OPTIMIZATION OF PRODUCTION VARIABLES GOVERNING YIELD AND STABILITY OF FACTOR VIII IN CRYOPRECIPITATE

by

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I declare that this thesis is my own work. It is being submitted for the Masters Diploma in Technology (Medical Technology) at the Cape Technikon, Cape Town.

It has not previously been submitted for any diploma, degree or examination at any other institution. This study was carried out at Western Province Blood Transfusion Service.

In this research the statistical planning and analyses, and recommendations arising from these analyses, have been done with the support of the Institute for Biostatistics of the Medical Research Council.

.....C.J.Collett..... .....4:11:97.....

Carol Joan Collett **Date**

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"The people who get on in life are the people who get up and look for the circumstances they want and if they can't find them, they make them"

George Bernard Shaw (1856 - 1950)

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LIST OF ABBREVIATIONS

ABBREVIATIONS	FULL NAME
AABB	American Association of Blood Banks
ACD	Acid citrate dextrose
ALB	Albumin
ALT	Alanine Aminotransferase
Ca	Calcium
Ca^{++}	calcium ions
$CaCl_2$	Calcium chloride
Cl	Chloride
CPD	Citrate phosphate dextrose
CPDA-1	Citrate phosphate dextrose adenine
CPDA-2 (0.5)	Citrate phosphate dextrose adenine (half strength)
CPU	central processing unit
°C	degrees celsius (unit of temperature)
da	daltons
C.V.	coefficient of variation (statistical term)
DCL	Diagnostic Chemical Limited
DF	degrees of freedom (statistical term)
EDTA	Ethylenediaminetetraacetic acid

ABBREVIATIONS	FULL NAME
ELISA	Enzyme linked immunoassay
factor	component of the fractional factorial design
FDP	Fibrin degradation products
FVIII	Factor VIII
FIX	Factor IX
F value	Variance ratio (statistical term)
FpA	Fibrinopeptide A
g	gram (unit of mass)
g/l	gram per litre
<	less than
HMK	high molecular weight kininogen
HCL	Hydrochloric acid
IS	International standard
IU/ml	International unit per millilitre
kb	kilobase
K	Potassium
>	greater than
M	Molar (unit of concentration)
mEq/l	milli equivalent per litre
Mg	Magnesium

ABBREVIATIONS	FULL NAME
mg	milligram (unit of weight)
mol/l	mole per litre (unit of volume)
ml	millilitre (unit of volume)
mm Hg	millimetre of mercury
mM	milli Molar (unit of concentration)
mmol/L	millimole per litre (unit of concentration)
MRC	Medical Research Council
mRNA	messenger Ribonucleic acid
NaN_3	Sodium nitrate
Na	Sodium
NIBSC	National Institute for Biological Standards and Control
nm	nanometre (unit of wavelength)
%	percent
p	probability (statistical term)
PBS	phosphate buffered saline
pH	negative logarithm of hydrogen ion activity
PL	phospholipid
SD	standard deviation (statistical term)
sec	seconds

ABBREVIATIONS	FULL NAME
TP	Total protein
SE	standard error (statistical term)
µl	micro litre (unit of volume)
µg/ml	micro gram per millilitre
U/dl	units per decilitre
U/ml	units per millilitre
variable	key elements being measured in the study
VIII:Ag	Factor VIII antigen
VIII:C	Factor VIII activity
VIII	Factor VIII protein
v/v	volume per volume
vWF	von Willebrand Factor
vWF:Ag	von Willebrand Factor antigen
w/v	weight per volume
WFH	World Federation of Haemophilia
WPBTS	Western Province Blood Transfusion Service
XL-FDP	cross-linked fibrin degradation products

SUMMARY

Cryoprecipitates are used as the raw material for the preparation of Factor VIII (FVIII) for replacement therapy for haemophiliacs. Routinely, cryoprecipitate only recovers 50% of the Factor VIII in the plasma. The purpose of this study, production of cryoprecipitate, was to investigate those variables which play a key role in determining the yield of Factor VIII present in cryoprecipitate. Cryoprecipitate production involves a wide range of variables which could effect the final outcome of the product. These vary from the donor blood group, time of donation, exercise levels of the donor, to a time delay prior to processing, temperature storage conditions, to the method utilised for plasma freezing and thawing. The objective was to explore which combination of variables in the procedure would lead to a process which would optimize the preparation of cryoprecipitate in a routine environment, to yield the highest levels of Factor VIII.

Frequently in scientific investigations, particularly when a practical approach has to be adopted, questions arise in which the effects of a number of different variables in a process, require evaluation. Such questions can usually be most economically investigated, by arranging the analysis according to an ordered plan in which all the factors are viewed in a regular way. Provided the plan has been correctly chosen, it is possible to determine not only the effect of each individual variable, but also the way in which each effect depends on the other factor, by means of an interaction. This makes it possible to obtain a more complete picture of what is happening, than would have been obtained by varying each of the

variables one at a time while keeping the others constant. Designs of this sort lend themselves well to statistical analysis, and provide their own estimates of experimental error. This type of statistical analysis called, 2^K Fractional Factorial Experimental Design, forms the basis of this study in which 14 key variables in the production process of cryoprecipitate were defined as possible areas in which Factor VIII levels in the cryoprecipitate are effected.

Key variables have been identified on an individual basis in previous studies (Burka *et al.*, 1975), however this blended approach to optimise the key variables within the production environment, and define further combinations which could be incorporated into the production, has never been attempted.

The statistical design used in the study was compiled by the Institute for Biostatistics of the Medical Research Council (MRC). Units of blood were collected and processed, from blood donors under the stipulated criteria, corresponding to the study design. The pre, post and final samples obtained were analyzed against selected appropriate assays. The results of all the assays were statistically analyzed by the Institute for Biostatistics at MRC. The results are all tabulated by way of a statistical analysis, with their probability significance portrayed. Significant interactions between the factors were identified.

It is clear from this work that certain factors within the variables are more critical to the cryoprecipitate production than others. Significant variables identified in the FVIII

assay, prior to separation of the plasma were, the type of primary anticoagulant used, being that of citrate phosphate dextrose (CPD), in conjunction with a pre-processing time of under six hours with the whole blood maintained at a temperature of 20°C. Of secondary importance, was the duration of the bleed, only if consideration was also given to the donor blood group. Of benefit during the production phase, was a rapid freezing temperature of >-60°C.

The fibrin degradation product assay (FDP) demonstrated the usefulness of performing this assay as an indication of FVIII activation at the time of donation, caused by the technique utilised to insert the donation needle into the vein.
The results highlighted that there are seventeen (17) steps in the FVIII production process, to which attention to detail should be paid to optimize yields. Interestingly, WPBTS already comply with twelve (12) of the conditions. Further improvement could be obtained if:

* only blood Group A donors were bled
* blood were allowed to reach 20°C prior to separation
* Fibrinopeptide A (FpA) assays were performed
* a fast freezing rate (>-60°C) was implemented
* the starting plasma volume was uniform.

Achievement of the above variables and their factors, not currently used, would determine the optimal production cycle and provide the increased yield of Factor VIII in cryoprecipitate production, this study set out to achieve.

CHAPTER 1

INTRODUCTION

Blood of a haemophiliac is deficient in Antihaemophilic Factor, or better known as Factor VIII. This deficiency can be partially corrected by transfusions of a plasma component, termed cryoprecipitate which contains the Factor VIII. Cryoprecipitate is the cold soluble precipitate obtained from thawed plasma. Factor VIII concentrates are prepared from a number of pooled cryoprecipitate, used in home treatment for haemophiliacs. The better the FVIII quality and yield, the fewer number of cryoprecipitates are required per pool, thus reducing cost and decreasing the donor exposure rate per infusion. This in turn, reduces the infusion risk to the patient.

Factor VIII is a labile coagulation factor which deteriorates rapidly when removed from the human body. Variables influencing the recovery of cryoprecipitate from the plasma are numerous. Improvements in transfusion and fractionation techniques in recent years, have made it possible to produce Factor VIII (FVIII) from blood, while preserving other components in the plasma for fractionation into further products, for use by other patients. The cost of haemophilia treatment relates mainly to the costs involved in producing a product that is of adequate quality, and sufficient in supply (Biggs *et al.*, 1974).

Cryoprecipitate production involves a wide range of variables which affect the final outcome of the product. These extend from variables pertaining to the donation criteria through to the final freezing stages in the production cycle. Variables specific to the blood donation procedure are, donor blood type, exercise levels of the blood donor prior to donation, type of primary anticoagulant used, length of time taken to bleed the unit of blood, time delay prior to separation of the red cells and plasma, and temperature of the blood at the time of separation. Variables which influence the production processing cycle revolve around freezing and thawing temperature, freezing and thawing techniques, precipitation and centrifugation methods. These variables listed above were my choice for the study. Variables chosen for the study were those that could be controlled, whereas those that could not be controlled or remained constant, were excluded.

Since the advent of cryoprecipitate production initiated by Pool in 1964, there has been an active interest by a myriad of researchers, on various aspects of Factor VIII production. Variables influencing the recovery of labile coagulation from human plasma are numerous. Over the years, many attempts have been made to improve the final yield of FVIII by modifying the method of preparation. Cryoprecipitate production involves many variables, as outlined above (Smith and Hodges, 1984).

Additional variables not included in the study ranged from, donor age, type of collection container, volume of the starting plasma, pH of the plasma, the level of anxiety of the donor, the time from collection to freezing, temperature during centrifugation during the final precipitation of the cryoprecipitate, heat exchange,

and determination of the end point of production, all of which can affect the level of coagulation elements present. These were excluded, as the production specifications for these criteria could be determined and regulated to remain constant throughout the study. As many as 90 variables with their appropriate factors have been identified, which could conceivably affect the final cryoprecipitate yield and quality of FVIII levels. Many of these variables and factors have systematically been individually evaluated by investigators since the discovery by Pool in 1964. Each step of the production process must be meticulously carried out, to ensure optimum proficiency, as it could have a cumulative effect on the final yield. To date, no single investigation on FVIII has ever been reported, where as many as fourteen variables were evaluated simultaneously, by a fractional factorial experimental design. A total of 128 regular, volunteer blood donors all meeting the donation criteria were used in this study. The relevant blood donor base in a specified geographical area was stipulated.

The purpose of this study was to identify and assess those selected variables with dominant factors, which play a key role in determining the yields and recovery of Factor VIII in cryoprecipitate. In order to achieve this objective fourteen key variables in the bleeding and production process were selected. Six of the variables relate to the blood donor, donation and collection techniques and the handling of the whole blood prior to the separation of the red cells and plasma. The other eight variables are concerned with the production process.

The experimental design used in this study was a 2^K Fractional Factorial Design

compiled up by the Department for Biostatistics at the Medical Research Council. The design was compiled in such a manner that enabled all fourteen variables to be compared with each other, simultaneously by using opposite factors. This type of design requires only a fraction of the total number of treatments in a full factorial design. The design would enable the more significant and dominant factors to be highlighted and provide knowledge on how variables effect the cryoprecipitate quality. With this approach it is possible, in addition to optimizing the key variables and their factors, may reveal any previously unobserved interactions between variables which could further enhance FVIII quality.

The study was conducted within the routine cryoprecipitate production environment at the component processing laboratory of the Western Province Blood Transfusion Service, with the intention of providing guidelines which would be suitable in a routine large scale production process.

Assays selected to evaluate the samples were FVIII and von Willebrand Factor (vWF) assays, fibrinogen, pH determination and fibrin degradation product (FDP) assays. Plasma proteins and electrolyte assays were also performed. All the data was analyzed by means of the statistical method, Analysis of Variance (ANOVA). The Fractional Factorial Analysis resulted in the analysis of effects and interactions which in turn determined the significant variables with the probability confidence limits set at $p = 0.05$. The results for significant effects and interactions are presented in a table of means. Selection of FVIII, vWF, pH and fibrinogen assays were based on experience over the years. The selection of the FDP assay was

established as a result of a previous study we researched, whereby early activation of FVIII at the time of phlebotomy can be confirmed. The choice of performing the plasma protein and electrolyte assays was due to the fact that no mention of their effects on FVIII yield had yet been reported. Samples for assay were taken from the blood donors prior to donation (termed pre), after the separation of the red cells and plasma (termed post) and from the cryoprecipitate (termed final).

Factor VIII content of cryoprecipitate varies from one unit to the next, with the average potency fluctuating from day to day, resulting in variations in the production process.

This study was undertaken in order to resolve the **objectives** and answer the **hypothesis:**

* can the Factor VIII content within the packs of cryoprecipitate prepared from individual blood donors, be made uniform?

* which variables, such as primary anticoagulant, conditions of freezing and thawing, the interval of time between blood collection and processing and donor variation in FVIII level, affect the quality of FVIII in cryoprecipitate and can the FVIII levels be increased?

* what combination of variables in all the technical steps involved in the procedure, will lead to a process which optimizes the preparation of cryoprecipitate, in a practical blood bank environment i.e. were there any variables which had a dramatic effect upon the Factor VIII yield, such that modification or control of them would protect against a poor quality product?

It has been shown by this work that certain variables with their factors, are more critical to the cryoprecipitate production than others. Significant variables identified in the FVIII assay, prior to separation of the plasma were, the type of primary anticoagulant used, that of citrate phosphate dextrose (CPD), and a pre-processing time of under six hours, with the whole blood maintained at a temperature of 20°C. Of secondary importance, was the duration taken to bleed the unit of blood only if consideration was given to the donor blood group. During the production phase, of benefit was a rapid freezing temperature of more than -60°C.

The Fibrin degradation product assay (FDP) demonstrated the usefulness of performing this assay as an indication of FVIII activation at the time of donation. If activation of FVIII was evident, it could have been caused by the technique utilised to insert the donation needle into the vein.

The results highlighted that there are seventeen (17) steps in the FVIII production process, to which attention to detail should be paid to optimize yields. Interestingly, WPBTS already comply with twelve (12) of the conditions. Further improvement could be obtained if:

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* Fibrinopeptide A (FpA) assays were performed
* a fast freezing rate (>-60°C) was implemented
* the starting plasma volume was uniform.

Implementation of the above variables, would determine the optimal production cycle and provide the increased yield of Factor VIII in cryoprecipitate production, this study set out to achieve.

Study aim : to standardize the production of cryoprecipitate taking into account the critical variables and their factors in order to define a STANDARDISED PRODUCTION METHOD.

CHAPTER 2

LITERATURE REVIEW

2.1 CLASSIC HAEMOPHILIA (HAEMOPHILIA A)

2.1.1 INTRODUCTION

Haemophilia is one of the commonest of the hereditary haemorrhagic disorders resulting from an abnormality in the activity of one of the blood clotting ingredients. Without normal activity, clotting cannot take place and bleeding is prolonged (Biggs *et al.*, 1974). Knowledge of bleeding disorders affecting male members in certain families was recognized as early as the second century, and also documented in the Talmud (Foster and Zimmerman, 1989).

Haemophilia, at the turn of the century was explained by geneticists, as the presence of an abnormal haemorrhagic disorder, however it was unknown whether the cause of the condition was a cellular or plasma deficiency. In 1911 Addis demonstrated that a prolonged clotting time could be rectified by using the globulin fraction of plasma, although at that time, lack of adequate blood banking facilities made whole blood the product frequently used to treat bleeding episodes. Due to the volumes required, patients' systems were often overloaded causing unsatisfactory recoveries (Aronson, 1990). It is a well known fact that Queen Victoria was responsible for the spread of haemophilia among the European royalty. The first successful treatment of haemophilia patients recorded was with

intravenous infusions of plasma in 1924 (Smit Sibinga, 1996).

Step by step through the twentieth century this inherited sex-linked bleeding disorder, its onset and method of treatment was unravelled. It is now known that haemophilia is caused by the partial or complete absence of an essential coagulation component in the blood, called Factor VIII or Antihaemophilic Globulin. The absence of Factor VIII (FVIII) affects males almost exclusively, knows no geographical boundaries and is transmitted in a sex-linked recessive manner. At present there are two forms of haemophilia recorded: Haemophilia A or Classical Haemophilia, characterized by a deficiency of clotting Factor VIII and Haemophilia B or Christmas Disease which is caused by a deficiency in Factor IX. Approximately one in every 10,000 males in all parts of the world suffers from Haemophilia A. Without treatment, very few boys with haemophilia can survive to adulthood. With proper treatment however, they can expect to lead full, normal lives (World Federation of Haemophilia :WFH, 1994).

