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DETECTION OF MEASLES-SPECIFIC IgM ANTIBODIES: COMPARISON OF 2-MERCAPTOETHANOL TREATMENT, DENSITY GRADIENT CENTRIFUGATION, PROTEIN A-SEPHAROSE AFFINITY CHROMATOGRAPHY, ION-EXCHANGE CHROMATOGRAPHY AND HAEMADSORPTION TECHNIQUES

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Summary. — The sensitivity, specificity and effectivity of 5 methods for detection of measles-specific IgM antibodies were compared. A total of 371 sera from non-immunized as well as immunized measles patients was included into the study. The highest positive rate was achieved by the haemadsorption-immunosorbent (HIST) test. The density gradient (DG) centrifugation and the ion-exchange chromatography (IERCR) were 10—15% less effective. Protein A-Sepharose affinity chromatography (ACR) and 2-mercaptoethanol (2-ME) treatment showed positive results in the half of samples which had been positive in the HIST. The titres were in significant correlation ($r = 0.90$ to $r = 0.8793$ at $P = 0.01$). Specificity and reproducibility of the tests were good. Rheumatoid factor did not influence the results. In general, HIST was found as most sensitive and effective.

Key words: measles; IgM class antibody; serum fractionation; capture immunoassay

Introduction

Detection of IgM class antibodies has become of increased importance in serological work. It allows the diagnosis of acute virus infection in a single serum sample early at the onset of disease (Doerr *et al.*, 1980; Roggendorf *et al.*, 1980; Kangro *et al.*, 1984). This is of interest not only in single cases, but also by measles under the conditions of vaccination (Schluederberg *et al.*, 1973; Smith *et al.*, 1982; Nagy *et al.*, 1984). IgM determination was of special interest in our analyses of measles outbreaks among vaccinees (Gerike *et al.*, 1982; 1986). In order to improve the vaccination strategy it was necessary to know whether the vaccinated persons with serologically confirmed measles had a primary or a secondary immune response. The recommended technique should be not only specific and sensitive, but also suitable for

screening of many serum samples. Having this in mind, the following procedures have been performed and compared:

- 2-mercaptoethanol (2-ME) treatment of total serum before haemagglutination inhibition test (HIT)
- separation of IgG and IgM by density gradient (DG) centrifugation, by protein A-Sepharose affinity chromatography and by ion-exchange chromatography with consecutive examination of the IgM fraction in HIT before and after 2-ME treatment
- haemadsorption immunosorbent test (HIST).

Materials and Methods

Serum samples. A total of 371 paired serum samples was collected from patients with confirmed measles. The sera were taken at the beginning of disease (sample 1) and 14 days later (sample 2); they were stored at −20 °C. Before testing, the sera were inactivated at 56 °C, the nonspecific agglutinins were removed by overnight incubation with 2.5% monkey erythrocytes at 4 °C.

Virus antigens. Measles virus strain Boston grown in FL cells and split by ether treatment (Norrby, 1962) was used as antigen.

Microhaemagglutination inhibition test (HIT). The pretreated sera were tested by the standard HIT using 4 haemagglutinating (HA) units of virus and 0.5% monkey erythrocytes (*Cercopithecus aethiops*). Inhibition was regarded for positive from the dilution $\geqslant$ 1 : 8. A difference of $\geqslant$ 2 steps in the titre of sample 1 and 2 was assumed to confirm the clinical diagnosis. Serum fractions obtained by the three separation methods were tested starting at a dilution 1 : 2 and using 1 HA unit to enhance the sensitivity of HIT.

2-ME treatment. The serum samples pretreated as described above or the separated serum fractions were saturated with 2-ME to the concentration of 0.1 mol/l, incubated for 1 hr at 37 °C and finaly examined by HIT. Titre reduction by $\geqslant$ 2 dilution steps in comparison with the untreated sample indicated measles-specific IgM. Serum sample 1 was used for 2-ME treatment. All other procedures were performed with the sample 2.

Density gradient centrifugation. Separation of serum IgM was performed as described (Gerike *et al.*, 1982); 0.2 ml of serum diluted 1 : 2 was layered over a discontinuous sucrose gradient in 3 × 5 ml swing-out rotor (VAC 601, VEB Zentrifugenbau, G.D.R.) and centrifuged for 18 hr at 130 000 × g. To reduce the time consuming analysis of each fraction, only fractions previously shown to contain IgM were examined in HIT. The IgG rich fractions were tested in a pool.

