FACTORS AFFECTING THE SENSITIVITY AND REPRODUCIBILITY OF PASSIVE HAEMAGGLUTINATION TEST FOR THE QUANTITATION OF MEASLES-SPECIFIC ANTIBODIES

S. KUMAR[1], J. SOKHEY[1*], D.K. SOOD[1], S. SINGH[1], H. SINGH[2]

[1]Central Drugs Laboratory, Central Research Institute, Kasauli 173204;
[2]Department of Microbiology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Received May 18, 1994; revised August 19, 1994

Summary. – Various factors affecting the passive haemagglutination test (PHA) for the quantitation of measles-specific antibodies have been evaluated with the aim to obtain maximum sensitivity and reproducibility of the test. The antigen used for sensitization was prepared in Vero cells using Edmonston 245 strain of measles virus. Sheep red blood cells (SRBCs) were found most sensitive for use in PHA test. The optimum dilution of tannic acid was found to be 1:40,000 for tanning of fixed and 1:10,000 for unfixed SRBCs, when the tanning was carried out at 4 °C overnight. Fixed and tanned SRBCs sensitized with 32 HA units of measles HA antigen at 56 °C for 30 mins were found optimal. SRBCs from different sheep affected the sensitivity of the assay. Stability study of SRBCs showed that storage at -70 °C of glutaraldehyde-fixed and sensitized SRBCs gave better results as compared to those stored at -20 °C and +4 °C. Tanned SRBCs could be stored at -70 °C only up to 15 days. Sensitized SRBCs with-stood two cycles of freezing and thawing after removal from -20 °C and -70 °C. Sensitized SRBCs could be stored for 120 days without any significant loss of titer at -20 °C, +4 °C or 22 °C; when lyophilized with stabilizers, there was a slight decline in the titer after exposure at 37 °C for 30 days. The lyophilized sensitized SRBCs after reconstitution were found to be stable at +4 °C for 3 days without any loss in the titer. The use of stabilized and lyophilized SRBCs made the performance of PHA test simple, quick, economical and reproducible. Measles-specific antibodies of 192 serum samples of 6 – 9 months infants and serum samples obtained from 99 healthy rhesus monkeys were determined by PHA and haemagglutination-inhibition (HAI) tests. The PHA titres were higher than the HAI ones and the correlation coefficient between the two methods was 0.909 in case of the serum samples of infants and 0.966 for the monkey serum samples. Chi square (x^2) test revealed that the PHA test is more sensitive and specific test than the HAI one for detection of measles antibodies.

Key words: measles virus antibodies; passive haemagglutination ; haemagglutination-inhibition; sensitization of erythrocytes

Introduction

Several methods have been described for the assay of measles-specific antibodies (Norrby, 1962; Albrecht *et al.,* 1981; Voller and Bidwell, 1976; Ruckle and Rogers, 1957; Palosuo *et al.,* 1971; Rapicetta *et al.,* 1983; Heberling and Kalter, 1986; Kleiman *et al.,* 1981; Neumann *et al.,* 1985). All the methods have some advantages and disadvantages.

The most commonly employed method is the haemagglutination-inhibition (HAI) test, but it has an inherent disadvantage of being less sensitive (Albrecht *et al.,* 1981; Rapicetta *et al.,* 1983; Weigle *et al.,* 1984; Kramer and Cremer, 1984).

The major drawback of HAI test is that it requires monkey RBCs and moreover, a variation exists between the individual monkeys of a given species (Yeager *et al.,* 1977). One study showed that antibodies against measles antigen are present in laboratory housed rhesus and bonnet monkeys (Saha *et al.*, 1979). The PHA test for quantitation of mea-

*Corresponding author

sles-specific antibodies has been studied by few workers (Ghyka *et al.*, 1973; Sakata and Sugiura, 1988). However, no standard procedure was employed in these studies.

In the present study variables affecting the PHA test have been investigated with a view to establish the optimal conditions for fixing, tanning, sensitizing and stabilizing sheep RBCs and thus obtaining the maximum sensitivity and reproducibility of the test. After standardization of various factors the PHA test was applied for the detection of measles-specific antibodies in serum samples obtained from 192 infant and 99 healthy rhesus monkeys. All the samples were also titrated by the conventional HAI test. The results obtained by both the methods were compared and evaluated statistically.

