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Single Radial Haemolysis for the Determination of Antibody to Reoviruses

Brief Report

By

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With 1 Figure

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Summary

A single radial haemolysis (SRH) for reovirus antibody determination was developed and compared to standard haemagglutination-inhibition (HI) and complement fixation (CF) techniques. SRH appeared more simple and sensitive than CF, but less sensitive and less specific than HI.

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Single radial haemolysis (SRH) was originally developed for measuring antibody to influenza (9, 10), but has been successfully applied to other haemagglutinating and non-haemagglutinating human viruses (2, 3, 5—7, 14, 15). Quantitation of antibody to reoviruses in human and animal sera has been usually performed by HI or neutralization (11, 12), CF being group-specific and of low sensitivity. In this paper we describe the detection of reovirus antibody by a newly developed SRH technique, in comparison with HI and CF (13).

Human O red blood cells (HRBC), without any coupling agent, were used. Freshly prepared "aged" chromium chloride-treated (1), glutaraldehyde-stabilized (4) or "aged" chromium chloride-treated glutaraldehyde-stabilized HRBC (5) was no better than untreated erythrocytes. Sheep red blood cells, if treated with chromium chloride, could be substituted for HRBC, but a few sera produced non-specific haemolysis, which never happened with HRBC. Reovirus type 1 (Lang), type 2 (D5 Jones) and type 3 (Abney) were grown on monolayers of monkey kidney cell lines (CV-1, LLC-MK, Vero) in serum-free Eagle's Minimal Essential Medium; the cells were disrupted by sonication. Antigen (HA titre $\geqslant 160$) was mixed with an equal volume of 5 per cent HRBC suspension in PBS and incubated at room temperature for 30 minutes; the erythrocytes were then washed 3 times and resuspended at 5 per cent in PBS. Each empty Hyland immunoplate received a mixture of 0.3 ml antigen-coated HRBC suspension,

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0.1 ml normal guinea pig serum (as a source of complement), and 2.6 ml molten 1.5 per cent agarose (Indubiose A 37) in PBS (10). Five μl undiluted heat-inactivated serum was added to 2 mm wells and the plates were incubated overnight at 37° C: a clear haemolysis zone of ⩾3 mm diameter was considered a positive reaction. Wells filled with PBS in reovirus SRH plates, and plates prepared with HRBC incubated with supernatant of sonicated uninoculated cells served as negative controls.

Of the 32 post-immunization samples from animals receiving any reovirus type, antibody response was detected by CF in 24 (75.0 per cent), by SRH in 26 (81.2 per cent) and by HI in 100 per cent. Table 1 shows that the results with SRH were less specific than those with HI—for example the sera of animals immunized with type 3 did not react by HI with type 1 or 2, but 3 of 5 reacted by SRH and CF. When 68 randomly selected human sera were tested (Table 1), SRH detected, as for guinea pig sera, mainly group-specific antibody, but was more sensitive than CF. HI detected antibody in 57 (83.8 per cent), SRH in 36 (52.9 per cent), and CF in 17 (25.0 per cent) sera. When the haemolysis ring

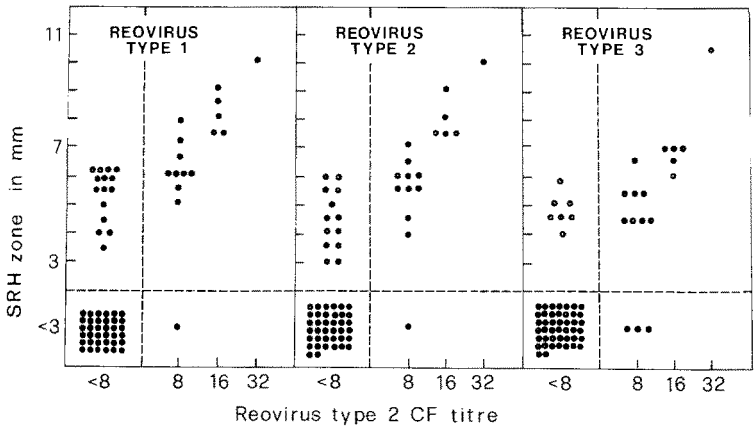

Fig. 1. Measurement of reovirus antibody in human sera: comparison between CF and SRH in 68 serum samples

Table 1. *Comparison of HI, SRH, and CF tests for determination of reovirus antibody in 32 serum samples from 15 immunized guinea pigs*[a]