2.1.2 GENETIC TRANSMISSION

When a haemophilia male marries a normal woman, a defective X chromosome is transmitted to all of his daughters, with the result that they will all be carriers of the defective X chromosome, while all the sons will be normal. However, males born to carrier daughters, have an equal chance of being either haemophiliacs or being normal, and daughters born to female carriers, have an equal chance of being either normal or carriers. It is possible that if a carrier female marries a haemophiliac

that a female child with haemophilia can be produced, however this situation is extremely rare. There are two possible explanations for about a third of haemophiliacs having no family history of the disease. A genetic mutation in either the patient or his mother might have occurred. The other possibility is that the family had very few, or no male children in previous generations, and the defective gene which was carried by female children, did not have the opportunity to express itself in the males (Eipe, 1971).

2.1.3 BLEEDING MANIFESTATIONS

Haemophilia is present at the time of birth and a low Factor VIII level can be detected in the umbilical cord blood of a baby boy with haemophilia. In most instances the first bleeding episode only manifests itself at the age of six to twelve months once the child starts to become mobile. The first injuries are in the form of deep bruises and superficial cuts. Haemarthrosis of the knees and ankles and haemorrhages into skeletal muscles occur when the child begins to walk. People with haemophilia suffer painful episodes of bleeding into the joints and muscles over the years. This eventually results in chronic arthritis in the affected areas, whilst brain haemorrhages can be fatal. With the introduction of r liable methods for determining the level of Factor VIII in the blood, it was found that the severity of the disease correlated with the degree of Factor VIII deficiency. Table 2.1 shows the relationship of blood levels of Factor VIII to the severity of haemorrhagic manifestations (Eipe, 1971). Haemophilia can be classified as severe, moderate or mild according to the FVIII level. The average normal level of FVIII is 100 U/dL; the

normal range is about 50 to 180 U/dL (Kasper, 1995).

Level of Factor VIII (%)	Haemorrhagic Manifestations
50 - 100	none
25 - 50 (mild)	tendency to bleed after major injury
5 - 25 (mild 5 -30 U/dL)	moderate bleeding after operations/injury
1 - 5 (moderate 2-4 U/dL)	severe bleeding after minor injury
0 (severe 1 U/dL)	severe haemophilia with spontaneous bleeding into muscles/joints with crippling effect

Table 2.1. *Severity of Factor VIII Blood Levels (Eipe, 1971)*

In industrialised first world countries boys born with haemophilia start receiving treatment at an early age. This eliminates life-threatening bleeding episodes and allows them to lead normal, productive lives (WFH publication ,1994). In countries where diagnosis and treatment are not available, boys continue to suffer and develop orthopaedic problems and die at a young age. It is estimated that 80% of the world population with haemophilia receive inadequate care. Replacement therapy is provided in the form of intravenous infusions of either processed or fractionated plasma products. The raw plasma product is called cryoprecipitate, in which Factor VIII is found. This product raises the patient's Factor VIII concentration to a level of haemostasis, for that particular bleeding episode and maintains this status until healing is achieved.

2.2 FACTOR VIII

2.2.1 NOMENCLATURE

In 1985, new recommendations for the nomenclature of FVIII and von Willebrand Factor (vWF) were formulated by an International Committee on Thrombosis and Haemostasis (Marder *et al.*, 1985). The symbol for Factor VIII protein is VIII and not VIII:C. The symbol for Factor VIII antigen is VIII:Ag and is used for the immunologic measurement of FVIII. The symbol for FVIII activity is VIII:C to be used for the functional measurement of cofactor activity.

The symbol for von Willebrand factor is VWF and the antigen VWF:Ag (Foster and Zimmerman, 1989).

2.2.2 STRUCTURE AND SYNTHESIS

Coagulation FVIII is produced from a 289 kb gene consisting of 26 exons which give rise to a 9 kb mRNA. FVIII is a glycoprotein present in the plasma at a low concentration (0.1μg/ml), and has a molecular weight of 2 x 10^6 daltons. Its absence or deficiency results in Haemophilia A. . The primary structure contains 2332 amino acids and exhibits a triplicated region of 330 amino acids (A domains) an unique region of 983 amino acids (B domain), and a carboxyl-terminal duplicated region of 150 amino acids (C domain), that are arranged in the order A1-A2-A3-C1-C2 (Bihoreau *et al.*, 1989 and Masure *et al.*, 1978). Figure 2.1 shows a schematic representation of the structure of FVIII.

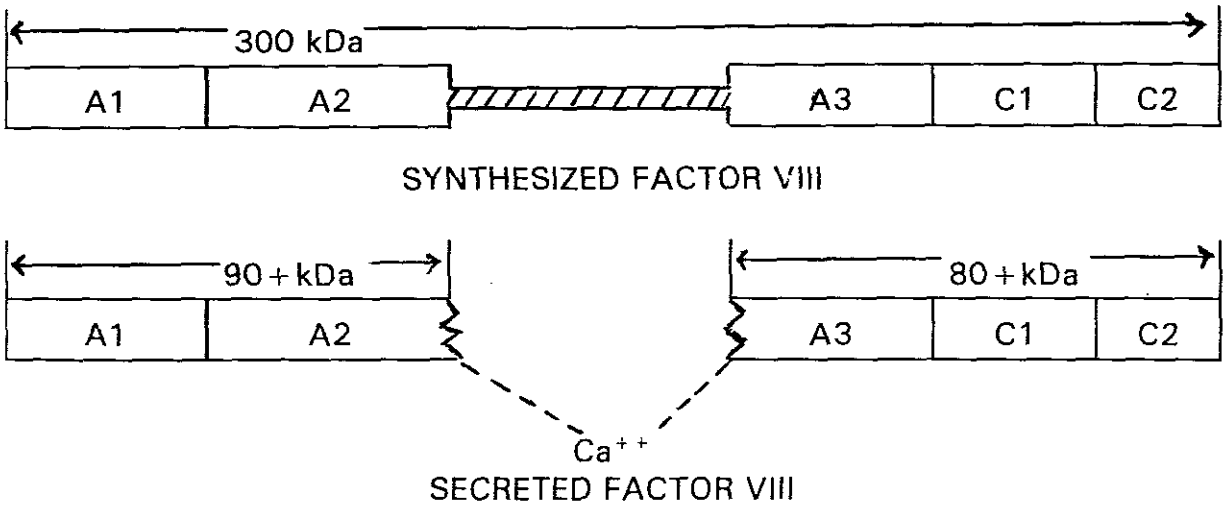

Figure 2.1. *Linear representation of the amino acid sequence of human Factor VIII. There are three A domain repeats (A1, A2 and A3) and two C domain repeats (C1 and C2) (Foster and Zimmerman, 1989).*

The gene controlling FVIII is on the X chromosome. The site of synthesis is unknown. Patients with Haemophilia A lack FVIII and usually lack VIII:Ag. Equally poorly understood is how and where FVIII, which is synthesized as a single- chain polypeptide, is converted to the two-chain molecule found in plasma, with a variable heavy chain molecular weight of 210,00 to 90,000 and a light chain of molecular weight of 80,000. The half-life of FVIII after transfusion is eight to twelve hours (White and Shoemaker, 1989), however *in vivo* investigations have indicated a relatively longer biological half -life of FVIII:C in less purified material such as plasma and cryoprecipitate, than in more purified concentrates. It varies between 10 and 20 hours (mean 13.9 hours) for less pure products, and 4.8 to 20 hours (mean 12 hours) in the more purified group. A Factor VIII half life of less than 7 is not acceptable (Allain, 1984).

FVIII circulates in association with von Willebrand Factor (VWF) which stabilises it. Von Willebrand Factor is found in the plasma, megakaryocytes, platelets and

endothelial cells, and is required for normal adherence of platelets to injured vessel walls. It is produced under control of autosomal genes. When measured by an immunological test using specific antibodies, it is known as von Willebrand Factor antigen : VWF:Ag (Kasper, 1995).

2.2.3 COFACTOR FUNCTION

FVIII is involved in the activation of Factor IX. Its function is measured by the FVIII activity assay, in which the end point is the formation of fibrin strands. The molecule can be detected as Factor VIII antigen (VIII:Ag, also called Factor VIII coagulant antigen, VIII:CAg) by an immunologic test using homologic antibodies (inhibitors) to human FVIII. Activated FVIII (FVIIIa) is an essential participant in the intrinsic pathway of coagulation (Foster and Zimmerman, 1989). FVIII is the co-factor of IXa in the activating step that converts X to Xa. Phospholipid and calcium are necessary components of this complex (Kabi Vitrum, 1987). The catalytic efficiency of FVIII is greatly increased by activation of thrombin or Factor Xa. FVIII requires thrombin for activation, but does not result in conversion to an active protein. It is inactivated by further exposure to thrombin and activated protein:C (White and Shoemaker, 1989). The endpoint of the cascade is the rapid conversion of fibrinogen into insoluble fibrin to form a clot around a platelet plug (Burke *et al.*, 1986).

The schematic representation in Figure 2.2, portrays the intrinsic coagulation pathway.

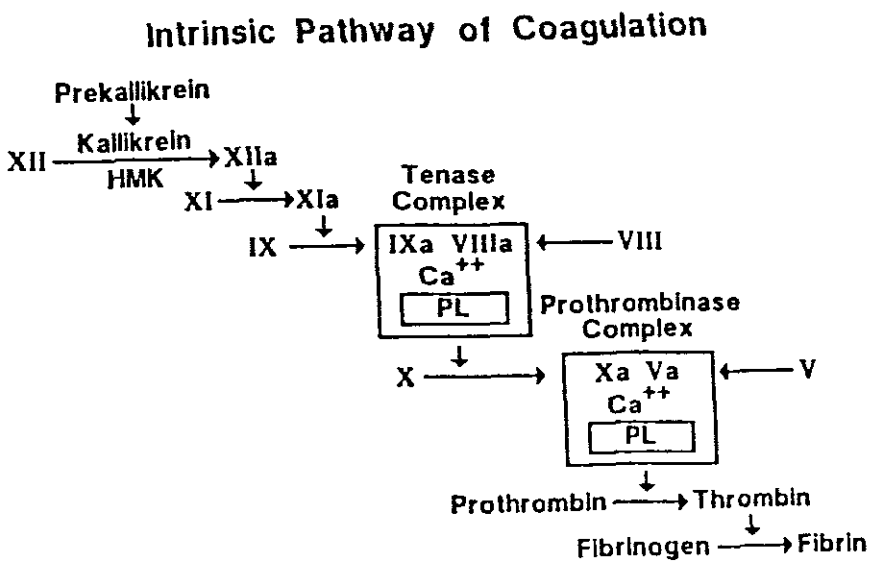

Figure 2.2. *Schematic representation of the intrinsic pathway of coagulation illustrating the components of the tenase complex and the prothrombinase complex. Coagulation factors are represented by their Roman numerals. Activated coagulation factors are represented by their Roman numerals followed by a small case a. Other abbreviations: high molecular weight kininogen (HMK); calcium ions (Ca^{++}); phospholipid (PL) (Foster and Zimmerman's Scheme, 1989).*

2.3 IMPROVING FACTOR VIII STABILITY AND YIELDS IN PLASMA

Until 1960, the only effective treatment available for haemophilia was the infusion of plasma. In 1964 cryoprecipitate became available as a product for treatment (Brodniewicz-Proba, 1991). The endeavour to enhance the levels of FVIII in plasma was pursued. Pool *et al.* (1964) discovered that the insoluble gummy material formed in plasma which had been stored at refrigerator temperatures, contained a high proportion of FVIII when measured. Pool realized that the same phenomenon occurred during the thawing of frozen plasma. The precipitate when reduced to a low volume, provided better therapy for Haemophilia A, than plasma. (Pool *et al.*, 1964). The development of cryoprecipitate and improved production techniques

now made available a treatment that was less costly and easier for haemophiliacs to administer (Aledort, 1988). The next two decades saw numerous researchers attempt to enhance the FVIII activity in cryoprecipitate, by improving the numerous variables involved in the production of cryoprecipitate. In presenting the final product in an acceptable form, the formulation of the plasma protein must preserve its biological activity during processing. The production process must maintain the product as close as possible to the normal physiological state and minimize physical loss of activity or structural damage to the proteins during manufacture. These aspects of formulation are important in the preparation of cryoprecipitate, because FVIII is a biological labile component of plasma (McIntosh and Foster, 1990). Changes in the collection and processing of plasma have a direct impact on the FVIII concentration in the cryoprecipitate.

Variables influencing the recovery of FVIII from the plasma are numerous (International Forum, 1983). In the production of cryoprecipitate, there can be up to 90 variables involved , ranging from the blood group of the blood donor to the temperature of the centrifuge during final processing, involving numerous technical production steps. Some of these variables and their corresponding factors influence the final quality of the cryoprecipitate to a larger extent than others. (Pool, 1975). Elimination of the less critical stages are advantageous, thus reducing the number of stages, which require vigilant awareness in the attempt to produce a consistently good product. If the production criteria for the preparation of cryoprecipitate is not accurately and adequately controlled, effective recovery of all the Factor VIII present in the plasma is not possible. Modification of the mode

of preparation, over the years, has been attempted to improve the final yield of FVIII in the cryoprecipitate (Reiss and Katz, 1976). Each step in the production process must be carefully accomplished, in order to obtain optimum proficiency at every step of the operation, which in turn, will have a cumulative effect on the FVIII yield.

2.3.1 HIGH POTENCY CRYOPRECIPITATE FROM EXERCISED BLOOD DONORS

FVIII activity increases significantly as a result of short durations of intense muscular exercise. The mechanism of the rise of FVIII activity after exercise is uncertain, but it is thought it may be due to elevated synthesis of FVIII, activation of FVIII already in circulation or a release of FVIII from body stores (Strand *et al.*, 1974). The elevation of FVIII levels peak shortly after exercise is ceased. The benefit of exercising blood donors prior to venisection as a method to raise FVIII, is acceptable to blood donors and achieves results 40% higher than normal (Van Gastel *et al.*, 1973).

2.3.2 PRE-SELECTION OF DONORS BY BLOOD GROUP

Variations in plasma levels of FVIII in individuals with different ABO groups have been reported in the literature (McLellan *et al.*, 1988). In 1964 Preston and Barr demonstrated that blood group O persons have notably lower FVIII levels than those persons with blood groups A, B or AB. The reason for the variation between

the ABO blood groups is not understood , as there is no relation between the H secretor status and FVIII levels.(Preston and Barr, 1964).

The plasma of group O blood has been reported to contain only 75% of the activity present in the other blood groups (Tomasulo *et al.*, 1980). Again no explanation for the association between the ABO blood group and FVIII in the plasma could be furnished. The FVIII molecule is a glycoprotein containing the A, B, and H antigenic determinants, and the removal of the carbohydrate may affect FVIII and its related activities (Tomasulo *et al.*, 1980). Similar observations were made by Jeremic *et al.* (1976) and Wahlberg *et al.* (1980). Observations were also made that group A female donors, on oral contraception, yielded the highest FVIII levels (Jeremic *et al.*, 1976). Blood group studies carried out by Carlebjork *et al.* (1986a), confirmed previous findings that blood group O persons, had Factor VIII levels of 20% lower than those of other blood groups (Carlebjork *et al.*, 1986a). Plasmapheresis of individuals with blood groups other than O was recommended as a collection method to obtain plasma with higher Factor VIII levels.

2.3.3 BLOOD COLLECTION

Venipuncture procedures require that blood collection should be performed with a sterile unused donation blood bag system, containing a new needle. The needle entry into the vein should be a clean cut with a blood flow that is rapid throughout donation, and that the mixing of the blood with the anticoagulant, be continuous. The start and end of the bleeding procedure is critical. In the event of activation of

the coagulation system, as a result of the first millilitre of blood being left without anticoagulant, activation of FVIII could follow (Britten *et al.*, 1966). Activation of the coagulation system can best be detected by the Fibrinopeptide A and Fibrin Degradation Products (FDP) assays. During blood coagulation, fibrinogen is converted to fibrin by the action of thrombin which in turn activate FVIII and FXIII. Assay methods have made it possible to monitor whether activation has occurred (Skjonsberg *et al.*, 1986). Random assay of units of blood provides an indication in the accuracy of the bleeding procedure. It has been shown that there is some correlation between the Fibrinopeptide A (FpA) content in plasma and donation time (Prowse *et al.*, 1984). The amount of Fibrinopeptide A present, serves as an indication of the amount of thrombin generated and indirectly as to the extent of activation of FVIII (Pflugshaupt and Kurt, 1983).

