JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1976, p. 450-452
Vol. 4, No. 5
Copyright © 1976 American Society for Microbiology
Printed in U.S.A.

NOTES

Hemolysis-in-Gel Test for Demonstration of Chlamydia Antibodies

ERIK LYCKE* AND MARGRETH PETERSON

Department of Virology, Institute of Medical Microbiology, University of Göteborg, Göteborg, Sweden

Received for publication 17 June 1976

Sheep erythrocytes were coated with antigens of a strain of *Chlamydia trachomatis* serotype D. Chlamydial antibodies in sera from patients with salpingitis or pneumonia were demonstrated by passive hemolysis in agarose gel.

Hemolysis-in-gel or single radial hemolysis has been successfully applied for demonstration of antibodies against gamma globulin (5) and *Toxoplasma gondii* (6) and against influenza (1, 7; G. C. Schild, J. S. Oxford, and J. L. Virelizier, 47th Symp. Int. Assoc. Biol. Soc., London, 1974), rubella (8, 9), and mumps viruses (3). We have, by means of sheep erythrocytes (RBC) treated with tannic acid and coated with chlamydia antigen from infected McCoy cells, developed a hemolysis-in-gel test for demonstration of antibodies against chlamydia.

Cultures of irradiated McCoy cells (2) were harvested 3 days after inoculation with a strain of *Chlamydia trachomatis* serotype D. The antigen was released by freezing and thawing of the cells and concentrated by pelleting at 35,000 $\times g$ for 60 min. The antigen was resuspended in 5 ml of 2-SP medium (2) containing 0.6% bovine albumin, clarified by low-speed centrifugation, and adsorbed onto sheep RBC.

A 2.5% suspension of washed RBC was treated with tannic acid (1:40,000) at 37°C and pH 7.2 for 15 min. Subsequently, the suspension of RBC was washed once in phosphate buffer, pH 7.2, and a second time in a buffer of pH 6.7. Antigen and RBC were mixed, 0.4 ml of RBC per ml of antigen, and kept at room temperature for 30 min. The cells coated with antigen were washed in phosphate buffer, pH 7.2, and 10 ml of the 2.5% suspension was added to a mixture of 35 ml of 0.03 M sodium azide and 30 ml of a 1.6% (wt/vol) agarose solution in saline, heated to 47°C.

The agarose containing the antigen-coated RBC was poured onto 9.5-cm^2 plastic plates, 11 ml/plate, and allowed to solidify in the cold. After solidification of the gel, 36 3-mm holes were punched out, and each hole was filled with 0.5 μl of serum or diluted serum. Before use, the sera had been absorbed with sheep RBC. The plates were incubated in the refrigerator for 24 h, and then 4 ml of normal guinea pig serum, diluted 1:4 in barbital buffer, pH 7.2, was added. After subsequent incubation of the plates at 37°C for 2 h, the hemolytic zones appearing were measured with a precision caliper. Controls with an antigen prepared from noninfected McCoy cells were used in each run.

The relationship between the size of the hemolytic zones and the antibody concentration (dilution of antiserum) is presented in Fig. 1. A linear relationship between the diameters of the zones in millimeters and the log antibody concentration was demonstrable within the range of 8- to 12-mm zones. Below this range the diameters of the zones deviated from linearity. Less accurate determinations were obtained when the zones were more than 15 or less than 6 mm in diameter. Within the 8- to 12-mm range, the standard deviation of measurements was 0.15 mm. A difference of 0.5 mm between diameters of two zones might thus be considered significant.

In seven women diagnosed as cases of salpingitis, *C. trachomatis* was isolated, and three of the isolates were typed as belonging to serotype D. Results of a small serological study with complement fixation (CF) and hemolysis-in-gel tests are presented in Table 1. The antigen used in the CF test was a commercially available ornithosis antigen prepared from mouse tissue and boiled (Universiteit van Amsterdam, Laboratorium voor de Gezondheidsleer). All but three of the sera studied displayed chlamydia antibody activity by both types of tests. Three sera were positive in hemolysis but negative in CF.

In 17 other patients clinically diagnosed as

salpingitis cases, but in whom attempts to isolate chlamydia had been unsuccessful, 13 were negative in CF as well as in hemolysis-in-gel tests. In one woman a rise in antibody titer from the acute phase to convalescent phase was observed, and in the remaining three cases the sera caused hemolytic zones of <6 mm but did not react in CF.

Seven patients from an outbreak of ornithosis were studied serologically using the hemolysis-in-gel test and CF (Table 2). Acute-phase as well as convalescent-phase sera were available from these patients. Seroconversion was detected in all cases by both types of tests. CF titers of <4 in acute-phase sera corresponded to negative findings in hemolysis-in-gel tests. In one case a low CF acute-phase titer was associated with negative hemolysis, and in another patient from whom several serum samples were tested a drop in CF titer over a period of 1 year was not accompanied by decreasing titers in the hemolysis-in-gel tests.