Materials and Methods

Reference standard anti-measles serum, obtained from Statens Serum Institute, Copenhagen, Denmark, was diluted with PBS pH 7.2 to contain 2 IU/ml.

Negative control serum. Ten rhesus monkey sera which were found seronegative for measles-specific antibodies by HAI and neutralization tests were pooled and used in the assay as negative control.

Measles HA antigen. The Edmonston-245 strain of measles virus was cultivated for 10 days at 34 °C in Vero cells in MEM containing 2% calf serum. Infected fluid was harvested and treated with 0.125% Tween-80 and equal volume of ether. The mixture was centrifuged at 2,000 rpm for 30 mins in cold. The clear supernatant was collected and ether was removed by passing nitrogen gas. The supernatant containing the virus was distributed in small aliquotes and stored at 4 °C. The concentration of the measles HA antigen was standardized to 128 HA units/ml.

Serum samples of 192 infants aged between 6 to 9 months were collected on filter paper strips and processed by the method described by Saha and Saxena (1983). Briefly, 10 mm discs were cut from filter paper strips and put in 0.1 ml of PBS pH 7.2. After 45 mins, discs were squeezed and removed. The separated serum gave a final dilution of 1:8. The method would not detect antibodies below 1:8. Before testing sera were inactivated at 56 °C for 30 mins to remove non-specific inhibitors and adsorbed with monkey RBCs for performance of HAI test only. For the PHA test the latter step is not required.

Serum samples collected from healthy 99 rhesus monkeys were obtained for the study from Polio Vaccine Testing Laboratory of the Institute. Serum samples were stored at -20 °C and before testing inactivated at 56 °C for 30 mins. The sera were screened for measles antibodies by PHA and HAI tests.

RBCs. One volume of blood was mixed with 1.2 volume of Alsever's solution and stored at 4 °C.

RBCs were washed thrice in PBS and centrifuged at 2,000 rpm for 7 mins. The packed RBCs were resuspended in PBS to 5% suspension.

Normal rabbit serum (NRS). Blood was drawn by cardiac puncture of healthy rabbits. Serum was separated and inactivated at 56 °C for 30 mins. It was then adsorbed with washed and packed sheep RBCs at room temperature for 30 mins and stored at -20 °C in small aliquotes until used.

Fixation of SRBCs was done with glutaraldehyde by the method described by Daniel *et al.* (1963). RBCs of other animals were fixed with glutaraldehyde only.

Tanning of SRBCs. SRBCs were treated with various dilutions (twofold) of tannic acid ranging from 1:10,000 to 1:80,000. A 5% suspension of RBCs was prepared in PBS. Equal volumes of RBCs suspension and tannic acid were mixed and incubated overnight at 4 °C, and for 30 and 60 mins at 22 °C, 37 °C and 56 °C with occasional shaking. RBCs of other animals were treated only with 1:40,000 dilution of tannic acid at 4 °C overnight. After tanning, RBCs were washed with PBS and made 5% using PBS.

Sensitization of SRBCs with measles HA antigen. Equal volumes of 5% SRBC suspension and various concentrations of measles HA antigen ranging from 128 to 1 HA unit per ml were mixed in duplicate in PBS. The mixtures were incubated at 4 °C, 22 °C, 37 °C and 56 °C for 30 and 60 mins, respectively. Then the sensitized RBCs were centrifuged, washed and resuspended in PBS containing 0.5% NRS.

PHA test. Serial twofold dilutions of test and reference serum samples were made in PBS with 0.5% NRS. Titrations were performed in V-shaped microtiter plates using 0.05 ml of each serum dilution and 0.05 ml of 0.5% sensitized RBCs.

Unsensitized RBCs were added to the first dilution of the test serum and standard serum to serve as controls. The plates were shaken gently and incubated at 22 °C for 2 hrs. Negative serum controls were also included in each test. The highest dilution of serum which gave complete haemagglutination was taken as the endpoint.

Stability study of glutaraldehyde-fixed, tanned and sensitized SRBCs. After standardization of the PHA test, glutaraldehyde-fixed, tanned and sensitized SRBCs were kept at +4 °C, -20 °C and -70 °C for a study period of 120 days. On the day of test, fixed SRBCs stored at -20 °C and -70 °C were immediately thawed at 37 °C and washed twice with PBS. Thereafter, SRBCs were tanned with tannic acid and finally sensitized in the standard way. Similarly tanned SRBCs stored at -20 °C and -70 °C were thawed immediately at 37 °C, washed with PBS and sensitized by the standard procedure. The sensitized SRBCs stored at -20 °C and -70 °C were immediately thawed at 37 °C before testing.