Standards recommended by the American Association of Blood Banks (AABB), require that blood collected for the preparation of cryoprecipitate and platelet concentrates, be collected from blood donors bled within eight minutes or less. To date no further recommendations to alter this have been proposed (AABB Standards, 1988). It was felt that the FVIII activity and platelet yield might be drastically diminished if the blood was collected over a period of time, i.e. longer than 8 minutes due to activation of clotting factors (Reiss and Katz, 1976).

The Fibrinogen/Fibrin degradation products (FDP) and D-Dimer are regarded as one of the most useful parameters available to evaluate the fibrinolytic status and degree of coagulation (Sato *et al.*, 1995). Detection of the different fibrinogen and

fibrin derivatives can help identify whether processes involving thrombin, Factor XIII and plasmin, have occurred in the donated blood unit (Proietti *et al.*, 1990). Fibrin formation can be determined indirectly by the determination of released fibrinopeptides, however these can be difficult to analyze as they have short half-lives. The results do not give the same information as direct determination of soluble fibrin (Wiman and Ranby, 1986).

2.3.4 EFFECT OF ANTICOAGULANTS ON FACTOR VIII

2.3.4.1 TYPE OF ANTICOAGULANT

FVIII is a calcium linked protein which may dissociate in the presence of calcium-chelating anticoagulants such as citrate, with substantial loss in activity. FVIII:C in plasma anticoagulated by citrate, may be stabilised by restoring plasma ionised calcium to physiological levels, combined with secondary anticoagulation by heparin, in other words, by anticoagulant exchange to heparin from citrate (Cumming *et al.*, 1990). Similar findings were reported by Rock *et al.* (1983) and by Krachmalnicoff and Thomas (1983).

Citrate-phosphate-dextrose-adenine (CPDA-1), used in a 1:8 ratio, is a registered anticoagulant for the collection of whole blood. However, reports by Rock *et al.* (1988) indicate, that by using this ratio allows the citrate present to be far in excess of the requirement to prevent clotting. By reducing the citrate level, the yield of FVIII is increased (Rock *et al.*, 1988). The safety of red cells prepared by

half strength citrate anticoagulant (0.5 CPDA-2) as a therapeutic agent was investigated, with the findings of the study being that CPDA-1 and CPDA-2 were comparable (Murphy *et al.*, 1991). These findings are comparable to those of Prowse *et al.* (1987) who found that the use of half strength citrate, did not impair standard blood donation, collected into CPDA-2, nor were there any incidents of formation of blood clots. To be a useful anticoagulant, half strength citrate (0.5) should have the ability to maintain red cell viability on storage, to the same standard as is currently available with other anticoagulants (Prowse *et al.*, 1987). Griffin and co-workers (1988) were able to show that by collecting blood into 0.5 CPD-A2, the stability of FVIII in plasma or whole blood stored at room temperature was greatly improved. Recent studies have shown that half-strength citrate CPD solution, improves the maintenance of erythrocyte, 2,3 diphosphoglycerate during storage of red cell concentrates, as well as provides a FVIII output of increased stability and concentration (Suontaka *et al.*, 1996). Contrary to the findings of Rock and Palmer (1985), Smit Sibinga and Das (1984), Krachmalnicoff and Thomas (1983) and Morgenthaler *et al.* (1985) which all found ACD to be a better primary anticoagulant, an independent study by De Wit *et al.*(1986), concluded that CPD remained the superior anticoagulant over ACD (acid citrate dextrose) and heparin.

The anticoagulant concentration in ACD and CPDA solutions is almost identical, although the higher FVIII activity in CPDA seems to be due to the preferable pH in the CPDA medium. The pH on collection in both solutions are similar, but FVIII loss occurs in ACD, possibly due to the lack of its buffering capacity (Weisert and Jeremic, 1973). In a study carried out by Foster *et al.* (1988), the loss of FVIII in

the final product was due to a citrate induced inactivation, which was avoided by controlling the concentration of ionised calcium.

FVIII travels as a calcium-linked complex which loses activity in the presence of citrate. When subjected to a stronger chelating substance, this complex dissociates completely and all activity is lost. FVIII has shown increased stability when prepared from plasma containing physiological levels of ionised calcium, obtained by the use of heparin as the anticoagulant. No difference in pH was observed in plasma prepared from heparin or citrate (Cumming *et al.*, 1987). Heparin is a potent, rapid acting anticoagulant that catalyses the inactivation of thrombin (Oman and Tejidor, 1993). It acts to prevent the conversion of prothrombin to thrombin in the final step of the clotting procedure. In 1980, Rock patented the technique of using heparin or sodium heparin as the mode of achieving improved FVIII yields in the production of cryoprecipitate. Heparin as an initial anticoagulant furnishes the means for maximum preservation of FVIII:C (Smit Sibinga *et al.*, 1984). When blood is collected into heparin, and combined with the methodology of thaw-siphon and fast-thaw techniques, an even greater improvement in the yield of FVIII is experienced (Smit Sibinga *et al.*, 1984). Collection of blood in heparin, instead of chelating anticoagulants or neutralization of citrate by the addition of Ca^{++} to heparinized CPD plasma, renders VIII:C significantly stable (Mikaelsson *et al.*, 1983). A too high a concentration of Ca^{++} forms a clot after freezing and thawing, instead of the usual precipitate. FVIII:C loses less activity, if blood is collected either directly into heparin or if citrated blood is heparinized, it must be recalcified within 12 hours of blood collection (Morgenthaler *et al.*, 1985). In order to be able

to recalcify the plasma, it is critical to determine a concentration of calcium chloride, which is as low as possible. A concentration of 10 - 14 mM of calcium chloride is the concentration recommended, higher concentrations catalyze fibrin formation (Adolfsen, 1986).

Fibrinogen and fibronectin are major elements in cryoprecipitate in the presence of heparin. Fibronectin molecules have binding sites for heparin, and in conjunction with the vWF, help amplify the concentration of the precipitate. The fibrinogen content is elevated by heparin (Smit Sibinga *et al.*, 1988). Wallevik *et al.* (1989) proved that by collecting blood into heparin, FVIII activity is improved and that the red cell concentrates and remaining plasma products when infused, show no adverse effects *in vivo.*

FVIII produced from cryoprecipitate collected in heparin, is able to produce a high yield intermediate purity product that is able to be heat treated, however, with detrimental consequences to stability and solubility (Palmer *et al.*, 1990). Measurement of FVIII:C activity can only be carried out in the presence of heparin, if the heparin is removed by neutralization with either polybrene or Cellex T (Morgenthaler *et al.*, 1985). FVIII prepared from heparinized plasma was found to have poor solubility, whereas cryoprecipitates from citrated plasma with the heparin added after plasma separation has a far better solubility (Morgenthaler *et al.*, 1985). The presence of heparin has an inhibitory effect during the measurement of FVIII. Neutralization of heparin can also be achieved by the use of either protamine sulfate or anion exchange filters. The concept of using enzymes such as

heparinase (Hepzyme) also achieves neutralization of heparin (Oman and Tejidor, 1993). Efficient removal of heparin can be achieved by the use of an anion-exchange resin in the form of a tablet (Heparsorb^{R}). Heparsorb^{R} incubated with 1 ml of heparinized plasma is capable of rapid removal of heparin from the plasma to allow further evaluation of blood coagulation factors (Cowan *et al.*, 1981). Due to reliability of results reported in the literature, and ease of use, Heparsorb^{R} is recommended as the assay to follow (Thompson and Counts, 1976).

0.5 CPD-A2 is not a licensed anticoagulant in South Africa, so had to be excluded from the study. On the other hand, although not used as the primary anticoagulant, heparin was considered for use as a comparative factor.

2.3.4.2 INFLUENCE OF pH

Factor VIII is stable in a narrow pH range around 7. It dissociates reversibly in the presence of high salt concentrations (International Forum, 1983). pH and salt concentration can influence the thrombin-fibrinogen reaction (Morrison, 1966). The pH of the plasma influences the stability of FVIII during the collection and storage of blood and maximum stability is achieved at a pH of 6.9. The pH of the anticoagulant solution used is important and should be maintained between 6.7 and 7.0 (Vermeer *et al.*, 1976a). The precipitation stage of the FVIII production should be performed at a slightly acidic pH instead of the neutral pH of 7.0, as an improved product is obtained having better solubility and greater stability (Liu *et al.*, 1980). Similar conclusions were made during a study in Norway (Skjonsberg *et al.*,

1987). pH of heparin solutions are 6.5, while the pH of ACD solutions are 4.9 and that of CPD solutions are 5.5 (Rock *et al.*, 1979b). 40% loss of FVIII activity was experienced if cryoprecipitates were prepared from acidified plasma (Shanberge, *et al.*, 1972).

A rise in pH from 7.2 to 7.7 is experienced when fresh frozen plasma is stored at room temperature for an extended amount of time. This can be explained by the possible diffusion of CO_2 from the polyvinylchloride bags containing the plasma (Smak-Gregoor *et al.*, 1993).

2.3.5 EFFECT OF DELAYED PROCESSING PRIOR TO PRODUCTION

The effect of delaying the separation of the red blood cells from plasma is one of the many variables affecting the final outcome of the recovery of FVIII in the production of cryoprecipitate.

Standards recommended by the American Association of Blood Banks (AABB) require that blood collected for the preparation of cryoprecipitate and platelet concentrates, be collected from blood donors and the plasma separated from the red blood cells, and frozen within 6 hours (Hughes *et al.*, 1988).

Several reports by Avoy *et al.* 1978; Burka *et al.* 1975; Slichter *et al.* 1976 and Sohmer *et al.* 1982 demonstrated that delaying refrigeration for up to eight hours only minimally influences the final activity of FVIII. Extension of the holding time

for the units of whole blood, from six to eight hours at room temperature can provide enhanced flexibility in the preparation of cryoprecipitates (Moraff *et al.*, 1990). However, other researchers failed to demonstrate any significant difference between four to six hour old plasma, as well as eighteen hour old plasma as raw materials for cryoprecipitate. The possible explanation is the longer second phase half-life of FVIII (Wensley and Snape, 1980). The delay in the separation of red blood cells and plasma does not prove to be significant unless related directly to whether the whole blood was stored at 4°C or 20°C (Pepper *et al.*, 1978). Storage of whole blood at between 1°C - 6°C is inferior to storage at between 20°C - 24°C, when these temperatures are rapidly imposed on the blood by forced cooling, which in turn could have a detrimental effect on the cryoprecipitate present as well as the resultant FVIII. Storage at 20°C - 24°C does not result in higher risks of microbiological contamination (Allersma *et al.*, 1996). A low citrate content in 0.5 CPD improves the stability of FVIII plasma of whole blood held at room temperature for 8 hours prior to separation (Suontaka *et al.*, 1996).

2.3.6 EFFECT OF PRE-PROCESSING STORAGE TEMPERATURES

FVIII at 4°C decays in a bi-phasic fashion. A rapid decrease of FVI'. with a half-life of five hours is followed by a more stable phase. This accounts for the rapid loss of activity within the first five hours after collection of up to 39%, decreasing to 14% at eighteen hours, with the whole blood being stored at 4°C (Pepper *et al.*, 1978).

In a study by Slichter *et al.* (1976), it was shown that the FVIII yield in cryoprecipitate made from whole blood stored for six hours at 4°C was reduced by 10% . This was also indicated by the instability of FVIII by *in vivo* and *in vitro* studies. Pietersz *et al.* (1989) and Booth *et al.* (1982) reported benefits from the storage of whole blood in a controlled environment at 20°C, prior to the preparation of cryoprecipitate. The temperature of the whole blood was rapidly cooled to the ambient temperature of 20°C. FVIII levels were well preserved at 24 hours with recoveries of 80%, compared to a yield of only 65% at 4°C. This was a significant finding, as for the first time it permitted blood collected at donation facilities, to be transported overnight to the production centres and processed the next day, without refrigeration of the whole blood.

2.4 IMPROVING FACTOR VIII RECOVERIES DURING PROCESSING

The potency of cryoprecipitate is affected partially by the technique of processing and partially by donor elements (Kasper *et al.*, 1975).

2.4.1 THAWING OF FACTOR VIII IN RELATION TO TEMPERATURE.

The thawing conditions of frozen plasma determine the FVIII recovery. When thawing is carefully controlled in rate, heat exchange and timing, the non-cold precipitable constituents redissolve, leaving the cryoprecipitate in a gel state. The cold-insoluble cryoprecipitate shows a temperature range over which solubility changes from the gel phase to complete resolution. Literature reports that the

differences observed in the FVIII activity when using the "refrigerator-thaw" technique, compared to the "bath thaw" technique, are vast (Orthner and MacPherson, 1984). The "bath thaw" method using circulating water at 4°C, for up to 90 minutes is accepted as the route to follow.

2.4.2 FREEZING RATE OF SEPARATED PLASMA

Fast freezing under conditions of optimal heat exchange, yield an improved FVIII recovery compared to slow freezing. An important criterion for consideration, is the rate at which the core of the plasma mass is solidly frozen. During slow freezing, the water crystallizes from the outer layers towards the inside, increasing the concentration of ions, proteins and other substances, resulting in changes to osmolarity and pH. The freezing point drops and the FVIII molecular structure is altered (Smit Sibinga, 1986). Components which influence freezing are, rate of freezing, heat exchange, eutectic point, temperature control and time. The shape of the frozen packs is an important consideration, as uniformity would allow an optimal thawing process of the plasma mass. In 1978, Mason introduced an improved freezing technique whereby the plasma packs, prior to freezing were placed between metal sheets in order to achieve plasma slabs of equal and minimum thickness. A slow freezing rate results in a cryoprecipitate with a FVIII yield which is twice that of a fast frozen cryoprecipitate (International Forum, 1983). A heavier cryoprecipitate results in a FVIII with lower activity. It is however recommended that plasma be completely frozen within 30 minutes of separation.

2.4.3 THE STABILITY OF FACTOR VIII IN THE FROZEN STATE

The optimal temperature for preserving FVIII in plasma is minus 30°C. The eutectic point of plasma is minus 23°C. At any temperature below minus 23°C, there is an irregular mixture of water/ice and pools of unfrozen concentrated protein in hypertonic salt solution (Britten and Grove-Rasmussen, 1966). Attempts to bring frozen plasma from a storage temperature of minus 40°C to a complete thaw within 30 minutes results in the preparation of a poor quality cryoprecipitate. If the frozen plasma is warmed to minus 10°C prior to thawing the quality of the product is enhanced (Foster *et al.*, 1984).

Storage temperature conditions of fresh-frozen plasma have a significant effect on fibrinogen levels in cryoprecipitate. The fibrinogen is a major protein constituent in cryoprecipitate, as cryoprecipitate is used as a fibrinogen supplement as well as for its FVIII properties. Enhancement of fibrinogen can be achieved by allowing the frozen plasma to "warm" over a period of sixteen hours to minus 5°C prior to thawing (Farrugia *et al.*, 1992). The estimated range of fibrinogen is between 100 - 300 mg per 10 ml volume of cryoprecipitate. There is no evidence to indicate that the fibrinogen content is directly proportional to the amount of FVIII present in any given cryoprecipitate (Ness and Perkins, 1980).

2.4.4 FREEZING TECHNIQUE OF PLASMA

Temperature changes in plasma during freezing can be separated into three parts, namely a very slow decrease near freezing point, followed by two rapid events before and after freezing. The final stage, close to the freezing point takes the most time. The first stage is a rapid fall to freezing point. At freezing point the change from liquid to solid occurs and a temperature plateau is formed, the plateau is caused by the need of a large amount of energy transport at the conversion. Hence the requirement for competent freezing equipment, in order to minimise the delay in temperature decline. Handling of the frozen plasma must be quick and efficient to avoid temperature fluctuations and better freezing conditions are experienced when ethanol baths are utilised. FVIII recovery depends mainly on the time of the phase change at the freezing point, therefore rapid freezing rates result in higher recoveries of FVIII (Carlebjork *et al.*, 1984 & 1986b).

Placement of plasma in a freezer, with a temperature below minus 40°C has been found not to be suitable as a freezing technique (Farrugia and Prowse, 1985). Plasma frozen in a minus 70°C ethanol bath or below minus 85°C produce a better quality cryoprecipitate. When using a freezer, care must be taken not to stack the plasma bags on top off each other as freezing will be prolonged. The time taken to freeze the plasma, under 60 minutes, is more critical than the type of freezing equipment used (Slichter *et al.*, 1976).

