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CONCENTRATION OF RIFT VALLEY FEVER VIRUS BY ALCOHOL PRECIPITATION

Frederick Klein
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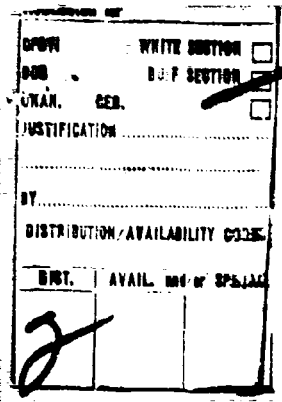
Hobert B. Bonner
Ralph E. Lincoln

APRIL 1970

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



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DEPARTMENT OF THE ARMY
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Process Development Division
AGENT DEVELOPMENT & ENGINEERING LABORATORIES

Project 1B563603DE32

April 1970

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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ABSTRACT

Methanol concentrated Rift Valley fever virus approximately 100-fold without appreciable loss of infectivity. The method showed potential both for scale-up and for the concentration of other viruses.

CONCENTRATION OF RIFT VALLEY FEVER VIRUS BY ALCOHOL PRECIPITATION*

Poliovirus preparations have been successfully concentrated by precipitation with methanol and further purified by elution, ultracentrifugation, and enzymatic treatment.[1,2] They also have been successfully concentrated by chromatography on cellulose ion-exchange columns[3,4] and on calcium and aluminum phosphate gels.[5]

Earlier, we observed that when Rift Valley fever virus (RVFV) was precipitated with potassium aluminum sulfate (alum),[6] the volume of viral supernatant fluid was reduced rapidly to 2% of its original volume, yet approximately 100% or more of its original infectivity was retained. In the continuation of these studies, a single-step method for concentration and partial purification of RVFV preparations by precipitation with methanol is described. The method is an adaptation of one that has already proved successful with foot-and-mouth disease virus.[7] The RVFV particles remained highly infectious after 100-fold concentration.

The origin and maintenance of the wild pantropic van Wyk strain of RVFV[8] used in this study were described earlier.[8] Tissue cells (L-DR) grown in suspension to near the peak of the log phase were diluted to approximately 2 x 10^5 cells/ml in Eagle's minimum essential medium[9] supplemented with 10% bovine serum. A multiplicity of inoculum of 0.01, seeded directly into the tissue cell cultures, was used. Flasks were routinely incubated at 37 C for 72 hours on a reciprocating shaker (100 3-inch strokes/min).

In concentrating the virus, tissue cells and cellular debris were first removed by low-speed centrifugation. The supernatant fluid was then cooled to -1 C in a dry ice and water mixture, and precooled absolute methanol (-10 C) was added in a ratio of 1:5 (alcohol to virus supernatant). The virus-alcohol mixture was held at -10 C overnight or approximately 16 to 18 hours, after which the mixture was centrifuged for 30 minutes at 6,000 x g in the SS-34 rotor of a Sorvall RC-2 centrifuge operating at -10 C. The supernatant fluid was decanted and the precipitate was resuspended in an equal volume of 199 peptone medium supplemented with 10% bovine serum.

Amount of infective virus was determined by titration in 6- to 8-g Swiss-Webster mice, using four mice per log dilution. The probit method[10] of calculating mouse intracerebral lethal dose$_{50}$ (MICLD$_{50}$) values was used. The concentrations achieved by this process were calculated on both per-milliliter and total-volume bases. Results are reported showing both the degree of concentration and percentage recovery of infectivity.

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Results reported here were obtained by using one concentration of alcohol (20%), one holding period (18 hours at -10 C), and one centrifuge speed (2,000 rpm) for the concentration of RVFV particles. This method resulted in concentration of infective virus about 100-fold with little loss of infectivity (Table 1). The supernatant liquid was completely clarified and void of infectious virus particles after one centrifugation, thereby eliminating the time-consuming task of additional centrifugation. Quantitation of the precipitate showed 50 to 80% recovery of infectivity after concentration by this process.

TABLE 1. CONCENTRATION OF RIFT VALLEY FEVER VIRUS BY ALCOHOL PRECIPITATION

		Original Viral Suspension	Concentrated Viral Suspension	Recovery, per cent
Virus Suspension I				
Repl 1.	Volume, ml	100	3.8[b]	68
	Log_{10} $MICLD_{50}$/ml	7.9	9.2	
	Log total $MICLD_{50}$[c]	9.9	9.8	
Repl 2.	Volume, ml	100	4.2	51
	Log_{10} $MICLD_{50}$/ml	7.9	9.0	
	Log total $MICLD_{50}$	9.9	9.6	
Virus Suspension II				
Repl 1.	Volume, ml	100	2.8	73
	Log_{10} $MICLD_{50}$/ml	7.8	9.2	
	Log total $MICLD_{50}$	9.8	9.6	
Repl 2.	Volume, ml	100	3.1	81
	Log_{10} $MICLD_{50}$/ml	7.8	9.2	
	Log total $MICLD_{50}$	9.8	9.7	
Repl 3.	Volume, ml	100	3.5	62
	Log_{10} $MICLD_{50}$/ml	7.8	9.0	
	Log total $MICLD_{50}$	9.8	9.6	

a. For all five cultures, supernatant volume was 100 ml. Likewise, value for all five supernatants was $<10^{-1}$ $MICLD_{50}$/ml, total $MICLD_{50}$ and concentration.

b. Volume represents the original precipitate plus an equal volume of suspending medium.

c. Volume x $MICLD_{50}$/ml = total $MICLD_{50}$.

This concentration method has the advantages of simplicity and potential scale-up for any volume of culture, and our results on concentration achieved and percentage loss of infectivity seem very promising. Certain possibilities for its use are: (i) the reduction of large volumes of virus suspensions without appreciable loss of infectivity, thereby making storage and further purification possible; (ii) the preparation of antigens (live, dead, or attenuated) for vaccine production; and (iii) if some purification is achieved, the removal of potentially harmful and/or interfering substances originating from destruction of the tissue cells. We are presently attempting to consider these possibilities and to assess the effect of continuous centrifuge flow instead of batch treatment utilizing this concentration process. This method also seems to offer potential for concentrating other viruses that contain essential lipoid material and are sensitive to ether or other fat solvents.

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