HYLAND
DIVISION BAXTER TRAVENOL LABORATORIES

Interoffice correspondence

L. K. Meeting Copy

To: Distribution

From: L. Kriley

Subject: PROJECT DECISION COUNCIL BOOKLET

Date: June 14, 1979

cc: G. Harding
G. Phelps

Attached is a copy of the subject booklet, containing project prooosals for:

1. Hepatitis Risk Removal - Hemofil
2. Hepatitis Risk Removal - Proplex
3. AHF Yield Improvements (Ultrafiltration)
4. Hemofil Quality Improvements (Ultrafiltration)

for review at the P.D.C. Meeting on June 15, 1979.

GRO-C: L Kriley

:sh

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WORLDWIDE COURT REPORTERS INC

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HEPATITIS B AND NON-A, NON-B HEPATITIS INFECTIVITY-FREE HEMOFIL

I. OBJECTIVE

The objective is to substantially differentiate Hemofil from other Factor VIII concentrates by developing a Hepatitis B and Non-A Non-B Hepatitis Infectivity-Free Hemofil. It is desirable that other product characteristics remain equivalent to those of the existing product, although if other characteristics are slightly negatively affected, the loss would be more than offset by the gains from the removal of Hepatitis risk.

II. RECOMMENDATION

On the basis of the following project evaluation, it is recommended that:

A. Approval be given to proceed with all necessary work packages outlined on the attached project critical path analysis (Attachment I) for the development and implementation of Hepatitis B and Non-A, Non-B Hepatitis Infectivity-Free Hemofil.

B. The project be designated as the #1 "A" priority, and a project team established.

C. PDC review be held in three months to review data from the evaluation of heat treated and gamma radiation treated Method IV Hemofil and ultrafiltered product to determine if license approval for one of the methods will be requested or if other approaches must be evaluated.

III. BACKGROUND INFORMATION

A. Project History

In June, 1977, a study of the feasibility of removing Hepatitis from Hemofil was approved by the PDC. Much background information on Hepatitis was presented at that time and this information is available from L. Kriley upon request. Project monthly summaries were prepared and distributed by A. J. Lazos from the initiation of the project until June 29, 1978. As a result of this previous study, Research and Development indicates that heat inactivation of virus in Hemofil, gamma radiation treatment of Hemofil, and the ultrafiltration process Factor VIII concentrate are the three Hepatitis risk removal approaches which should first be seriously considered within this project.

B. Hemofil Availability

It has been projected that Hemofil inventories worldwide will begin to accumulate in the fourth quarter of 1979. A detailed projection of the Hemofil inventory status is attached. (Attachment II)

The removal of the risk of Hepatitis transmission by Hemofil is needed in order to allow sales of the projected excess inventories at the highest possible selling price.

C. Competitive Activities

Behringwerke and Immuno are reportedly working actively on the development of a Factor VIII replacement product that is "safe" from the risk of Hepatitis. We have information to the effect that Behringwerke is approaching the completion of the clinical evaluation of its product.

IV. PROJECT BENEFITS - HEPATITIS-FREE HEMOFIL

A. The significant demonstrable product differentiation that will be accomplished by the development and implementation of a Hepatitis Infectivity-Free Hemofil will result in dramatic increases in unit sales and in average selling price. Total incremental revenue, resulting from both sales volume and A.S.P. increases, is projected at approximately $39 million in 1981 increasing to $47 million in 1983. Attachment III shows a detailed derivation of these projections.

Because of the difficulty of estimating the production costs associated with the removal of Hepatitis infectivity from Hemofil, the current Hemofil standard cost has been used to calculate the projected effect on gross profit, $87,370,000. from 1981 through 1983.

B. The unit sales and average selling price projections in Attachment III were developed from forecasts which assumed that neither Hyland nor a competitor would have a Hepatitis-free product before 1983. The upside return to Hyland in these projections assumes that Hyland develops a Hepatitis-free product substantially before a competitor does. To the contrary, if a competitor would develop a Hepatitis-free product before Hyland, the downside risk is approximately equally dramatic. ~~A projection of this downside risk is included as Attachment IV.~~

Another downside risk that we are currently facing results from the large Hemofil price differentials between European countries. If we are not able to substantially differentiate Hemofil from competitive products, as would be achieved by having a Hepatitis-free Hemofil, all European prices for Hemofil could drop as low as 20¢, as is currently seen in the U. K.. Attachment IV shows a summary calculation indicating that the potential revenue loss from such a price drop could be as large as $18 million to $20 million per year. Hepatitis-free Hemofil would be relatively certain protection against this downside risk.

C. International and domestic sales personnel report that the improvement in concentrate products that is most requested by patients and the medical community is the removal of the risk of the transmission of Hepatitis. Accomplishment of this objective would be viewed as a major contribution to the treatment of hemophiliacs and would significantly enhance Hyland's image and brand loyalty to Hemofil.

D. If ultrafiltration is selected as the method for Hepatitis removal, other product improvements, which are the subject of the Hemofil Quality Improvements - Ultrafiltration project proposal, may result.

E. The heat inactivation method to be evaluated in the first three months is the method that is used by Behringwerke. If our data after three months shows that it does not remove the risk of Hepatitis transmission, an article can be published by Research, giving Hyland a strong counter argument against Behringwerke's claim.

V. SUMMARY OF PROJECT ACTIVITIES AND COSTS

A. As a brief summary, the most expeditious and probably successful processes for accomplishing a Hepatitis risk-free product are the heat inactivation and gamma radiation treatment of the current Hemofil product after it is dried. If in-vitro testing of treated H B_sA_g and Non-A, Non-B Hepatitis-positive product shows that it is negative, tests in chimpanzees (where the incubation time should be 14-21 days) will be undertaken. This will take three months, and during this period the ultrafiltration Factor VIII concentrate will be characterized and further developed so that evaluation of it by this process in the next three months can be undertaken if the heat inactivation or gamma radiation treatment approaches are not effective. Research will then evaluate other potential and more time consuming methods, such as immuno adsorption and chemical treatments, if the above methods fail.

When a method is shown to be successful, data will be submitted to the Bureau and the licensing activity required by the Bureau will be conducted as the process is being developed within the plant. It is estimated that product could be available for sale as early as 14 months after the initial P.D.C. project approval, at a cost of $621,000.

