I M M U N O L T D

Eingegangen am:
11. Mai 1987
PRODUCT MANAGEMENT

TELEFAX NO: 87/87

NUMBER OF PAGES INCLUDING COVER SHEET: 3

DATE: 11.5.87

TO: DR. SCHOPPMANN

FROM: MR.R.NICHOLSON

SUBJECT:

TWO-STAGE FACTOR VIII ASSAY AND
KJELDAHL TEST

FAX No: 0732 455175

TEL No: GRO-C

F A X

11.5.87

To: Dr. Schoppmann

Following our discussions last Friday, please find attached the questions and proposed response.

I have now had a chance to discuss these further with Peter Coombes and the DHSS are giving apparent priority to our Kryobulin application at the present time in the hope that it will be considered in July. If possible a reply by Tuesday would be much appreciated and as discussed if you can let us have some preliminary statement on the Hepatitis re-challenge test in relation to Kryobulin then we would very much like to mention these results at this time.

Thank you again for your help last week.

With best wishes.

GRO-C

R. Nicholson,
Immuno Ltd

1. What is the assay for specific activity and what are the limits of error?

The assay for specific activity is the Two-Stage Factor VIII assay and Kjoldahl Test. The limits of error for the Factor VIII assay are 72 to 128% and for the protein assay 96 to 104%.

2. What is meant by "Stability satisfactory"?

This means in accordance with the requirements of the BP and EP.

3. Is the antibody used in the Two-Dimensional Laurell Electrophoresis a specific antibody to the intact molecule or will it react with fragments?

If fragments or aggregates were generated during the vapour heat treatment process then a different pattern would be found in the Two-Dimensional Laurell Electrophoresis as the generation of fragments and aggregates would affect the electrophoretic mobility. The SDS page electrophoretic separation is in any case a much more sensitive method for detecting neoprotein formation and the data presented in the analytical report indicates that significant amounts of fragments or aggregates are not generated during the vapour heat treatment process.

4. Please comment on the inactivation data given for Kryobulin heat treated, steam treated and vapour heated with reference to the apparent differences shown due to the purity of the product.

Inactivation data is presented for three products in order to allow comparison with products previously submitted for licensing and to show the improvements in inactivation shown by Kryobulin vapour heated.

Kryobulin heat treated is our current licensed product (10 hours 60°C Dry heat). Kryobulin steam treated was the subject of our unsuccessful variation submitted on 5th March 1986 (1 hour 60°C increased moisture and pressure). Kryobulin vapour heated is the subject of this variation (10 hours 60°C increased moisure and pressure).

The difference in log steps inactivation of HTLVIII due to freeze drying is not attributed to the differing purity of the steam and vapour heated products but reflects a small variability in the resolution of the reverse transcriptase assay. Irrespective of the product tested, in all assays performed on the "steam" or "vapour" heated product, the inactivation shown by freeze drying and one hour heating at 60°C always showed a four log step reduction overall. However, the reverse transcriptase assay whilst retaining a sensitivity of one infectious unit HTLVIII has a resolution of ± 1 log step. The difference between 4 and 3 log steps reduction reflects this feature of the assay.

The figure of zero HTLVIII activity shown at the end of the 10 hours of vapour heat treatment is however not called into question as the absolute sensitivity remains at one infectious unit due to the way the assay is performed as outlined in the sections relating to the assay procedure.

TOTAL P.03