When the temperature of plasma is lowered, tiny ice crystals form and solutes such as the proteins and salts become concentrated in the inter crystalline spaces. If the temperature is lowered further, the salt concentration reaches a stage at which some of the protein elements become insoluble and precipitate. Cryoprecipitation is a process of salting out at subzero conditions where sodium chloride is the predominant salt in the plasma with a eutectic point at minus 23°C. At temperatures above this, small salt pockets will remain in the liquid solution (Rock and Tittley, 1979). When plasma is frozen in different thicknesses and not in slabs, the thinner portions thaw more quickly, thus losing FVIII content before the rest of the plasma reaches its liquid state (Rock and Tittley, 1977).

2.4.5 SEPARATION OF RED BLOOD CELLS AND PLASMA BY CENTRIFUGATION

Red cell contamination during separation of the plasma for cryoprecipitation production is an important consideration. One of the side effects of frequent infusion of cryoprecipitates is immunization of the patient. A high spin centrifugation of 4500 - 5000 x g for 20 - 30 minutes is suggested as a crucial production criteria (International Forum, 1983). FVIII levels in the cryoprecipitate were compared between platelet rich plasma, prepared by a relatively soft centrifugation spin and that of fresh frozen plasma, prepared at high speed centrifugation. Both centrifugation techniques produced acceptable FVIII levels (Smith and Hodges, 1984).

2.4.6 POST-THAW HOLDING TIME OF CRYOPRECIPITATE

It is the period instantly post-thaw, when the physico-chemical change from the solid form has just transpired, that is the determining factor in FVIII recovery (Rock and Tittley, 1977).

2.4.7 THAWING TECHNIQUE OF FROZEN PLASMA

FVIII can be lost in two ways during thawing. Dissolution of the precipitate occurs when the temperature at the solid-liquid interface exceeds the FVIII solubility limit. The second way involves inactivation of FVIII by enzymic degradation or other instabilities occurring from hostile physico-chemical environments (Burka *et al.*, 1975). In order to prevent the above from happening, thawing must be achieved immediately with temperature control being vital. The problem is that overheating occurs and in order to avoid this, an optimal thaw/temperature rate is necessary. In order to maximise thawing, a large surface area at the point of exposure to the heat, with even distribution is required, to prevent excessive heat on any one point of the surface area (Foster *et al.*, 1982 and 1984).

The presence of ice crystals in the plasma during thawing is a critical phase, to which close attention must be given (Burka *et al.*, 1975). An increase of 20% in FVIII yield was obtained by the careful regard for detail.

Rapid thawing can be obtained by rocking the frozen plasma packs in a water bath in which the temperature of the water is held constant. This method is known to enhance the yield and activity of FVIII (Margolis and Eisen, 1986). Rapid thawing of the frozen plasma is usually achieved by submerging the packs in a circulating water bath at temperatures between 4°C and 8°C, with a thaw time of 120 minutes. A major drawback of this technique is that the end-point of thawing "soft ice" stage, associated with high yields, can simply be overlooked (Wensley and Snape, 1980).

According to Kang (1980), the thaw-siphon technique yields the best recovery of FVIII above all other thawing methods, although it is labour intensive on a large production scale, with a critical stage being the end stage at the "soft ice" phase. The fast-thaw is an adequate thawing technique also requiring attention during the final thawing stages.

2.4.8 PRECIPITATION

The thaw siphon thawing technique described by Mason (1978), has become a well accepted thawing technique over the years. The procedure exploits the Le Chatelier equilibrium principle, in which the equilibrium of the two-phase system (ice - water) is continually displaced to the right by constant removal (by siphoning) of the thawed phase. There is little contact between FVIII and the liquid phase, because the contents of the pack remain frozen. *In vivo* studies of cryoprecipitate, prepared by this technique, confirm that the survival of FVIII compares well to that

of an intermediate purity concentrate (Toolis *et al.*, 1980).

In the thaw-siphon procedure, the temperature gradient between the "hot" 3°C bath water and the frozen plasma is responsible for a continuous flow of heat , via the walls of the plastic packs from the water to the frozen mass. Pressure from the water supports the contact with the reducing plasma mass. The thin plastic layer of polyvinyl chloride foil intensifies the continued heat exchange. The outflow of the thawed plasma is sometimes interrupted as the volumes reduce, and the sides of the plastic bags are sucked together. This is overcome by inducing puckering, by the use of elastic bands (Mason *et al.*, 1981). This siphoning technique has benefits in that the plasma protein is removed during the initial stages, and the precipitate is washed free from any trapped soluble plasma protein, thus enhancing the final purity. The fast processing by the continuous thaw-siphon technique by Mason *et al.* (1981) allows fast processing at controlled low temperatures without the use of a centrifuge. The rise in FVIII experienced, is due to reduced redissolution and reduced regradation of FVIII, due to maintenance of temperatures below 4°C and the fast processing. Trapping of the cryoprecipitate within the plasma mass, also contributes to the improved yield (Prowse and Mc Gill, 1979). Alternately, the thawed plasma can be sedimented by a 4°C centrifugation step for 15 minutes at 4733 x g. (Tomasulo *et al*, 1980). Sedimentation of cryoprecipitate is completed after 5 minutes centrifugation at 1500 x g. Further centrifugation for an augmented period produces no further precipitation and only prolongs production time (Vermeer *et al.*, 1976a).

Cryoprecipitate appears to form a dense and heavy solid phase, which should be easy to separate from the liquid, but attention to the type of centrifugal equipment is important. Fine precipitate particles are sometimes present in the supernatant plasma and if not precisely centrifuged, by the use of adequate separation forces, cryoprecipitate can be wasted (Foster *et al.*, 1984). Another important factor is the requirement to control temperature at the point of sedimentation. If too cold, freezing will occur, thus reducing FVIII recovery. If too warm, the precipitate will redissolve. The supernatant plasma is drained from the precipitate in a 4°C cold room to minimize the redissolving of the precipitate to a final volume of 10 ml (Slichter *et al.*, 1976).

The final volume of the cryoprecipitate has a direct influence on the final FVIII and fibrinogen content. A final volume of 10 - 12 ml produces a cryoprecipitate with high FVIII and fibrinogen content, diminished of excess of plasma proteins. A final volume less than 10 ml results in lower recoveries (Hoffman *et al.*, 1987). Frozen cryoprecipitates once thawed and pooled, have an expiry time of six hours, due to bacterial contamination possibilities and also due to the unstable nature of the FVIII. Fibrinogen on the other hand is an extremely stable plasma protein if stored at 1 - 6°C for several days (Howard *et al.*, 1991).

The AABB recommendation prior to 1989, was that thawed cryoprecipitate should be stored at room temperature for up to six hours, if not used immediately. This was subsequently changed after researchers showed that cryoprecipitate and fibrinogen were better stored at 1 - 6°C (Spivey *et al.*, 1992). Adequate levels of

Factor VIII are preserved in thawed cryoprecipitated FVIII that has been thawed at room temperature for up to 24 hours. Precipitation of fibrinogen and FVIII occur at temperatures 1°C - 6°C. Loss of FVIII occurs on thawing, so if required for accurate FVIII determination, samples should be tested immediately (Saxena *et al.*, 1991).

2.5 STANDARDIZATION OF FACTOR VIII ASSAYS

The determination of Factor VIII proagulant activity (this means the total or partial lack of production of the FVIII protein), is the most common specific coagulation factor assay performed and is used to diagnose Haemophilia A (Brandt *et al.*, 1988), as well as determination of FVIII activity in cryoprecipitates.

As the emphasis of this research work revolves around Factor VIII activity, it was important to review the literature and the problems encountered with FVIII assays and assay types available, before commencing the study in order to determine reproducibility, accuracy and reliability of results.

The number of variables that may affect the Factor VIII results are well documented in the literature. Elements such as clot detection methods, technical staff, specimen handling, data detection methodology and analytical parameters are but a few of the criteria to be carefully considered when choosing the assay type to be used. Analytical parameters related to type of buffer systems, aPTT reagent, substrate plasma and reference standards are major determinants of the precision and

accuracy of the assay. One of the most important options is the selection of FVIII:C deficient substrate plasma. The use of different types of deficient plasma has been implicated as the cause of variability of one stage assays (Wasi *et al.*, 1994). Clotting assays for the determination of Factor VIII activity suffer from low precision and limited accuracy (Carlebjork *et al.*, 1987).

It is of great significance that the appropriate kind of FVIII:C standard, according to the sample being assayed, be used. In other words, a concentrate standard when assaying FVIII concentrates and a plasma standard when assaying plasma samples should always be used (Lusher *et al.*, 1984). Concentrate standards should be used for the assay of therapeutic concentrates. Calibration of the plasma standard against the international standard concentrate has led to discrepancies between assay methods: one and two stage assays, (Barrowcliffe, 1984). Several collaborative studies organized by the National Institute for Biological Standards and Control (NIBSC) in Great Britain, have demonstrated that one-stage and two-stage methods do not give the same results when comparing dissimilar preparations: plasma and concentrates, (Mikaelsson and Oswaldsson, 1984). Standardization of the FVIII plasma standard used, is either frozen or freeze dried normal plasma which has been calibrated against the International Reference Standard Plasma or other national standards. The plasma standard should always be stored at below minus 30°C. The first International Standard (IS) for Factor VIII established in 1971 by Bangham and colleagues was a major step forward in the standardization of measurements of Factor VIII clotting activity.

Most investigators use the one-stage Factor VIII:C method, although the two-stage method gives higher yields (Kjellman, 1984). The estimation of specific activity depends on the Factor VIII assay which varies significantly between the two-stage and one-stage assay (Allain *et al.*, 1980). It is generally accepted that the two-stage assay method provides a more precise and less artificial evaluation of FVIII content. The two-stage assay has not been routinely used as it is difficult to automate, and the test plasma requires absorption prior to assay (Rock and Palmer, 1985). Brandt *et al.* (1988) recommended that whatever plasma sample is used to construct the standard curve, it should be standardized against a reference plasma with known activity, preferably one based on the International Reference Plasma.

In an investigation by Mikaelsson and Oswaldsson (1984), a comparison of various assay methods was undertaken which included a chromogenic substrate assay. The findings were that the new chromogenic substrate assay provides both accurate and precise measurement of FVIII:C. The assay was found to be superior to all other FVIII coagulation assays. Validation of the Kabi chromogenic assay against the one-stage clotting assay was carried out, with good correlation between the assays. The chromogenic assay was found to be both cost effective and less labour intensive (Pool, 1992).

The advent of chromogenic substrates has led to great progress concerning methods for determining important coagulation parameters. A method was developed that is simple to perform, requires no defibrination, contains reagents with stability and produces results that would be accurate and reproducible. Hence

the Coatest^{R} for FVIII detection was developed ((Rosen, 1984).

The Coatest^{R} Factor VIII Kit was evaluated by a collaborative clinical survey (4 laboratories participated) against the one-stage clotting assays. Precision was higher with the chromogenic method (Rosen *et al.*, 1985). The Coatest^{R} was adapted to a microplate technique which is inexpensive and convenient. Many plasma samples are able to be tested at the same time, hence less labour intensive. This test kit is not dependent on the use of Haemophilia A plasma as test substrate (Lethagen *et al.*, 1986). The Coatest^{R}, however requires maintaining the temperature at 37°C, concise timing of reagent addition and in pipetting techniques. The technique has a further advantage in that all the reagents are of animal or synthetic origin. Variations in use of the kit, by diluting reagents which result in longer incubation times, can be utilized, thus allowing up to 96 tests, compared to 50, if used according to the manufacturer's instructions (Prowse *et al.*, 1986). This assay design is now in routine use in the quality control of the production of FVIII. The simplicity of the microplate assay, in combination with the high resolution at all Factor VIII levels, makes it quite suitable for screening blood donors. Selection of donors with high Factor VIII levels for plasmapheresis, would increase the quality of plasma intended for the production of cryoprecipitates. The microplate method is advantageous over classical coagulation assays. No reagents of human origin are used, thus decreasing the risk of virological contamination. Large amounts of samples can be assayed in a short period of time. The technique is easy to perform with high precision, even with personnel not trained in blood coagulation assays (Carlebjork *et al.*, 1987).

2.6 2^k FRACTIONAL FACTORIAL EXPERIMENTAL DESIGN

A full 2^k factorial design requires all combinations of two versions of each of k variables. If a variable is continuous, the two versions become the high and low level factors of that variable. The notation using plus and minus signs are used and the list of experimental runs is called the design matrix. The design matrix contains k columns and $N = 2k$ rows. There is a column for each of the k variables, and each row gives the combination of versions for each run (Box and Hunter, 1961a & b). A complete factorial experiment is one in which all possible combinations of all the levels of the different variables, are investigated. This would involve a large number of tests when the number of variables is greater than five. It is possible to investigate the main effects of the variables and their interactions in a fraction of the number of tests required for the complete factorial experiment (Box *et al.*, 1963). The object of fractional factorial designs is to obtain information on the main effects, and as many of the interactions with a smaller number of tests than would normally be required. This type of design is particularly valuable in a production environment when there are a large number of variables implicated. It is a worthwhile means of establishing which variables are playing a dominant role in producing an effect. It is used where certain variables, which may interact, are to be studied simultaneously with other variables whose influence, if any, can be described by main effects only.

A weakness of this design is that it provides no way of detecting all interactions, especially if several variables are playing a dominant role in producing an effect.

Nevertheless it is effective in identifying dominant variables in a cost effective manner (Hendrix, 1979). When a number of variables are to be compared, they should be compared under the same conditions. Any extraneous variation will add to the experimental error. Replication of an experiment is a means of providing an unbiased estimate of the experimental error variance and more reliable estimates of the effects (Dykstra, 1959). The basis of all interpretations in this type of experimental design is the assumption that the variables which have high loadings of a factor, have something in common. Fractional factorial designs depend just as much upon the formulation and testing of the hypotheses as any other form of research (Adcock, 1954). Confounding is the process by which unimportant comparisons are deliberately confused for the purpose of assessing the more important comparisons with greater precision.

Statisticians can therefore be of major help in study design. The more the researcher knows what is going on, the more the statistician can be of help in study design (Boen, 1993).

CHAPTER 3

MATERIALS AND METHODS

This chapter describes the experimental design utilized and covers, sample collection, bleeding procedures, production variables with their factors and the assay techniques employed in an attempt to produce an improved quality cryoprecipitate.

3.1 EXPERIMENTAL DESIGN

This study was based on a statistically recognized experimental design called ***Fractional Factorial Experimental Design***. Fractional Factorial Experimental Design is employed when a myriad of variables with their factors are used in any one production process, all of which could have an influence on the outcome of the product. To commence a table was compiled of the production variables, using opposing criteria,called factors. Units of blood were collected and processed into cryoprecipitate, *from blood donors under the stipulated criteria, corresponding to* the study design. The pre, post and final samples obtained were analyzed against selected appropriate assays. The results of all the assays were statistically analyzed. The results are presented in the form of tables of means together with standard errors and least significant differences. Interactions between the significant variables were identified. In selecting this design, the OBJECTIVE was to:

* gain understanding on how the variables affect product properties
* uncover variables which influence the FVIII yield in the cryoprecipitate, in order to achieve an improved product
* find "winning" combinations of variables and their factors.

The statistical design was proposed, compiled and analyzed by the Institute for Biostatistics at the Medical Research Council (MRC). The variables were coded as a chart of experiments with (+) and (-) signs, representing high and low levels of each factor (Hendrix, 1979). The chart is shown in Table 3.1

Fourteen production variables were identified as critical steps in the production process. These are indicated as V1 to V14 in Table 3.2. The purpose of the Fractional Factorial Experimental Design in Table 3.1 is to enable all two-factor interactions to be estimated. In order to achieve this, a total of 32 production runs were required. For the purpose of obtaining reliable estimates of factor means, the size of the donor base of 52,000 blood donors was considered. It was accepted that the 32 production runs would have to be repeated four times over, to be of value. The study variables selected are shown in Table 3.2, with their specific factor for each of their high and low levels. The first six variables relating to blood donation were, donor blood group, time of donation, exercise levels of the donor, primary anticoagulant, delay prior to processing, and temperature storage conditions, all conditions relating to pre commencement of the production of cryoprecipitate.

