

4. Title: Microculture Assay of Reovirus Antibody

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OBJECTIVE

To investigate the utility of micro-neutralization culture systems to measure serum antibody to reoviruses.

DESCRIPTION

In an attempt to develop a microculture system for the assay of reoviruses and their antibodies, the growth of viruses in various cells grown in wells of microtiter plates was tested. It was found that after appropriate incubation periods, while reovirus-infected MK2 cells showed no evidence of CPE, the presence of virus could be detected by the presence of hemagglutinins in growth media. A suspension of red cells dropped directly into the well will form a hemagglutination pattern in the presence of virus. Further, the finding that the sensitivity of the test is increased ten-to a hundred-thousand fold if growth media is serumfree prompted further investigation of this system.

PROGRESS

The sensitivity of the microsystem compared to conventional assay systems is shown in Table 1 as determined on a single pool of each serotype. Microculture titers were determined on day 7. Assayed titers were exceeded in sensitivity only by tube titrations employing a single blind pass after 21 days.

Sequential virus titers with incubation time were determined by setting up virus titrations of each type in triplicate plates and adding red cells to one titration each day. Results are shown in Table 2 and illustrate that reovirus types 1 and 2 attain peak titers by day 8 whereas type 3 appears to grow more slowly. Titers are expressed as HA<sub>50</sub> of the original inoculum in this case of 0.05 ml. 1HA<sub>50</sub> being that amount of virus resulting in hemagglutination in 50% of the wells tested.

Table 1. Virus Titer Determined by Various Assay Systems.

| Virus | Titer log <sub>10</sub> /1.0 ml |        |                |              |
|-------|---------------------------------|--------|----------------|--------------|
|       | Micro                           | 7 days | Tube Titration |              |
|       |                                 |        | 28 Days        | Plaque titer |
| Reo 1 | 8.8                             | 6.5    | 9.5            | 7.8          |
| Reo 2 | 8.8                             | 6.5    | 9.5            | 7.8          |
| Reo 3 | 6.8                             | 5.5    | 8.5            | 6.7          |

Table 2. Virus titer determined by Hemagglutinin end points on consecutive days.

| Virus | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Reo 1 | 3.8 | 4   | 5.7 | 6.7 | 7.0 | 7.3 | 7.5 | 8.0 | 8.2 | 7.8 |
| 2     | 2.5 | 4.5 | 4.5 | 5.3 | 6.2 | 6.5 | 7.3 | 7.3 | 7.5 | 7.5 |
| 3     | 2.0 | 2.0 | 3.5 | 3.5 | 3.5 | 4.5 | 4.5 | 4.5 | 5.8 | 5.5 |

Table 3. Serum antibody titers of immunized guinea pigs.

| Type | Titer Determined by |                |               |                     |
|------|---------------------|----------------|---------------|---------------------|
|      | HAI                 | Micro—<br>Neut | Tube<br>Neut. | Plaque<br>Reduction |
| 1    | 320                 | 640            | 160           | 730                 |
| 2    | 160                 | 320            | 80            | 340                 |
| 3    | 160                 | 320            | 40            | 98                  |

Since this microculture system seemed to be a rapid and sensitive method of assaying reoviruses, studies of its efficacy in measuring serum antibody were carried out. With conventional methods, each has its disadvantage—the non-specific lipoprotein inhibitors of the hemagglutination-inhibition test, the prolonged incubation time or large virus dose with "break-through" in the tube neutralization test, or the inconvenience of the plaque neutralization test. On the other hand since the end-points of the microneutralization test are determined by hemagglutination, whether non-specific inhibitors originally present in the serum being tested will interfere had to also be determined.

Antisera were prepared in guinea pigs by a single injection IM of virus and serum collected 21 days later. Homologous antibody titers were determined by the several methods, and representative results are shown in Table 3. Sensitivity is comparable between the micro-neutralization and plaque reduction tests. Further assays by microculture against the heterologous serotypes for each of these sera were negative for heterotypic antibody, despite the presence of non-specific inhibitors. Similar tests with a series of human sera confirmed the sensitivity, specificity and lack of influence of non-specific inhibitors in the microneutralization test.

Finally, the influence of virus dose on serum antibody titer was tested. In general, a ten-fold increase in virus dose resulted in only a two-fold reduction of titer. However, the reproducibility of the test dose used is such that as little as 10 HA<sub>50</sub> of virus can be accurately employed to assay antibody with a high degree of sensitivity.

#### SUMMARY

A microculture system for the assay of reovirus and its antibody has been developed and evaluated. It has been found to be rapid and very sensitive for the detection of virus. Its utility in measuring antibody is suggested by its sensitivity, degree of specificity, reproducibility and freedom of influence by non-specific serum inhibitors. The test would appear to have application in large scale testing of sera for reovirus antibody and possibly also as a detection system for the presence of reovirus in clinical specimens.