Preservation of sensitized SRBCs. Sensitized SRBCs were divided into three batches and three types of stabilizers were added to each batch. In batch A 5% SRBC suspension was made in PBS containing 4% lactose, 2% D-sorbitol, 0.5% NRS, 0.2% L-histidine hydrochloride and 0.08% L-alanine hydrochloride. In batch B 5% SRBC suspension was made in PBS containing 0.2% Hepes, 10% mannite, 1% sodium glutamate, 1% bovine serum albumin, 5% sucrose and 0.5% NRS. The three mentioned stabilizers were previously sterilized by filtration using membrane filter. Batch A, B and C of 5% sensitized and stabilized SRBCs were lyophilized.

For the test, lyophilized sensitized SRBCs were reconstituted in PBS containing 0.5% NRS for batches A and C and PBS containing 0.2% Hepes for batch B to get 0.5% SRBCs. Similar

dilutions of reference and test sera were also made in the above mentioned buffers.

HA test. Titer of haemagglutinin or measles HA antigen was determined by HA test. Twofold serial dilutions of antigen were prepared in PBS using microtiter plates of V-shaped wells (0.025 ml of diluted antigen per well). To each well 0.025 ml of 0.5% rhesus monkey RBCs were added. The plates were gently shaken and incubated at 37 °C for one hour. The highest dilution of antigen which gave complete HA was taken as the endpoint and it contained 1 HA unit (HAU).

HAI test. Serial twofold dilutions of serum samples were prepared in microtiter plate of V-shaped wells in PBS (0.025 ml of diluted serum per well). Four HAU of antigen in 0.025 ml was added to each well. Plates were kept at 22 °C for 45 mins, after which 0.050 ml of 0.5% rhesus monkey RBCs were added. Plates were gently shaken, kept at 37 °C for 1 hr. Titers (the highest serum dilutions which gave a complete inhibition of HA) were recorded. Controls included sera without antigen at the lowest dilution, uninfected tissue culture fluid and 0.5% monkey RBCs.

Results

Fixation, tanning and sensitization of SRBCs

Fixation of SRBCs with glutaraldehyde was found to be better and easier than that with formaldehyde (data not shown). Hence the former was used throughout the study.

Fixed SRBCs, when tanned with 1:10,000, 1:20,000, 1:40,000 and 1:80,000 dilutions of tannic acid exhibited titer differences of not more than twofold dilution with reference anti-measles serum (2 IU/ml). The titers were highest at 1:40,000 dilution of tannic acid in case of fixed SRBCs. Unfixed SRBCs exhibited highest titers at 1:10,000 dilution of tannic acid when tanned at 4 °C overnight (Table 1).

Table 1. Effect of dilution of tannic acid for tanning SRBCs

Dilution of tannic acid	Titers of reference anti-measles serum		
	Glutaraldehyde fixed SRBCs	Formaldehyde fixed SRBCs	Unfixed SRBCs
1:10,000	1:80	1:40	1:40
1:20,000	1:160	1:20	1:20
1:40,000	1:320	1:80	1:20
1:80,000	1:80	1:20	<1:5

The other factors affecting the sensitivity of the PHA test were the concentration of sensitizing measles HA antigen (Table 2), the period of exposure of tanned SRBCs to the measles HA antigen and the temperature of sensitization. The optimal concentration of measles HA antigen for sensitization was 32 HAU for all types of fixed and unfixed SRBCs. The maximum titer of the reference serum was obtained when the exposure of tanned SRBCs to sensitizing measles HA antigen proceeded for 30 mins at 56 °C. There was two- to fourfold fall in titer when fixed cells were sensitized at 37 °C, 22 °C or 4 °C. Temperature of 56 °C was unsuitable for sensitizing unfixed SRBCs, as they tended to lyse very rapidly at this temperature with a change in colour from reddish to brown. Moreover, the unfixed SRBCs showed a tendency to clump, giving nonspecific agglutination. However, when the unfixed SRBCs were sensitized at 22 °C and used, very low titers were obtained.

