INFECTED BLOOD INQUIRY

BRENDON GRAY WITNESS STATEMENT

EXHIBIT WITN6984112

MILES

Gutter Biological
Berkeley, CA

Memorandum

Date: December 19, 1989

Subject: Koate HP - PL

From: Elias L. Greene EG - 9.897

cc: C. Moore
R. Hein
R. Victor

To: Craig Simpson - Bayer U.K.

A. In reply to your questions in NE/SC/321, enclosed are the following:

1) **The following pages of the PLA for Koate HP were missing from our copy. Please provide them:**

Pages between 4-1/2 and 4-5 (4-2 details specifications for aluminum hydroxide gel)
Pages between 5-3/7 and 5-9.

Attachment 1 is the pages between 4-1/2 and 4-5 and between 5-3/7 and 5-9.

2) **Specification of starting materials (Koate HP PLA Page 4-1/2).**
(i) If tested, would those starting materials that are said to comply with the current USP revision plus pyrogen test meet the requirements of the BP/EP Monographs? Are the pyrogen tests and dosages used for the starting materials the same as those that have been notified to us previously for these starting materials when used in the manufacture of Koate HS?

The tests are not identical to USP. We will test raw materials and respond by the end of January, 1990.

(ii) If tested, would Polysorbate 80 comply with the BP Monograph?

We will carry out the special BP tests by the end of January to verify whether our material conforms to the BP.

(iii) Please confirm that the supplier of heparin is the same as that used in the manufacture of Koate HS and if tested it would meet the requirements for Heparin Sodium Injection USP and Heparin Sodium BP.

Heparin is the same as used in Koate HS. Testing will be performed to verify whether this material meets BP.

(iv) **Full specifications including tests and limits are required for:**
Tri-N-Butylphosphate (TNBP) - including limits for TLC characterization.
Aluminum hydroxide gel.
Polyethylene glycol - including nominal molecular weight. If tested, would it comply with the relevant BP monograph.

TNBP. Attachment 2 is the tests and limits.

Aluminum hydroxide gel. Attachment 3 is the tests and limits.

Polyethylene glycose. Attachment 4 is the tests and limits. The nominal weight PEG that is used is not one given in the BP, thus test limits based on weight would not correspond (hydroxyl value and freezing point) to our material. All other parameters meet BP.

3) **Manufacture.**

(i) **Preparation of AHF concentrate for viral inactivation (BPR iv. 20-33B page 3). (Attachment 2).**
Why is dilution of the AHF concentrate with 0.01NHCl or histidine buffer necessary and how is the decision made as to which diluent and which dilution (1:50 or 1:100) should be used? Please provide full specifications, including tests and limits for 0.01N HCl and Histidine buffer.

The AHF concentrate is not diluted at this point. A sample of 1.0 ml or less is removed and can be diluted 1/50 or 1/100 so it can be on scale in the UV spectrophotometer. This is only to determine the A280 of the concentrated solution at this step. Since the combined A280 is approimately 25, it must be diluted to get it on scale, i.e., between 0 to 2.0 absorbance units. For convenience, the dilution can be made with either 0.01 N HCl (routine lab chemical for A280 dilution) or Histidine column buffer if available. (Attachment 5 is test and limits for Histidine buffer.)

(ii) Preparation of TNBP treated AHF solution for column loading (BPR IV. 20-33C Page 5). Attachment 3. Please provide the names of the filters that may be used to filter the AHF solution prior to leading on the Biogel A5M Column.

The virus inactivation step of the AHF concentrate with the TNBP/TWEEN-80 solution manages to result in a mainly homogenous solution with only a few very small pieces of denatured protein. We found early that we could not filter this material through even a 0.45 u filter. We are currently using a stainless steel screen (100 mesh) to remove only the denatured protein. Once through the screen the AHF concentrate added to the column is homogeneous with no debris.

(iii)Gel filtration of TNBP treated AHF solution-collection of peak 1. BPR IV. 20-33C states that collection of pool 1 is completed when the peak "bottoms out" and begins to rise again to an A280 of 1.2-2.0. This appears to be a broad A280 range between which collection may be stopped. However, the additional data provided by you on March 11, 1989, states that the cut-off point is when an A280 of 1.8-2.0 is reached. Is validation data available which supports the chosen cut-off point and is a continuous monitor/recorder trace for the gel filtration procedure available?

The BPR has been changed to end Pool 1 at the discretion of the supervisor. The rule of thumb used is that when the A280 on the chart recorder reaches 0.5 A280 units above the valley floor, Pool 1 is ended. (see xerox of recorder trace for GF022 column run - Attachment 6). Coagulation assays have verified our decisions because >75% of the applied AHF can be recovered in AHF Pool 1, whereas less than 5% is detected in Pool 2. We routinely cannot account for remaining approximately 15% of the applied AHF.

