DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
Atlanta GA 30333

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Dr. Feldman:

Thank you for your inquiry concerning experiments on heat inactivation of LAV in clotting concentrates performed at CDC. Several preliminary experiments were undertaken to determine the approximate time needed for inactivation. The method for quantitation of viral inactivation is based on a double antibody recapture assay developed by Steve McDougal. This method will soon be published in the Journal of Immunologic Methods. I have enclosed a preprint of that assay for your information.

LAV virus was added to the reconstituted factor VIII concentrate to achieve a final concentration of 10^6 to 10^7 viable virus particles per ml. Glycine and sucrose were added for stabilization of protein and the material heated at various periods ranging from 1 minute to several hours at 60°C. Virus was rapidly inactivated and no detectable virus grew in inoculated cultures after 3-4 minutes of heating. The detection of limit of viable viruses in these assays is about 10^1/ml.

Subsequently two experiments were performed using lyophilized material. LAV virus was then added to reconstituted factor VIII material to achieve a final concentration of about 10^6 per ml. That material was lyophilized in half ml aliquots for 24 hours and subjected to assay for viable virus. At the end of lyophilization the unheated material contained a little more than 10^3 virus per ml. When heated for various periods of time at 60° no viable virus was detected after 4-5 minutes of heating. No data was available on residual moisture in these preparations.

The next experiment was performed using lyophilized material. Bulk plasma was obtained and LAV virus was introduced to achieve a final concentration of $10^{6.2}$ per ml. After lyophilization, viable virus in the reconstituted material was $10^{4.27}$ per ml. Material was heated at 68°C for periods of 24, 48, 60, 72, 96 hours. In all samples following heat treatment, no viable viruses were detected in assay system.

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The second series of experiments were performed on factor VIII and factor IX concentrates obtained from another company. Virus was introduced into liquid concentrates and then lyophilized. Initial titer of virus in Factor VIII was $10^{5.26}$ viable viruses per ml and in Factor IX $10^{5.25}$. After lyophilization, the viral titer was $10^{3.68}$ in Factor VIII, and in Factor IX, $10^{4.4}$. After heating both groups at 60^{o} for 24 hours, no viable viruses were detected in either factor VIII or factor IX concentrates.

Because LAV appeared to be extremently heat labile, we believe that the procedures presently used by manufacturers for heat treatment of hepatitis virus would adequately inactivate LAV virus. We are collaborating with the FDA on a generic study to examine the effects of heating concentrate preparations in liquid and dry form for various periods and heat ranges. The data from these studies will be made available as soon as possible.

I hope this information will be useful to you. If I can be of any further help, please do not hesitate to ask.

Sincerely yours,

GRO-C

Bruce L. Evatt, M.D.
Director
Division of Host Factors
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cc:
Dr. J. Steven McDougal

Enclosure

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