Table 2. Optimum concentration of measles HA antigen for sensitization of SRBCs

Concentration of measles HA antigen (HAU/ml)	Titers of reference anti-measles serum	
	Glutaraldehyde fixed SRBCs	Formaldehyde fixed SRBCs
128	1:40	1:20
64	1:80	1:20
32	1:160	1:80
16	1:80	1:20
8	1:20	1:5
4	1:20	<1:5
2	<1:5	<1:5
1	<1:5	<1:5

Suitability of RBCs belonging to different animals

The sensitivity of PHA test was influenced by the use of RBCs of different animals. SRBCs (originating from sheep) were most suitable for use in the PHA test, as maximum titer of the reference anti-measles serum was obtained with them, while RBCs from rabbit, horse, fowl or guinea pig gave much lower titers, and nonspecific agglutination with goose RBCs was obtained (Table 3).

Table 3. Susceptibility of RBCs belonging to different animals

RBCs from	Titers of reference anti-measles serum
Sheep	1:320
Rabbit	1:40
Guinea pig	1:20
Fowl	<1:5
Horse	1:40
Goose	NSA

NSA – non-specific agglutination.

Table 4. Effect of RBCs from various sheep on the sensitivity of PHA test

RBCs from sheep No.	Titers of reference anti-measles serum
1	1:160
2	1:80
3	1:80
4	1:80
5	1:40

SRBCs from different sheep varied greatly in susceptibility and reproducibility of the PHA test. Up to fourfold differences in titers were observed. Hence, throughout the study SRBCs from a single animal were used (Table 4).

Stability of fixed, tanned and sensitized SRBCs

It is very essential to determine the stability of fixed, tanned and sensitized SRBCS at various temperatures of storage and for different time intervals. Table 5 shows the stability of fixed, tanned and sensitized SRBCs at +4 °C, -20 °C and -70 °C during 120 days. At -70 °C, there was no loss in titer with fixed and sensitized SRBCs. However, in case of tanned SRBCs, eightfold loss in titer was observed after 15 days of storage at -70 °C, The loss in titer was higher when fixed, tanned and sensitized SRBCs were stored at +4 °C or -20 °C. To see the effect of freezing and thawing of sensitized SRBCs stored at -70 °C and -20 °C on titers, the sensitized SRBCs were subjected to five cycles of rapid freezing and thawing, and the titers were determined. It was observed that with two cycles of freezing and thawing there was no fall in the titer at -70 °C or -20 °C. However, the pattern of decline in the titer was the same after third freezing and thawing at both these temperatures.

Stability of lyophilized sensitized SRBCs

An attempt was made to improve the stability of lyophilized sensitized SRBCs with stabilizers (Table 6). The lyophilized sensitized SRBCs of batches A, B and C showed nonspecific agglutination after reconstitution in PBS. The problem of nonspecific agglutination was overcome by reconstitution of the lyophilized sensitized SRBCs using PBS supplemented with 0,5% NRS (batches A and C) or 0.2% Hepes (batch B). It is evident from the Table 6 that

Table 5. Stability of glutaraldehyde-fixed, tanned and sensitized SRBCs

No. of days	Titers of reference anti-measles serum								
	Fixed SRBCs			Tanned SRBCs			Sensitized SRBCs		
	+4°C	-20°C	-70°C	+4°C	-20°C	-70°C	+4°C	-20°C	-70°C
0		1:320			1:320			1:320	
15	1:160	1:160	1:320	1:160	1:160	1:320	1:80	1:160	1:320
30	1:80	1:160	1:320	1:40	1:20	1:20	1:40	1:160	1:320
60	1:80	1:160	1:320	1:20	1:20	1:20	1:40	1:80	1:320
120	1:80	1:160	1:320	NSA	1:20	1:20	1:20	1:80	1:320

NSA – non-specific agglutination.

Table 6. Effect of lyophilization on stability of sensitized and stabilized SRBCs

Temperature of storage	Time period of storage (days)	Titers of reference anti-measles serum		
		Stabilized batches [a]		
		A	B	C
-20°C	15	1:320	1:320	1:320
	30	1:320	1:320	1:320
	120	1:320	1:320	1:160
+4°C	15	1:320	1:320	1:320
	30	1:320	1:320	1:320
	120	1:320	1:320	1:160
22°C	15	1:160	1:320	1:80
	30	1:80	1:320	1:40
	120	1:80	1:160	1:20
37°C	15	1:160	1:160	1:80
	30	1:80	1:160	1:40
45°C	15	1:80	1:80	1:40
	30	1:40	1:80	1:40

