

## Antibodies to Human Coronavirus OC43 Measured by Radial Haemolysis in Gel

Hellevi Riski, Tapani Hovi, Pertti Väänänen & Kari Penttinen

To cite this article: Hellevi Riski, Tapani Hovi, Pertti Väänänen & Kari Penttinen (1977) Antibodies to Human Coronavirus OC43 Measured by Radial Haemolysis in Gel, Scandinavian Journal of Infectious Diseases, 9:2, 75-77, DOI: [10.3109/inf.1977.9.issue-2.04](https://doi.org/10.3109/inf.1977.9.issue-2.04)

To link to this article: <http://dx.doi.org/10.3109/inf.1977.9.issue-2.04>



Published online: 02 Jan 2015.



Submit your article to this journal [↗](#)



View related articles [↗](#)



Citing articles: 1 View citing articles [↗](#)

---

# Antibodies to Human Coronavirus OC43 Measured by Radial Haemolysis in Gel

HELLEVI RISKI,<sup>1</sup> TAPANI HOVI,<sup>1</sup> PERTTI VÄÄNÄNEN<sup>2</sup>  
and KARI PENTTINEN<sup>1</sup>

From the <sup>1</sup>Department of Virology, University of Helsinki and <sup>2</sup>Orion Diagnostica, Helsinki, Finland

**ABSTRACT.** An application of the haemolysis-in-gel (HIG) technique was developed to quantitate antibodies against the human coronavirus OC43. Preinfection and convalescent sera from two patients with verified OC43 infection showed a significant increase in antibody titres measured by HIG as well as by haemagglutination-inhibition (HI). 241 of the other 306 sera tested (80 %) caused radial haemolysis in gels containing viral antigen-sensitized erythrocytes and complement. No haemolysis was seen in gels prepared with uninfected material. Correlation of the diameter of the haemolysis ring to the respective HI titre was highly significant. However, 62 sera (20 %) with HI titres between 8 and 320 were negative in the HIG. Attempts to show that these sera contained nonspecific inhibitors of haemagglutination were unsuccessful. OC43 HI and HIG probably measure different antibody populations.

## INTRODUCTION

McIlmurray (4) and Weiler et al. (10) were the first to use passive haemolysis-in-gel (HIG) for quantitating antibodies in serum samples. Recently this method has been applied for measuring antibodies against influenza and certain other viruses (6, 7, 9). Serum samples to be tested are applied into prepunched wells in agarose gel which contains the viral antigen attached to erythrocytes and complement. Radial diffusion of the antibodies and the subsequent antibody-antigen reaction in the presence of complement result in circular areas of haemolysis with diameters proportional to the concentration of antibodies in the tested serum (6, 7, 9).

Compared to the conventional serological tests used in the routine virus serology, haemagglutination inhibition (HI) and complement fixation (CF), HIG offers several advantages. For instance, serial serum dilutions are unnecessary and the test appears to be completely unaffected by nonspecific inhibitors of haemagglutination (6, 7, 9). The test is also accurate and reliable and seems to be suitable for routine diagnostics of influenza or mumps infections (9). Chemical coupling of the viral antigens to erythrocytes (9) makes this novel method, at least in principle, applicable to diagnostics of any virus group or strain.

In this paper we describe an application of the

HIG test for measuring antibodies against the human coronavirus OC43.

## MATERIAL AND METHODS

### *Antigen*

OC43 virus (8) was supplied by Dr Timo Estola, State Veterinary Medical Institute, Helsinki, and was passaged in brains of suckling mice (2). The brains were harvested by the appearance of typical encephalitis symptoms, generally 72-90 h after inoculation. Infected brains were homogenized as a 10 % suspension in Difco nutrient broth and the homogenate was clarified by brief centrifugation. Haemagglutinating (HA) activity in the supernatant was quantitated employing a 0.5 % suspension of chicken erythrocytes as described previously (2, 5). The supernatant was stored in small aliquots at -70°C.

### *Sera*

Pre- and post-inoculation sera from two patients with experimental OC43 infection were kindly provided by Dr D. A. J. Tyrrell.

306 acute phase or convalescent sera from patients (age 1-80 years) with suspected viral infection were selected for this study on the basis that the aetiology of the disease was not revealed by the standard virus serological techniques (or routine screening test employs 15-20 different CF antigens). The sera were stored at -20°C.

### *Haemagglutination inhibition (HI) test*

Two-fold serial dilutions of the sera were mixed with 4 HA units of the OC43 antigen and incubated for 10 min at room temperature. After adding the chicken erythrocytes the incubation was continued for further 30 min (5).