Reovirus test antigen	No. of sero-positive animals after immunization against											
	Type 1			Type 2[b]			Type 3			None		
	HI	SRH	CF	HI	SRH	CF	HI	SRH	CF	HI	SRH	CF
Type 1[b]	5	4	3	2	5	5	0	3	3	1	0	0
Type 2[b]	2	3	3	5	5	5	0	3	3	0	0	0
Type 3[b]	3	3	3	4	5	5	5	3	3	0	0	0

[a] Each group of 5 animals was inoculated intranasally (8) with live reovirus type 1, type 2, or type 3. Sera were drawn after 5 and 6 weeks; results were identical except that SRH zones were slightly larger at 6 weeks

[b] These animals were sero-positive by HI against type 2 before inoculation

diameters were plotted against CF titres (Fig. 1), a good correlation was observed between the two techniques; similar results (not shown) were obtained for guinea pig sera. It is not clear why the antibody response by SRH, using haemagglutinating antigen attached to RBC, appears different from that measured by HI —using influenza virus the results are very similar though anti-neuraminidase antibody can be detected as well as HI. Since all sera were pre-treated with kaolin, it is unlikely that HI was detecting non-specific inhibitors.

SRH is nevertheless a useful technique for work with reoviruses. It is simple, uses small quantities of heat-inactivated serum, without need for dilutions, and is easy to read; in addition, the plates can be fixed with formaldehyde (9) or glutaraldehyde as a permanent record. It might be applied to non haemagglutinating human viruses, such as herpesvirus instead of CF.

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References

1. Goding, J. W.: The chromic chloride method of coupling antigens to erythrocytes: definition of some important parameters. J. immunol. Meth. **10**, 61—66 (1976).
2. Grillner, L., Blomberg, J.: Haemolysis-in-gel and neutralization tests for determination of antibodies to mumps virus. J. clin. Microbiol. **4**, 11—15 (1976).
3. Grillner, L., Strannegaord, Ö.: Evaluation of the hemolysis-in-gel test for the screening of rubella immunity and the demonstration of recent infection. J. clin. Microbiol. **3**, 86—90 (1976).
4. Habicht, G. S., Miller, F.: Preparation of conjugated erythrocytes for long term use in hemolytic plaque assays, complement fixation studies, or passive hemagglutinations: a comparative study of several methods. J. immunol. Meth. **11**, 117—127 (1976).
5. Hierholzer, J. G., Tannock, G. A.: Quantitation of antibody to nonhemagglutinating viruses by single radial hemolysis: serological test for human coronavirus. J. clin. Microbiol. **5**, 613—620 (1977).
6. Odelola, H. A.: Application of single-radial-haemolysis test for the detection of antibodies to togaviruses. Arch. Virol. **60**, 325—328 (1979).
7. Riski, H., Hovi, T., Väänänen, P., Penttinen, K.: Antibodies to human coronavirus OC43 measured by radial haemolysis in gel. Scand. J. inf. Dis. **9**, 75—77 (1977).
8. Rosen, L.: Reoviruses. In: Lennette, E. H., Spaulding, E. H., Truant, J. P. (eds.), Manual of clinical Microbiology, 2nd ed., 735—739. Washington, D. C.: American Society for Microbiology 1974.
9. Russell, S. M., McCahon, D., Beare, A. S.: A single radial haemolysis technique for the measurement of influenza antibody. J. gen. Virol. **27**, 1—10 (1975).
10. Schild, G. C., Pereira, M. S., Chakraverty, P.: Single-radial-haemolysis: a new method for the assay of antibody to influenza haemagglutinin. Bull. W.H.O. **52**, 43—50 (1975).
11. Schmidt, N. J., Lennette, E. H., Hanahoe, M. F.: Microneutralization test for the reoviruses. Application to detection and assay of antibodies in sera of laboratory animals. Proc. Soc. Exp. Biol. Med. **121**, 1268—1275 (1966).

12. SCHMIDT, N. J.: Antibody assays for enteroviruses and reoviruses. In: ROSE, N. R., FRIEDMAN, H. (eds.), Manual of Clinical Immunology, 488—493. Washington, D.C.: American Society for Microbiology 1976.

13. SEVER, J. L.: Application of a microtechnique to viral serological investigations. J. Immunol. **88**, 320—329 (1962).

14. VÄÄNÄNEN, P., HOVI, T., HELLE, E.-P., PENTTINEN, K.: Determination of mumps and influenza antibodies by haemolysis-in-gel. Arch. Virol. **52**, 91—99 (1976).

15. WESSLEN, L.: Demonstration of antibodies to measles virus by hemolysis-in-gel (HIG) test. Scand. J. Infect. Dis. **10**, 15—20 (1978).

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