Affinity chromatography (ACR). Based on the data of Field *et al.* (1980), protein A-Sepharose-CL 4B (Pharmacia F. CH., Sweden) was prepared in a vol of 1 ml. The gel was suspended in HEPES buffer (25 mmol/l, pH 6.2). Serum diluted 1 : 3 and with 0.1% Dextran blue (Serva, F. R. G.) was applied on the column in a vol of 0.15 ml and eluted with HEPES buffer. The eluate containing Dextran blue was collected in 3 fractions and examined by HIT. The gel was regenerated by washing with 1.25 ml of 1 mol/l acetic acid. After washing with HEPES buffer, the gel was again ready for use. If the buffer contained 0.1% sodium azide, the gel could be repeatedly used for up to 1 year. To test the adsorption capacity of the gel, the immunoglobulin content in selected serum samples was quantitatively determined before and after ACR fractionation by means of immunoplates (Tri-Partigen or LC-Partigen, Behring, F.R.G.).

Ion-exchange chromatography (IECR). The method described by Nagy *et al.* (1984) was further modified. QAE-Sephadex-A 25 (Pharmacia F. Ch., Sweden) was suspended in 30 mmol/l Tris-HCl buffer pH 8.8 to prepare a 1.5 ml column. Serum diluted 1 : 4 was applied in 0.1 ml vol. The IgG fraction was eluted with Tris-HCl buffer of increasing molarity and increasing pH value (3.5 ml of 120 mmol/l Tris-HCl pH 8.1 and then 2.5 ml of 210 mmol/l pH 7.7). By applying 0.5 ml of 0.3 mol/l Tris HCl of pH 6.6 the IgM fraction was eluted. After repeated elution with the same buffer, IgM was removed from the column. The fractions were tested for specific measles antibodies as described above.

Haemadsorption immunosorbent test (HIST). The capture technique described by several authors for detection of rubeola-specific IgM antibodies (Krech and Wilhelm, 1979; Denoyel

et al., 1981; Logt *et al.*, 1981) was modified for measles IgM determination. Polystyrene micro titration plates (KOH-I-NOOR, Č.S.S.R. or Greiner, F.R.G., U-shaped wells) were coated with goat antibody to human IgM (μ-chain specific, Behring, F.R.G.). The incubation of antiserum followed in dilution 1 : 500 (carbonate buffer pH 9.6, vol 0.1 ml) for 2 hr at 37 °C. Such plates could be stored for about 4 weeks at 4 °C. The coating solution was removed before use, the plates were dried by shaking, then 0.1 ml of 25 mmol/l HEPES buffer pH 6.2 containing 1% bovine serum albumin was added to each well and incubated for 1 hr at room temperature. The pretreated samples of the patients were added in two-fold dilution series starting from 1 : 40 or 1 : 80. After incubation at 37 °C for 2 hr, the plates were washed in cold tap water, dried and then the antigen was added (2—3 HA units in 25 μl per well). After incubation at 4 °C overnight a 0.07% monkey erythrocyte suspension (prepared according to photometric measurements) was added in 0.1 ml vol, shaked and incubated for 3 hr room temperature. After centrifugation of the plates for 10 min at 80 × g the results were red either immediately or 24 hr later. Haemadsorption was manifested by a thin, erythrocyte layer coating the surface of wells if the measles-specific IgM was present in sera diluted 1 : 40—80. The test was regarded as negative, if the erythrocytes formed a firm knob-like sediment at the bottom of the well.

Rheumatoid factor (RF). The latex agglutination test (DAB 7, SSW Dresden, G.D.R.) was performed to evaluate the possible influence of RF on the results of individual tests.