Table I. Rise of virus-specific antibodies in verified OC43 infections

Heat-inactivated serum samples were absorbed with chicken erythrocytes and assayed in HIG and HI tests as described in Material and Methods

Patient and serum	HIG (diameter of haemolysis ring)	HI titre
S.A.K.		
Preinfection	7	8
Convalescent	9	32
J.H.S.		
Preinfection	6	8
Convalescent	9	32

For routine HI tests the sera were heated for 30 min at 56°C and absorbed with chicken erythrocytes. In certain experiments the sera were also treated with acetone, heparin, kaolin or periodate, trypsin-periodate or RDE to remove possible nonspecific inhibitors of haemagglutination, using standard techniques (3).

#### Haemolysis-in-gel (HIG)

OC43 antigen or similar homogenate prepared from uninfected mouse brain ("control antigen") was coupled to chicken erythrocytes by  $\text{CrCl}_3$  (1, 9). The sensitized erythrocytes were mounted in agarose gels together with complement (1:30 dilution of quinea pig serum). 5  $\mu\text{l}$  samples of undiluted sera were applied into prepunched wells (diameter 3 mm) in the gel and the plates were incubated for 20 h at 37°C. The sera were heated for 30 min at 56°C before the HIG.

#### Serum fractionation

To investigate antibody activity in different Ig classes 0.2 ml samples of 1:2 dilutions of the sera were layered on 5 ml gradients of 12.5%–37% w/v sucrose in Dulbecco's phosphate buffered saline and centrifuged for 17 h at 30000 rpm in a Spinco SW 50.1 rotor at 4°C. Fractions of about 0.25 ml collected dropwise from the bottom were tested for HI and HIG activities as described above. Sera containing Paul-Bunnell reactive IgM (19 S) antibodies and rubella HI antibodies of the IgG class (7 S) were run in parallel gradients as sedimentation markers.

#### Statistical analysis

A standard regression analysis was kindly performed by Mr S. Sarma, M.Sc., to compare the results obtained by using the two serological methods.

## RESULTS AND DISCUSSION

The two serum pairs from verified OC43 infections showed titre increases in both HIG and HI tests (Table I). (A 2 mm increment in the diameter of the haemolysis ring was found to represent significant increase in antibody levels (9).) This finding suggests that OC43 HIG may be used as a diagnostic

test for OC43 infections. However, greater numbers of similar serum pairs should be assayed before conclusions.

80% of the other sera tested (241/306 sera) caused circular areas of haemolysis in the gels containing OC43 antigen. No haemolysis was found in gels of the control antigens. The completeness of the haemolysis within the rings was more variable than what we previously found with influenza or mumps virus (9). Nevertheless, the edge of the haemolysis ring was sharp allowing exact measuring of the diameter.

35 sera were tested twice or more often using different batches of gel plates. The diameter of the haemolysis ring induced by a given serum was well reproducible in different batches and the observed variation of the diameter did not exceed 2 mm. This finding supports the earlier conclusion that HIG is a relatively accurate serological method (9). Some of the sera produced rings of complete haemolysis in some plate batches and areas of partial haemolysis in some others. The reason for this variation is not known.

All the sera assayed by HIG were also tested for HI antibodies against OC43 virus. Only 4 out of the 306 sera were without measurable HI antibodies (Fig. 1). The number of patients having measurable HI antibodies was greater in this study than what was reported previously from Finland (5), while the age distribution of titres higher than 40 was similar in both studies (data not shown). We do not know the reason for this difference, but it is noteworthy that the actual titrations were made in different laboratories and that different hens were used as red cell donors, which might have influenced the results.

Correlation between the HI titre and the diameter of the haemolysis ring was highly significant, as studied by regression analysis ( $r=0.291$ ;  $P<0.001$ ). This was true even when the HIG-negative sera were included in the test ( $r=0.238$ ;  $P<0.001$ ). It is, however, evident from Fig. 1 that the HI activity in the sera can be divided into two classes: one which is associated with the ability of the serum to cause haemolysis in OC43-HIG plates, and the other which seems to be independent of the latter activity. 62 sera (20%) with HI titres 8–320 were negative in HIG.

