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Brief Communication

THE IBR-IPV VIRUS-SERUM NEUTRALIZATION TEST

STUDIES ON THE INFLUENCE OF THE VIRUS-SERUM INCUBATION PRIOR TO INOCULATION

A modification (*Bitsch* 1973) of the IBR-IPV virus-serum neutralization test gave an essential improvement of the sensitivity of this test compared to the conventional test. However, the titer distribution of sera with virus-neutralizing antibodies (VNA) suggested that an additional improvement by 1 or 2 steps of the titer scale would be desirable. With a view hereto, 2 experiments were carried out to elucidate to what extent titers were influenced by preinoculation incubation (P) of the virus-serum mixture.

In experiment I, virus-serum mixtures were preincubated in air for periods varying from 1 to 24 hrs. at 37°C or at room temperature (abt. 22°C). In experiment II, P varied from 30 min. to 3 hrs. at 37°C in air or water. The resulting VNA titers (with 100 TCID50 of virus) are shown in Figs. 1 and 2, respectively. Four tissue culture tubes were inoculated per dilution. The above reference gives further information about methods and materials.

The possible influence of P at 37°C on the survival of virus was examined simultaneously in both experiments. Virus suspensions in PBS with 2 % SPF calf serum with calculated titers of 100, 10, 1, and 0.1 TCID50 were incubated at 37°C for up to 48 hrs. Titration was made by inoculation of 6 tubes from each suspension. The results are given in Table 1.

Table 1. Experiments I and II. Titers (TCID50, $\log_{10}$ values) of working suspensions of virus after varying periods of incubation at 37°C.

Incubation period (hrs.)	0	1	2	3	4	6	12	24	48
Experiment I		2.0		1.8		1.8	1.8	2.0	1.8
Experiment I	1.8	2.0	1.7	2.3	2.0				

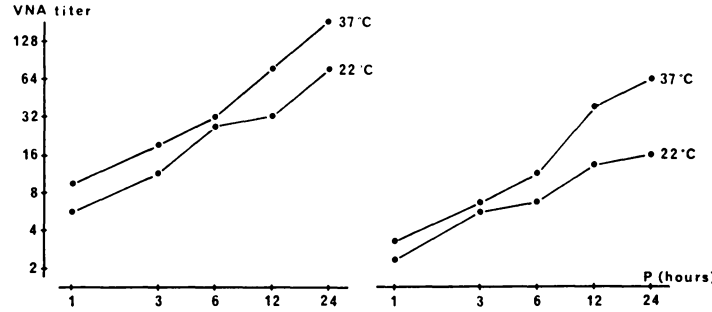

Figure 1. VNA titers of two sera after varying periods of preinoculation incubation (P) of the virus-serum mixtures in air at 37°C and 22°C.

Fig. 1 shows that considerable rises in VNA titer occurred at $P > 1$ hr. and that the effect of the incubation was greater at 37°C than at 22°C. Fig. 2 shows that incubation in water instead of air made no particular difference to the VNA titers, not even with a short P. Finally, it appears from Table 1 that the higher VNA titers obtained after $P > 1$ hr. are by no means due to a reduction in virus titer during incubation.

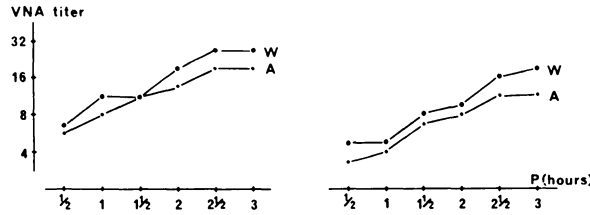

Figure 2. VNA titers of two sera after varying periods of preinoculation incubation (P) of the virus-serum mixtures in water (W) and air (A) at 37°C.

Fourty IBR-IPV-positive sera were then titrated simultaneously with P_1 (1 hr.) and P_{24} (24 hrs.). Only 2 tissue culture tubes were used per dilution. The results, given in Table 2, show that with P_{24} there is an almost constant titer rise over the whole titer scale, the mean rise being 4.8 steps. For a few sera, it was found that P_{48} gave a further titer rise of 1 to 2 steps.

Finally, in order to evaluate a possible unspecific virus neutralization (VN) by P_{24}, the method was used in an examination of sera which had previously been found VNA-negative. Hank's

Table 2. VNA titers of 40 IBR-IPV-positive sera with preinoculation incubation periods of 1 hr. (P_1) and 24 hrs. (P_{24}).

P_1 titers	< 1	1—1.4	2—2.8	4—5.6	8—11	16—22	32—45	64
Number of samples	7	5	10	6	7	4	0	1
P_{24} titers	11—45	22—45	32—90	90—360	128—512	360—720	—	1024
Improvement (titer steps)	4—6	4.5—5.5	3.5—5.5	4.5—6	4—5.5	4—5.5		4

balanced salt solution with 2 % serum was used for virus suspensions and serum dilutions. Of 279 sera, 245 (87.8 %) gave no VN in any of the 2 tubes inoculated with undiluted serum, while 22 gave VN in 1 of 2 such tubes, and 12 (4.3 %) in both tubes. One sample gave VN in 1 of 2 tubes with serum dilution ½, but in no tube with dilution ¼.

Thus, preincubation of the virus-serum mixtures for longer than 1 hr. seems to bring about a considerable improvement in the sensitivity of the test. With P_{24} the improvement would be of the order of at least 3 steps of the titer scale. The working-out of a final procedure will require further studies.

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REFERENCE

Bitsch, V.: The IBR-IPV virus-serum neutralization test. Sensitivity and significance of the tissue culture tube test. Acta vet. scand. 1973, *14*, 683—690.

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