USE OF COMPLEMENT FIXATION SCREENING TECHNIQUE TO IDENTIFY POLIOVIRUS IN ENTEROVIRUS ISOLATES

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SINCE the introduction of tissue culture methods for isolation of viruses, many previously unknown viruses of human origin have been characterized. Of these, the largest number classified to date fall into the enterovirus group. The usual procedure for identification of these viruses by neutralization test (NT) is a time-consuming, expensive task.

Less expensive and less time-consuming complement fixation (CF) techniques have been reported for identification of certain enterovirus isolates (*1–4*). Few laboratories performing diagnostic tests on viruses have incorporated a CF procedure into their routine identification of enterovirus isolates.

At the beginning of the 1961 poliomyelitis season, a screening procedure by CF for identification of the three types of poliovirus was initiated by the Immuno-Serology Unit, Communicable Disease Center, to simplify typing of suspected enteroviruses. This report presents the findings obtained with the procedure, and correlates these results with identification by NT or by history of the type of oral vaccine fed.

Materials and Methods

Specimens for identification. Fecal and rectal swab specimens for isolation were collected during the 1961 poliomyelitis season from the following sources: (*a*) an evaluation of the Sabin oral vaccine in infants; (*b*) a poliomyelitis, type 3, epidemic preceding the Atlanta oral vaccine program; (*c*) enterovirus surveillance following the Atlanta oral vaccine program; and (*d*) enterovirus surveillance specimens from six areas of the United States.

Virus isolation. Since the procedures employed in this laboratory for virus isolation are used routinely in various laboratories, they are mentioned only briefly here. Monkey kidney tissue culture tubes containing 1.0 ml. of maintenance medium were each inoculated with 0.25 ml. of fecal extract prepared in Hanks' BSS solution and treated with antibiotics. Inoculated tubes, incubated at 37° C., were read at 2- to 3-day intervals and held for a minimum of 10 days or until a 4+ cytopathic effect was noted. Fluid from tubes showing cytopathic effects was harvested when the cells were completely degenerated and was kept frozen until typed.

Neutralization tests. For neutralization tests a 10^{-3} dilution of each isolate was incubated with an equal volume of specific immune serum for 1 hour at 37° C. Tissue culture tubes were inoculated with 0.2 ml. of the serum-virus mixture per tube, and 0.1 ml. of the diluted isolate was used as the virus control.

Complement fixation technique. The diluent for the complement fixation technique consisted of veronal buffered saline containing 0.1 percent gelatin. One lot of complement (guinea pig serum), from a commercial source, was used throughout. The complement was titered and used in a dilution corresponding to seven 50 percent hemolytic (7C'H50) units in 0.4 ml. (*5*). The optimal dilution of hemolysin was selected according to the method of Osler, Strauss, and Mayer (*5*). Poliomyelitis hyperimmune serums were prepared in monkeys, using the CMRL strains of the three types of poliovirus. A dilution of each serum representing four units of antibody was used.

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Virus isolates were used as antigens. Tissue culture fluid harvested from monkey kidney cells that demonstrated cytopathic effect was thawed and used undiluted as antigen. These antigens were not inactivated or centrifuged. When it was necessary to test an isolate after further passage in monkey kidney cells, a 1:2 dilution of the tissue culture fluid was used as antigen.

Procedure for test. Two-tenths ml. of hyperimmune monkey serum containing 4 antibody units, 0.2 ml. of undiluted antigen, and 0.4 ml. of complement containing 7C'H50 were combined and incubated overnight at 4° C. The tests were removed from the refrigerator and left at room temperature for 15–20 minutes while the sheep cells were sensitized (equal volumes of optimally diluted hemolysin and standardized 2.8 percent cells). Two-tenths ml. of sensitized sheep cells was added to all tubes. After thorough mixing, the tubes were placed in a 37° C. water bath for 30 minutes. Upon removal from the water bath, all tubes not showing complete hemolysis were centrifuged and read by comparison with color standards.

A three-tube complement control was set up with each isolate to check for anticomplementary activity. All three tubes contained 0.2 ml. of the isolate. One tube contained 7C'H50 (0.4 ml.), the second tube 3.5C'H50 (0.2 ml.), and the third tube 1.75C'H50 (0.1 ml.). Sufficient diluent was added to each tube to give a total volume of 0.8 ml. before refrigeration.