Results

The examination of 63 sera from measles patients for IgM after fractionation in density gradient, by affinity and ion-exchange chromatography showed in 43 cases (68.3%) corresponding results (Table 1). Comparing the sucrose gradient (SG) with IECR, the agreement was considerably higher (90.4%). The ACR yielded two times more negative results than SG separation or IECR. When comparing the geometric mean titres (GMT) of the IgM fractions prepared by the three fractionation methods, the lowest titre were found after ACR (Fig. 1). The GMT of IgM fractions, which had been negative after ACR fractionation but positive after the other two separation techniques, were significantly lower (P = 0.01) than titres of the fractions of

Table 1. Results of measles-specific IgM detection by means of sucrose gradient centrifugation, ion-exchange and affinity chromatography and haemadsorption immunosorbent test in convalescent sera of measles patients

Samples No.	Procedure				
	SG	ACR	IECR	HIST	
26	+	+	+	+26	− 0
17	−	−	−	+ 6	−11
14	+	−	+	+14	− 0
4	−	−	+	+ 4	− 0
2	+	−	+	+ 2	− 0
63	+42	+26	+44	+52	−11
Positive rate	66.7%	41.3%	69.8%	82.5%	

+ positive result; − negative result

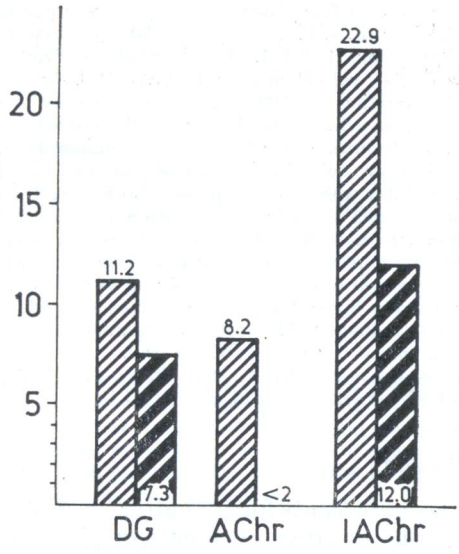

Fig. 1.
GMT in haemagglutination inhibition test for measles antibodies as found in IgM fractions after serum fractionation in sucrose gradient, by affinity and ion-exchange chromatography
Dashed columns (thin lines): GMT in the IgM fractions positive after DG, IECR (IAChr) and ACR (AChr).
Striated columns (thick lines): GMT in the IgM fractions positive after DG and IECR, but negative after ACR.
Ordinate: GMT in IgM fractions as determined by HIT

sera revealing anti-measles IgM also after ACR fractionation. The binding capacity of the protein A-Sepharose columns for IgG was in average 91.5%. The loss of IgM ranged widely from a minimum of 4.1% to the maximum of 73.4% (mean 44.5%).

The correlation between the IgM titres calculated after fractionation on SG, by ACR and IECR was significant (SG/ACR $r = 0.90$; SG/IECR $r = 0.89$ at $P = 0.01$). Reproducibility of titre determinations was good. The standard deviation from the GMT was 10% after SG and IECR separations and 18% after ACR.

Comparison of the results of IgM determination by HIST with SG, ACR and IECR showed that HIST was the most sensitive procedure (Table 1),

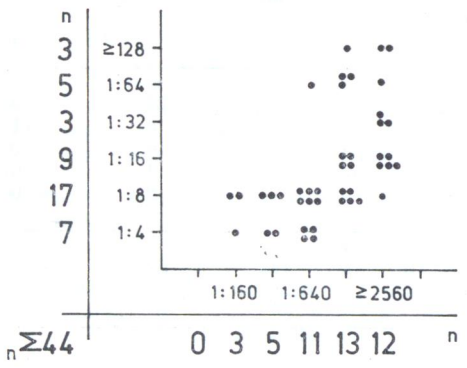

Fig. 2.
Measles antibody haemagglutination inhibition titres of IgM fractions prepared by ion-exchange chromatography as compared to titres of antimeasles IgM obtained by HIST
$r = 0.8793$
Abscissa: haemadsorption immunosorbent titre; ordinate: HIT titre of IgM fraction after IECR

which allowed to achieve the highest positive rate. The latter test confirmed in each case the positive results of other tests. The titres as determined by HIST were considerably higher (GMT = 1010.5) than the titres of IgM fractions after IECR (GMT = 13.9), the multiplication factor was 72.7. The correlation with r = 0.8793 was significant at P = 0.01 (Fig. 2). Relative low titres (1 : 80—160) in HIST were recorded even in sera which were IgM-negative after SG or after IECR separations; their HIT titre was usually about $\geqslant$ 1 : 128.

The specificity of HIST was examined as described by Logt *et al.* (1981). Only the samples containing IgM detectable after SG centrifugation revealed positive titres in HIST which were reduced by 2-ME treatment.