B. Attachment I, Project Status, shows a detailed list of project activities and scheduling with a critical path analysis.

C. Total cost for all evaluations within the project will be approximately $1,749,000 and it is estimated that 29 months will be required to complete the project. Because of the technical complexity of the various approaches, and because no capital expenditures will be required before the three month review by the P.D.C., it is suggested that capital expenditure projections be evaluated at that time. With the full time utilization of 15 R & D personnel during this three month period, expenditures will be approximately $187,500. Note that this is a 100% commitment of all of the development personnel who have experience with Hemofil.

VI. REGULATORY

Because the Hemofil manufacturing process must be modified to accomplish the project's objective, a license amendment must be obtained. Prior to that, an I.N.D. must be filed and clinicals must be performed. The use of a chimpanzee model to indicate Hepatitis non-infectivity would be required and Dr. Rodell indicates half-life and recovery studies in humans would probably also be required by the Bureau of Biologics.

VII. KEY CONSIDERATIONS, RISKS

A. Non-A, Non-B Hepatitis has not been characterized and the number of types of Non-A, Non-B Hepatitis remains a question. Therefore, a high risk factor is associated with the removal of Non-A, Non-B infectivity from Hemofil. The probability of success associated with the removal of Non-A, Non-B Hepatitis and Hepatitis B infectivity from Hemofil is 60%.

B. Diagnostic tests that are currently available for Hepatitis B surface antigen are not as sensitive as chimpanzees. A diagnostic test for Non-A, Non-B Hepatitis has not yet been developed and Lou Liddy, Corporate Director of Hepatitis Test Market Development, estimates that it will be at least three years before such a test is commercially available. Proof of non-infectivity must be accomplished in chimpanzees and it is increasingly difficult and expensive to obtain chimpanzees.

C. Any claim of Hepatitis risk removal short of complete freedom from infectivity by Hepatitis B and Non-A, Non-B Hepatitis will have a significantly smaller benefit to the Company.

D. The heat inactivation of Hepatitis virus will result in the metabolism and discoloration of the dextrose that is in the current Hemofil; therefore, another stabilizer, perhaps albumin, must be added.

E. Product half-life and yields may be reduced by the process that removes Hepatitis risk.

PROJECT AUTHORIZATION REQUEST (PAR) FOR MAJOR DEVELOPMENT PROJECTS

Project Control Number: _____ ☐ Original Request (Date 6/15/79) ☐ Updated Request (Date _____) ☐ New Product

Date: 6/15/79 Total Project Cost ($) _____ ☐ Other than New Product Development (Specify Below)

Project Leader: _____ Marketing Division: Hyland Therapeutics

☐ Round Lake Development ☐ Other Development Site (Specify) _____

PROJECT NAME: Hepatitis B and Non-A, Non-B Hepatitis Infectivity-Free Hemofil

PROJECT DESCRIPTION: (Major functions product must perform)

Produce a Hepatitis Infectivity-free Hemofil in which other product characteristics remain equivalent to those of the existing product, although if other characteristics are slightly negatively effected, the loss would be more than offset by the gains from the removal of Hepatitis risk.

MARKET FACTS: (Competition, market size, market segment expected, marketing advantages, marketable selling price, standard manufacturing cost for product to be marketable)

Currently there is no Hepatitis risk-free Factor VIII concentrate available. Being the first manufacturer with a Hepatitis-free product would be a significant marketing advantage because it is the single most requested product improvement.

PRODUCT INFORMATION: (For new product or major modification)

EXPECTED PRODUCT LIFE: (Years) _____

ESTIMATES:

Incremental Hemofil Sales:

Replaces Cat. # _____ CURR. ANN. SALES & PROFIT		UNITED STATES AND INTERNATIONAL					
		First Year 1981		Second Year 1982		Third Year 1983	
		Probable		Probable		Probable	
	Domestic	International	Domestic	International	Domestic	International	Domestic	International
Unit Sales ..... MM A.U.			101	47	113	55	125	63
Unit Average Selling Price			+25%	+15%	+25%	+15%	+25%	+15%
Total Sales ($) ... (000)			14,300	24,500	15,600	27,200	16,800	30,300
Market Share (%)			+20%	+12%	+20%	+13%	+20%	+13%
Total Selling & Marketing Exp. ($) ..								
Unit Standard Cost ($)								
Material								
Labor								
Overhead								
Total ..... ¢/A/U.			8.2¢	8.2¢	8.2¢	8.2¢	8.2¢	8.2¢
Unit Marketing Exp. ($)								
Gross Profit ($) .... (000)			6,020	20,650	6,330	22,690	6,550	25,130
Gross Profit (%)			42%	84%	41%	83%	39%	83%

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PROJECT AUTHORIZATION REQUEST (PAR) FOR MAJOR DEVELOPMENT PROJECTS

PROJECT CONTROL NUMBER: HEPATITIS RISK REMOVAL EMOFIL

DATE: PLAN A

PROJECT EXPENSE DETAIL ($000's)

Major Milestones	Start Date	Comp. Date	Division R & D Material	Division R & D Labor & OH	Division R & D Total	Mfg. Engineering Material	Mfg. Engineering Labor & OH	Mfg. Engineering Total	Other CLINICAL Material	Other CLINICAL Labor & OH	Other CLINICAL Total	Round Lake Preproduction Material	Round Lake Preproduction Labor & OH	Round Lake Preproduction Total	Grand Total	Est. Salable Units	Equip. & Molds
A. FEASIBILITY FOR CLINICALS	0	4	41	51					270						361		
B. PREPARE CLINICAL/ STABILITY LOTS	5	7	43	53					20						116		
C. CLINICAL TRIALS & STABILITY TESTING	8	14	20	29					70						119		
D. MFG IMPLEMENT'N	12	14	11	13					—						24		
E.																	
F.																	
G.																	
H.																	
I.																	
J.																	
K.																	
TOTAL			115	146					360						421		

Deduct Value of Released Products expected before Decision to Terminate Development Form is signed. (____ Units @ $ ____ each) $(____)$(____)

Form is expected to be signed ____/____/____.