[a]See Materials and Methods.

after storage of lyophilized SRBCs at -20 °C or +4 °C for 15 days there was no loss in titer. The results of storage for 15 days at 22 °C, 37 °C or 45 °C were also better. However, exposure of stabilized sensitized SRBCs at 37 °C for 30 days resulted in twofold drop in titer. Lyophilized, sensitized and stabilized SRBCs of the batch B, which were found to be highly stable, were reconstituted with PBS containing 0.2% Hepes and its stability was observed for five days at +4 °C. The results indicated that the reconstituted SRBCs retained their original titer of 1:320 up to 3 days. On the fourth and fifth day, the titer declined two and eight times, respectively.

Application of PHA test

After standardizing the PHA test, it was applied to detect the level of antibodies in serum samples obtained from 192 infants aged between 6 to 9 months and healthy 99 rhesus monkeys. Simultaneously, these sera were also titrated by HAI test. The correlation between titers of individual monkey sera obtained by PHA and HAI tests is presented in Figs. 1 and 2. Out of 99 monkey serum samples tested 45 samples were found positive for measles antibodies by PHA test, whereas only 30 samples were found positive by HAI test. In case of 192 serum samples of infants, 143 serum samples were negative by HAI test as compared to 65 samples by PHA test proving a higher sensitivity of the PHA test. The antibody level in the negative serum samples could be detected only up to 1:8 because of the limitation of the filter paper technique. The titer in 127 infant serum samples ranged from 1:8 to 1:512 by PHA test in contrast to 49 samples exhibiting antibody titer in the range of 1:8 to 256 by HAI test. The correlation coefficient between the two

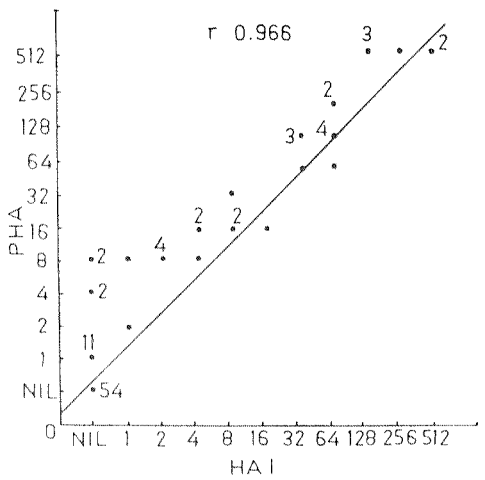

Fig. 1
Relationship between antibody titers of 99 monkey sera estimated by HAI and PHA tests
Reciprocals of titers indicated on the abscissa and ordinate. Numbers of serum samples shown at points. r – correlation coefficient.

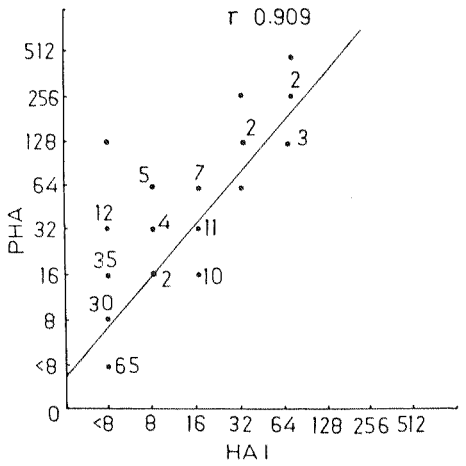

Fig. 2
Relationship between antibody titers of 192 sera of infants estimated by HAI and PHA tests
For legend see Fig. 1.

methods was 0,966 in case of monkey sera and 0.909 in infants sera. Chi square (x^2) test applied on 127 PHA and 49 HAI titers of positive infant serum samples, and similarly on 45 PHA and 30 HAI titers of positive monkey serum samples revealed that the PHA test was more sensitive than the HAI one. The calculated value of x^2 was based on one degree of freedom and found to be significant ($p < 0.05$).

Discussion

An attempt has been made in this study to develop a quick and sensitive method for the quantitation of measles-specific antibodies. Factors influencing the PHA method involved source and the conditions of fixation and sensitization. Regarding the fixation procedure, that by glutaraldehyde was found to be better and easier than fixation by formaldehyde.

