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Brief communication

IMPROVED SENSITIVITY OF THE IPV/IBR VIRUS-SERUM NEUTRALIZATION TEST

The serum neutralization (SN) test is still the most important method for the demonstration of infectious pustular vulvovaginitis/infectious bovine rhinotracheitis (IPV/IBR) antibodies. However, several authors have reported a relatively low sensitivity of the SN test, titers often being very low.

The aim of this publication is to propose a modification of the SN test, which should give an improved sensitivity and yet significant results.

For illustration of the improved sensitivity, the results of examination of sera from 2 experimentally infected bulls are given in Fig. 1.

IPV virus was suspended in phosphate-buffered saline (PBS) in concentrations of 10, 100, and 1000 TCID50 per 0.1 ml. Twofold dilutions of the 2 sera in PBS were tested against those 3 virus concentrations in the usual way, i.e. with 0.1 ml virus suspension and 0.1 ml serum dilution, but also with 0.2 and 0.4 ml serum dilution. For each virus dose and serum dilution 4 primary calf kidney cell culture tubes with 1.8 ml maintenance medium were inoculated after incubation of the virus-serum mixture at 37°C for 1 hr. Titers were recorded as 50 % endpoints (reciprocals of serum dilutions).

The inverse linear relationship between virus dose and antibody titer appears from the figure. But the most important thing, which it not surprising, is that with increased amount of serum it was possible to demonstrate very low antibody concentrations. In the examples shown the sensitivity of the SN test was improved more than 4 times when 10 TCID50 and 0.4 ml of serum was used instead of 100 TCID50 and 0.1 ml serum.

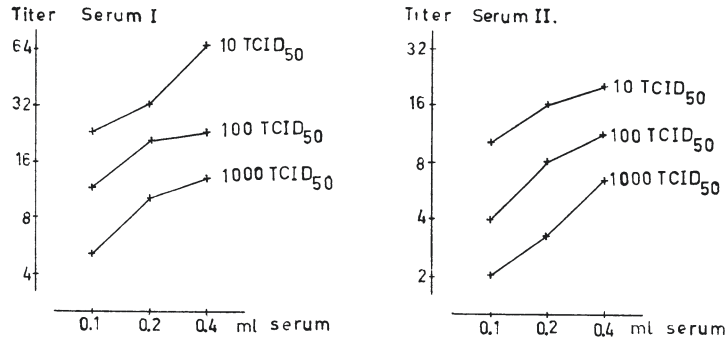

Figure 1. IPV SN titers of 2 sera in relation to varying doses of virus and serum.

The only objection to using a lower virus and a higher serum dose in the SN test is that it might lead to unspecific inhibition of virus growth. Since February 1969, however, the writer has examined about 3900 blood samples from cattle, and in no case there was any evidence of unspecific results.

Groups of animals examined were as follows:

I. Bulls at AI centres. Of 1335 bulls at the 45 Danish bull centres 139 were found to be antibody carriers (*Bitsch et al.* 1970), all with relatively high titers. All but 7 were stationed at 4 centres within which a spreading of the disease among the bulls had occurred. The remaining 7 were imported or had been in contact with bulls from the above-mentioned 4 centres.

II. Young bulls before introduction to AI centres. One-hundred-and-thirty animals were examined for antibodies with a negative result.

III. Pregnant heifers and cows. These samples were, for reasons other than IPV, sent to the laboratory from all over the country, and usually herds were represented by 1 to 3 samples each. Of a total of 1948 samples 24 were found to inhibit growth of virus. Titers ranged from 1 when using 10 TCID50 and 0.1 ml of serum to 16 when using 100 TCID50 and 0.1 ml of serum. Reacting animals were found only in herds where semen from bulls stationed at the above-mentioned 4 IPV-infected centres had been used for insemination.

IV. Imported cattle. Of 478 animals, mainly pregnant heifers and cows, 3 had titers ranging from 2 to 4 when using 100 TCID50 and 0.1 ml of serum. The rest were negative.

Routine procedure. For screening, 4 tissue culture tubes are inoculated per sample: 2 tubes each with 10 TCID50 of IPV virus and 0.5 ml of serum, and 2 tubes each with 100 TCID50 and 0.1 ml of serum, the appropriate virus-serum mixture having first been incubated at 37°C for 1 hr. Serum samples are inactivated for 30 min. at 56°C. When a great number of samples are to be examined, and the results are expected to be negative, examination with 100 TCID50 is omitted.

In cases of virus growth inhibition the titer of the serum is determined and given in relation to the amount of virus and serum used.

Known positive and negative controls need not be included while controls on the virus suspensions are essential. From each of the virus suspensions calculated to contain, respectively, 10, 1, and 0.1 TCID50 per 0.1 ml, 6 tubes are inoculated each with 0.1 ml. Final reading of inoculated tubes is made after 6 or 7 days.

Virus suspensions and serum dilutions are made in PBS with 2 % serum from a colostrum-deprived calf reared in isolation.

Since with this procedure the writer has never found any evidence of unspecific results, it is proposed that no SN test should be considered "not positive", unless 0.5 ml of serum will not inhibit growth of 10 TCID50 of IPV virus.

This modified SN test is used by the writer also for the detection of antibodies against pseudorabies (0.25 ml of serum per 10 TCID50), and equine rhinopneumonitis viruses. Also here the modification is considered to be an essential improvement.

V. Bitsch
The State Veterinary Serum Laboratory,
Copenhagen, Denmark.

REFERENCES

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