Net Round Lake Preproduction Expense $ ____ $ ____ Net Project Expense

SPECIAL FACILITIES, EQUIPMENT & MOLDS REQUIRED: ($000's)

Facilities: ____ Square feet @ $ ____ per square foot ..... $ ____

Equipment: (Description and Cost Detail on Backup Exhibit ____) ..... ____

Molds: (Description and Cost Detail on Backup Exhibit ____) ____

Total Facilities, Equipment, & Mold Expenditures: ..... $ ____

PROJECT AUTHORIZATION REQUEST APPROVAL:

Milestone	Net Project Expense	Facilities Equipment and Mold Expenditures	Total Project Cost	Product Development Committee Chairman	Product Development Committee Date	New Products Committee Chairman	New Products Committee Date

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PROJECT AUTHORIZATION REQUEST (PAR) FOR MAJOR DEVELOPMENT PROJECTS

HEPATIT. RISK

PROJECT CONTROL NUMBER: REMOVAL HEMOPE-

DATE: PLAN 2

PROJECT EXPENSE DETAIL ($000's)

Major Milestones	Start Date	Comp. Date	Division R & D			Mfg. Engineering			Other CLINICAL			Round Lake Preproduction			Grand Total	Est. Salable Units	Equip & Molds
			Material	Labor & OH	Total	Material	Labor & OH	Total	Material	Labor & OH	Total	Material	Labor & OH	Total			
1. FEASIBILITY																	

I. Background and Objectives

Hepatitis in patients receiving whole blood has been well documented (Wallace, 1976). While clinical hepatitis after transfusion of blood products is less common in hemophiliacs (Lewis, 1970; Biggs, 1974). Several reports have appeared in the literature documenting the presence of persistent abnormalities of liver function tests in the majority of these patients (Mannucci et al., 1975; Hasiba et al., 1977; Hilgartner and Giardina, 1977; Levine et al., 1977; McVerry et al., 1977; Yannitsiotis et al., 1977). These studies suggest that abnormal liver function tests are: 1) present in over 50% of patients; 2) not associated with symptomatic evidence of liver disease; 3) not related to the intensity of F-VIII or F-IX replacement therapy, the age of the patient or underlying nature of the hemostatic defect, i.e. Hemophilia A, Hemophilia B, or von Willebrand's disease (Hilgartner and Giardina, 1977); and 4) persistent over at least a one-year period. One study suggested that abnormal liver function was more frequent in patients receiving lyophilized factor concentrates than in those receiving cryoprecipitate (Hasiba, et al., 1977), but this has not been found by others (Levine et al., 1977; McVerry et al., 1977). The dosage of the infectious agents in donor plasma and concentrates is considered the most influential factor in the incidence of posttransfusion hepatitis but the recipient's hereditary factors, immunological competence and age also play a role (Barker et al., 1970).

Transmission of posttransfusion hepatitis continues to be one of the major complications associated with transfusion of plasma products. Several means have been used to minimize the hepatitis problem. They include: 1) rejection of donors whose plasma has been associated with the transmission of the

disease; 2) utilizing a simple but reliable test for detecting the infectious agents in plasma (Blumberg et al., 1968; Dane et al., 1970; Shulman, 1970; Gerety et al., 1975; Goeke et al.); 3) passive immunization with hyperimmune gamma globulin; 4) isolating the causitive agents and developing an appropriate vaccine (Price, 1968; Prince et al., 1974; Feinstone et al., 1975); and 5) neutralizing or inactivating the virus (Lo Grippo, 1969; Lo Grippo and Hayashi, 1973).

Hepatitis viruses are known to be highly resistant to various concentrations of many chemical and physical agents such as, phenol and ether (Bebson, et al., 1943), merthiolate (Beeson et al., 1944), and chlorine (Neefe et al., 1945). The viruses remain active for several years in the frozen state and withstand repeated thawing and refreezing (Neefe et al., 1946). They are, however, effected by heat and irradiation. The pioneering work of Gellis and others (1947) showed that Hepatitis B infectivity was removed from albumin solutions which were heated at 60°C for 10 hours. A recent publication by DeFlora (1978) indicated that the loss of activity of purified HBsAg in phosphate buffer at 20, 37, 44, 56, 70 and 98°C was linear with respect to time and the half life of inactivation ranged from 6 min. at 98°C to over 52 days at 20°C. Temperature inactivation required the most time at neutral pH. Heat inactivation occurs at faster rate at pH values below 5.0 or over 8.0 and divalent cations such as magnesium enhance HBsAg stability. The conditions which result in inactivation of purified HBsAg solutions may not be the same conditions which cause inactivation of the Hepatitis B found in a therapeutic product. However, the stated conditions (temperatures and times) provide a guideline for experimental studies.

The objectives of this proposal will be to outline plans for development of hepatitis-free F-VIII and F-IX preparations which have acceptable characteristics relative to other preparations and result in satisfactory yields.

II. Specific Aims

1. To modify the current Hyland Preparative procedures in order to facilitate complete removal and/or inactivation of both Hepatitis B and non-A non-B so that patients can be treated with products which are free from hepatitis infectivity.
2. To provide F-VIII and F-IX concentrates which have acceptable characteristics of potency, stability and solubility relative to Hemofil and Proplex so they are effective in treatment of the hemophilias.
3. To achieve both acceptable F-VIII and F-IX yields and definite economic advantages in sales.

III. Rationale

Travenol sales personnel have indicated that the most desired characteristic of new F-VIII and F-IX concentrates would be lack of hepatitis infectivity. This characteristic would distinguish Hyland products from those manufactured by most other companies except Behring which will be marketing a F-VIII preparation it claims is free of Hepatitis B infectivity. Hepatitis-free F-VIII and F-IX concentrates will provide advantages to patient and company.

IV. Preparation of F-VIII Concentrate

Three different methods for preparation of a F-VIII concentrate have been outlined in the following section. The specific procedures have been utilized in other documented investigations and are designed to achieve removal of large amounts of hepatitis associated antigen with inactivation (by heat or irradiation) of remaining infective hepatitis.