No influence of rheumatoid factor was found on the antibody titres determined by either method. RF positive sera were added to antimeasles IgM positive and also to antimeasles IgM negative sera. The virus-specific IgM titers were not changed in any case.

Out of 286 serum pairs which were included into the comparison of 2-ME effect and IECR results, the sample 1 showed negative HIT titres ($<$ 1 : 4) in 62 cases (21.7%). The serum sample 2 of these patients were positive for measles-specific IgM in 43 cases (69.4%). In the rest of 224 patients, 2-ME treatment of whole serum showed corresponding results with IECR in 51% of cases. The positive rate of 2-ME treatment decreased, if the serum sample 1 was taken later than 5 days from the onset of disease. In such case 2-ME treatment yielded positive results only in 22.1% of cases positive for IgM by IECR procedure. No false positive reaction was found after 2-ME treatment.

The time consume for individual testing methods varied considerably. For 2-ME treatment and HIST we needed the shortest time, which lasted longer by 1 or 2 hr than the HIT only. The additional time required for separation methods was 20 hr for SG centrifugation of 3 serum samples, 1.5 hr for ACR or IECR of 30 samples, respectively.

Discussion

To detect virusspecific IgM, 2-ME treatment of whole serum is the simplest and oldest routinely used technique (Banatvala *et al.*, 1967). Our results, in accordance with previous experience, confirmed that this method is succesful only under certain limited conditions. 2-ME splits the disulphidic bonds of IgM molecules converting them into immunologically inactive peptide chains. The significant titre difference between untreated and treated samples should be $\geqslant$ 2. To achieve effective reduction of the antibody titre as result of this procedure, virusspecific IgM must take about 75% of viral antibody. Such conditions in serum are fulfilled only for a short period of acute disease. Our data demonstrate the limited probability to meet exactly that interval in out-patients. Corresponding to this, the antibody detection rate is relatively low as already described by Forghani *et al.* (1973). The method is suitable, therefore, for rapid screening only when no other techniques are available.

From the separation techniques for obtaining the IgM fraction, SG had been introduced first and was most frequently used since that (Schluederberg, 1965; Vesikari and Vaheri, 1968). It has been regarded for reliable and upmost sensitive technique (Dibbert, 1976; Leidel *et al.*, 1977; Bringuier *et al.*, 1978). Employed mostly in rubeola diagnostic, it served as standard for introducing other IgM detection methods (Meurmann *et al.*, 1977; Field *et al.*, 1980; MMWR, 1982). We used it also in this sense, because it cannot be applied routineously due to high demands on time and equipment.

The original chromatographic procedures are also time consuming (Bürgin-Wolff *et al.*, 1971; Pattison *et al.*, 1978). The use of little amounts of gel allows to save time and material with IECR (Johnson and Libby, 1980; Nagy *et al.*, 1984) as well as with protein A-Sepharose ACR. Prot A of *Staphylococcus aureus* binds human IgG of subclasses 1, 2 and 4 to the Fc region. The application of prot A-Seph yields better results than that of bacterial suspension (Field *et al.*, 1980). In comparison to SG separation, ACR on prot A-Seph is less sensitive (61.9%). The reason was the reactivity of human IgM molecules with prot A by the $F(ab)_2$-gamma receptors (Vidal and Conde, 1982). In sera with relatively low IgM content after prot A separation it decreases under the sensitivity threshold of HIT (Fig. 1). A similarly decreased positive rate was described also by other authors (Roggendorf *et al.*, 1976; MMWR, 1982). The relatively wide range of absorbed IgM registered with different serum samples in our experiments could be explained by different proportion of "prot A-reactive" respectively "prot A-nonreactive IgM molecules" (Grov, 1975).

