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PAH/EB

14 MAR 1986

13 March, 1986

CONFIDENTIAL

Dr B T Colvin
Department of Haematology
The London Hospital
Whitechapel Road
LONDON E1 1BB

Dear Dr Colvin;

HTLV-III/LAV INACTIVATION

Over recent months Haemophilia Centre Directors have requested the HTLV-III/LAV inactivation data relating to our heat treatment process for Factorate (Armour AHF Concentrate). In response to those requests and having regard to recent media comment, we set out below details of the viral inactivation data along with other important information.

It is now generally accepted that the maximum HTLV-III contamination that could be expected in any coagulation factor concentrate before processing would be of the order of 5 logs. Processes that can be shown to inactivate in excess of this virus challenge are therefore likely successfully to effect viral elimination.

The recently completed study by our US research laboratories, using a highly sensitive assay method, gave the following results after seeding of a Factorate solution with 6.3 logs of HTLV-III. The solution was lyophilised, and the resulting dried product heated at the Armour standard of 60°C for 30 hours. A reduction of 2.3 logs was shown on lyophilisation and a further 3.2 logs on heating, giving a total elimination of 5.5 logs of virus particles. A more complete synopsis of this study is attached to this letter.

There has been no reported case of AIDS, and no reported sero-conversion associated with the administration of Factorate to a virgin patient not at risk for AIDS. Furthermore, a recent publication(1) has described an evaluation of Factorate in a group which included 46 HTLV-III sero-negative patients, none of whom sero-converted in the short term follow-up. Finally, live HTLV-III virus has never been isolated from heat treated Factorate.

As you may already know, all our plasma collection centres are situated in the American mid-west away from the known areas of high risk for AIDS. Our typical donor is a multiple visitor and undergoes thorough medical examination and follow-up at each attendance. Each donation is now specifically screened for HTLV-III antibody and all product being supplied is donor tested.

Contd/...

Before donor testing it was estimated that the risk of including an HTLV-III contaminated donation in a plasma pool was from 0.25 - 0.3%. By introduction of our donor testing, it may be assumed that this risk has been minimised.

However, it should not be overlooked that there may be material in centres, or in the home that is not derived from donors tested for anti-HTLV-III. We do appreciate that this information would aggravate the potential for distress to the haemophiliac, because of the patient's inference that non-donor tested material may be less safe with regard to the AIDS risk. Further, we recognise that any decision to give a patient this information rests with you as the unit director.

If there is any further information or help which you think I may be able to provide, I hope that you will not hesitate to telephone me. In my absence, our Director of Clinical Sciences, Mr Robert Christie, can give immediate advice or help.

I am sending this letter to UK Haemophilia Centre Directors who are likely to have used 'Factorate' in the preceding 12 months.

Yours sincerely

GRO-C

Dr P Harris
Medical & Technical Director

Reference

1. Fielding P, Nilsson I M & Hansson B G
Lancet, 1985; ii: 832

Enc.

Internal
Correspondence

DATE:

TO:

FROM:

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.

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