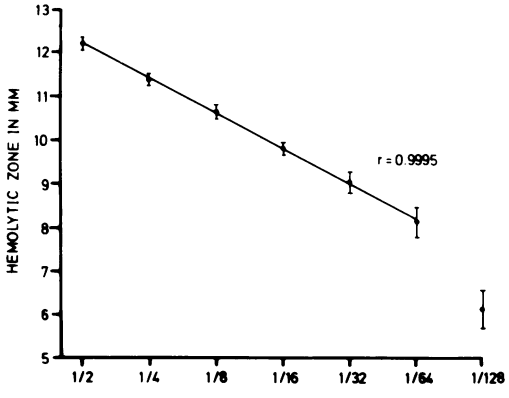

FIG. 1. *Hemolysis-in-gel test for demonstration of chlamydia antibodies. Regression line was calculated from results of eight tests with each of six dilutions of a human serum. Bars denote standard deviations of means, and r is the correlation coefficient.*

TABLE 1. *Demonstration of chlamydia antibodies in seven patients with salpingitis*[a]

Patient	Acute phase		Convalescent phase	
	CF	HIG	CF	HIG
MO	ND	ND	8	8
HO	16	17	128	18
GEn	8	11	32	12
AS	ND	ND	128	14
AS			256	14
AL	<4	9	4	9
EF	<4	10	<4	11
HO	4	10	8	12
HO			8	12

[a] Comparison of CF titers and results of hemolysis-in-gel (HIG) tests. Sera from the acute phase were collected at the time of admittance to the hospital. ND means that serum was not available.

TABLE 2. *Chlamydia antibodies in seven patients with suspected ornithosis*[a]

Patient	Acute phase		Convalescent phase	
	CF	HIG	CF	HIG
MM	<4	0	16	13
EA	4	10	64	14
BG	<4	0	64	13
KB	4	10	16	13
HS	<4	0	4	16
EL	<4	0	8	9
GE	8	0	256	14
GE			32	14

[a] CF titers and results of hemolysis-in-gel tests (HIG). Sera from the acute (first week of disease) and convalescent phases were tested simultaneously. The third serum sample from patient GE was obtained 1 year after recovery from the disease.

The hemolysis-in-gel test seems well suited for demonstration of antibodies against chlamydia. Since the test is sensitive and accurate (4) and simple to perform, it may be of value especially for serological screening in epidemiological studies on chlamydial infections. The results of the present report suggest that a difference between diameters of hemolytic zones greater than 0.5 mm would correspond to a significant change in the antibody concentration.

A number of findings indicated the specificity of the chlamydia hemolysis-in-gel test. Seroconversions were observed in all cases of ornithosis. These changes occurred in parallel with changes in CF titers against an ornithosis antigen. Sera from seven cases of salpingitis, from which *C. trachomatis* was isolated, all displayed antibody activity. In a group of 17 cases of salpingitis, from which chlamydia could not be cultivated, 13 were negative, 1 demonstrated a rise in antibody titer, and 3 showed very weak reactions.

The antigen used by us was produced with a strain of *C. trachomatis* serotype D. Therefore, the hemolytic activity of sera from patients with ornithosis must have been caused by group-specific antibodies. However, it is probable that type-specific chlamydial antibodies are also detectable. With proper antigens, type-specific antibodies against influenza A strains are demonstrable in the single radial hemolysis test (1, 7).

We are indebted to John D. Treharne, who kindly performed the serological typing.

LITERATURE CITED

1. **Callow, K. A., and A. S. Beare.** 1976. Measurement of antibody to influenza virus neuraminidase by single radial hemolysis in agarose gels. Infect. Immun. **13**:1-8.
2. **Gordon, F. B., I. A. Harper, A. L. Quan, J. D. Treharne, R. St. C. Dwyer, and J. A. Garland.** 1969. Detection of chlamydia (bedsonia) in certain infections of man. I. Laboratory procedures: comparison of yolk sac and cell culture for detection and isolation. J. Infect. Dis. **120**:451-462.
3. **Grillner, L., and J. Blomberg.** 1976. Hemolysis-in-gel and neutralization tests for determination of antibodies to mumps virus. J. Clin. Microbiol. **4**:11-15.
4. **Grillner, L., and Ö. Strannegård.** 1976. 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.
5. **Hall, J. M.** 1971. Specificity of antibody formation after intravitreal immunization with bovine gammaglobuline and ovalbumin. I. Primary response. Invest. Ophthalmol. **10**:775-783.
6. **Jackson, W. B., G. R. O'Connor, and J. M. Hall.** 1974. Plate hemolysin test for the rapid screening of toxoplasma antibodies. Appl. Microbiol. **27**:896-900.
7. **Russel, S. M., D. McCahon, and A. S. Beare.** 1975. A single radial haemolysis technique for the measurement of influenza antibody. J. Gen. Virol. **27**:1-10.
8. **Skaug, K., I. Ørstavik, and J. C. Ulstrup.** 1975. Application of the passive haemolysis test for the determination of rubella virus antibodies. Acta Pathol. Microbiol. Scand. Sect. B **83**:367-373.
9. **Strannegård, Ö., L. Grillner, and I.-M. Lindberg.** 1975. Hemolysis-in-gel test for the demonstration of antibodies to rubella virus. J. Clin. Microbiol. **1**:491-494.