The simplest explanation for this divergence would be nonspecific inhibitors of haemagglutination present in the HIG-negative sera. However,

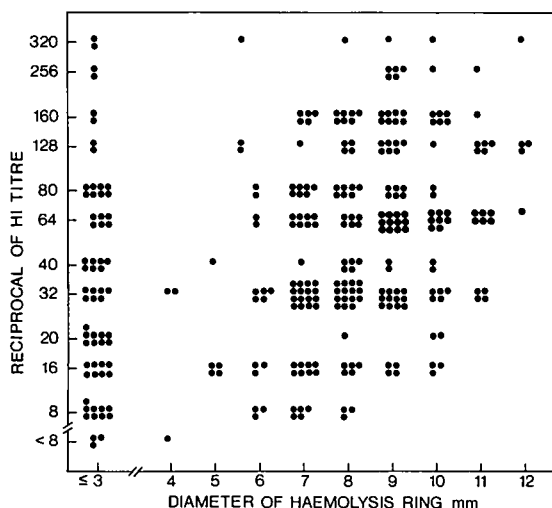


Fig. 1. OC43 antibodies in human sera; correlation between HI titre and HIG diameter. For details see the text.

there is no evidence for nonspecific inhibitors of OC43 haemagglutination in the literature. 12 of the HIG negative sera with HI titres 80 or more were treated with kaolin, heparin, RDE, acetone, periodate or trypsin-periodate. None of these treatments reduced the apparent HI titre of the serum more than by one or sometimes by two dilutions. Similar effects on HI titres were observed in HIG-positive sera after the treatments. Furthermore, when two HIG-negative-HI-positive sera were analyzed in gradient centrifugation a vast majority of the HI activity cosedimented with IgG in both cases, like that in the HIG-positive sera.

By these studies we cannot exclude nonspecific inhibitors of OC43 haemagglutination sedimenting at 7 S and resistant to the methods used above to remove the inhibitors. We can, however, envisage other possible explanations for these findings as well. For instance, the classes and specificities of the OC43 antibodies reacting in HI and HIG may be different. Coupling of the haemagglutinin to erythrocytes may mask some viral determinants involved in the haemagglutination reaction per se. Patients with HI antibodies but without HIG antibodies may have been exposed to the virus longer times ago than those having also HIG antibodies. If any of these suggestions is true remains to be shown by further studies.

In conclusion, we have developed an application of the haemolysis-in-gel technique to measure antibodies against OC43, a human coronavirus. A

good general correlation is seen between the results of the HIG test and the respective HI titres. However, a significant proportion of human sera containing HI antibodies against OC43 does not induce haemolysis in the gels. Sensitivity of the HIG method to detect seroconversion and the relation, if any, of the HIG-reactive antibodies to immunity against OC43 virus require further studies.

#### ACKNOWLEDGEMENTS

This work was supported by a grant from the Orion Research Foundation to Hellevi Riski; Kari Penttinen was supported by the Sigrid Juselius Foundation. Technical assistance of Mrs Anja Kallionpää, Miss Raili Saloranta and Mrs Terttu Väänänen is gratefully acknowledged.

#### REFERENCES

1. Faulk, W. P., Vyas, G. N., Phillis, C. A., Fudenberg, H. H. & Chism, K.: Passive haemagglutination test for antirhinovirus antibodies. *Nature (New Biol)* 231: 101, 1971.
2. Kaye, H. & Dowdle, W. R.: Some characteristics of haemagglutination of certain strains "IBV-like" virus. *J Infect Dis* 120: 576, 1969.
3. Lennette, E. H. & Smith, N. J.: Diagnostic procedures for viral and rickettsial infections, 4th ed., pp. 264-265, 393-394, 428-429. Am Public Health Assoc 1969.
4. McIllmurray, M. B.: Characterization of anti-influenza antibody. M.Sc. Thesis, University of Manitoba 1965.
5. Riski, H. & Estola, T.: Occurrence of antibodies to human coronavirus OC43 in Finland. *Scand J Infect Dis* 6: 325, 1974.
6. Russel, S. M., McCahon, D. & Beare, A. S.: A single radial haemolysis technique for the measurement of influenza antibody. *J Gen Virol* 27: 1, 1975.
7. Schild, G. C., Oxford, J. S. & Virelizier, J.-L.: Immunity of influenza. *Dev Biol Stand* 28: 253, 1975.
8. Tyrrell, D. A. J. & Bynoe, M.: Cultivation of a novel type of common cold virus in organ cultures. *Br Med J* 1: 1467, 1965.
9. Väänänen, P., Hovi, T., Helle, E.-P. & Penttinen, K.: Determination of mumps and influenza antibodies by haemolysis-in-gel. *Arch Virol* 52: 91, 1976.
10. Weiler, E., Melletz, E. W. & Breuninger-Peck, E.: Facilitation of immune haemolysis by an interaction between red cell sensitizing antibody and gamma-globulin allotype antibody. *Proc Natl Acad Sci USA* 54: 1310, 1965.

#### Address for reprints:

H. Riski, M.D.,  
Dept of Virology,  
University of Helsinki,  
Haartmaninkatu 3,  
SF-00290 Helsinki 29,  
Finland