1. Method A

The current Method IV Hemofil process could be utilized with or without the addition of albumin as a stabilizer. Albumin (1-3%) may be added to the F-VIII concentrate at the final process step (dilution step). This product would be filled, frozen and dried as is the current Hemofil. The final lyophilized product would be heated to various temperatures (45°-60°) for a time period or treated with γ-radiation (Barker et al.,). After heating or irradiating, the product will be analyzed for potency, solubility, stability and other characteristics (see Plan A). If successful, this plan would offer the most rapid means of developing a concentrate which is not hepatitis infective

If Plan A is unsuccessful, Plan B (Methods B and C) includes a feasibility study to choose a F-VIII procedure which results in a hepatitis-free preparation with other satisfactory product characteristics. Both plans can be undertaken concurrently without additional laboratory personnel, assuming no interruption in currently scheduled Coagulation Research activities.

2. Method 8

The following procedure could be used starting with dissolved cryoprecipitate or Cohn I: 1) decrease fibrinogen content by precipitation with 0.3 - 0.6 M glycine (Blomback, Wagner ,); 2) purify F-VIII by batch adsorption to DEAE A-50 and selective elution to reduce hepatitis risk (Haystak et al., 1973;); 3) concentrate the F-VIII by either PEG, ultrafiltration, hollow fiber or gel filtration to adjust potency and remove unwanted small molecular weight proteins; 4) add 1-3% albumin and/or other substance(s) to stabilize F-VIII and enhance solubility of preparation ( ); 5) fill in bottles, freeze and lyophilize; 6) heat and/or with γ-irradiate lyophilized preparation. Various temperatures will be used over a period of time in an attempt to neutralize or inactivate the hepatitis virus, e.g. according to DeFlora (1978) the half-life of purified HBsAg at pH 7.0 is: a) 2 days and 4 hours at 44°C; b) 6 hours and 41 min. at 56°C and; c) 1 hour and 53 min. at 70°C. Steps 1-4 could be partially interchanged to facilitate an improvement in yield and product characteristics.

3. Method C

Cryoprecipitate or Cohn I could be utilized as the starting precipitate from plasma and would subsequently be processed as follows: 1) batch adsorption and elution of F-VIII from heparin-sepharose to reduce amount of fibrinogen and hepatitis associated with the F-VIII (Maderas, Bell and Castaldi, 1978); 2) further purification and concentration by PEG, ultrafiltration or hollow fiber; 3) addition of 1-3% albumin and/or other substance(s) to stabilize F-VIII and improve solubility of the final

product ( ); 4) fill, freeze and dry preparation; and 5) heat or irradiate as in Method A or B to inactivate the hepatitis virus.

Preparations would include a Hepatitis marker to ascertain whether the hepatitis is removed and/or inactivated. In-process and final container samples would be screened for the marker. Ultimately, the final product will be administered to chimpanzees to determine the presence or absence of infectivity.

All process steps and final containers would be examined for: 1) F-VIII antigen (VIIIR:Ag), coagulant (VIII:C); 2) activation by Kingdon time, S-2160 and S-2251 assays; and 3) fibrinogen,FSP(including fibrinopeptide A). In addition, final containers would be analyzed by other tests in the current Hemofil product specifications to ascertain limits of acceptability.

B. Removal of Hepatitis Infectivity from F-IX Concentrates

Two different methods for preparation of a F-IX concentrate are summarized in the Methods A and B (see project plans A and B, pp.). Method A utilizes the current Proplex process while Method B involves an ion exchange procedure currently being used by Coagulation Research to produce a F-IX concentrate. Both methods are designed to minimize the amount of hepatitis associated antigen in the final product. Heat or γ-radiation will be used to inactivate remaining infective hepatitis.

The current RIA Negative Proplex procedure, a licensed method, can be assessed for removal of hepatitis infectivity by stated methods and with success can be most expeditiously marketed. However, if success is not achieved with

Method A, then hepatitis removal can be examined by Method B.

1. Method A

The current RIA Negative Proplex procedure could be utilized and the final product heat (45-60° over a time period ( )) or radiation treated to inactivate remaining hepatitis associated activity. For improvement of product stability and solubility, albumin (1-3%) and/or other substance(s) may be added to the final bulk prior to sterile filtration. The characteristics of the final product including potency, solubilization, stability, other characteristics (mentioned below) and removal of hepatitis infectivity will be examined.

2. Method B

At present, a project plan has been initiated for recovery of F-IX concentrate from cryo-free plasma. This process utilizes DEAE Sephadex A-50 to isolate the F-IX concentrate. It has been shown that this process greatly reduces the amount of hepatitis associated antigen ( ). Hepatitis removal from the preparation is scheduled to be minimally assessed on the current project plan. However, additional experiments could include heat and/or radiation treatment of the final product in an attempt to inactivate the hepatitis. Once again, albumin (1-3%) and/or other substance(s) may be needed in the preparation to enhance product stability during heating or irradiation

The entire processes and final containers from Methods A and B would be analyzed for: 1) coagulation factors II/IIa, VII, IX/IXa and X/Xa, XII/XIIa and 2) activation by Kingdon Time and S-2160. Short term (3 hr.) stability of the reconstituted product will be examined by the same assays. Accelerated stability studies will be performed on final containers also

using the same assays above. The final container will be analyzed for the same characteristics as the current Proplex product with a view toward determining satisfactory specifications. In addition, the final product will be analyzed for PKA and bradykinin content as well as by disc gel electrophoresis (basic and SDS), I.E.P. (with polyvalent antiserum)); double diffusion (with monspecific antisera) and R.I.A.,for HBsAg, anti-HBs, HBcAg, anti-HBc. These analyses will be determined throughout the processes and on the final containers. Ultimately, the presence or absence of hepatitis infectivity in the final containers will be ascertained in chimpanzees. The sequence of activities for either Method are seen in Plans A and B on pages

The project which concerns removal of hepatitis from F-IX concentrates would require additional laboratory personnel (3-4 people) in order to accomplish this concurrently with both the current Coagulation Research projects and the project for removal of hepatitis from a F-VIII concentrate.

