APPLIED MICROBIOLOGY, Oct. 1973, p. 617-619
Vol. 26, No. 4

Microcomplement-Fixation Inhibition: a Rapid and Economical Test to Detect Non-Complement-Fixing Antibodies

RICHARD G. OLSEN, LARRY E. MATHES, AND DAVID S. YOHN

Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio 43210

Received for publication 15 June 1973

Complement-fixation inhibition test was adapted to the microtiter system. When cat and monkey non-complement-fixing antibody were used, the microtest was shown to be as sensitive as the conventional tube complement-fixation inhibition test.

Although the complement-fixation (CF) test has been shown to be sensitive and reliable in diagnostic serology, a major limitation of the CF test is that immunoglobulins from some animal species do not fix guinea pig complement. It has been long recognized (3), for example, that avian antibody, except pigeon and parrot, is incompatible with guinea pig complement. In addition, many investigations (2, 6, 7) have shown that antibodies associated with non-complement-fixing immunoglobulin (IG)G subclasses limits the application of the CF test in diagnosis of some mammalian diseases.

To augment the CF test, Rice (5) introduced the CF inhibition (CFI) test. In principle the CFI test is based on the principle that non-complement-fixing antibody may bind with specific reference antigen determinants, thereby inhibiting the subsequent reaction between the reference antigen, reference complement-fixing antibody, and complement. The advantage of the CFI test over other non-complement-dependent tests (i.e., precipitin and fluorescent antibody tests) is the ability to standardize all CFI reagents.

The objective of the study reported here was to adapt the CFI test to the microtiter system and to compare the sensitivity of the microtiter CFI test to the conventional tube CFI test.

The conventional tube CFI test was performed by the procedures described by Rice (5). The CFI tube and microtiter tests consisted of incubating twofold dilutions of heat-inactivated experimental serum with 2 CF units of reference antigen for 1 h at 37 C. The above reactants were subsequently mixed with 2 CF units of heat-inactivated (56 C for 15 min) reference antibody plus $5C'H_{50}$ units of guinea pig complement and incubated for an additional 16 h at 4 C. Optimally sensitized sheep red blood cells (SRBC) (1.25%) were added to the test and incubated for an additional 30 min at 37 C. In the microtiter CFI tests, experimental serum titers were reported as the reciprocal of the highest serum dilution that visually reduced the CF reaction between reference antibody and reference antigen by 50%, i.e., from a 4+ reaction to a 2+ reaction. In the tube test, 50 and 25% inhibition were determined spectrophotometrically at 554 nm.

The tube CFI test employed 0.25 ml each of experimental serum, reference antibody, reference antigen, and sensitized SRBC, and 0.50 ml of complement. The microtiter CFI test used 25 μliters each of experimental and reference sera, reference antigen, and SRBC, and 50 μliters of complement.

Determination of units of complements, reference antibody, reference antigen, and hemolytic amboceptor were all performed under conditions which simulated those of actual CFI test procedures. Figure 1 illustrates the test results of microtiter CFI tests that were performed parallel with standard microtiter CF tests.

Two reference complement-fixing antiviral serum and reference virus antigen systems were used in this study: (i) anti-Yaba poxvirus monkey serum #3215 and soluble Yaba poxvirus antigens, and (ii) rabbit antiserum prepared toward the interspecies antigen from the feline leukemia virus (Kawakami-Theilen strain) and interspecies antigen from the Rauscher murine leukemia virus. Both anti-virus-virus antigen systems have been shown in previous reports (1, 4) to be good CF systems and to be virus specific.

CFI experimental antibody was obtained from three Yaba virus-infected rhesus monkeys

(YV-B, YV-L, YV-T) 52 weeks post virus inoculation and four Snyder-Thelien feline sarcoma virus-induced, tumor-bearing-specific, pathogen-free cats. All cat and monkey sera were previously shown (1, 4) to contain CFI virus-specific antibodies.

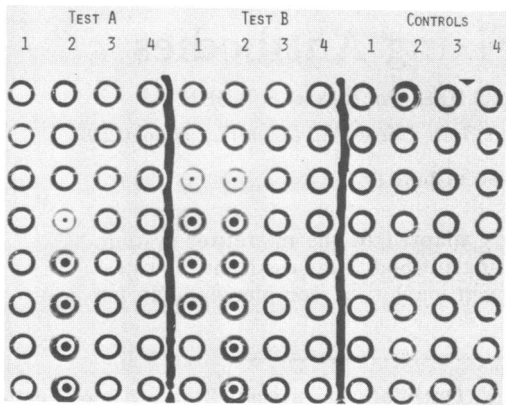

FIG. 1. *Microtiter complement-fixation and complement-fixation inhibition tests for antibodies in two samples of experimental rhesus monkey serum to the Yaba poxvirus. Row 1 of test A and row 1 of test B are direct CF tests whereas row 2 of each test is a CFI test. Serum on test A has a CF titer of <1:2 and a CFI titer of 1:16. Serum tested in test B has a CF titer of 1:64 and CFI titer of 1:8. Reagents in row 3 of both tests consist of reference antibody and varying dilutions of experimental antibody plus complement. This control determines whether there is a reaction between the two serum systems. Row 4 is an anti-complementary test for experimental antibody. Other controls (top 4 wells) include (1) anti-complementary control for 2 CF units of reference antibody, (2) the positive control which consists of incubating 2 CF units of reference antibody and 2 CF units of reference antigen and complement, (3) anti-complementary control for 2 CF units of reference antigen, and (4) complement ($5C'H_{50}$) control. The positive control should always produce a 4 + CF reaction. To perform both the CF and CFI tests one needs only 100 μliters of serum.*