(iv) Has the operational lifespan of the A5M column been established and characterized in terms of volume throughput before column replacement is necessary and is validation data available?

To date (10-27-89), we have performed 60 column runs on the 140L BIO Gel A-5M production column. After column run #28, we repacked the bottom stack and after column run #55, we repacked stacks 1, 2, 3. It appears that

approximately every 25 column runs, we will have to repack the bottom stack. The two items we monitor very closely each run are the Void Volume Buffer pool (approx. 42 kg) before the Pool 1 begins to elute, and the shape of the conductivity (salt peak). All of the final container lots processed to date from these 60 column runs (20 domestic and 7 CRC lots) have passed rabbit pyrogen test (at least 50 IU/kg) and both bulk sterility and final container sterility. After each column run 0.1M NaOH (approx. 2 column volumes) is pumped through the column and, if it is stored, at least 1.8 column volumes of 0.05M NaOH is pumped into the column prior to isolation. This regimen keeps the column gel clean. As to the ultimate column runs "possible" before use of "new" gel is necessary - no estimate is possible.

(v) **Final container filling (Aseptic).**
Details of biological validation i.e. media runs sufficient to demonstrate an acceptable low level of contamination in the filling lines used for final container filling.

A sample of the sterile filtered AHF Bulk is submitted for bulk sterility testing. These are usually the first bottles out of the filling line. All final container lots to date have passed bulk sterility testing in QA.

(vi) **Details of the lyophilization process including details of time and vacuum are required; batch production records would be a suitable source of information.**

For freeze drying of Koate-HP lots check the enclosed freeze-drying cycle chart (Attachment 7). Also enclosed are the new (not yet instituted) reduced freeze dryer cycles for Koate-HP. The reduced, shorter cycles will be the same regardless of either 5 or 10 ml fills.

4) **Finished product specification.**

(i) **As was necessary for Koate HS, can the limit for solubility be dropped from 20 to 10 minutes and would it meet the BP requirements if tested?**

Yes. However, please note that the BP requirement for solubility is solution within 30 minutes.

(ii) What are the limits for fibrinogen content and as was the case with Koate HS would the product meet the BP requirements with regard to fibrinogen content, i.e. not more than 80% of the total protein?

No limits have been set for fibrinogen since the levels remaining from the process are so low (0.6mg/mL). Fibrinogen content is less than 80% of the total protein.

(iii)There are no limits for albumin content, however as for Koate HS is the level monitored by cellulose acetate electrophoresis (informative test)?

There is a specification for albumin of no more than 10.0 mg/mL for Koate HP, and albumin content is measured on every lot by radial immunodiffusion.

(iv) If tested would Koate HP comply with the requirements of the BP?

Yes.

5) Batch analytical data for 3 batches of Koate HP, including batch size and date of manufacture together with full test limits and quantitative results. Details are required of the batches used in the toxicological studies (Batches PR64F001 Y64E001) and the clinical study (Batch Y64F001) reported in the PLA for Koate HP. If possible, please provide batch analytical data for these batches.

Attachment 8 is the batch analyses for lots PR64F001 and Y64E001. PR64F001 and Y64F001 are the same; the letters PR were changed to Y after the IND was approved.

6) Stability data for 3 batches of Koate HP sufficient to support the claimed shelf life. Test data must include conditions of storage together with specification, test methods, limits and quantitative results.

Attachment 9 is stability data for 3 batches of Koate HP.

7) Packaging.
A full specification for the stoppers and aluminum seals used in primary packaging is required together with details of the sterilization procedures used for bottles, stoppers and aluminum seals.

Attachment 10A is the specifications for bottles, stoppers and

aluminum seals. Attachment 10B is the procedures used to rinse and sterilize the bottles and stoppers.

8) **Administration equipment provided with Koate HP.**
Please confirm that the manufacturer of the administration equipment is as detailed for Koate HS. Under the DHSS Procurement Directorate's single-use sterile device scheme manufacturers must have a registered UK division. If the manufacturer differs from that supplying Koate HS administration equipment, please provide their name and address together with that of their UK division.

The manufacturer is the same as for HS.

9) **Please provide an updated list of Source Plasma (Human) centers.**

Attachment 11.

10) **Phase I single dose pharmacological study.**
Please confirm that the batch of Koate HP used in the Phase I study reported in Attachment IV of the Koate HP PLA is Batch No. Y64F001.

Yes.

B. The information requested in NE/SC/324 are below:

1. The completed tables containing the data for 3 lots - Attachment 12.

2. Printout of all reactions reported with Koate HP to date - there is only 1. Attachment 13.

3. Original data on which poster of Dr. Louie et. al. are based is Attachment 14.

I hope this will be what is needed to complete the submission. Please let me know if anything further is needed.

Sincerely yours,

GRO-C

Elias L. Greene