The optimal dilution of tannic acid for tanning of fixed RBCs overnight at 4 °C was found to be 1:40,000. However, it was 1:10,000 for unfixed RBCs. Peel (1980) had also observed maximum titers at 1:40,000 and 1:80,000 dilution of tannic acid, while standardizing the PHA test for titration of tetanus antitoxin. The concentration of measles HA antigen for sensitization has a great effect on the final sensitivity of RBCs. Sensitization of tanned RBCs was best achieved at 56 °C for 30 mins using 32 HA units of measles HA antigen.

The validity of the optimal conditions found in this study was confirmed by the consistency of findings when other batches of sensitized RBCs were prepared from fresh samples of RBCs taken from time to time from the same animal.

The need to use RBCs from a single animal that was mentioned earlier (Peel, 1980; Galazka and Abgarowicz, 1967) was confirmed in the present study. Although some variations in results were observed with RBCs from different sheep as expected, yet, sheep RBCs (SRBCs) were found to be the most susceptible and sensitive for the PHA test as compared to RBCs of other animals. Fixed RBCs were used because of their resistance to lysis, stability on storage and ease of handling. Moreover, a maximum titer of the reference anti-measles serum was obtained with fixed RBCs, but not with unfixed RBCs. This was due to the sensilization of fixed RBCs at 56 °C which withstood this temperature. Hence, when SRBCs were processed under the optimal conditions, maximum sensitivity and reproducibility of results was achieved. After standardization of the PHA technique using glutaraldehyde-fixed, tanned and sensitized SRBCs, it was essential to see the stability of all the reagents used during each step of the test. It was evident that fixed and sensitized SRBCs could be stored at -70 °C without any significant loss in titer up to 120 days. On the other hand, the sensitivity of the sensitized SRBCs decreased 2 – 8 times at +4 °C or -20 °C after storage for 15 or more days. Our results are in accordance with the earlier findings of Hubert *et al.* (1963), who has made similar observations using various bacterial antigens for sensitization of RBCs and storing them up to 180 days at -70 °C. Tanned SRBCs could be used up to 15 days when stored at -70 °C without any decrease in sensitivity and reproducibility of the test. When unlyophilized sensitized SRBCs were stored at -70 °C or -20 °C, one cycle of freezing and thawing was unavoidable before testing. The results obtained clearly indicate that two cycles of freezing and thawing after removal from -70 °C or -20 °C did not alter the susceptibility and sensitivity of the sensitized SRBCs. To increase further the stability of sensitized SRBCs, it was considered important to lyophilize them in the presence of stabilizers. The lyophilization, using above mentioned combination of stabilizers increased the stability of SRBCs even when tested after storage for 120 days at -20 °C, +4 °C or 22 °C. However, the use of stabilizers as lactose, D-sorbitol, L-histidine, L-alanine and NRS helped in retaining the titer of sensitized SRBCs at +4 °C or -20 °C for 120 days. These results confirm the earlier findings of Cook (1965) and Gupta *et al.* (1987) who emphasized the use of stabilizers in the lyophilized preparations of sensitized SRBCs. Our lyophilized sensitized SRBCs of batch B reconstituted could be used up to 3 days without any decline in titer.

The second part of the study was to compare the sensitivity and correlation of the PHA with the HAI test for assay of measles-specific antibodies. It was observed that titers obtained by the PHA test were higher than those by the HAI test. Similar results were reported by Sakata and Sugiura (1988). From our results with the PHA and HAI tests it is clear that the PHA method is quite specific and sensitive for quantitating measles-specific antibodies.

Therefore, the PHA method can be adopted and is recommended in the day-to-day routine work in laboratories engaged in the sero-epidemiological studies of measles.

Acknowledgements. The authors are thankful to Mr. K. Kumar and Mr. G. Chand for technical assistance, and to Mr. P.C. John for preparation of the manuscript.

References

Albrecht, P., Herrmann, K., and Burns, G.S. (1981): Role of virus strains in conventional and enhanced plaque neutralization test. *J. virol. Methods* **3**, 251–260.

Cook, R.J. (1965): Reversed passive haemagglutination systems for the estimation of tetanus toxin and antitoxin. *Immunology* **8**, 74–80.

Danill, T.M., Weyand, J.G.M., and Stavitsky, A.B. (1963): Micromethods for the study of proteins and antibodies IV. Factors involved in the preparation and use of stable preparation of formalinized, tannic acid treated proteins sensitized erythrocytes for detection of antigen and antibody. *J. Immunol.* **90**, 741–750.

