

DETERMINATION OF THE SENSITIVITY OF THE PLAQUE VIRUS
NEUTRALIZATION TEST: COMPARISON WITH THE SENSITIVITY
OF ANTIBODY DETECTION IN HAEMAGGLUTINATION AND COMPLEMENT
FIXATION SYSTEMS

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The sensitivities of basic serological methods were compared by Grabar (1): precipitation, as the least sensitive method, requires 3–20 μg AbN/ml serum, while the complement fixation (CF) test 0.1 μg AbN/ml; bacterial agglutination is somewhat more sensitive (0.05 μg AbN/ml). By passive haemagglutination (PHA) with adsorbed protein or bacterial antigen it is possible to detect up to 0.005 μg AbN/ml serum and by haemolytic reaction under standard conditions about 0.01 μg AbN/ml serum. There have been no reports about the sensitivity of the virus neutralization test. By comparing the sensitivities of the CF and PHA reactions with the sensitivity of the 50% plaque virus neutralization (PVN) test it is possible to estimate the least amount of antibody necessary for accomplishment of the latter reaction.

To determine experimentally the sensitivity of the PVN test, the small spherical f 2 phage was used because of its extreme antigenic simplicity (2). The surface of this bacterial virus namely represents a single antigen, the individual surface antigenic sites being identical. Due to this advantage it was possible to use the f 2 phage in three types of serological reaction (CF, PHA and PVN tests), because only a single and the same surface antigen of the viral capsid becomes involved in either of the reactions.

The CF test was carried out by the routine diagnostic procedure (8 antigenic units of phage f 2, 2 units C'H₅₀ of guinea pig complement, 1% suspension of sheep erythrocytes sensitized by haemolytic amboceptor). The reaction was evaluated as positive in case of at least 50% haemolysis inhibition. The PHA test with f 2 phage adsorbed onto sheep erythrocytes was carried out by the method of Stavitsky and Arquilla (3). The optimal ratio, i.e. a positive reaction scored 4+ in the presence of antiserum excess, was found to be at least 100–200 PFU of f 2 phage per 1 erythrocyte. The arrangement of the PVN test was described (4).

The titres of anti-phage antibody in hyperimmune rabbit antiserum against f 2 phage, determined in the CF, PHA and PVN tests were 32, 512 and 1,600,000, respectively (mean values from 5 experiments).

It is evident that by the PVN test it is possible to detect an about 50,000 times smaller amount of antibody than by the CF test and a 2,000 times smaller amount of antibody than by PHA. Controls with normal rabbit serum were negative. By the method of the PVN test it is thus possible to detect 10⁻⁵–10⁻⁶ μg antibody N of anti-phage antibody. Therefore, the PVN test can be placed among the most sensitive serological methods, like the bactericidal (5) and the microhaemolytic (6) reactions, by which it is possible to detect approximately the same values.

References

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