DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

Communicable Disease Center
Atlanta 22, Georgia

November 2, 1960

Refer to:

Albert B. Sabin, M.D.
Children's Hospital Research Foundation
Elland Avenue and Bethesda
Cincinnati 29, Ohio

Dear Dr. Sabin:

Your letter to Dr. Kokko was given to me to fill in some of the technical procedures used in our viremia study.

In response to your questions, firstly, rhesus monkey kidney cells have been used throughout the entire study. The growth medium consisted of LAH in Hank's with 5% calf serum added. The calf serum was obtained from a single lot and had been pretested by CPE against the three polio types. No evidence of neutralization was observed in serum dilutions up to 10%. The growth medium was changed on the 3rd or 4th day of incubation and the cell cultures were used on the 6th or 7th day of incubation. Antibiotics were used in growth and maintenance media in the following concentrations:

Streptomycin - 100 µg/ml
Penicillin - 200 units/ml
Nyasin - 100 units/ml

Maintenance medium consisted of LAH in Earle's at a pH of 7.8 to 7.9.

Our method of inoculation involved removal of the growth medium from the bottle cultures, and addition of 5 ml of the pooled A.M. and P.M. sera. Contact with the cell monolayer was maintained for one hour at room temperature with agitation at 10 minute intervals. At the end of this period, the serum was poured off and 15 ml maintenance medium added. Cultures were observed for eight days.

Regarding the titration of the vaccine, dilutions were prepared in half log intervals and 0.2 ml of each dilution inoculated in each of 6 tissue culture tubes. I would like to add that we do not consider this procedure as yielding accurate titers. Our intention was to confirm viability and the approximate range of virus concentration. We realize that a mean of a series of tests would be more accurate and, therefore, we submit our results as approximations only.

Dr. Sabin -- 11/2/60

Titrations of positive specimens have not been completed but the following results may be of some interest.

Number	Virus Fed	Results	2.5 ml	1.0 ml	10^{-1}	10^{-2}	10^{-3}
#38	Cox Type 1	Pool	+				
		AM		0	0/4		
		PM		0	0/4		
#281	Sabin Type 2	Pool	+				
		AM		+	2/4	1/4	
		PM		+	1/4	0/4	
#425	Sabin Type 2	Pool	+				
		AM		+	4/4	2/4	0/4
		PM		+	4/4	1/4	0/4

We shall be happy to forward additional information as it becomes available. It was very pleasant to see you again during your recent visit to our new facilities.

With very best wishes,

Andrew R. Fodor, Ph.D., Chief
Virus Diagnostic Unit