Controls on the hyperimmune serums were set up using five dilutions of these serums and a known poliovirus complement-fixing antigen. The normal monkey kidney cell controls, which were harvested and frozen at the same time as the isolates, were tested against the three types of hyperimmune serum for nonspecific reactivity. Three-tube complement controls were set up on each lot of normal cells to check for anticomplementary activity of the particular lot of monkey kidney cells.

The following criteria were used to interpret CF results. If all controls were satisfactory, tubes showing 3+ or 4+ fixation were considered positive. Table 1 shows the CF reading and typing results of some isolate-antigens. Isolates showing more than ± (less than 85 percent hemolysis) in the 3.5-unit complement control were considered anticomplementary. Further passage of the anticomplementary isolates often removed the anticomplementary activity. Isolates showing 1+ or 2+ fixation with a single type of serum were passed again

Table 1. Complement fixation reading and typing results of selected isolate-antigens

Antigens	Amount of fixation with hyperimmune serum			Complement controls			Results of CF typing
	Poliovirus 1 1:128	Poliovirus 2 1:128	Poliovirus 3 1:64	Number of 50 percent units			
				7	3.5 [1]	1.75	
Isolate 2757	0	0	4	0	±	3	Poliovirus 3
Isolate 2760	0	0	4	0	±	4	Poliovirus 3
Isolate 3011	0	3	0	0	±	3	Poliovirus 2
Isolate 3048	0	0	4	0	±	4	Poliovirus 3
Isolate 3068	4	0	0	0	0	3	Poliovirus 1
Isolate 3074	0	4	0	0	0	2	Poliovirus 2
Isolate 3085	4	0	0	0	±	3	Poliovirus 1
Isolate 0456	0	0	2	0	0	2	Pass and repeat
Isolate 0614	2	2	4	2	4	4	Anticomplementary
Poliovirus 1	4	0	0	0	±	3	
Poliovirus 2	0	4	0	0	0	3	
Poliovirus 3	0	0	4	0	±	4	
Control monkey kidney cells.	0	0	0	0	0	2	

[1] When more than ± was noted in the 3.5-unit control, the isolate was considered anticomplementary.
NOTE: 0=no fixation; 4=complete fixation.

in tissue culture in order to increase the virus titer. Isolates showing no fixation in any of the three types of hyperimmune serum were identified as nonpoliovirus.

Results

A total of 980 isolates were screened by CF for identification of poliovirus. Of these, 468 isolates were typed by both CF and NT. The remaining 512 isolates were typed by CF only. These 512 isolates were obtained from persons who had been fed one of the three types of oral poliomyelitis vaccines and should have been positive for poliovirus. The CF typing of these isolates agreed in every instance with the type of oral vaccine fed.

Table 2 shows the correlation between isolates identified by both CF and NT. Two hundred and forty-two isolates were identified as nonpoliovirus by both tests. Of these, 165 were found to be other types of enteroviruses, and 77 isolates were unidentified, although they were screened also by NT for poliovirus. Of the 165 isolates identified as nonpoliovirus, 115 were identified as Coxsackie B_4 by NT, and 50 were identified as other types of Coxsackie, several types of ECHO, one adenovirus, and one reovirus 3.

Twelve isolates were incorrectly identified originally by CF. Five were first-passage isolates which were identified as nonpoliovirus by the CF screen and as poliovirus by NT. After further tissue culture passage these isolates were identified as one of the three types of poliovirus by CF.

The other seven incorrectly identified isolates gave fixation with poliovirus type 3 serum, but were subsequently identified as Coxsackie B_4 by NT. However, by both the CF and NT it was found that the poliovirus 3 typing serums were reactive with the Coxsackie B_4 isolates. In NT, both the poliovirus 3 and Coxsackie B_4 serums protected the monkey kidney cells against the cytopathic effect of Coxsackie B_4 isolates for 3 days. Then a breakthrough was noted with the poliovirus 3 serum while the B_4 serum continued to show protection. This was a definite delay of cytopathic effect by poliovirus 3 serum in comparison with the virus control.

