VIRAL IMMUNOASSAY FOR MASS SCREENING OF NANOGRAM AMOUNTS OF ANTIGEN

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For mass determinations of an antigen (screening of clinical and laboratory material) present in exceedingly small amounts in test samples, highly sensitive methods of radioimmunoassay and enzyme immunoassay are widely used [1, 6]. Although these methods have been elaborated in detail they possess various disadvantages, and for that reason efforts are still being made to develop modifications of these techniques and also to find other methods. The writers previously suggested a method of determining an ultramicromethod of assaying antibodies to bacteriophage. In this method of determination the phage acted simultaneously as both antigen and "label," for phage–antibody complexes, extracted by the immunosorbent, gave separate zones of lysis, the number of which could be used to judge the quantity of antibodies [2]. Later the phage, as label, was used to prepare conjugates with antibodies against diptheria toxin for quantitative determination of an antigen by the competitive principle (viral immunoassay) [3]. The method of obtaining conjugates was later modified: Conjugates of phage with Fab'-fragments of antibodies, attached in a regular manner were prepared [4]. This led to a new version of viral immunoassay for direct determination of an antigen extracted by an immunosorbent. The sensitivity of this method exceeded that of the competitive version, and also of radioimmunoassay and enzyme immunoassay [5]. However, this test was laborious and inconvenient for mass screening.

In this paper we suggest a version of viral immunoassay (SVIA) suitable for mass determinations of small quantities of an antigen. This method is simple, clear, highly sensitive, and enables qualitative and quantitative determination of the antigen to be combined over a wide range of concentrations. It requires no special apparatus, for the results are read visually.

$F(ab')_2$-fragments of rabbit antibodies against rat IgG, in a volume of 50 µl, or IgG fraction of immune serum (initial concentration of both was 4 µg/ml), or whole rabbit immune serum in a dilution of $2 \cdot 10^{-3}$ in 0.01 M Tris-HCl buffer, pH 8.0, with 0.14 M NaCl, each in a volume of 50 µl, was introduced into 96-well flat-bottomed plates (Leningrad Medical Polymers Factory). After incubation overnight (all subsequent operations were performed at room temperature) the material from the wells was shaken and 100 µl of 0.5% egg albumin in the same buffer was introduced into each well. After incubation for 1 h the albumin was shaken off and the plate washed 10 times by immersing it in the same buffer with 0.05% Tween-20. Next, different quantities of antigen (rat IgG) were added to the wells in a volume of 50 µl in buffer with Tween-20, whereas buffer alone was added to the control samples, which were incubated for 2 h. Unbound antigen was washed off 10 times by the method described above and various quantities of conjugate of phage ØX174 with Fab'-fragments of antibodies against rat IgG [5] in buffer with Tween-20 were added in a vlume of 50 µl to the wells. The unbound conjugates were washed off 10 times by the method described above, and to each well was added 50 µl of 0.01 M dithiothreitol in 0.1 M Tris-HCl buffer, pH 8.0, to separate the attached conjugates. After incubation for 30-40 min material from each well was applied in a volume of 20 µl to a layer of 0.9% with 1% peptone in 0.14 M NaCl and 2 ml of *E. coli* C, with a concentration of $2 \cdot 10^9$ bacterial cells/ml, which had solidified on glass; the total quantity of the mixture of agar and microorganisms applied to a glass plate measuring 13 × 19 cm was 25-26 ml. The glass plate was incubated at 37°C, and in order to reduce evaporation and drying of the agar, it was

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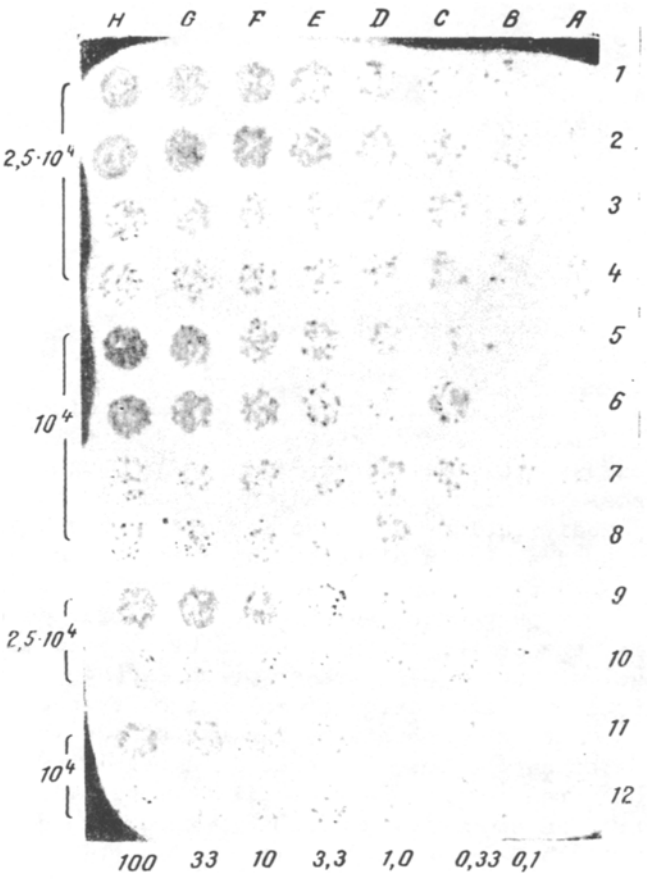

