Internal Correspondence

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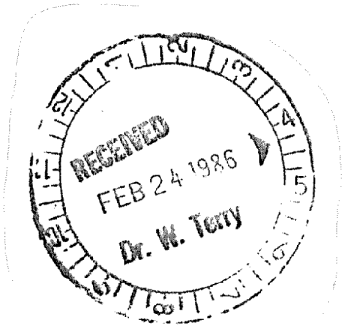

DATE: December 3, 1985

TO: Mike Hrinda, Ph.D.

FROM: Criss Tarr, Sc.D.

SUBJECT: Results of LAV infectivity assay of AHF Generation I samples.

Samples were received from Will Curry of AHF Generation I that had been spiked with LAV then either frozen, lyophilized, or lyophilized and heated at 60°C.

The source of the virus was about 3 liters of filtered cell culture fluid from a routine LAV production run (lot #7006-034). The virus was pelleted by centrifugation and resuspended as a concentrate in citrate-glycine buffer containing about 0.1% human albumin. The buffer was provided by Will Curry. The volume of the concentrate was 9.5 ml.

Samples of these spiked materials were removed for assay. The remaining spiked materials were vialed, frozen, and lyophilized according to a procedure specified by Will Curry. (Please refer to his documentation for the details.) Upon completion of the lyophilization, the vials were sealed and delivered to Will. After appropriate heating, the samples were returned for assay. They were stored at ambient temperature until used on 10/17/85.

This assay included the spiked, frozen AHF, lyophilized AHF, and the lyophilized AHF heated at 60°C for 30 hours. The assay was performed as follows. The lyophilized samples were reconstituted with sterile water for injection and the frozen samples were thawed then titrated by serial 10-fold dilutions in LAV culture medium. T25 culture flasks were seeded with sufficient CEM cells to give a final concentration of about $2x10^5$ cells/ml in a volume of 8 ml. Next 0.5 ml of the appropriate dilution of sample was added to replicate flasks. The flasks were gassed with 5% CO_2 then incubated at about 37°C. The cultures were observed twice a week for evidence of cytopathology and cell growth. Approximately twice weekly, cultures showing evidence of heavy growth were diluted by half with fresh LAV culture medium. The cultures were maintained for 6 weeks at which time positive control cultures had been killed by virus and negative control cultures continued to grow actively.

The estimation of virus presence is based on cytopathology and death of inoculated cultures.

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The results of the 6 week cultures were summarized in the following table:

Sample	log 10 $TCID_{50}$ titer[a]	log 10 reduction[b]
AHF, frozen	6.30	—
AHF, lyophilized	<4.00	>2.30
AHF, heated 30 hours	0.78	≤3.22

a 50% tissue culture infectious doses.
b log_{10} reduction from previous step.

CT/jh

cc: W. Curry
A. Schreiber, M.D.
W.D. Terry, M.D.