1–3·3 units during this period. The IgM levels generally declined after the first month but there was wide individual variation, and low levels were still present in 10 out of 13 sera (77 %) taken 6–12 months after the rash. In general, patients with strong initial responses showed persistence of IgM for over 3 months, albeit at low levels, whereas others had briefer and weaker responses lasting only 2–3 weeks. In some patients these weak responses were not detected until after the second week, and thus the highest proportion of IgM-positive sera (94·4 %) was obtained 2–6 weeks after the rash.

Comparison of MACRIA and IF

Altogether 261 of the sera from patients with varicella were tested by both MACRIA and IF . Although MACRIA units and IF titres correlated poorly, the two tests showed good agreement in distinguishing between IgM-positive and-negative sera. Only 34 specimens (13 %) gave discordant results of which 32 were MACRIA-positive but IF-negative. Of these MACRIA-positive sera, 26 (81 %) contained less than 5 units of IgM and were taken either soon after the onset of the rash or late in convalescence; 6 (19 %) contained more than 5 units and were taken between 7 and 28 days after the onset. The two MACRIA-negative sera had IF titres of 8 and were taken on the day of onset and at 61 days.

Discordant results were more numerous in sera from patients with zoster and occurred in 62 out of 220 specimens (28 %), of which all except 5 were MACRIA positive but IF-negative. The results with these 62 sera are shown in Fig. 3. The majority contained less than 5 units of IgM but 18 (29 %) contained 5–28 units. Discordant results occurred throughout the period of study, with a distribution of

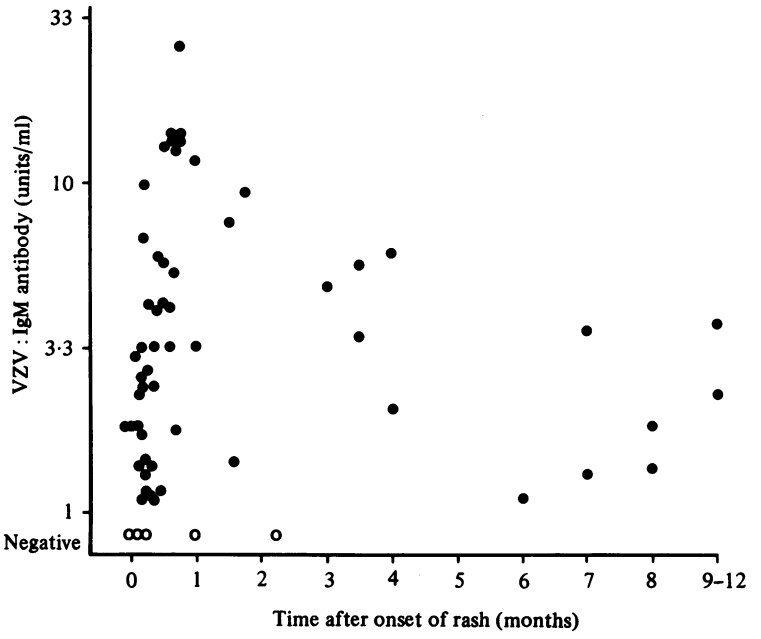

Fig. 3. MACRIA results with 62 sera giving discordant results in comparison with IF. Open circles denote MACRIA-negative/IF-positive sera.

titres similar to that of all zoster sera tested. The largest proportion occurred 3 or more months after the onset, when 12 out of 21 (57%) of the MACRIA-positive sera were IF-negative.

DISCUSSION

Indirect-type assays for antibody, in which antigen attached to a solid phase is reacted first with the test serum and then with a labelled reagent directed against human immunoglobulin, are usually satisfactory for detecting IgG antibody but not ideal for IgM, because the results can be either depressed by the preferential attachment of IgG or rendered falsely positive by IgM with anti-IgG activity. The antibody-capture method largely avoids these problems and has the additional advantage that the antibody level, which depends on the proportion of the total IgM that is specific, can be determined by testing a single dilution of serum (Brown, 1986). The use of a negative control antigen is not usually necessary, in contrast to the indirect-type tests.

Application of the IgM antibody-capture test to VZV infection has hitherto been hindered by extensive non-specific binding, due to the use of crude sonicated cell extract as an antigen. This problem can be minimized by using a purified antigen or a negative control antigen (Forghani *et al.* 1984) and by testing higher dilutions of serum, but at the cost of sacrificing two of the main advantages of the antibody-capture system. The MACRIA described here, based on a single selected monoclonal antibody to the virus, enabled us to use crude commercial antigen and a single dilution of the serum to be tested. An IgM response was detected in all

patients with varicella and followed the same course as in other acute virus infections, appearing shortly after the onset of symptoms, reaching a peak during the first month and declining to a low or undetectable level after 3 or more months. The MACRIA and IF tests both detected IgM in sera taken at optimum times, but the greater sensitivity of MACRIA enabled us to detect IgM in some additional specimens taken early or late when the levels were low. Two MACRIA-negative sera appeared to have IF titres of 8, but such weak IF reactivity is difficult to accept with confidence.