NO.	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
1.	+	-	+	-	+	+	+	-	-	-	+	+	-	+
2.	-	-	+	-	+	-	+	-	+	+	-	-	+	-
3.	+	-	+	+	+	-	-	+	-	+	-	+	+	-
4.	+	+	-	+	-	+	-	+	-	-	+	+	+	+
5.	+	-	+	+	-	+	+	-	+	-	-	-	-	+
6.	+	-	-	+	+	+	-	-	-	+	-	-	-	+
7.	+	+	-	+	+	-	+	-	+	+	+	-	-	-
8.	-	+	-	+	+	+	+	-	-	-	-	+	+	+
9.	+	-	-	-	-	+	-	-	+	+	+	+	-	+
10.	+	+	+	+	-	-	-	-	-	-	+	-	-	-
11.	-	+	-	-	+	-	-	+	-	+	+	+	-	-
12.	+	+	-	-	-	-	+	-	-	+	-	+	-	-
13.	+	+	+	-	-	+	+	+	-	+	-	-	+	+
14.	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15.	-	+	-	+	-	-	-	+	+	+	-	-	-	-
16.	-	-	+	-	-	+	-	+	-	-	-	+	-	+
17.	+	+	-	-	+	+	-	+	+	-	-	-	+	+
18.	+	-	+	-	-	-	-	+	+	+	+	-	+	-
19.	-	-	-	+	-	+	+	+	-	+	+	-	-	+
20.	-	-	+	+	-	-	+	-	-	+	+	+	+	-
21.	-	+	-	-	-	+	+	-	+	-	+	-	+	+
22.	-	+	+	-	-	-	+	+	+	-	+	+	-	-
23.	+	+	+	-	+	-	-	-	+	-	-	+	-	-
24.	-	+	+	+	-	+	-	-	+	+	-	+	+	+
25.	-	+	+	-	+	+	-	-	-	+	+	-	+	+
26.	+	-	-	-	+	-	+	+	-	-	+	-	+	-
27.	-	-	-	-	-	-	-	-	-	-	-	-	+	-
28.	-	-	-	+	+	-	-	-	+	-	+	+	+	-
29.	-	-	-	-	+	+	+	+	+	+	-	+	-	+
30.	-	-	+	+	+	+	-	+	+	-	+	-	-	+
31.	+	-	-	+	-	-	+	+	+	-	-	+	+	-
32.	-	+	+	+	+	-	+	+	-	-	-	-	-	-

Table 3.1 *Fractional Factorial Design. V1 - V14 are the different variables used and no 1 -32 the different production runs, the (+) is the high level factor (-) the low level factor of each variable.*

The other eight variables deal with the production stages of the cryoprecipitate process. They were, temperature for freezing and thawing of plasma, freezing and thawing techniques, centrifugation speeds, precipitation methods and rate of thawing. The variables and their factors were selected primarily, according to the current production process at WPBTS, but the latest technology outlined in the more recent literature also influenced our decision when making the selection. Variables which could be monitored to remain constant were not considered.

VARIABLE NAME		HIGH LEVEL (+) FACTOR	LOW LEVEL (-) FACTOR
V1	DONOR EXERCISE LEVELS	EXERCISE	NONE
V2	DONOR BLOOD GROUP	GROUP A	GROUP O,B,AB
V3	BLEEDING TIME	< 8 MINUTES	> 8 MINUTES
V4	ANTICOAGULANT	CPD	HEPARIN
V5	PRE-PROCESS DELAY	< 6 HOURS	12 HOURS
V6	PRE-PROCESS STORAGE	4°C	20°C
V7	THAWING TEMPERATURE	4°C	8°C
V8	FREEZING RATE	SLOW (2 hours)	RAPID (1 hour)
V9	FREEZING TEMPERATURE	>-60°C	-35°C
V10	FREEZING TECHNIQUE	ALCOHOL/DRY ICE	LIQUID NITROGEN (-60°C)
V11	CENTRIFUGATION	RAPID 4733 x g	SLOW 2415 x g
V12	HOLDING TIME	NONE	0°C (1 hour)
V13	THAWING TECHNIQUE	MOVING WATER	MOVING PACKS
V14	PRECIPITATION	SECOND SPIN	SYPHON

Table 3.2 *Study variables with their factors.*
V1 - V6 are the pre production variables with their factors - Stage A.
V7 - V14 are the production variables with their factors - Stage B.

Variables required to remain consistent throughout all the production runs were that:

* the same phlebotomist place the donation needle into the donors arms
* the donors donate at the same clinic
* there be regular mixing of the blood packs during donation
* the starting volume of 450 ml of each pack of blood be constant for each donor bled
* the final volume of the cryoprecipitate be 10 ml.

3.2 PRODUCTION VARIABLES AND FACTORS

3.2.1 DONOR FACTOR VIII (V1)

The blood donors with the requisite for the high (+) factor level for the variable of donor FVIII, were required to exercise for 5 minutes, using an ergometer set on a moderate difficulty rate, prior to donation. FVIII activity increases significantly as a result of short durations of intense muscular exercise (Strand *et al.*, 1974). No difference was allowed between male and female donors. The pulse rate and blood pressure of each of these donors was taken pre and post exercise. This was to ensure that the exercise did not elevate the physical state of the donc. to outside the normal specifications required for blood donation. The pulse and blood pressure of each donor varied according to the fitness and age of the donor. Criteria for blood donation at WPBTS are a pulse rate of between 60 - 84 beats per minute and blood pressure of a minimum of 100/60 mm Hg and a maximum of 160/95 mm Hg.

The requisite for the low (-) factor level was no exercise, an immediate medical check was performed, followed by the donation of blood. The medical investigation involved checking the medical history, taking the blood pressure and pulse rate and performing the iron test for determination of adequate iron levels prior to donation.

3.2.2 DONOR BLOOD GROUP (V2)

Group A blood donors were given the high level (+) factor for this variable. Blood Groups O, B and AB were given the low level (-) factor. On attendance at the clinic, the donors, if agreement was obtained to participate in the study, were allocated a design number according to their blood group.

3.2.3 BLEEDING TIME (V3)

This variable was induced by the application of a sphygmomanometer cuff at varying pressures. Normal bleeding time was considered to be < 8 minutes with the pressure at 60 mm Hg. This was allocated the high level (+) factor for the variable of bleeding time. In order to induce a condition for the bleeding time of low level (-) factor, the pressure of the sphygmomanometer was reduced to less than 40 mm Hg. This prolonged the bleeding time to greater than 8 minutes.

3.2.4 ANTICOAGULANT (V4)

The primary container of a triple blood pack contains regular Citrate-Phosphate-Dextrose anticoagulant/preservative (CPD) solution. This anticoagulant, first described by Gibson *et al.* (1957) has been recommended as an excellent blood preservative and is still used in modern transfusion services to-day. The formula is given in (3.1) below.

Formula (3.1)

Sodium Citrate	2.630 g
Citric Acid	0.327 g
Monobasic Sodium Phosphate Monohydrate	0.222 g
Dextrose	2.550 g
Distilled Water to make	100 ml

Volume for use in collecting 450 ml of blood is 63 ml CPD.

As CPD is the standard anticoagulant registered in South Africa for the collection of blood , it was automatic that it be utilised in the model of the high level (+) factor for the variable of anticoagulant. Heparin, a naturally occurring complex organic substance, possesses the property of retarding *in vitro* coagulation of blood. It was selected as the alternative anticoagulant for the low level (-) factor. Due to the configuration of the standard blood pack system, CPD was used as the primary anticoagulant. Heparin was added immediately after the blood donation was complete. The concentration of heparin (Pularin^{R} Injection, Glaxo) added, was 3 U/ml. This was followed by the addition of 0.5 Mm Calcium chloride ($CaCl_2$), Formula 3.2.

Formula (3.2) for $CaCl_2$:

Di-hydrate Calcium Chloride (mw 147.02)	0.735 g
Distilled Water up to	100 ml

An example for the method for the calculation of the amount of Heparin and $CaCl_2$ to be added to each unit of blood was as follows:

mass of whole blood plus pack	=	594 g	
minus mass of pack	=	30 g	
mass of whole blood	=	564 g	
density of whole blood	=	1.05 g/ml	
volume of whole blood	=	$\frac{\text{mass of blood}}{\text{density}}$	$\frac{564}{1.05}$
	=	537 ml	

Amount of Heparin added:

Pularin 5000 IU/5ml	ie.	1000 IU/ml	
want 3 U/ml final dilution factor		$\frac{1000}{3}$	
	=	333.3	
Heparin to be added	=	$\frac{\text{blood volume}}{333.3}$	$\frac{537}{333.3}$
	=	1.61 ml	

Amount of $CaCl_2$ to be added:

Stock solution 50 Mm
want 0.5 Mm final
dilution factor $= \frac{50}{0.5} = 100$

Volume of 50 Mm $CaCl_2$ to be added

$$= \frac{\text{blood vol./100} + \text{blood volume}}{100}$$

$$= \frac{(537/100) + 537}{100}$$

$$= 5.42 \text{ ml}$$

3.2.5 PRE-PROCESS DELAY (V5)

Units of blood for cryoprecipitate production should be processed within six hours according to the Standards for Blood Banking, to maintain adequate levels of Factor VIII.

The high level (+) factor for this variable was set at < 6 hours, i.e. the plasma had to have been separated from the red cells and frozen within that time. The low level (-) factor time delay for this variable was determined to be 12 hours. The blood during this delay was left at the temperatures decided on according to the next variable.

3.2.6 PRE-PROCESS STORAGE (V6)

After the units of blood had been collected, according to their required high and low factor level, those that pertained to the high level (+) factor were placed immediately in a blood hamper (WPBTS design) whose environment had been

cooled to 4°C. The blood remained at that temperature for the time duration as set out in V5, i.e. either processed in less than six hours or after twelve hours. The low level (-) factor specification for this variable was normal room temperature (20°C). Those units required to remain at their said temperatures for twelve hours, were all bled late in the afternoon and further processed the next morning.

3.2.7 THAWING TEMPERATURE (V7)

This phase of the process followed the freezing stages which were covered under variables V8, V9 and V10. The choices of temperature variety for this variable were limited, due to this crucial production stage where cryoprecipitate was lost when temperatures of extreme nature were utilized. The high level (+) factor for the thawing temperature was set at exactly 4°C, and that of the low level (-) factor at 8°C. This thawing step was carried out in thermostatically controlled stainless steel water-baths, into which the frozen plasma packs were placed. A further description is given under variable V13.

3.2.8 FREEZING RATE (V8)

Variables V8, V9 and V10 are closely interlinked. This variable deals with the speed at which the plasma was frozen. This stage followed the centrifugation step, variable V11), in the production cycle. After the blood had been centrifuged under the required specifications, the blood was placed in a plasma extractor. The sterile seal was broken and the maximum amount of plasma was transferred to the empty

No.1 satellite container (Figure 3.1). The tubing at the y-connector was sealed and the red cells disconnected from the plasma. The red cells were discarded for the purpose of the study, due to some of the abnormal conditions they would have had to tolerate. The plasma in satellite container 1 and empty satellite container 2, were placed in plastic overwrap bags (Fenwal). This procedure provided protection to the plastic containers during freezing. The plasma bags were compressed between two metal plates, held together with elastic bands on each end to obtain relative uniform thickness during freezing. The freezing rate for the high level (+) factor, was selected to be slow for a time period of two hours. The rate for the low level (-) factor, was designated to be rapid for one hour.

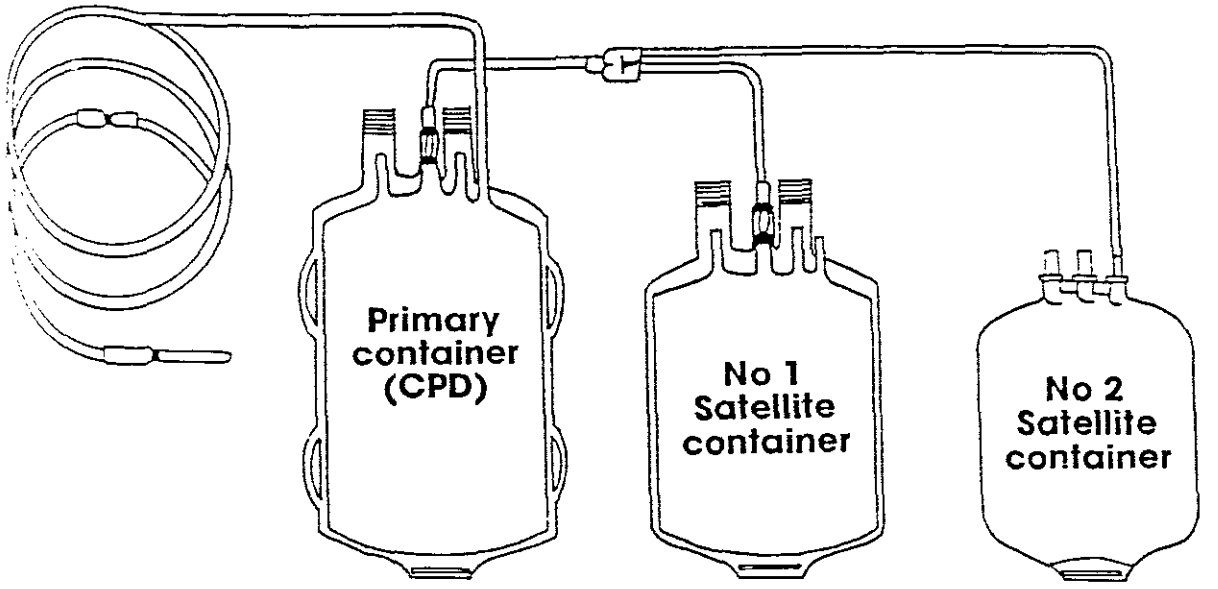

Figure 3.1 *Triple Blood Pack Configuration*

3.2.9 FREEZING TEMPERATURE (V9)

In relation to variable V8, the speed at which the plasma was frozen, was governed by the temperature at which it was stationed. The temperature for the high level (+) factor, was adopted to be -60°C and that for the low level (-) factor at -35°C.

3.2.10 FREEZING TECHNIQUE (V10)

The high level (+) factor freezing technique applied, was a freezing tank filled with a mixture of 70% ethanol prepared from a 96.6% solution (Analar) and nuggets of solid carbon dioxide (Afrox -Dry Ice). The amount of dry ice added was directly proportional to the temperature required, i.e. as indicated in V9, either -60°C or -35°C. The temperature at this phase was monitored by a digital thermometer. The low level (-) factor had two systems for this variable, due to difficulty in obtaining exceptionally low temperatures. When a temperature of -35°C was required, a mechanical freezer was used for the purpose. The compressed plasma slabs were placed onto the shelves under the compressor fans. When the temperature of below -60°C was needed, the plasma slabs were placed inside the upper layers of the liquid nitrogen tanks. The vapour present yielded the necessary cold environment, and the slabs were instantly snap-frozen.

3.2.11 CENTRIFUGATION (V11)

The plasma was separated from the red cells in the first of the processing stages. The normal centrifugation criteria employed routinely, was selected as the high level (+) factor. This was at a speed of 4733 x g for 25 minutes. A slower acceleration of 2415 x g for 45 minutes was utilized for the low level (-) factor of this variable. Temperature of the blood was vital at this point of the operation and was maintained at 4°C.

3.2.12 HOLDING TIME (V12)

This holding stage was the final step in the production process. The high level (+) factor for this variable received no holding procedure. The cryoprecipitate was immediately placed between metal plates and snap-frozen at -40°C in alcohol/dry ice for ten minutes. The low level (-) factor required the completed cryoprecipitate to be chilled at 0°C for an hour. Thereafter it was also snap-frozen. The final products were placed at below -23°C until required for assay.

3.2.13 THAWING TECHNIQUE (V13)

This stage of the operation followed after variable V7. The thawing technique was either one whereby the frozen plasma was placed in a water-bath at the required temperature viz 4°C or 8°C, and the water circulated by means of jet sprays within the enclosure. Alternatively the plasma was fixed to the moving arms of a water-

bath known as a "rocking water bath". This resulted in a motion of agitation of the plasma packs, without circulating the water. The duration of the thaw was controlled by the thawing temperature. When all the plasma was in a " soft ice slush", it was considered the end point of the thaw. The cryoprecipitate was visible as a solid matrix of firmly enmeshed strands in the semi-frozen fluid mass. The high level (+) factor for this variable was the system, whereby there was movement of water within the thawing bath, while the low level (-) factor was that of movement of the plasma packs, with no water movement. The thawing time with each of these methods was ± 90 minutes.

3.2.14 PRECIPITATION (V14)

The last stage in the production cycle was the precipitation of the cryoprecipitate containing the Factor VIII. It can be procured by centrifugation of the plasma at the soft ice stage. The plasma was centrifuged for a second time at 4733 x g for 15 minutes. The cryoprecipitate strands were forced to the bottom of the pack. After centrifugation, the supernatant plasma was drained into the second satellite container (Figure 3.1), leaving behind 10 ml of plasma to resuspend the cryoprecipitate. This process was used for the high level (+) precipitation factor. The low level (-) precipitation factor eliminated the centrifugation step by substituting it with a siphon method. During thawing the seal was removed from the satellite empty bag. As the plasma gradually thawed, siphoning commenced, and the supernatant plasma was transferred to the empty bag. The duration of the siphoning depended on the thawing technique and temperature, but on average

took ± 90 minutes to complete. The final product was further drained until 10 ml remained. The cryoprecipitate was then snap frozen at -60°C in alcohol/dry ice and stored at -30°C until required for assay purposes.

