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Typing of ECHO Viruses by a Complement Fixation Technic. (23954)

PEKKA HALONEN,* LEON ROSEN AND ROBERT J. HUEBNER

U. S. Dept. of Health, P.H.S., Nat. Inst. of Allergy and Infectious, Diseases, N.I.H., Bethesda, Md.

Typing of ECHO viruses(1) by standard neutralization tests is an inconvenient and laborious procedure because of large number of prototypes. Recent findings, that complement-fixing (CF) antigens for ECHO viruses could be prepared consistently from monkey kidney tissue cultures and that these antigens reacted type specifically with ECHO antisera prepared in guinea pigs and monkeys(2), suggested the possibility of typing ECHO virus isolates by means of a CF technic. This preliminary report describes simple methods which have been successful in typing 28 ECHO virus isolates by such a procedure.[†]

Methods. CF antigens were prepared by inoculating each of 4 rhesus kidney tissue culture tubes containing 1 ml of the following: lactalbumin hydrolysate 0.5%, calf serum 2%, Earle's solution 97.5%, with 0.1 ml of undiluted fluid from the original or an early monkey kidney passage of the ECHO isolate. After complete degeneration of tissue had taken place, the tubes were frozen and thawed, and pooled tissue culture fluid from these tubes was used as antigen without centrifugation. Of 28 ECHO virus isolates tested, 15 were from a Washington, D.C. welfare institution(3), and 13 were from other

sources in Washington, D.C., and from other localities in U.S. and England (ECHO type 9).[‡] All were typed originally by neutralization tests except the ECHO type 10 isolates. The latter, however, produced the unique cytopathogenic effect characteristic of the prototype strain of ECHO 10 virus. The CF typing tests for most strains were done without prior knowledge of the neutralization test data. ECHO antisera used in CF tests had been prepared in monkeys(4).§ Homotypic and heterotypic CF titers of these sera against CF antigens of ECHO prototype strains prepared in monkey kidney tissue culture are published elsewhere(2). Dilutions of monkey sera are shown in Table I and in each instance represented at least 4 units of antibody. These dilutions were determined by the desirability of conserving serum and the fact that 4 units of antibody have been used successfully for many years in this and other laboratories as the best working dilutions for purposes of titrating viral and rickettsial antigens. Complement fixation tests were performed by standard technic in this laboratory(2).

Results. Representative results of CF typing tests are shown in Table I. Each ECHO antigen reacted only with homologous antiserum, except the ECHO 8 antigens, which reacted with both type 1 and 8 antisera, and to

* Rockefeller Foundation Fellow and Visiting Scientist, N.I.H. Present address: University of Helsinki, Finland.

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§ The sera were obtained from Nat. Foundation for Infantile Paralysis.

TABLE I. Representative Results of ECHO CF Typing Tests. 4 = no hemolysis; 3, 2, 1 = different degrees of hemolysis; 0 = complete hemolysis.

Type of isolate by neutralization test	Type and reciprocal of dilution of ECHO antisera													
	E-1 256	E-2 128	E-3 128	E-4 128	E-5 256	E-6 256	E-7 256	E-8 256	E-9 128	E-10 128	E-11 128	E-12 128	E-13 256	E-14 256
ECHO type 3	0	0	4	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	4	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	3	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	2	0	0	0	0	0	0	0
8	4	0	0	0	0	0	0	4	0	0	0	0	1	0
9	0	0	0	0	0	0	0	0	4	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	4	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	4	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	3	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0	4

a lesser extent with type 13. This, of course, was not unexpected in view of other data on the relationship of ECHO types 1, 8 and 13 (2, 4). The positive reactions were 4+ fixation in the majority of cases. In those few cases where the positive reactions were only 2+ or 3+ fixation, they were clearly separable from completely negative reactions. Polio-myelitis and Coxsackie B isolates (from the same sources as the ECHO isolates) which reacted in CF tests with homologous antisera gave no reaction with any of ECHO antisera.

Table II summarizes results of ECHO CF typing experiments with 28 ECHO isolates.

TABLE II. Number and Type of ECHO Isolates Typed by CF Technic.

ECHO type	No. of isolates	No. of anti-gens prepared from isolates	No. of anti-gens typed by CF technics
3	1	1	1
4	1	2	2
6	4	5	4
7	7	9	9
8	5	5	5
9	1	1	1
10	3	5	5
11	1	1	1
12	2	5	5
14	3	3	3
Total	28	37	36

Two or 3 antigens were prepared from some of these isolates and hence the total number of antigens tested was 37. All 28 isolates were successfully typed by this CF technic. One antigen from an ECHO type 6 isolate, prepared by inoculating monkey kidney tubes with undiluted material, and which caused complete degeneration of tissue in less than 24 hours, did not give any reaction. However, another antigen from the same isolate, prepared by diluting original tissue culture material 1:10,000 before inoculation of monkey kidney tubes, gave 4+ fixation with ECHO type 6 antiserum. No antigens were anticomplementary to the extent that they could not be typed.

These preliminary results indicate that the complement fixation test may be a faster and less expensive technic for typing of ECHO viruses than is the neutralization test.

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