A comparison of the sensitivity of the tube CFI test with the CFI test adapted to the microtiter system is summarized in Table 1. As expected, at 50% inhibition the CFI titers in all experimental sera were nearly the same in both tests. By reducing, however, the percent inhibition in the conventional tube test from 50 to 25%, the sensitivity of CFI titers in both the cat and the monkey system was increased one- to threefold.

Because of the advantage of conventional spectrophotometric calculations of percent hemolysis with the larger volumes, the tube CFI test appears to be better suited for more accurate determination of weak antibody titers. Likewise, less subjective error is encountered in performing the tube CFI test. We have found, however, that the advantages of the microtiter CFI test over the conventional tube test are as follows: (i) economic use of serologic reagents, and (ii) the technical convenience which permits the investigator to perform a larger number of tests per given amount of time. The third and most important advantage is that the microtiter system permits the technician to perform both the complement-fixation test as well as CFI test on the same microtiter plate with only 100 μliters of serum sample. In this laboratory we have adopted the microtiter system to detect CF and CFI antibody in human serum to oncornavirus antigen (D. S. Yohn and R. G. Olsen, Abstr. Annu. Meet. Amer. Soc. Microbiol., 1972), CF and CFI antibody in dog serum to central myelin (A. Koestner et al., *In* Symposium on slow virus infections, William and Wilkins, 1973, in press), membrane protein, CF and CFI antibody in rhesus monkey serum

TABLE 1. *Comparison of the tube CFI test with CFI test adapted to microtiter system*

Type of CFI Test	Cat anti gs-3 CFI titers[a, b]				Monkey anti-Yaba virus CFI titers[c, d]		
	2882	R1912	R-770	R-386	YV-B	YV-L	YV-T
Microtiter at 50% inhibition[e]	1:8	1:2	1:2	1:8	1:16	1:8	1:16
Tube test[f]							
At 50% inhibition	1:8	1:8	<1:2	1:4	1:16	1:16	1:32
At 25% inhibition	1:32	1:16	1:4	1:16	1:32	1:32	1:64

[a] All cats were bearing a feline sarcoma virus (Snyder-Theilen strain)-induced fibrosarcoma.

[b] Reference antiserum was rabbit anti-FeLV-gs-3 serum and reference antigen was ether-disrupted Rauscher murine virus antigen (see Materials and Methods).

[c] Rhesus monkey serum 52 weeks postinoculation with Yaba poxvirus.

[d] Reference antibody was complement-fixing rhesus monkey serum #3215 and reference antigen was soluble antigen from Yaba tumor homogenate (see Materials and Methods).

[e] 50% inhibition was determined visually.

[f] 50 and 25% inhibition was determined spectrophotometrically (544 nm).

to poxvirus antigen (1) and CFI antibody in cats to the feline leukemia virus (4).

This work was supported in part by Public Health Service contract PH 43-1001 from the Special Virus Cancer Program and by grant CA 11613-02, both from the National Cancer Institute.

LITERATURE CITED

1. Hall, R. D., R. G. Olsen, S. P. Pakes, and D. S. Yohn. 1973. Differences in clinical and convalescent phase antibodies of rhesus monkeys infected with monkey pox, Tanapox and Yaba poxviruses. Infect. Immunity **7**:539-546.
2. McGuire, T. C., G. L. Van Hoosier, and S. B. Henson. 1971. The complement-fixation reaction in equine infectious anemia: demonstration of inhibition by IgG(T). J. Immunol. **107**:1738-1744.
3. Meyer, K. F., B. Eddie, and H. Yanamuna. 1939. Complement-fixation test with tissue-culture-antigens as aid in recognizing latent avian psittacosis (omithosis) Proc. Soc. Exp. Biol. Med. **41**:173-176.
4. Olsen, R. D., and D. S. Yohn. 1972. Demonstration of antibody in cat sera to feline oncornavirus by complement-fixation inhibition. J. Nat. Cancer Inst. **49**:395-403.
5. Rice, C. 1948. Inhibitory effects of certain avian and mammalian antisera in specific complement-fixation systems. J. Immunol. **59**:365-378.
6. Schmidt, N. J., and H. B. Harding. 1956. Demonstration of substances in human sera which inhibit complement-fixation in antigen antibody systems of lymphogranuloma nenereum, psittacosis, mumps, Q fever, and lymphocytic choriomengitis. J. Bacteriol. **71**:217-228.
7. Scott, R. M., and P. H. Russell. 1972. Complement fixation blocking activity of anti-Dengue antibody. J. Immunol. **109**:875-877.