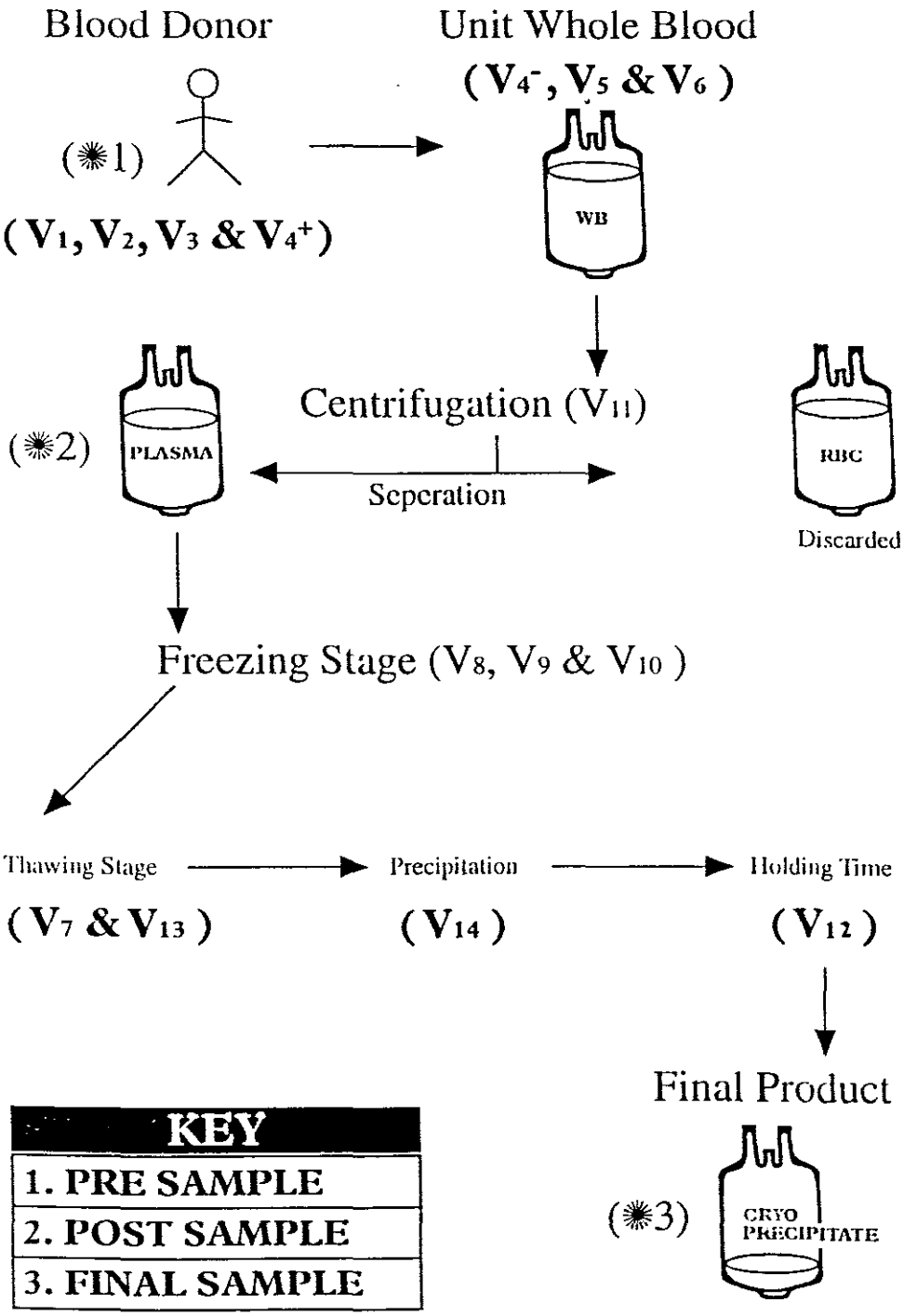

Figure 3.2 *Flow Chart summarizing the Production Cycle*

3.3 BLOOD COLLECTION

On presentation at the clinic, a worksheet for each donor was completed, capturing the relevant details such as age, sex and medical status (Appendix I). A production run number was allocated randomly (according to Table 3.1), and the design high (+) and low (-) level factor codes for the run recorded onto the worksheet. The donor was phlebotomized accordingly and the blood processed as indicated. Whole blood (480 - 500 ml) was collected from randomly selected blood donors at the Medipark Clinic, Foreshore, Cape Town. Blood was obtained from 128 regular donors in good health and between the ages of 16 and 65 years. The sample size chosen to achieve a level of reliability in the estimation of means was based on the numbers of donors on the HQ panel (5536 in total). No distinction was made between male and female donors. The donation was made into a 2,3-ethyl, hexyl-phthalate plasticized polyvinylchloride triple bag system with 70 ml CPD as the anticoagulant (Fenwal).

The donation procedure after complying with the medical check, followed the bleeding specifications outlined on the worksheet. The phlebotomy was performed by the same nursing sister throughout, and the needle was inserted into the arm in a single movement so as not to activate the haemostasis pathways. Adequate mixing of the blood was maintained throughout the donation. After donation, the weight of each pack was recorded.

3.4 SAMPLE COLLECTION

Blood samples were taken from each donor's non-donating arm, prior to any exercise or medical checks being performed. 5ml was taken into tubes containing no anticoagulant for determination of the electrolyte and plasma protein base lines of the donors. 10 ml of blood was taken into EDTA for the evaluation of the Factor VIII (FVIII), Fibrinogen, von Willebrand Factor (vWF), Fibrin degradation products (FDP) and pH base line levels. The plasma was alloquoted in 1 ml quantities in plastic tubes, labelled with the relevant design number and frozen at below -23°C, until the assays were ready to be performed. These samples were labelled the PRE specimens. POST samples (10 ml) were taken from each unit after the centrifugation (V11) stage of the production cycle, once the red cells and plasma had been separated. Again the plasma was alloquoted into 1 ml quantities and frozen. The cryoprecipitate was considered to be the FINAL sample, but was only alloquoted when the Factor VIII assay was ready to be performed. Each of the 128 production runs effectively, had a PRE, POST and FINAL sample for assay.

3.5 ASSAYS

The following analyses were performed on each sample: Factor VIII, Fibrinogen, von Willebrand Factor, Fibrin Degradation Products, Ph, Electrolytes and Plasma Proteins, including Sodium, Chloride, Total Protein ,Calcium, Magnesium, Potassium, Albumin and Alanine Aminotransferase. Testing was performed in such a way that all the specimens for a particular production run were completed before

commencing the assays for the next production run. A total of four production runs were carried out in order to obtain reliable estimates of the mean effects and interactions. FVIII, VWF and fibrinogen assays were included in the study, due to the fact that, from experience, they played an important role in determining the yields in the cryoprecipitate. Ph determination is not routinely included as a routine assay, but due to the variation in anticoagulants, I felt it important to include. FDP assay provides a clear indication to whether FVIII activation has occurred, at the time of phlebotomy. Plasma proteins and electrolytes have not been reported on in previous studies and I felt significant findings might come to light by including this set of assays in the study.

3.5.1 FACTOR VIII ASSAY (VIII:C)

3.5.1.1 *PRINCIPLE*

A chromogenic assay technique was selected for the determination of Factor VIII coagulant activity (VIII:C), in plasma and blood fractions. The assay was designed to give a linear correlation between Factor Xa, generated and Factor VIII:C content of the sample. In the presence of calcium and phospholipids, Factor X was activated to Factor Xa by Factor IXa. This generation was greatly stimulated by Factor VIII, which may be considered as a cofactor in this reaction. By using optimal amounts of Ca^{2+} and phospholipids and an excess of Factor Ixa and X, the rate of activation of Factor X was solely dependent on the amount of Factor VIII:C content of the sample. FXa hydrolyses the chromogenic substrate S-2222,

liberating the chromophore p-nitroaniline (pNA). The chromophore absorbs light at 405 nm. This reaction is simplified below.

Factor X		Factor IXa, Ca^{2+}, phospholipid Factor VIII	Factor Xa
Substrate	Factor Xa	peptide	+ PNA

The colour intensity was considered proportional to the Factor Xa in the reaction mixture. Factor Xa was considered proportional to the Factor VIII:C content in the sample. The colour intensity of the reaction was thus an indirect measure of the Factor VIII:C content of the sample. The hydrolysis of S-2222 by thrombin in the reaction mixture was prevented by the addition of the synthetic thrombin inhibitor, I-2581, to the reaction mixture. The reaction is displayed diagrammatically in Figure 3.3.

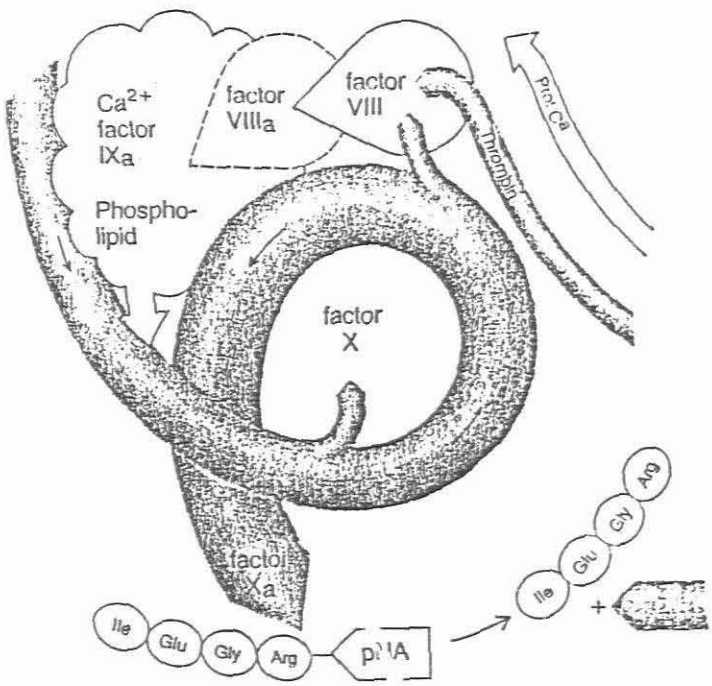

Figure 3.3 *Diagrammatic presentation of the Chromogenix Reaction where FVIII is a cofactor in the activating step that converts X to Xa. Phospholipid and Ca^{2+} are components in the complex.*

3.5.1.2 *REAGENTS*

The assay kit used was the Chromogenix Coatest^{R} Factor VIII (Kabi Vitrum, Sweden).

(a) **S-2222 + I-2581**

Chromogenic substrate (Bz-Ile-Glu(γ-OR)-Gly-Arg-PNA), 20 mg and synthetic thrombin inhibitor, 335 μg with mannitol added as a bulking agent. These were reconstituted with 10 ml sterile water to obtain a concentration of 2.7 mmol/L. This reagent remained stable for 6 months at 2 - 8°C.

(b) **Factor Ixa + Factor X**

Lyophilized bovine Factors Ixa and X with bovine albumin added as a stabilizing agent were reconstituted with 10 ml sterile water. This reagent was stable for 12 hours at 2 - 8°C. This reagent can be alloquoted into small volumes and if frozen at -20°C, remained stable for a month.

(c) **Calcium chloride ($Ca Cl_2$)**

Calcium chloride was supplied in a 0.025 mmol/L solution, remained stable if stored at a temperature of between 2 - 8°C.

(d) **Stock Buffer Solution**

A new buffer working solution was prepared each day. One volume of stock buffer solution was diluted with 9 volumes of sterile water before utilizing. The

constituents of the diluted buffer are Tris 0.05 mmol/L, Ph 7.3 and 0.2% bovine albumin. The opened stock solution vial remained stable for one month at 2 - 8°C.

(e) **Phospholipid**

This reagent consisted of a phospholipid emulsion prepared from porcine brain, with sodium azide added as a preservative. An open vial remained stable for one month at 2 - 8°C.

3.5.1.3 *EQUIPMENT*

The following additional materials were required for this assay:-

plastic test tubes (5 ml)
vortex mixer
laboratory assay timer
polystyrene microtitre plates (flat-bottomed)
heat incubator 37°C
Titertek Multiskan PLUS plate reader (405 nm filter)
Automatic microliter pipettes and tips

Multiskan PLUS is an eight-channel vertical light path filter photometer designed to measure the absorbances of liquids. In vertical photometry, the absorbance of light is proportional to the amount of light absorbing substance in the well. The Multiskan plate reader has a programmable calculation mode. Recall Programme 1 being that for the cryoprecipitate and Recall Programme 0 for the plasma. The calculation mode was set as follows :-

Standard 1	0%	0.00 U/ml FVIII	0.000	0 000
Standard 2	25%	0.25 U/ml FVIII	0.237	5 000
Standard 3	50%	0.50 U/ml FVIII	0.475	10 000
Standard 4	100%	1.00 U/ml FVIII	0.950	20 000

Once the programme has been recalled, the start key is pressed, the plate inserted and enter selected. The user then enters the concentrations and Multiskan PLUS calculates a standard line with the help of concentrations and absorbances of these standards, using the method of least squares. Measured absorbances of the samples are converted to concentration units by the use of this line. Concentrations obtained and the standard line are sent via the interface. A printout of the results can finally be obtained.

3.5.1.4 *STANDARD PREPARATION*

A standard curve was prepared for each microtitre plate. Standards were prepared using either plasma (WP plasma std, in-house), or cryoprecipitate, depending on the samples to be assayed. The FVIII standard was prepared according to WPBTS standard operating procedures. The pre-assayed standard was pre-diluted to 1 U/ml Factor VIII in buffer. This was further diluted as shown below in Table 3.3 for the plasma standard, and Table 3.4 for the cryoprecipitate standard. All the dilutions were made in plastic tubes. Each dilution was mixed on the vortex mixer before being used for the next dilution.

Plasma Standard				
Pre dilution	(1)	100 µl plasma std	+	600 µl buffer
100%	(2)	25 µl of dil.1	+	1000 µl buffer *
50%	(3)	500 µl of dil.2	+	500 µl buffer *
25%	(4)	500 µl of dil.3	+	500 µl buffer *

* these dilutions were used in the assay

Table 3.3 *Dilutions for plasma standard.*

Cryoprecipitate Standard				
Pre dilution	(1)	100 µl cryo std	+	1450 µl buffer
Pre dilution	(2)	50 µl of dil.1	+	300 µl buffer
100%	(3)	25 µl of dil.2	+	1000 µl buffer *
50%	(4)	500 µl of dil.3	+	500 µl buffer *
25%	(5)	500 µl of dil.4	+	500 µl buffer *

* these dilutions were used in the assay

Table 3.4 *Dilutions for cryoprecipitate standard*

3.5.1.5 *SAMPLE PREPARATION*

Samples were thawed at 37°C. They were assayed within 30 minutes of the dilutions being made. As a result only one plate was set up in a run. The dilutions for the plasma and cryoprecipitate samples were made as shown in Table 3.5.

Plasma Sample				
Pre dilution	(1)	50 µl test sample	+	300 µl buffer
100%	(2)	25 µl of dil. 1	+	1000 µl buffer *
Cryoprecipitate Sample				
Pre dilution	(1)	7.2 µl test sample	+	1000 µl buffer
100	(2)	25 µl of dil. 1	+	1000 µl buffer *
* denotes the dilution used in the assay				

Table 3.5 *Sample dilutions for plasma and cryoprecipitate.*

15 µl Cryoprecipitate sample was applied into each of duplicate wells on a 96 well plate. 15 µl of each standard was applied to each well, commencing with buffer (0%) to act as a control, followed by the 25%, 50% and 100% standard dilutions. The reader had been programmed to read in that order. The last two wells of the plate were filled with a back standard (100% standard dilution), which acted as control and check versus the standard's calibrated value. For the cryo samples this consisted of the undiluted cryo standard, whereas for the plasma the 100% dilution was used. The plate arrangement is displayed in Figure 3.4.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0%	3	7	11	15	19						
B	25%	3	7	11	15	19						
C	50%	4	8	12	16	20						
D	100%	4	8	12	16	20						
E	1	5	9	13	17	21						
F	1	5	9	13	17	21						
G	2	6	10	14	18	STD						
H	2	6	10	14	18	STD						

Figure 3.4 *Layout of microtitre plate. The plate is numbered 1 - 12, horizontally, and A - H vertically for easy identification when reading results.*

3.5.1.6 *ASSAY*

The assay is demonstrated by a diagrammatic flow chart in Figure 3.5.