The current major Coagulation Research projects include: a) DEAE prepared F-IX concentrate; b) AT-III; c) AUTOPLEX (involves 3 portions which are: 1) screening IV-1 paste, 2) ascertaining mechanism of action of concentrate, and 3) monitoring the process during manufacture of first 10 lots made by Production Personnel); 3) characterization of Development F-VIII preparation;d) Reagent preparation for Glendale and Research. Certain priority realignments may allow pursuit of hepatitis removal projects for both F-VIII and F-IX concentrates with little additional manpower. For example, the elimination of both the AT-III project and the portions of the AUTOPLEX project which involve both IV-1 screening and ascertaining the mechansim of action of the concentrate would result in the need for only one additional person to implement both hepatitis removal projects.

Attachment I

RESEARCH PROPOSAL - TIME REQUIREMENTS

	ACTIVITY	MAN-MONTHS REQUIRED
1.	Literature Search	1 mo.
2.	Physical Methods	
	A. Filtration	2 mo.
	B. Nonspecific Adsorption	2 mo.
	C. Centrifugation	------
	D. Selective Precipitation	3 mo.
3.	Specific Binding Techniques	
	A. Charm technique	3 mo.
	B. Other	3 mo.
	1. Adsorption chromatography	
	2. Ion exchange chromatography	
	3. Other	
4.	Inactivation	
	A. Ionizing Radiation	3 mo.
	B. Visible Light Radiation	2 mo.
	C. Chemicals	2 mo.
	D. Heat	3 mo.
	E. Chemical Inhibitors	-----

HEPATITIS REMOVAL

I. Literature Search - 1 month then ongoing

A. Physical Removal

1. Centrifugation
2. Filtration
3. Nonspecific adsorption
4. Selective precipitation

B. Specific Binding Techniques

1. Immunoadsorbents for ant gen
2. Adsorption chromatography

C. Inactivation

1. Radiation
   a. Ionizing
   b. Light sensitizing dye
2. Chemical
3. Heat (dry powder)
4. Chemical inhibition

II. Physical Methods

A. Filtration

Evaluation of efficiency of various filter systems employing Biological Marker.

B. Nonspecific Adsorption

Determine if nonspecific adsorption offers any possibility based upon literature review. If so, apply technique to HBsAg.

C. Centrifugation

Initial studies and literature review previously indicated that centrifugation would not be a fruitful method. If review of literature does not indicate some method(s) of improvement, would recommend that this avenue not be pursued further.

D. Selective Precipitation

This technique would be a reevaluation of studies initiated by Garcia and as published by Johnson (1976, J. Lab. Clin. Med. 88: 91-101). May be further expanded upon. May be able to use biological marker for initial work, however, HBsAg and HBV may react differently from each other and any marker that could be selected.

III. Specific Binding Techniques

A. Reevaluation of the charm technique should be included in any study of this type. The efficiency of the method may be evaluated by the use of biological markers since studies with HBsAg will not offer the sensitivity needed.

B. Other techniques such as adsorption chromatography and ion exchange should be evaluated. Much has been reported in the literature on methods for purification and concentration of HBsAg. This information may be used to study the removal of HBsAg from the products.

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II. Specific Aims

1. To modify the current Hyland Preparative procedures in order to facilitate complete removal and/or inactivation of both Hepatitis B and non-A non-B so that patients can be treated with products which are free from hepatitis infectivity.
2. To provide F-VIII and F-IX concentrates which have acceptable characteristics of potency, stability and solubility relative to Hemofil and Proplex so they are effective in treatment of the hemophilias.
3. To achieve both acceptable F-VIII and F-IX yields and definite economic advantages in sales.

III. Rationale

Travenol sales personnel have indicated that the most desired characteristic of new F-VIII and F-IX concentrates would be lack of hepatitis infectivity. This characteristic would distinguish Hyland products from those manufactured by most other companies except Behring which will be marketing a F-VIII preparation it claims is free of Hepatitis B infectivity. Hepatitis-free F-VIII and F-IX concentrates will provide advantages to patient and company.

IV. Preparation of F-VIII Concentrate

Three different methods for preparation of a F-VIII concentrate have been outlined in the following section. The specific procedures have been utilized in other documented investigations and are designed to achieve removal of large amounts of hepatitis associated antigen with inactivation (by heat or irradiation) of remaining infective hepatitis.

1. Method A

The current Method IV Hemofil process could be utilized with or without the addition of albumin as a stabilizer. Albumin (1-3%) may be added to the F-VIII concentrate at the final process step (dilution step). This product would be filled, frozen and dried as is the current Hemofil. The final lyophilized product would be heated to various temperatures (45°-60°) for a time period or treated with γ-radiation (Barker et al.,). After heating or irradiating, the product will be analyzed for potency, solubility, stability and other characteristics (see Plan A). If successful, this plan would offer the most rapid means of developing a concentrate which is not hepatitis infective

If Plan A is unsuccessful, Plan B (Methods B and C) includes a feasibility study to choose a F-VIII procedure which results in a hepatitis-free preparation with other satisfactory product characteristics. Both plans can be undertaken concurrently without additional laboratory personnel, assuming no interruption in currently scheduled Coagulation Research activities.

2. Method 8

The following procedure could be used starting with dissolved cryoprecipitate or Cohn I: 1) decrease fibrinogen content by precipitation with 0.3 - 0.6 M glycine (Blomback, Wagner ,); 2) purify F-VIII by batch adsorption to DEAE A-50 and selective elution to reduce hepatitis risk (Heystak et al., 1973;); 3) concentrate the F-VIII by either PEG, ultrafiltration, hollow fiber or gel filtration to adjust potency and remove unwanted small molecular weight proteins; 4) add 1-3% albumin and/or other substance(s) to stabilize F-VIII and enhance solubility of preparation ( ); 5) fill in bottles, freeze and lyophilize; 6) heat and/or with γ-irradiate lyophilized preparation. Various temperatures will be used over a period of time in an attempt to neutralize or inactivate the hepatitis virus, e.g. according to DeFlora (1978) the half-life of purified HBsAg at pH 7.0 is: a) 2 days and 4 hours at 44°C; b) 6 hours and 41 min. at 56°C and; c) 1 hour and 53 min. at 70°C. Steps 1-4 could be partially interchanged to facilitate an improvement in yield and product characteristics.