Fig. 1. Qualitative and quantitative parameters obtained by SVIA, depending on quantity of antigen and of conjugates added to the sample. Horizontally below — antigen concentration (in ng/ml); vertically: on right — No. of row, on left — quantity of conjugate added (PFU). Rows 1-8 — wells covered with $F(ab')_2$-fragments of antibodies (rows 9-12 - wells covered with diluted whole immune serum; rows 1 and 2, 5 and 6, 9-11 — conjugates after separation were not diluted; rows 3, 4, and 10 — conjugates diluted after separation by 2, 3, 4, 5, and 6 times from C to H respectively; rows 7, 8, and 12 — conjugates diluted after separation by 2, 3, 4, and 5 times from E to H respectively; vertical row A indicates corresponding controls.

covered with a lid. The results were read 2.5-3 h later or next day, the plate being kept at 4°C.

The results of one experiment are given in Fig. 1. The dark dots are zones of lysis of the phage, formed on the culture of micro-organisms in the agar layer on the glass (plaque-forming units — PFU). Clearly the number of PFU detected increases with an increase in the quantity of antigen added to the sample, compared with the control. Single plaque-forming units were discovered in the control; in this case a significant difference between experiment and control could be seen when the antigen concentration reached 0.1-0.3 ng/ml. When the antigen concentration was 10-100 ng/ml, the PFU could no longer be counted, because confluent lysis was observed. However, if samples with conjugates corresponding to antigen concentrations of 1 to 100 ng were diluted beforehand actually in the wells on the plate by 2, 3, 4, 5, and 6 times respectively, the PFU could now be counted (Fig. 1; rows 3, 4, 7, 8, 10, and 12). On the basis of data thus obtained a calibration curve can be plotted. Curves for antigen concentrations from 1 to 1000 ng/ml, when the wells were covered with the IgG-fraction of immune serum and different amounts of conjugates, are given in Fig. 2. The use of diluted whole immune serum to cover the wells also proved effective (Fig. 1, rows 9-12). The error

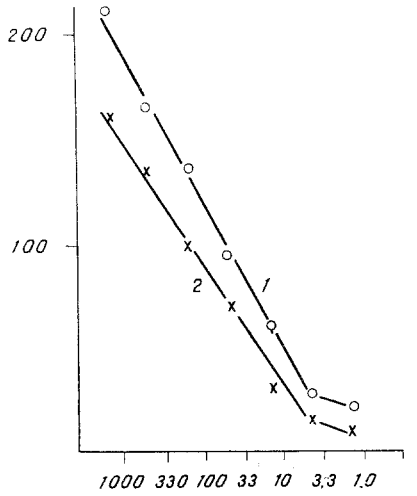

Fig. 2. Calibration curves for determining antigen with the aid of SVIA. Abscissa, quantity of antigen (in ng/ml); ordinate, number of PFU discovered. 1) Quantity of conjugates per sample — 10^4; 2) quantity of conjugates per sample — 5×10^3.

between parallel determinations of the same experiment and between different experiments did not exceed 15%.

Thus besides radioimmunoassay and enzyme immunoassay, the suggested method can also be used for antigen screening. Besides its relative simplicity in use, SVIA also has definite advantages over the other methods mentioned. With the use of isotopes or enzymes as markers, particular care must be taken throughout the work and some expensive equipment is needed. The process of formation of a sufficient number of zones of lysis for determination when SVIA is used, runs a stable course, although it does require at least 2.5 h, and to stop an increase in the number of zones of lysis, all that is necessary is to cool the preparation; the number of PFU can easily be counted visually or under low power, and they can also be recorded photographically. It is a simple matter to read the results qualitatively, for confluent lysis or a larger number of PFU than in the background is obvious. The high sensitivity of SVIA is due to the properties of the conjugate: The phage "labels" single molecules of antigen, and the results obtained are therefore not relative values, as for radioimmunoassay and enzyme immunoassay, but absolute, for each PFU corresponds to one antigen molecule. By varying the number of Fab'-fragments in the conjugate or the number of conjugates themselves, the sensitivity of the method can be increased or reduced depending on the aim of the analysis. The range discovered in the present experiments extended from 0.3 ng to 1 μg/ml of antigen, and this is especially important in clinical practice. The facts described above indicate that our suggested method can be widely used both for clinical antigen screening and for laboratory investigations.

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LITERATURE CITED

1. Immunology: Methods of Investigation [Russian translation], Moscow (1983).
2. V. T. Skvortsov and V. M. Bondarenko, Vopr. Virusol., No. 5, 603 (1971).
3. V. T. Skvortsov and Yu. V. Vertiev, Lab. Delo, No. 4, 437 (1981).
4. V. T. Skvortsov and A. E. Gurvich, Byull. Éksp. Biol. Med., No. 2, 179 (1984).
5. V. T. Skvortsov, Byull. Éksp. Biol. Med., No. 8, 251 (1986).
6. T. Chard, Introduction to Radioimmunoassay and Related Techniques, Amsterdam (1978).