We observed an equal sensitivity by SG and IECR, but the last method is more effective. According to Nagy *et al.* (1984), it is possible to prepare IgM-fractions of 50—100 sera at the same time without any expensive equipment. For testing of the fractions in HIT, 2-ME treatment was inevitable because no absolute separation of IgG and IgM is possible. The application of 2-ME is recommended also at testing of SG and ACR serum fractions to avoid false-positive results. In contrast to HIST, false-negative results, as a rule, may occur by SG and/or IECR fractionation of samples containing low levels of measles-specific IgM. Such sera usually contain relative high IgG levels. The IgG/IgM ratio may be then so unfavourable, that 2-ME treatment does not sufficiently reduce the antibody titre. However, HIST may reliably show the virus-specific IgM titres even under such conditions. As compared to other techniques used in our experiments, the latter method revealed the highest positive rates. False-positive results caused by virus-specific IgG avoided by means of μ-chain specific antibodies as well as due to the blocking effect of bovine serum albumin present in the buffer applied before incubation with the tested serum. As already shown by Krech (1979) and Logt *et al.* (1981), RF does not influence the IgM capture immunoassay. This assumption was confirmed by Briantais *et al.* (1984) provided that erythrocytes are used as indicator cells. In addition to the specificity and sensitivity of the HIST one must consider its easy performance. It needs a few serum only, the duration of test does not considerably exceed that of the simple HIT.

Summing up HIST can be regarded for a safe and effective method of measles-specific IgM antibody testing suitable for routine work. As shown by others, it has turned out useful not in rubeola diagnostic, but also in detection of IgM antibodies to other haemagglutinating viruses (Logt *et al.*, 1982; Roussel *et al.*, 1984). In the case of not haemagglutinating viruses, IECR can be recommended. Its sensitivity is close to that of SG centrifugation, but it is considerably less expensive. Both methods in question were useful (under consideration of their limitations) in our serologic studies (Gerike *et al.*, 1986).

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References

Banatvala, J. E., Best, J. M., Kennedy, E. A., Smith, E. E., and Spence, M. E. (1967): A serological method for demonstrating recent infection by rubella virus. *Br. med. J.* **3**, 285—286.

Briantais, M.-J., Grangeot-Keros, L., and Pillot, J. (1984): Specifity and sensitivity of the IgM capture immunoassay: studies of possible factors inducing false positive or false negative results. *J. virol. Meth.* **9**, 15—26.

Bringuier, J. P., Andre, J., and Sohier, R. (1978): The use of IgM antibody responses in the diagnosis of primary infections to measles, rubella, mumps and *M. parainfluenzae* viruses. *Med. Microbiol. Immunol.* **164**, 299—305.

Bürgin-Wolff, A., Hernandez, R., and Just, M. (1971): Separation of rubella IgM, IgA and IgG antibodies by gel filtration on agarose. *Lancet* **2**, 1278—1280.

Denoyel, G. A., Gaspar, A., and Peyramond, D. (1981): Diagnosis of recent rubella virus infection by demonstration of specific immunoglobulin M antibodies: comparison of solid-phase reverse immunosorbent test with sucrose density gradient centrifugation. *J. clin. Microbiol.* **13**, 698—704.

Dibbert, H.-J. (1976): Antikörper der Immunoglobuline 19 S und 7 S bei der Röteln-Diagnostik. *Zentbl. Bakt. ParasitKde Abt.* I **234**, 145—158.

Doerr, H. W., Fretschner, R., and Geisen, H. P. (1980): Virusspezifischer IgM-Nachweis mit Routinemethoden. *Zentbl. Bakt. ParasitKde I Abt.* **246**, 158—166.

Field, P. R., Shanker, S., and Murphy, A. M. (1980): The use of protein A-sepharose affinity chromatography for separation and detection of specific IgM antibody in acquired rubella infection: a comparison with absorption by staphylococci containing protein A and density gradient ultracentrifugation. *J. immunol. Meth.* **32**, 59—70.

Forghani, B., Schmidt, N. J., and Lennette, E. H. (1973): Demonstration of rubella IgM antibody by indirect fluorescent antibody staining, sucrose sensity gradient centrifugation and mercaptoethanol reduction. *Intervirology* **1**, 48—59.

Gerike, E., Hülsse, Ch., and Tischer, A. (1982): Serologische Analyse eines Masernausbruches in einem Kinderkollektiv mit hohem Durchimpfungsgrad. *Dt. Gesundh.-Wes.* **37**, 255—259.

Gerike, E., Tischer, A., Manuwald, O., Gilberg, J., Kohlhaas, H., Pöhle, W., König, E., and Horn, H. (1986): Seroepidemiologische Analyse von Masernherden in geimpften Populationen. *Z. klin. Med.*, in press.