3. Method C

Cryoprecipitate or Cohn I could be utilized as the starting precipitate from plasma and would subsequently be processed as follows: 1) batch adsorption and elution of F-VIII from heparin-sepharose to reduce amount of fibrinogen and hepatitis associated with the F-VIII (Maderas, Bell and Castaldi, 1978); 2) further purification and concentration by PEG, ultrafiltration or hollow fiber; 3) addition of 1-3% albumin and/or other substance(s) to stabilize F-VIII and improve solubility of the final

product ( ); 4) fill, freeze and dry preparation; and 5) heat or irradiate as in Method A or B to inactivate the hepatitis virus.

Preparations would include a Hepatitis marker to ascertain whether the hepatitis is removed and/or inactivated. In-process and final container samples would be screened for the marker. Ultimately, the final product will be administered to chimpanzees to determine the presence or absence of infectivity.

All process steps and final containers would be examined for: 1) F-VIII antigen (VIIIR:Ag), coagulant (VIII:C); 2) activation by Kingdon time, S-2160 and S-2251 assays; and 3) fibrinogen,FSP(including fibrinopeptide A). In addition, final containers would be analyzed by other tests in the current Hemofil product specifications to ascertain limits of acceptability.

B. Removal of Hepatitis Infectivity from F-IX Concentrates

Two different methods for preparation of a F-IX concentrate are summarized in the Methods A and B (see project plans A and B, pp.). Method A utilizes the current Proplex process while Method B involves an ion exchange procedure currently being used by Coagulation Research to produce a F-IX concentrate. Both methods are designed to minimize the amount of hepatitis associated antigen in the final product. Heat or γ-radiation will be used to inactivate remaining infective hepatitis.

The current RIA Negative Proplex procedure, a licensed method, can be assessed for removal of hepatitis infectivity by stated methods and with success can be most expeditiously marketed. However, if success is not achieved with

Method A, then hepatitis removal can be examined by Method B.

1. Method A

The current RIA Negative Proplex procedure could be utilized and the final product heat (45-60° over a time period ( )) or radiation treated to inactivate remaining hepatitis associated activity. For improvement of product stability and solubility, albumin (1-3%) and/or other substance(s) may be added to the final bulk prior to sterile filtration. The characteristics of the final product including potency, solubilization, stability, other characteristics (mentioned below) and removal of hepatitis infectivity will be examined.

2. Method B

At present, a project plan has been initiated for recovery of F-IX concentrate from cryo-free plasma. This process utilizes DEAE Sephadex A-50 to isolate the F-IX concentrate. It has been shown that this process greatly reduces the amount of hepatitis associated antigen ( ). Hepatitis removal from the preparation is scheduled to be minimally assessed on the current project plan. However, additional experiments could include heat and/or radiation treatment of the final product in an attempt to inactivate the hepatitis. Once again, albumin (1-3%) and/or other substance(s) may be needed in the preparation to enhance product stability during heating or irradiation

The entire processes and final containers from Methods A and B would be analyzed for: 1) coagulation factors II/IIa, VII, IX/IXa and X/Xa, XII/XIIa and 2) activation by Kingdon Time and S-2160. Short term (3 hr.) stability of the reconstituted product will be examined by the same assays. Accelerated stability studies will be performed on final containers also

using the same assays above. The final container will be analyzed for the same characteristics as the current Proplex product with a view toward determining satisfactory specifications. In addition, the final product will be analyzed for PKA and bradykinin content as well as by disc gel electrophoresis (basic and SDS), I.E.P. (with polyvalent antiserum)); double diffusion (with monspecific antisera) and R.I.A.,for HBsAg, anti-HBs, HBcAg, anti-HBc. These analyses will be determined throughout the processes and on the final containers. Ultimately, the presence or absence of hepatitis infectivity in the final containers will be ascertained in chimpanzees. The sequence of activities for either Method are seen in Plans A and B on pages

The project which concerns removal of hepatitis from F-IX concentrates would require additional laboratory personnel (3-4 people) in order to accomplish this concurrently with both the current Coagulation Research projects and the project for removal of hepatitis from a F-VIII concentrate.

The current major Coagulation Research projects include: a) DEAE prepared F-IX concentrate; b) AT-III; c) AUTOPLEX (involves 3 portions which are: 1) screening IV-1 paste, 2) ascertaining mechanism of action of concentrate, and 3) monitoring the process during manufacture of first 10 lots made by Production Personnel); 3) characterization of Development F-VIII preparation;d) Reagent preparation for Glendale and Research. Certain priority realignments may allow pursuit of hepatitis removal projects for both F-VIII and F-IX concentrates with little additional manpower. For example, the elimination of both the AT-III project and the portions of the AUTOPLEX project which involve both IV-1 screening and ascertaining the mechansim of action of the concentrate would result in the need for only one additional person to implement both hepatitis removal projects.

Attachment I

RESEARCH PROPOSAL - TIME REQUIREMENTS

ACTIVITY	MAN-MONTHS REQUIRED
1. Literature Search	1 mo.
2. Physical Methods	
A. Filtration	2 mo.
B. Nonspecific Adsorption	2 mo.
C. Centrifugation	------
D. Selective Precipitation	3 mo.
3. Specific Binding Techniques	
A. Charm technique	3 mo.
B. Other	3 mo.
1. Adsorption chromatography	
2. Ion exchange chromatography	
3. Other	
4. Inactivation	
A. Ionizing Radiation	3 mo.
B. Visible Light Radiation	2 mo.
C. Chemicals	2 mo.
D. Heat	3 mo.
E. Chemical Inhibitors	-----

HEPATITIS REMOVAL

I. Literature Search - 1 month then ongoing

A. Physical Removal

1. Centrifugation
2. Filtration
3. Nonspecific adsorption
4. Selective precipitation

B. Specific Binding Techniques

1. Immunoadsorbents for ant gen
2. Adsorption chromatography

C. Inactivation

1. Radiation
   a. Ionizing
   b. Light sensitizing dye
2. Chemical
3. Heat (dry powder)
4. Chemical inhibition

II. Physical Methods

A. Filtration

Evaluation of efficiency of various filter systems employing Biological Marker.

B. Nonspecific Adsorption

Determine if nonspecific adsorption offers any possibility based upon literature review. If so, apply technique to HBsAg.