Table 2. Correlation between isolates screened for identification of poliovirus by complement fixation and neutralization tests

Neutralization test	Complement fixation test		Total
	Poliovirus positive	Poliovirus negative	
Poliovirus positive	214	5	219
Poliovirus negative	7	242	249
Total	221	247	468

Approximately 80 percent of the isolates were identified as poliovirus or nonpoliovirus on the first CF testing. The remaining 20 percent showed either anticomplementary activity or low-level virus titer. After further passage in tissue culture, the majority of the isolates tested by CF were identifiable as poliovirus or nonpoliovirus.

Conclusions

The complement fixation technique described here was tested with a sufficient number of enterovirus isolates to indicate that it is an extremely useful tool in screening isolates suspected to be poliovirus. Neutralization tests must be held for 7 days before a final interpretation can be made, whereas CF tests are read after overnight fixation of complement. The rapidity of the CF screen test makes it a practical addition to the NT in the initial stages of identification of unknown viruses. Although the correlation demonstrated between the NT and CF was quite good, the CF test cannot be considered a substitute for NT because almost 20 percent of the isolates tested required additional tissue culture passage to obtain a definitive result and a number of isolates remained anticomplementary.

With high-titered hyperimmune serums prepared against the clinically important types of ECHO and Coxsackie viruses, the CF technique will be an even more useful tool in virus diagnostic laboratories.

Summary

Isolates from 980 fecal and rectal swab specimens were used as complement-fixing antigens and screened for the three types of poliovirus.

Of these isolates, 726 (74 percent) were identified as type 1, 2, or 3 poliovirus. Twelve isolates, or 1.2 percent, were incorrectly identified by complement fixation. Five of these isolates were subsequently found to be poliovirus, and seven were shown to be Coxsackie B_4 by neutralization test.

REFERENCES

(*1*) Godtfredsen, A.: Typing of freshly isolated strains of polio, ECHO, and Coxsackie viruses by complement fixation. Acta Path Microbiol Scand 49: 189–198 (1960).

(*2*) Halonen, P., Rosen, L., and Huebner, R. J.: Typing of ECHO viruses by a complement fixation technique. Proc Soc Exp Biol Med 98: 105–106 (1958).

(*3*) Schmidt, N., and Lennette, E. H.: An inquiry into the use of the complement fixation test for the typing of poliomyelitis viruses. Amer J Hyg 66: 119–130 (1957).

(*4*) LeBouvier, G. L., Lawrence, G. D., Parfitt, E. M., and Jennens, M. G.: Typing of poliomyelitis viruses by complement fixation. Lancet 2: 531–532 (1954).

(*5*) Osler, A. G., Strauss, J. H., and Mayer, M. M.: Diagnostic complement fixation; I.A. method. Amer J Syph Gonor Ven Dis 36: 140–153 (1952).

The Older American

The past 10 years have seen astonishing improvements in the life of our older citizen, but a gap remains between what that life is and the democratic goal of maximum independence, according to the first annual report of the President's Council on Aging. The council, set up in May 1962, has been directed to make studies, disseminate information, and offer recommendations for Federal action on problems of the aged.

The council's report, entitled "The Older American," briefly portrays the situation of the nation's nearly 18 million people over 65. Emphasizing that there can be no composite picture drawn of the older American, it sets forth facts about his income, his health, his employment, and his housing, and discusses his needs and his wants.

Among the facts:

- The average two-person family headed by a person over 65 has a yearly income of $2,530, substantially lower than the "modest but adequate" budget of $3,010 devised by the Bureau of Labor Statistics for such a family.
- Less than one-third of the total income of older people comes from earnings.
- More than 12 million have at least one chronic condition, and more than half of these have some limitation on their activities.
- The average annual medical care expenses for an older person, including payments from both public funds and personal resources, are $315.
- Of the one in six aged persons who is hospitalized in a year, the hospital bill averages $525.
- According to recent surveys of firms with pension plans, 9 out of 10 companies employing 1,000 or more workers have mandatory retirement policies.
- Of the people 65 or older who head households, about one-third live in dilapidated or deteriorated housing or housing without some or all plumbing facilities.

The council's report also describes the many advances made in these and other areas. Notable among the contributions of the Federal Government are the expansion of the social security program, an increase in the amount spent on research, and efforts to increase housing specifically built for older people. The report states that 80 percent of the recommendations for specific Federal action of the 1961 White House Conference on Aging have been carried out, either wholly or substantially.

Copies of the report may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C., 20402, for 50 cents.