

# DETECTION OF THE ANTIGEN-ANTIBODY REACTION BY LIGHT SCATTERING SPECTROSCOPY

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The possibility of recording the results of serologic reactions not yielding visible phenomena by means of light scattering spectroscopy (LSS) was studied. Influenza virus and *Mycoplasma pneumoniae* antigens and the corresponding antisera were used as the test objects. It was shown that, in principle, LSS can be used to detect the antigen-antibody complex in serologic reactions not yielding visible phenomena.

KEY WORDS: serologic reaction; light scattering spectroscopy.

As a rule serologic reactions take place in two phases: In the first phase molecular antigen-antibody complexes are formed, after which the second phase is observed as a visible phenomenon such as agglutination, precipitation, etc. However, there are certain serologic reactions in which the formation of the antigen-antibody complex cannot be detected visually, and can be found only by means of a second indicator system. In particular, such reactions include the complement fixation test (CFT), the hemagglutination inhibition test (HIT), etc.

The necessity of using a second system to record the results of the serologic test makes it much more complicated, more time-consuming, and less stable, and this is a particular disadvantage for mass investigations. It is therefore important to seek methods of recording the formation of the antigen-antibody complex objectively and quantitatively in serologic tests not yielding visible phenomena. It was shown previously that light scattering spectroscopy (LSS) can be used to record the results of some immunologic reactions [1, 4]. The sensitivity of this method for recording the precipitation test was found to be higher than visual assessment and enabled the antigen-antibody complex to be detected in dilutions of the reaction components for which no visible precipitation was observed [1]. LSS has also been successfully used to determine the average size of submicroscopic virus particles [2].

On the basis of these results it was decided to undertake the present investigation in which LSS was used to detect antigen-antibody complexes in serologic tests not yielding visible phenomena.

## EXPERIMENTAL METHOD

Antigen of influenza A<sub>2</sub> virus, coronavirus OC-43, and also of *Mycoplasma pneumoniae* and the corresponding sera were used as the test objects. Titers of antibodies against virus antigens were determined by the HIT and titers of *M. pneumoniae* antigen and of antibodies against it by the CFT.

The HIT was carried out in plates of a Takachi microtitrator in a volume of 0.1 ml by the usual method; the CFT was carried out in a micromodification by the method described previously [3].

To determine the titer of the same antibodies by LSS a mixture of equal volumes of corresponding dilutions of the sera with the working dilution of antigen in a total volume of 0.5 ml was used. When the titer of *M. pneumoniae* antigen was determined a mixture of equal volumes of double dilutions of antigen (up to 1:1024) and of hyperimmune rabbit serum, in a

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TABLE 1. Results of Titration of Sera with Dry Influenza Diagnostic Serum of Strain A<sub>2</sub>/Hong Kong/68 (8 antigenic units) and Coronavirus OC-43 Antigen (4 antigenic units)

Antigen	Serum	Dilution of serum							Titer	Control serum
		1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
Influenza A <sub>2</sub> diagnostic serum (8 antigenic units)	Homologous immune rabbit	—	—	—	—	—	4+	4+	1:320	—
Coronavirus OC-43 antigen (4 antigenic units)	Control	4+	4+	4+	4+	4+	4+	Not tested	0	—

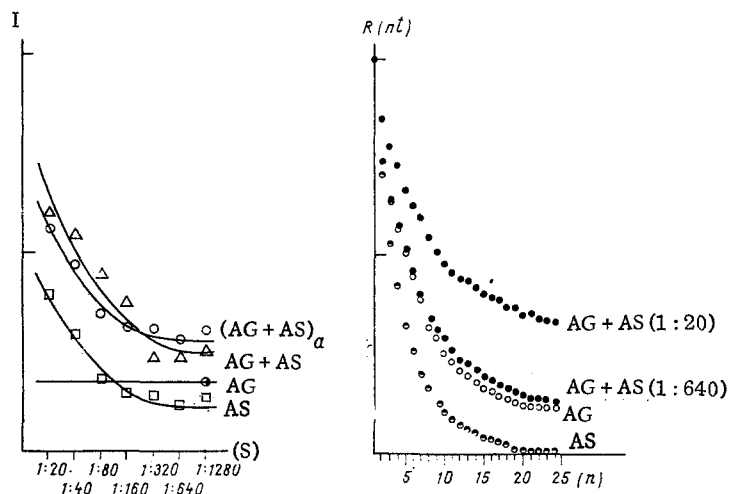


Fig. 1. Results of measurement of integral intensity (I) and correlation function  $[R(nt)]$  of scattered light for a system consisting of influenza A<sub>2</sub> antigen — homologous antiserum. Abscissa, dilution of antiserum (s) and No. of correlator channel (n). t) Sampling time, 200  $\mu$ sec. AG) Antigen, AS) antiserum,  $(AG + AS)_\alpha$  additive curve obtained by addition of intensities of AG and AS; AG + AS — curve actually observed.