Grov, A. (1975): Human IgM interacting with staphylococcal protein A. *Acta path. microbiol. scand.* **83**, 173—176.

Johnson, R. B., and Libby, R. (1980): Separation of immunoglobulin M (IgM) essentially free of IgG from serum for use in systems requiring assay of IgM-type antibodies without interference from rheumatoid factor. *J. clin. Microbiol.* **12**, 451—454.

Kangro, H. O., Booth, J. C., Bakir, T. M., F., Tryhorn, Y., and Sutherland, Sh. (1984): Detection of IgM antibodies against cytomegalovirus: comparison of two radioimmunoassays, enzyme-linked immunosorbent assay and immunofluorescent antibody test. *J. med. Virol.* **14**, 73—80.

Krech, U., and Wilhelm, J. A. (1979): A solid phase immunosorbent technique for the rapid detection of rubella IgM by hemagglutination inhibition. *J. gen. Virol.* **44**, 281—286.

Leidel, J., Mertens, T. H., and Eggers, H. J. (1977): Auftreten un Persistenz rötelnspezifischer IgM-Antikörper. *Dt. med. Wschr.* **102**, 1418—1421.

Meurmann, O. H., Viljanen, M. K., and Granfors, K. (1977): Solid-phase radioimmunoassay of rubella virus immunglobulin M antibodies: comparison with sucrose density gradient centrifugation test. *J. clin. Microbiol.* **5**, 257–262.

Morbidity and Mortality Weekly Report (1982): Serologic diagnosis of measles. **31**, 396–402.

Nagy, G., Kosa. S., Takatsy, S., and Koller, M. (1984): The use of IgM tests for analysis of the causes of measles vaccine failures: experience gained in an epidemic in Hungary in 1980 and 1981. *J. med. Virol.* **13**, 93–103.

Norrby, E. (1962): Hemagglutination by measles virus. 4. A simple procedure for production of high potency antigen for hemagglutination-inhibition (HI) tests. *Proc. Soc. exp. Biol. Med.* **134**, 889.

Pattison, J. R., Jackson, C. M., Hiscock, J. A., Cradock-Watson, J. E., and Ridehalgh, M. K. S. (1978): Comparison of methods for detecting specific IgM antibody in infants with congenital rubella. *J. med. Microbiol.* **11**, 411–418.

Roggendorf, M., Schneweis, K. E., and Wolff, M. H. (1976): Zum Nachweis rötelnspezifischer IgM im Hämagglutinationshemmungstest. Vergleichende Untersuchungen mit der Absorption von IgG durch Protein-A-haltige Staphylokokken und der Dichtegradienten-Ultrazentrifugation. *Zentbl. Bakt. ParasitKde Abt.* I **235**, 363–372.

Roggendorf, M., Frösner, G. G., Deinhardt, F., and Scheid, R. (1980): Comparison of solid phase test systems for demonstration antibodies against hepatitis A virus (anti-Hav) of IgM-class. *J. med. Virol.* **5**, 47–68.

Schluederberg, A. (1965): Immune globulins in human viral infections. *Nature (Lond.)* **205**, 1232–1233.

Schluederberg, A., Lamm, St. H., Landrigan, Ph. J., and Black, F. L. (1973): Measles immunity in children vaccinated before one year of age. *Am. J. Epidem.* **97**, 402–409.

Smith, F. R., Curran, A. S., Raciti, K. A., and Black, F. L. (1982): Reported measles in persons immunologically primed by prior vaccination. *J. Pediatr.* **101**, 391–393.

Van der Logt, J. T. M., van Loon, A. M., and van der Veen, J. (1981): Hemadsorption immunosorbent technique for determination of rubella immunoglobulin M antibody. *J. clin. Microbiol.* **13**, 410–415.

Van der Logt, J. T. M., Heessen, F. W. A., van Loon, A. M., and van der Veen, J. (1982): Hemadsorption immunosorbent technique for determination of mumps immunoglobulin M antibody. *J. clin. Microbiol.* **15**, 81–86.

Vesikari, T., and Vaheri, A. (1968): Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. *Br. med. J.* **1**, 221–223.

Vidal, M. A., and Conde, F. P. (1982): Interaction between staphylococcal protein A and human immunoglobulin M takes place through the F (ab')$_2$ gamma-receptor on the protein A molecule. *Immunol. Lett.* **5**, 75–78.