C. Centrifugation

Initial studies and literature review previously indicated that centrifugation would not be a fruitful method. If review of literature does not indicate some method(s) of improvement, would recommend that this avenue not be pursued further.

D. Selective Precipitation

This technique would be a reevaluation of studies initiated by Garcia and as published by Johnson (1976, J. Lab. Clin. Med. 88: 91-101). May be further expanded upon. May be able to use biological marker for initial work, however, HBsAg and HBV may react differently from each other and any marker that could be selected.

III. Specific Binding Techniques

A. Reevaluation of the charm technique should be included in any study of this type. The efficiency of the method may be evaluated by the use of biological markers since studies with HBsAg will not offer the sensitivity needed.

B. Other techniques such as adsorption chromatography and ion exchange should be evaluated. Much has been reported in the literature on methods for purification and concentration of HBsAg. This information may be used to study the removal of HBsAg from the products.

IV. Inactivation

A. Ionizing Radiation

Employ biological markers to study the effect of:

1. Protein concentration
2. Exposure time
3. Number of virus particles
4. Product stability

B. Light radiation - study the characteristics of HBsAg and Dane particle rich material after exposure to dye and light. Study:

1. Time of exposure
2. Dye concentration
3. Various photosensitizing dyes
4. Product stability

C. Chemicals

On the front end these do not appear to offer much help. However, minimal treatment may result in increased sensitivity of the particle to inactivation by other methods.

D. Heat

Evaluate the effect of heat on the stability of dry (powder) product. Employ biological marker to study the inactivation rates in powder. The key will be in the identification of a biological marker that is similar to HBV for use in this study.

The final investigation will be to subject some known infectious material to treatment and then demonstrate it

is noninfectious by injection into chimps.

E. Chemical Inhibitors

This will primarily be a literature search, review and constant update. A number of new antivirals are being investigated and if these are evaluated with a "hepatitis like" virus, note of it should be made.

V. Other Studies

Similar investigations will have to be made employing non-A, non-B.

Attachment I

EQUIPMENT

	ITEM	QUANTITY
1.	Incubator, Bacteriological, CO_2 Floor Model - Reach-in	1
2.	Incubator, Bacteriological, CO_2 Counter top - Reach-in	1
3.	Incubator, Cell Culture Floor Model - Reach-in	1
4.	Microscope, Inverted cell culture	1
5.	Microscope, Fluorescent	1
6.	Centrifuge, Superspeed (Beckman J-21 or equivalent and rotors)	1
7.	Centrifuge, Laboratory table top	1
8.	Ultracentrifuge, Preparative (Beckman L5-50 with rotors)	1
9.	Autoclave - Amsco (Chamber approx. dim. - 2 x 2.5 x 3 ft)	1
10.	pH Meter	1
11.	Biohazard Hood (Baker Biogard or equiv.)	2
12.	Waterbath, dual chamber	2
13.	Balance, top-loading (0-1000 gm capacity - electronic)	1
14.	Hyland nephelometer with fluorescent module	1
15.	Laboratory refrigerator - freezer (Household type)	2
16.	Spectrophotometer (Beckman Model 24 with recorder)	1
17.	Hot-plate - stirrer	6
18.	VirTis Homogenizer	1

	ITEM	QUANTITY
19.	Polaroid MP-3 Camera	1
20.	Hyland Electrophoresis Power Supply	4
21.	Hyland Immuno-Illuminator	1
22.	Laboratory Carts (Stainless Steel)	3
23.	Laboratory Cart (for Spectrophotometer)	1
24.	Laboratory Chairs	7
25.	Storage cabinet with doors (approx. size - 2 x 4 x 7 ft.)	3
26.	Refractometer Abbe 3-L (B&L)	1
27.	Fraction Collector	1
28.	Laboratory shaker, Orbital, Variable	1
29.	Double pan balance (Harvard Trip balance)	1
30.	Gamma Counter (300 sample - automatic)	1
31.	Revco Freezer	1

SPACE REQUIREMENTS

Space requirements would be similar to that presently occupied by Microbiology Research. However, the laboratory should be resesigned to accommodate a biohazard area.

PERSONNEL

The proposed staff should reflect the project objective and requirements. Since a portion of the project will rely upon microbiological (virology and immunology) techniques, the staff should include persons trained in these disciplines. For the Hepatitis Removal project, the group should include 2 to 3 technicians 1 or 2 of which should be microbiologists. One of these should be at the Masters degree level or have sufficient experience to qualify as a Research Associate. The other 1 or 2 may be at the Sr. Research Assistant to Research Assistant level.

PLAN A - HEPATITIS-FREE F-VIII CONCENTRATE USING CURRENT HEMOFIL Attachment I

	ACTIVITY	RESPONSIBILITY	MONTHS REQUIRED (RELATIVE TO OTHER ACTIVITIES IN PLAN)
I.	ASCERTAIN EFFECT OF HEAT-TREATMENT OR IRRADIATION ON LOTS OF CURRENT HEMOFIL WITH/WITHOUT HEPATITIS MARKER.	KOVACS/TAYLOR	2.5
II.	CHARACTERIZE HEAT-TREATED HEMOFIL (RELATIVE TO UNTREATED)	TAYLOR/KOVACS/ LETTELIER/ WEYAND/McDONALD	3.0 (Concurrent with #I)
	• SOLUBILITY		
	• FIBRINOGEN, FSP		
	• VIII:C (ONE- AND TWO-STAGE)		
	• VIII:Ag		
	• HBsAg, anti-HBs, HBcAg, anti-HBc		
	• DISC GEL (SDS AND BASIC)		
	• DOUBLE DIFFUSION ANALYSIS		
	• OTHER CHARACTERISTICS (RELATIVE TO THOSE IN CURRENT PRODUCT SPECIFICATION)		
	• RECONSTITUTED STABILITY		
III.	CHIMPANZEE INFECTIVITY STUDY	HOLLINGER	2.0 (Concurrent with #I)
IV.	ANALYSIS OF DATA	TSE/TAYLOR/KOVACS	0.7
V.	DECISION TO USE THIS PROCESS OR PROCEED WITH ANOTHER METHOD (IF POSITIVE RESULTS WITH CURRENT HEMOFIL PROCESS PROCEED TO #VI ON THIS PLAN, OTHERWISE USE PLAN B)		
VI.	PREPARE 3 CLINICAL LOTS INCLUDING ASSAY SUPPORT (incl. analysis of data)	OLIVAREZ/KOVACS/ TAYLOR/LETTELIER	~~1.5~~ 2.2
VII.	DEFINE STABILITY PROGRAM	NICHOLSON/TSE/ANDARY	1.0
VIII.	STABILITY TEST PROGRAM	NICHOLSON	7.0
IX.	DEFINE CLINICAL PROGRAM AND DEVELOP CLINICAL PROTOCOL FOR SURVIVAL/RECOVERY TO SUPPORT HEPATITIS CLAIM	McCLURE/ANDARY/THOMAS	2.0 (Concurrent with #VI)