An IgM response was detected by MACRIA in 98% of patients with zoster but its height and duration were more variable than after varicella. In many patients the IgM response resembled that in varicella, but generally it was of significantly lower magnitude and more transient. Thus the diagnostic usefulness of an IgM test for confirmation of zoster depends greatly on the sensitivity of the test and the timing of the serum in relation to the onset of symptoms. These factors probably also account for the disagreement in the literature about the proportion of patients with zoster who produce IgM antibodies. In our study, also, discordant results, usually MACRIA-positive/IF-negative, were more frequent than after varicella and often occurred during the period of the peak response. It is unlikely that they were due solely to the lower IgM levels generally seen in the zoster patients and to the greater sensitivity of MACRIA, since many of the sera contained 5–28 units of IgM. Fluorescent staining with these specimens may possibly have been depressed by competition from IgG antibody of high avidity. From our data it appears that an IgM test can be used for confirmation of VZV infection, although the presence of IgM antibody in almost all cases of zoster clearly limits the ability of the laboratory to distinguish between primary infection and reactivation. Also, as with other virus infections, the persistence of low IgM levels for up to 1 year or more is not uncommon, and the incidence of asymptomatic VZV reactivation with IgM production is not known. Nevertheless we have found the IgM assay to be of value in confirming the clinical diagnosis on single specimens, especially from immunocompromised patients, for retrospective diagnosis during pregnancy and in hospital outbreak situations. Its usefulness for the diagnosis of congenital VZV infection is currently being investigated.

REFERENCES

ARVIN, A. M. & KOROPCHAK, C. M. (1980). Immunoglobulins M and G to varicella-zoster virus measured by solid phase radioimmunoassay: antibody responses to varicella and zoster infections. *Journal of Clinical Microbiology* **12**, 367–374.

BROWN, D. W. G. (1986). Viral diagnosis by antibody capture assay. In *Public Health Virology: 12 Reports* (ed. P. P. Mortimer), p. 92 London: Public Health Laboratory Service.

CAMPBELL-BENZIE, A., KANGRO, H. O. & HEATH, R. B. (1981). The development and evaluation of a solid-phase radioimmunoassay (RIA) procedure for the determination of susceptibility to varicella. *Journal of Virological Methods* **2**, 149–158.

CLARKE, J. R., RONALDS, C. J., KANGRO, H. O. & HEATH, R. B. (1984). Improving the varicella-zoster virus: IgG radioimmunoassay procedure by the use of purified antigen. *Journal of Virological Methods* **8**, 1–8.

CRADOCK-WATSON, J. E., RIDEHALGH, M. K. S. & BOURNE, M. S. (1979). Specific immunoglobulin responses after varicella and herpes zoster. *Journal of Hygiene* **82**, 319–336.

FORGHANI, B., MYORAKU, C. K., DUPUIS, K. W. & SCHMIDT, N. J. (1984). Antibody class capture assays for varicella-zoster virus. *Journal of Clinical Microbiology* **19**, 606–609.

GERSHON, A. A., STEINBERG, S. P., BORKOWSKY, W., LENNETTE, D. & LENNETTE, E. (1982). IgM to varicella-zoster virus: demonstration in patients with and without clinical zoster. *Paediatric Infectious Diseases* **1**, 164–167.

ROSS, C. A. & MCDAID, R. (1972). Specific IgM antibody in serum of patients with herpes zoster infections. *British Medical Journal* iv, 522–523.

SALACINSKI, P., HOPE, J., MCLEAN, C., CLEMENT-JONES, V., SYKES, J., PRICE, J. & LOWRY, P. J. (1979). A new simple method which allows theoretical incorporation of radio-iodine into proteins and peptides without damage. *Journal of Endocrinology* **81**, 131.

SCHMIDT, N. J. & LENNETTE, E. H. (1975). Neutralising antibody responses to varicella-zoster virus. *Infection and Immunity* **12**, 606–613.

TEDDER, R. S., MORTIMER, P. P. & LORD, R. B. (1981). Detection of antibody to varicella-zoster virus by competitive and IgM-antibody capture immunoassay. *Journal of Medical Virology* **8**, 89–101.