Galazka, A., and Abgarowicz, A. (1967): Assay of diphtheria and tetanus antibodies by the passive haemagglutination method. *Epidemiol. Rev.* **21**, 237–252.

Ghyka, G.R., Cernescu, C., and Cajal, N. (1973): Studies on the sensitivity of a passive haemagglutination test in the detection of measles antibodies. *Rev. Roum. Virol.* **10**, 295–300.

Gupta, R.K., Mehta, V.K., Gupta, V.K., Misra, C.N., and Saxena, S.N. (1987): The standardization of reverse indirect haemagglutination test for the assay of viral antigen of Japanese encephalitis vaccine. *J. biol. Stand.* **15**, 271–279.

Heberling, R.L., and Kalter, S.S. (1986): Rapid dot-immunobinding assay on nitrocellulose for viral antibodies. *J. clin. Microbiol.* **23**, 109–113.

Hubert, G.C., Kalmanson, G.M., and Guze, L.B. (1963): Preservation of antigen coated sheep erythrocytes by freezing for use in indirect haemagglutination procedure. *J. Bacteriol.* **86**, 569–572.

Kleiman, M.B., Blackburn, C.K., Zimmerman, S.E., and French, M.L.V. (1981): Comparison of enzyme linked immunosorbent assay for acute measles with haemagglutination, complement fixation and fluorescent-antibody methods. *J. clin. Microbiol.* **14**, 147–152.

Kramer, S.M., and Cremer, N.E. (1984): Detection of IgG antibodies to measles virus using monoclonal antibodies for antigen capture in enzyme immunoassay. *J. virol. Meth.* **8**, 255–263.

Neumann, P.W., Weber, J.M., Jessamine, A.G., and O'Shaughnessy, M.V. (1985): Comparison of measles antihaemolysin test, enzyme linked immunosorbent assay and haemagglutination inhibition test with neutralization test for determination of immune status. *J. clin. Microbiol.* **22**, 296–298.

Norrby, E. (1962): Haemagglutination by measles virus 4. A simple procedure for production of high potency antigen for haemagglutination inhibition HAI tests. *Proc. Soc. exp. Biol. Med.* **111**, 814–818.

Palosuo, T., Salmi, A.A., and Pettay, O. (1971): Measles antibodies detected by platelet aggregation and gel precipitation of patients with SSPE. *Arch. Ges. Virusforsch.* **35**, 35–45.

Peel, M.M. (1980): Measurement of tetanus antitoxin I. Indirect haemagglutination. *J. biol. Stand.* **8**, 177–180.

Rapicetta, M., D'Ambrosio, E.D., Proietti, E., and Morace, G. (1983): Antibody assay for measles virus: Comparison of immune adherence haemagglutination, single radial haemolysis, enzyme immunoassay and haemagglutination inhibition. *J. virol. Meth.* **6**, 303–310.

Ruckle, G.E., and Rogers, K.D. (1957): Studies with measles virus II. Isolation of virus and immunogenic studies in persons who have had natural disease. *J. Immunol.* **78**, 341–355.

Saha, S.M., Sharma, S.B., and Balasubramanyan, M. (1979): Antibodies against measles antigen in laboratory housed rhesus and bonnet monkeys. *Indian J. med. Res.* **69**, 213–216.

Saha, S.M., and Saxena, S.N. (1983): Filter paper disc method of blood collection for testing seroconversion after measles immunization. *Indian J. med. Res.* **77**, 421–424.

Sakata, H., and Sugiura, A. (1988): Passive haemagglutination test for measles immunity and serodiagnosis. *J. clin. Microbiol.* **26**, 636–640.

Voller, A., and Bidwell, D.E. (1976): Enzyme-immunoassay for antibodies in measles, cytomegalovirus infections and after rubella vaccination. *Br. J. exp. Path.* **57**, 243–247.

Weigle, K.A., Murphy, M.D., and Brunell, P.A. (1984): Enzyme-linked immunosorbent assay for evaluation of immunity to measles virus. *J. clin. Microbiol.* **19**, 376–379.

Yeager, A.S., Davis, J.H., Ross, L.A., and Harvey, B. (1977): Measles immunization. Successes and failures. *J. Am. med. Assoc.* **234**, 347–351.