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PLAN A - HEPATITIS-FREE F-VIII CONCENTRATE USING CURRENT HEMOFIL

Page Two

	ACTIVITY	RESPONSIBILITY	MONTHS REQUIRED (RELATIVE TO OTHER ACTIVITIES IN PLAN)
X.	FINAL PRODUCT TESTING	BACICH	1.5
XI.	ANALYSIS OF DATA	TSE/TAYLOR/KOVACS	1.0
XII.	FINALIZE CLINICAL PROTOCOLS AND SUBMIT IND TO B.o.B.	RODELL/McCLURE	2.0
XIII.	CLINICAL TRIALS	McCLURE	7.0 (Concurrent with XII)
XIV.	SUMMARIZE CLINICAL TESTING AND PREPARE B.o.B. SUBMISSION	McCLURE/RODELL/ THOMAS/TSE/ANDARY	7.0 (Concurrent with XIII)
XV.	PREPARE IMPLEMENTATION PLAN		6.0 (Concurrent with XIII)
	MANUFACTURING	OLIVAREZ	
	MARKETING	PHELPS	
XVI.	B.o.B. APPROVAL AND IMPLEMENTATION OF PROCEDURE IN PRODUCTION	PDC	

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PLAN B - HEPATITIS-FREE F-VIII CONCENTRATE BY A NEW METHOD

ACTIVITY	RESPONSIBILITY	MONTHS REQUIRED (RELATIVE TO OTHER ACTIVITIES IN PLAN)
I. PREPARE 6 FEASIBILITY LOTS UTILIZING METHODS OUTLINED IN PROPOSAL (INCLUDE HEPATITIS MARKER TO ASSESS ITS REMOVAL)	BERKEBILE/KOVACS	2.5
· ASSAY SUPPORT FOR 6 LOTS	KINLEY/LETTELIER/X	
· ESTABLISH CONDITIONS FOR LAB SCALE LYOPHILIZATION	TAYLOR/WEYAND	
· ESTABLISH HEAT OR IRRADIATION TREATMENT CONDITIONS	TAYLOR/WEYAND/X	
II. CHARACTERIZATION OF FINAL PRODUCTS		3 (Concurrent with #1)
A. QUANTITATIVE ANALYSIS		
1. BIOLOGICAL ASSAYS	KINLEY/McDONALD/X	
· VIII:C (One- AND TWO-STAGE)		
· PKA		
· FIBRINOGEN, FDP INCL. FPA		
· KINGDON TIME AND S-2160		
2. IMMUNOLOGICAL ASSAYS	LETTELIER/TAYLOR	
· MANCINI (R.I.D.) e.g. C.I.G.		
· LAURELL (ROCKET) VIIIR:Ag VIII:C		
· RIA HBsAg, anti-HBs, HBcAg, anti-HBc.		
B. QUALITATIVE ANALYSIS		
1. DISC GEL ELECTROPHORESIS	BERKEBILE/KOVACS/ WEYAND	
· BASIC · SDS		

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	ACTIVITY	RESPONSIBILITY	MONTHS REQUIRED (RELATIVE TO OTHER ACTIVITIES IN PLAN)
	2. I.E.P. (POLYVALENT ANTISERUM)		
	3. DOUBLE DIFFUSION (MONO-SPECIFIC ANTISERA)		
	C. OTHER CHARACTERISTICS FOUND IN CURRENT PRODUCT SPEC. E.G. pH, SOLUBILITY, APPEARANCE, ETC.	BACICH	
III.	ANALYSIS OF DATA AND SELECTION OF SINGLE PREPARATIVE METHOD	BERKEBILE/KOVACS/TSE ANDARY	0.7
IV.	PREPARE 5 (5L) LAB SCALE LOTS BY SELECTED METHOD (INCLUDE HEPATITIS MARKER):	KOVACS/BERKEBILE	2.0
	• ASSAY SUPPORT FOR 5 L LOTS	KINLEY/McDONALD/X	
	• LYOPHILIZE 5 L LOTS		
V.	CHARACTERIZE FINAL PRODUCT		2.5 (Concurrent with #IV)
	• QUALITATIVE AND QUANTITATIVE ASSAYS	KINLEY/McDONALD/X/ LETTELIER/TAYLOR/ BERKEBILE/KOVACS/ WEYAND	
VI.	ANALYSIS OF DATA FROM LOTS	BERKEBILE/KOVACS/ TSE/ANDARY	0.7
VII.	RECOMMENDATIONS FOR SCALE-UP, PREPARATION OF CTP's, Q.C. PRODUCT SPEC. AND MFG. PROCEDURE	BERKEBILE/KOVACS/ TAYLOR/TSE/ANDARY	0.7
VIII.	PROGRESS REPORT TO PDC	THOMAS	
IX.	PREPARE 5 R&D LOTS	BERKEBILE/KOVACS	3.0
	• ASSAY SUPPORT FOR 5 LOTS	TAYLOR/LETTELIER/X	
	• DEVELOPMENT SUPPORT	HOLST	
	• FINAL PRODUCT TESTING	BACICH	
X.	DEVELOP ANIMAL STUDY PROGRAM (IF REQUIRED BY B.o.B.)	WALSH/ANDARY	2.0
XI.	DEFINE CLINICAL PROGRAM AND DEVELOP CLINICAL PROTOCOL FOR SAFETY, EFFICACY; AND SURVIVAL/RECOVERY	McCLURE/THOMAS/ ANDARY	3.0 (Concurrent with #IX)