dilution of 1:128, was used. The dilutions of serum and antigens were made up in filtered sterile physiological saline. The technique of the measurements and the apparatus used were the same as in previous investigations [1].

#### EXPERIMENTAL RESULTS

The results of titration of the sera with dry influenza diagnostic serum for strain A<sub>2</sub>/Hong Kong/68 (8 antigenic units) in the HIT are given in Table 1. They show that inhibition of hemagglutination was observed with dilutions of immune serum of up to 1:320.

The results of titration of the same immune serum by means of LSS are given in Fig. 1. Simultaneously with measuring correlation functions, the integral intensity of light scattered at an angle of 90° was recorded. The results of measurement of the integral intensity of the scattered light in an antigen-antibody mixture (AG + AS) were compared with the total intensity of scattered light of the components  $(AG + AS)_\alpha$  and in that way the possibility of detecting the reaction from an increase in the integral intensity of scattered light compared with the additive values was investigated. Furthermore, measurements of the integral intensity by LSS enabled the relative contribution of the components to the resultant correlation curve to be estimated.

The data shown in Fig. 1 indicate that the presence of antigen-antibody complexes can be recorded from the intensity of scattered light up to a dilution of antiserum of 1:160. On the right side of Fig. 1 correlation curves are shown for AS (1:20), AG, and a mixture of

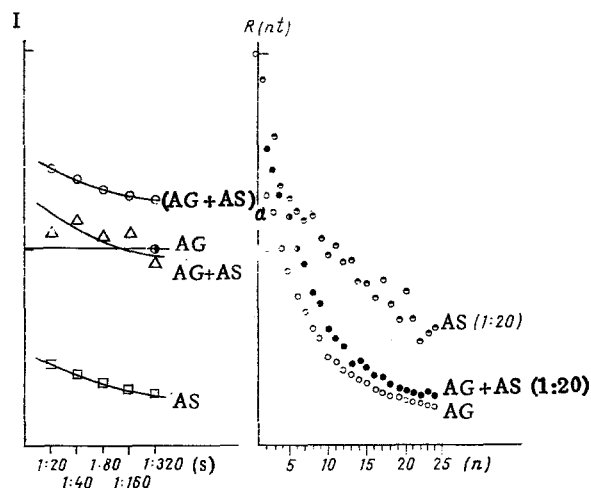


Fig. 2. Results of measurement of integral intensity (I) and correlation function [R(nt)] of scattered light for system consisting of coronavirus OC-43 antigen - control serum. Legend as in Fig. 1.

AG + AS. If no antigen-antibody complexes were formed in this mixture as a result of their specific interaction, the resultant curve would occupy a certain intermediate position between the AS and AG curves; moreover, since the intensity of scatter in AS (1:20) was greater than in AG, it would be closer to the AS curve.

This picture was observed when coronavirus OC-43 antigen was mixed with the control serum, which gave negative results in the HIT (Table 1, Fig. 2).

The influenza A<sub>2</sub> antigen was mixed with the homologous antiserum; the substantially widened correlation curve indicated the formation of an antigen-antibody complex. Widening of the correlation curve was observed with dilutions of serum up to 1:640. The causes of the effectively greater sensitivity of the spectral (correlation) method than of the method of measurement of integral intensity was discussed earlier [4].

Titration of *M. pneumoniae* antigen and antiserum against it in the CFT and measurements by the light-scattering method gave identical positive results up to maximal dilutions: (1:1024).

The investigation thus showed that, in principle, LSS can be used for the direct detection of antigen-antibody complex in serologic reactions not yielding visible phenomena, and also for quantitative evaluation. The sensitivity of this method for the models studied is comparable with that of the two-system serologic tests usually used for this purpose (CFT and HIT).

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