(i) The phospholipid solution and Factor IXa + X bovine mixture were mixed in the ratio 5:1 v/v. Three volumes of $CaCl_2$ were added to this blend.

(ii) 40 μl Of this mixture were added to each well.

(iii) The plate was then covered, gently tapped to mix, and incubated at 37°C for 10 minutes.

(iv) 30 μl Of the substrate solution (S-2222) was added to each well. The plate was covered, gently tapped to mix, and incubated at 37°C for a further 10 minutes.

(v) The plate was read on a Multiskan plate reader at wavelength 405 nm using the calculation mode.

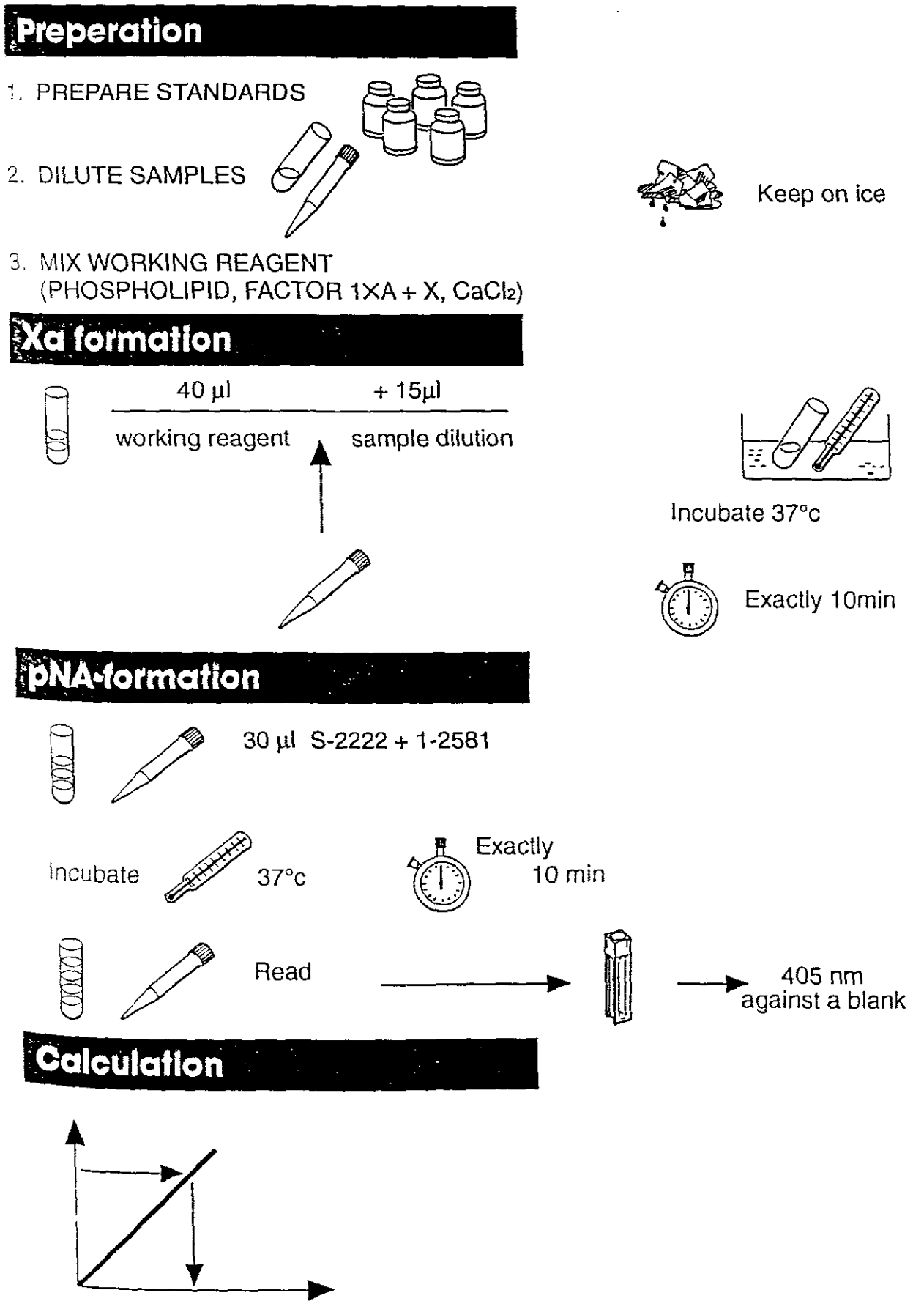

Figure 3.5 *Diagrammatic Flow Chart of FVIII Assay.*

3.5.1.7 *INTERPRETATION OF RESULTS*

Firstly, the standard readings obtained against the programme linear standard calculation, were checked to ensure that the results correlate. The back standard was checked to see that it fell between the range of 18 - 22 U/ml. If it was out of the specification range, a correction factor was calculated and the average of the sample readings adjusted accordingly. An example of a correction factor is given in Table 3.6. Duplicate sample readings were compared for accuracy and the mean result calculated and expressed in U/ml for each sample.

Back Standard Readings		20.49 24.64 45.13	
Average	=	22.57	
Actual Std Reading (100%)		18.52	
Correction Factor	=	$\frac{18.52}{22.57}$	= 0.82
Adjust each sample mean in on the plate by 0.82 U/ml.			

Table 3.6 *Calculation for correction factor.*

3.5.1.8 *THE EFFECT OF HEPARIN ON ASSAY*

The inhibitory effects of heparin require neutralization before an accurate F VIII assay can be performed. Those plasma and cryoprecipitate samples that were

spiked with heparin during the production process, were treated with Heparsorb^{R} (Organon Technika) in the following way:-

(i) 35 mg Heparsorb$_{R}$ was added to 0.5 ml plasma/cryoprecipitate sample;

(ii) incubated at room temperature for ten minutes while rotating on a rotator mixer;

(iii) centrifuged for five minutes at 1200 x g at room temperature, and

(iv) supernatant removed and assayed according to F VIII instructions above.

Expected Ranges: 0.5 - 1.0 U/ml

3.5.2 FIBRINOGEN ASSAY

3.5.2.1 *PRINCIPLE*

A rapid technique based on the Clauss technique for thrombin clotting time (Clauss, 1957) was employed. The principle of this method was that the clotting time of plasma was inversely related to fibrinogen concentration. The enzyme thrombin, converts the soluble plasma protein fibrinogen into its insoluble polymer, fibrin. At high thrombin concentrations (100 NIH units/ml), and low fibrinogen concentrations (5-80 mg/dl), the reaction was determined by the fibrinogen concentration. The use of diluted plasma and a relatively high concentration of thrombin, results in little interference by fibrin degradation products (FDP) or heparin, unless present in large amounts. The thrombin clotting time of test plasma was converted to fibrinogen

concentration by use of a calibration curve, derived from a range of fibrinogen concentrations, clotted by a fixed concentration of thrombin. When plotted on log-log paper, the thrombin clotting time was linear when compared to the fibrinogen concentration.

3.5.2.2 *REAGENTS*

a) **Data-Fi^R Thrombin Reagent**

This reagent is a lyophilized preparation of bovine thrombin (approximately 100 NIH units/ml) with stabilizers and buffers (Dade, Baxter Diagnostics - Ridge SA). 1 ml sterile water is used to reconstitute the reagent. The vial is re-stoppered and allowed to stand until dissolved. It is then inverted gently to mix. Shaking is not permitted. The reagent remains stable for 8 hours at room temperature or for 5 days at 2 - 8°C.

b) **Imidazole Buffer**

Formula (3.3)

Dissolve	3.40 g Imidazole(glyoxaline)
	5.85 g Sodium Chloride
make up to	500 ml
Add	186 ml Hydrochloric Acid (0.1 mol/l)
Make up to	1000 ml with Distilled Water
Check with a pH meter	pH 7.3

This reagent remains stable at 4°C and at room temperature for 24 hours if stored in a polystyrene tube.

(c) **Standard Plasma**

An in-house fibrinogen standard was used, which had been determined by replicate analysis of plasma by the Ratnoff and Menzie technique (Hall and Malia, 1974).

3.5.2.3 *EQUIPMENT*

The following additional materials were required for this assay:-

plastic test tubes (5 ml)
vortex mixer
timer
heat incubator at 37°C
Automatic microliter pipettes and tips
Log/log graph paper

3.5.2.4 *ASSAY*

Preparation of Standard Graph

(i) A range of dilutions of the plasma standard were prepared in the imidazole buffer. Dilutions of 1:5, 1:10, 1:20 and 1:40 were prepared.

(ii) 0.2 ml of each dilution were warmed to 37°C and 0.1 ml of thrombin was added.

(iii) The clotting times were recorded. The expected range was between 6 -20

seconds. Duplicate measurements were made.

(iv) Using log/log graph paper, thrombin clotting times were plotted against the fibrinogen content (g/l) of each dilution. A linear plot was obtained.

A new graph was prepared whenever a new batch of thrombin was utilized. Multiple clotting times of the control normal plasma were determined. The mean value ± 2 SD was obtained with the control plasma when each batch of tests was executed.

Preparation of Sample for Testing

(i) The test plasma was diluted 1:10 in imidazole buffer. Duplicate thrombin clotting times were obtained.

(ii) 0.2 ml of each test sample was warmed to 37°C

(iii) 0.1 ml of thrombin reagent added and the clotting time recorded.

3.5.2.5 *INTERPRETATION OF RESULTS*

The plasma fibrinogen value was derived by extrapolation of the mean of the duplicate values read against the standard graph. If the value of the test was outside the limits of the standard graph (6 - 22 seconds), the test plasma was diluted and a correction factor applied. This was determined in the same way as that of the FVIII assay. Example is given in Table 3.6. (page 68)

Expected Range: 2 - 4 g/l.

3.5.3 VON WILLEBRAND FACTOR ASSAY (vWFAg)

3.5.3.1 *PRINCIPLE*

Von Willebrand Factor is necessary in the primary haemostasis for the proper formation of the haemostatic plug and normal bleeding time. The presence of the antigen can be detected and measured by the double antibody sandwich enzyme-linked immunoassay (ELISA) technique. Wells of microtitre plates are coated with specific antibody and incubated with plasma dilutions. Antigen bound to the antibody is detected by a second antibody labelled with peroxidase.

3.5.3.2 *REAGENTS*

(a) **Buffers**

(i) **Coating Buffer: 0.05 M Carbonate Buffer; pH 9.6**

Formula (3.4)

Sodium Carbonate	1.59 g
Sodium Hydrogen Carbonate	2.93 g
Dissolve in distilled water	1000 ml
Add Thiomersal or Sodium Azide	0.02% w/v

(ii) **Phosphate Buffered Saline (PBS) 0.01 M; pH 7.2**

Formula (3.5)

Sodium Dihydrogen Phosphate	0.345 g
Disodium Hydrogen Phosphate	2.680 g
Sodium Chloride	8.474 g
Dissolve in distilled water	1000 ml

(iii) **Citrate Phosphate Buffer 0.1 M; pH 5.0**

Formula (3.6)

Citric Acid	7.30 g
Disodium Hydrogen Phosphate	23.87 g
Dissolve in distilled water	1000 ml

(b) **Enzyme Substrate**

Formula (3.7)

1.2 Ortho Phenylenediamine Dihydrochloride(OPD)	80 mg
Dissolve in Citrate Phosphate Buffer	15 ml
Add Hydrogen peroxide (20 vol) IMMEDIATELY BEFORE USE	10 µl

(c) **Sulphuric Acid(H_2SO_4) 1 M**

To obtain a 1 M concentration, 28 ml H_2SO_4 was added to 972 ml distilled water

(d) **Antisera**

(i) Rabbit Anti-Human von Willebrand Factor (2 ml)

Rabbit Anti-Human von Willebrand Factor, Dako-A082 (Denmark) is the purified immunoglobulin fraction of rabbit antiserum suspended in a solvent comprising of 0.1 M Sodium chloride (NaCl) and 15 mmol Sodium nitrate (NaN_3). The protein concentration is 5.4 g/l.

(ii) Peroxidase Conjugated Rabbit Anti-human von Willebrand Factor (2 ml)

Dako-P226 Peroxidase Conjugated Rabbit anti-Human von Willebrand Factor is the purified immunoglobulin fraction of rabbit antiserum conjugated with horseradish peroxidase of very high specific enzymatic activity. The solvent for this reagent is comprised of 0.05 M Tris/Hydrochloric acid (HCL) and 15 mmol Sodium nitrate (NaN_3). pH 7.2. The immunoglobulin concentration is 1.3 g/l.

3.5.3.3 *EQUIPMENT*

The following additional materials were required for this assay:-

plastic test tubes (5 ml)
vortex mixer
electronic timer
polystyrene microtitre plates and covers (flat-bottomed)
Titertek Multiskan PLUS plate reader with 492 nm filter
Automatic microliter pipettes and tips
Linear logarithmic graph paper
Tupperware container to serve as moist chamber

The principle of the Multiskan was previously explained under section 3.5.1.3 (see page 62), the only difference being that a different filter was selected viz. 492 nm.

3.5.3.4 *PREPARATION OF DILUTIONS*

Test plasma and cryoprecipitate samples were diluted in PBS, from 1:50 to 1:200 (i.e. 3 dilutions). Normal pooled plasma was diluted in PBS, from 1:25 to 1:3200 (i.e. 8 dilutions). These plasma dilutions were used to plot the standard curve on linear logarithmic paper.

3.5.3.5 *ASSAY*

(i) 100 µl Anti-vWFAg, diluted 1:500 in coating buffer was added to each well of a 96 well microtitre plate.

(ii) The plate was incubated in a moist chamber at room temperature for 2 hours.

(iii) The antiserum was then aspirated and the wells washed three times in PBS containing 0.05% (v/v) Tween 20.

(iv) 100 µl Of each plasma standard dilution was added to row A on the plate. 100 µl Of each diluted test sample was added as illustrated in Figure 3.6 below.

(v) The plate was re-incubated in the moist chamber at room temperature for a further hour.

(vi) The plasma samples were aspirated and the plates washed as in step iii.

(vii) 100 µl Of peroxidase-conjugated anti-vWFAg, diluted 1:500 in PBS containing 0.1% (v/v) Tween 20 was added to each well. The plate was again incubated in the moist chamber at room temperature for one hour.

(viii) The plate was aspirated and washed as in step iii.

(ix) The plate was given a further wash with Citrate Phosphate Buffer.

(x) 100 µl Of freshly mixed substrate solution (OPD) was added to each well. The plate was incubated at room temperature for 20 minutes.

(xi) At this stage colour development was observed. The reaction was stopped by adding of 150 µl 1 M Sulphuric acid to each well.

(xii) Absorbance readings were determined by reading the plate on the Multiskan

plate reader at 492 nm.

	1	2	3	4	5	6	7	8	9			
A	1:25	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400			
B	1	1	1	2	2	2	3	3	3			
C	4	4	4	5	5	5	6	6	6			
D	7	7	7	8	8	8	9	9	9			
E	10	10	10	11	11	11	12	12	12			
F	13	13	13	14	14	14	15	15	15			
G	16	16	16	17	17	17	18	18	18			
H	19	19	19	20	20	20	21	21	21			

Figure 3.6 *Layout of microtitre plate for vWFAg assay.*

3.5.3.6 *INTERPRETATION OF RESULTS*

The absorbance readings were plotted against vWFAg standard concentrations on linear-logarithmic graph paper. A straight line should be obtained with the pooled plasma (1.0 unit vWFAg per ml), diluted 1:8 to 1:256, indicating that the assay was sensitive to at least 0.01 unit vWFAg per ml. The absorbances of the test plasma samples were read off the graph and recorded as a percentage.

Expected Result : 100%

3.5.4 FIBRIN DEGRADATION PRODUCTS (FDP)

3.5.4.1 *PRINCIPLE*

During blood coagulation, fibrinogen is converted to fibrin by the action of thrombin. The resulting monomers polymerize to form a soluble gel of non-crosslinked fibrin. This fibrin gel is then converted to crosslinked fibrin by thrombin activated Factor VIII, to form an insoluble clot. The fibrinolytic enzyme plasmin, cleaves both fibrinogen and fibrin to yield degradation products (FDP). Only the degradation products from cross-linked fibrin (XL-FDP) contain D-dimer. This process is illustrated in Appendix IV. The green section is that of the Fibrinolytic System. Abnormal levels of XL-FDP in plasma indicate reactive fibrinolysis. The presence of XL-FDP in the circulation was first demonstrated by immunoprecipitation and gel electrophoresis techniques (Schifreen *et al.*, 1985). The Dimertest^{R} Latex Assay (Agen Biomedical Ltd, Australia) uses highly specific D-dimer monoclonal antibody attached to latex particles. Reactive fibrinolysis was demonstrated by latex agglutination at a plasma concentration of approximately 0.25 mg/l XI-FDP. The objective for performing this assay, was to determine whether the coagulation process had been activated at the point of the needle being inserted into the donor's arm, due to poor phlebotomy technique.

3.5.4.2 *REAGENTS*

(a) **Latex Reagent (2 ml)**

One dropper bottle containing a suspension of latex particles coated with mouse monoclonal Anti D-dimer antibody, containing 1 g/l Sodium azide as a preservative.

(b) **Positive Control (0.6 ml)**

A solution containing human D-dimer, stabilisers and preservative.

(c) **Negative Control (0.6 ml)**

A solution containing buffer and preservative.

(d) **Phosphate buffer Solution (PBS 20 ml)**

Formula (3.8)

Sodium Dihydrogen Phosphate	0.069 g
Disodium Hydrogen Phosphate	0.536 g
Sodium Chloride	1.695 g
Dissolve in distilled water	20 ml

(e) **Test Cards**

10 Disposable black 9 well reaction cards, with 60 disposable plastic stirrers for mixing. The reading surface of the test cards must not be touched with the

fingers.

Test reagents and samples are equilibrated at room temperature (20°C - 25°C), before testing commences. Reagents are mixed by inversion immediately prior to use in the assay. The reagents remains stable if stored at 2°C - 8°C.

3.5.4.3 *EQUIPMENT*

The following additional materials were also required:

Precision micropippettes and tips: 20 µl and 100 µl
Plastic test tubes and rack
Centrifuge

3.5.4.4 *ASSAY*

All the samples were tested by the Qualitative Screening Test (Koopman *et al.*, 1987) initially, and those that tested positive were then tested by the Semiquantitative Method (Mirshahi *et al.*, 1986).

Qualitative Screening Test

(i) Samples were thawed at 37°C and centrifuged before testing.

(ii) Positive and Negative controls were included in each batch of testing. The same assay procedure was followed for the controls as was for the test samples.

(iii) One disposable test card was selected, holding the dropper bottle vertical one drop of Latex reagent was added to the card.

(iv) 20 µl Of undiluted plasma or control solution were added adjacent to the latex droplet.

(v) The latex and plasma sample/control were mixed with the plastic stirrer, until the latex was uniformly distributed.

(vi) The card was gently rotated for 3 minutes.

(vii) At precisely 3 minutes the test was checked under a strong light source for agglutination. Agglutination was indicative of a positive result and no agglutination of a negative result.

Examples of the results are displayed in Figure 3.7

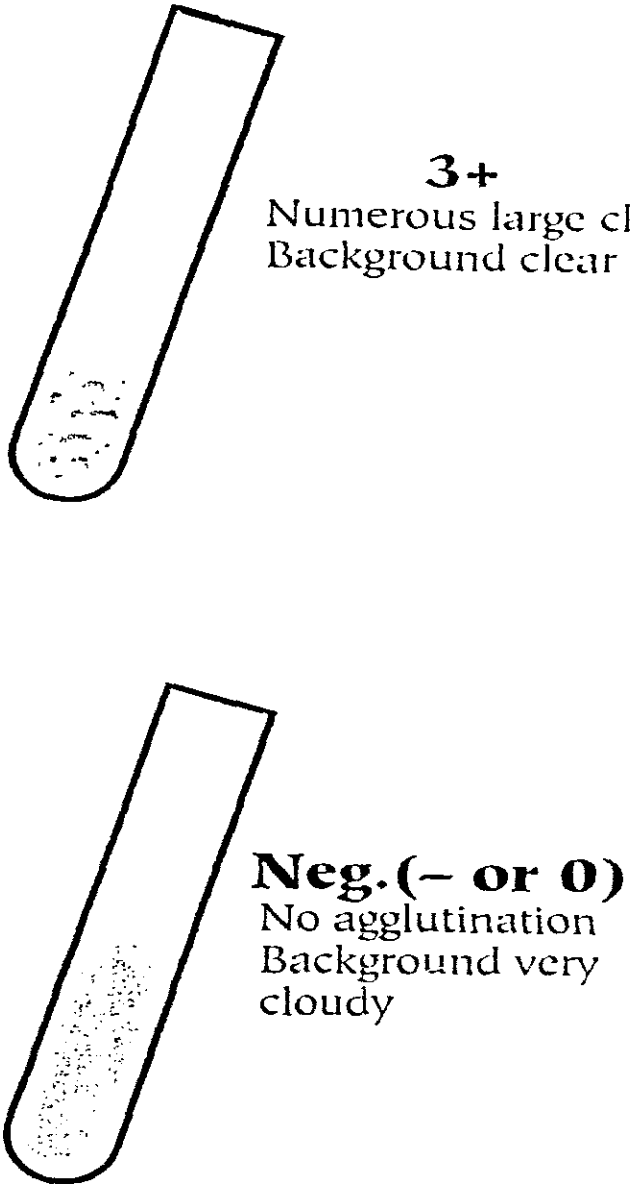

Figure 3.7 *Illustration of the FDP reaction results.*

Semiquantitative Screening Test

(i) If a positive result was obtained, the following procedure was followed:

(ii) Doubling dilutions of the test plasma were prepared as shown in Table 3.7.

DILUTION	PLASMA VOLUME (μl)	PBS VOLUME (μl)
UNDILUTED	100	NONE
1:2	100	100
1:4	100 of dil 1:2	100
1:8	100 of dil 1:4	100

Table 3.7 *Dilutions for the semiquantitative screening test*

(iii) Steps iii to vii for the Qualitative Screening test were then carried out on each dilution.

(iv) The highest dilution in which visible agglutination occurred was taken as the titre.

3.5.4.5 *INTERPRETATION OF RESULTS*

Elevated levels of plasma XL-FDP were indicative that activation of the coagulation system had occurred (reactive fibrinolysis),which will cause agglutination of Dimertest Latex as shown in Table 3.8.

TITRE	APPROXIMATE XL-FDP LEVELS (mg/l)	SAMPLE DILUTION			
		UNDIL.	1:2	1:4	1:8
0	NORMAL	-	-	-	-
1	0.25 - 0.5	+	-	-	-
2	0.5 - 1.0	+	+	-	-
4	1.0 - 2.0	+	+	+	-
8	2.0 - 4.0	+	+	+	+

Table 3.8 *Fibrin Degradation Products (FDP) Results for quantitative method "+" = agglutination "-" = no agglutination*

Levels greater than 4.0 mg/l were calculated by extending the dilutions beyond 1:8. The crosslinked fibrin degradation products, D-dimer, D-dimer E and high molecular weight derivatives were all recognized by the Dimertest monoclonal antibodies. No binding was found to the fibrinogen degradation products X, Y, D, E to 20 mg/l or to fibrinogen to 1000 mg/l.

Expected Result: Negative

3.5.5 pH DETERMINATION

3.5.5.1 *PROCEDURE*

pH value is the logarithm of the reciprocal of the hydrogen ion concentration; p for power and H for hydrogen. pH values range from 0 to 14, i.e. from very acidic to very alkaline. Water contains hydrogen and hydroxyl ions, the relative concentration of the ions are important. The product of the hydrogen and hydroxyl ion concentration in water at 25°C must always equal 10^{-14} gm/ion litre of

solution. If the hydrogen ion concentration exceeds the hydroxyl concentration the water is acidic. If the concentrations are equal, the water is neutral. When the hydroxyl ion concentration is greater than the hydrogen, the water is alkaline (Jackson and Morton, 1979).

The PRE, POST and FINAL samples' pH were measured using a Zeiss Model 300 pH meter, utilizing a N 60 A micro-electrode to determine the degree of acidosis in the samples.

Expected Value: 7.4

3.5.6 PLASMA PROTEINS AND OTHER SUBSTANCES

The chemistry parameters are preprogrammed on the Technicon Dax System. The Technicon Dax is a multiple chemistry analyzer which utilizes photometry techniques for the plasma protein and assays of other substances. The appropriate system values(SSVs) for the calibrators are entered into the calibrator parameter table and are shown in Table 3.9 for each of the assays selected. A quality control programme is performed daily on the machine to ensure the controls for each assay falls within the required specifications.

5.6.1 *PARAMETERS FOR PLASMA PROTEINS AND OTHER SUBSTANCES*

TEST	Mg	TP	ALT	ALB	CA
WAVELENGTH	660 700 nm	548 628 nm	340 380 nm	604 700 nm	572 628 nm
SAMPLE VOLUME	3.0 µl	5.0 µl	14.0 µl	2.5 µl	10 µl
REAGENT VOLUME	150/150µl	250 µl	250/50 µl	400 µl	300/100µl
INCUBATION TIME	150 sec.	330 sec.	288 sec.	30 sec	66 sec.
TEMPERATURE	37°C	37°C	37°C	37°C	37°C
TOTAL READ TIME	-	-	180 sec.	-	-

Table 3.9 *Plasma protein parameters and other substances*
Mg = Magnesium *TP = Total Protein*
ALB = Albumin *Ca = Calcium*
ALT = Alanine Aminotransferase

3.5.6.2 *ASSAY PRINCIPLES FOR PLASMA PROTEINS AND OTHER SUBSTANCES*

Magnesium

Magnesium ions form a red chelate with Xylidyl Blue in an alkaline medium which results in a spectral shift. The change of absorbance at 660 nm ,is directly proportional to the magnesium concentration, and can be quantified by an endpoint measurement.

(ii) **Total protein**

The peptide bond of proteins forms a coloured complex with Cu^{2+} ions in alkaline solution. The shift of spectral adsorption is measured photometrically, and is directly proportional to the protein concentration of the sample. This assay is based on the Biuret method.

(iii) **Alanine Aminotransferase**

The reaction is initiated by the addition of the reagent to the patient sample. The rate of decrease in the concentration of NADH, is directly proportional to the ALT activity in the sample. The reaction is monitored at 340 nm as a zero-order kinetic assay.

(iv) **Albumin**

BCG preferentially binds albumin at pH 4.2 causing a shift in the absorbance spectrum. The increase of absorbance measured at 604 nm after 30 seconds, is directly proportional to the concentration of complexed albumin.

(v) **Calcium**

Acidic calcium reagent is added to the serum sample and incubated to ensure the release of protein-bound calcium. An initial absorbance reading is taken before

adding reagent 2 to minimize the effects endogenous interfering substances. Addition of the second reagent, forms an alkaline medium wherein cresolphthalein complexone forms a coloured complex with calcium ions. Absorbance is measured at 572 nm. The 8-hydroxyquinoline in the reagent binds the free magnesium ions, thus minimising the possibility of their interference in the calcium assay.

3.5.6.3 *REAGENTS*

(i) **Magnesium**

Reagent 1 (diluent) containing 153 mmol Potassium carbonate.

Reagent 2 (colour reagent) containing Xylidyl blue-1 0.39 mmol; Buffer; Surfactant; Ethylene bis (oxyethylenenitrile) tetraacetic acid 0.09 mmol.

The Technicon Omnipak Magnesium Reagents are ready-to -use as supplied and no preparation is required. After opening, the reagent remains stable for fourteen (14) days at 2 - 8°C.

(ii) **Total Protein**

Total Protein Reagent contains Sodium hydroxide 200 mmol; Sodium potassium tartrate 50 mmol; Cupric sulfate pentahydrate 12 mmol; Stabilizers. The Technicon Omnipak Total Protein Reagent is ready-to-use as supplied and no preparation is required. After opening the reagent remains stable for thirty (30) days at room temperature.

(iii) **Alanine Aminotransferase**

ALT Reagent 1 contains Lactate dehydrogenase (porcine muscle) 3206 U; L-Alanine 675 mmol; NADH 0.32 mmol; Buffer; Stabilizer.

ALT Reagent 2 contains a-Ketoglutarate disodium 101 mmol.

These reagents must be reconstituted with the stipulated amount of deionized water before use. After opening, this reagent remains stable for twenty-one (21) days in the refrigerated compartment of the Technicon Dax system.

(iv) **Albumin**

Albumin Reagent contains Bromcresol green 0.30 mmol; Preservative; Surfactant and Buffer. The Technicon Omnipak Albumin Reagent is ready-to-use as supplied and no preparation is required. After opening, this reagent remains stable for thirty (30) days at room temperature.

(v) **Calcium**

Calcium Reagent 1 contains 8-Hydroxyquinoline 11.3 mmol; Cresolphthalein complexone 0.08 mmol.

Calcium Reagent 2 contains AMP Buffer. The Technicon Omnipak Calcium Reagents are ready-to-use as supplied and no preparation is required. After opening this reagent remains stable for fourteen (14) days in the refrigerated compartment of the Technicon Dax system.

3.5.6.4 *ASSAY PROCEDURE*

(i) Dilution of samples for these assays were not required. The frozen samples were thawed at room temperature before using.

(ii) Those samples which appeared turbid, were centrifuged prior to insertion into the analyzer.

(iii) The samples and reagents were placed into the various allocated holders and channels in the analyzer and the relevant assay programmes selected.

(iv) The readings were sent via the interface to the printer. They were calculated in the computer, and a printout containing all the assay results per sample, were printed on a separate page per sample.

Expected Values:

Magnesium	**1.8 - 2.6 mg/dl**
Total Protein	**6.4 - 8.8 g/dl**
Alanine Aminotransferase	**5.0 - 43.0 U/L**
Albumin	**3.0 - 5.5 g/dl**
Calcium	**9.2 - 10.9 mg/dl**

3.5.7 **ELECTROLYTES**

The chemistry parameters are pre-programmed on the Technikon Dax System. The Technicon Dax is a multiple chemistry analyzer which utilizes electrode analysis for the electrolyte determination. A quality control programme is performed daily on the machine to ensure the controls for each assay falls within the required specifications.

3.5.7.1 *PRINCIPLES FOR ELECTROLYTES*

(i) **Sodium (ISE)**

The Technicon Dax system sodium method is based on an indirect potentiometric procedure using an ion-selective electrode. The sodium ion-selective electrode responds selectively to sodium ions according to the Nernst equation (Eisenman, 1967). The serum sample is mixed with ISE buffer, thereby providing a constant pH and a constant ionic strength solution. As the buffered sample flows past the ion-selective electrode, changes take place in the electrical potential. These electrical potential changes are automatically measured against the potential of a reference electrode, in order to derive the correct analog value for that sample. The electrical potentials are proportional to the logarithm of the respective sodium ion concentrations in the sample. The analog/digital signals for each sample are processed and then sent to the Technicon Dax system CPU, where the software converts the data to reportable concentration units.

(ii) **Potassium (ISE)**

The Technicon Dax system potassium method is based on an indirect potentiometric procedure using an ion-selective electrode. The potassium ion-selective electrode responds selectively to potassium ions according to the Nernst equation (Eisenman, 1967). The serum sample is mixed with ISE buffer, thereby providing a constant pH and a constant ionic strength solution. As the buffered sample flows past the ion-selective electrode, changes take place in the electrical

potential. These electrical potential changes are automatically measured against the potential of a reference electrode, in order to derive the correct analog value for that sample. The electrical potentials are proportional to the logarithm of the respective potassium ion concentrations in the sample. The analog/digital signals for each sample are processed and then sent to the Technicon Dax system CPU, where the software converts the data to reportable concentration units.

(iii) **Chloride (DCL)**

The serum sample is mixed with chloride reagent. This reagent, an equilibrium solution of ferric, mercuric and thiocyanate ions, when combined with the chloride ions in the serum, undergoes a double displacement reaction, forming a red-brown chromophore, ferric thiocyanate. The absorbance is proportional to the concentration of the chloride in the sample.

$$2Cl + Hg(SCN)_2 \_\_\_\_\_ HgCl_2 + 2SCN$$

$$3SCN + Fe3 \_\_\_\_\_ Fe(SCN)_3 \text{ (red-brown)}$$

3.5.7.2 *REAGENTS*

(i) **Sodium and Potassium**

ISE Buffer contains buffer and preservative.

ISE Mid Range Calibrator contains 14 mmol Sodium; 0.4 mmol Potassium and a

Preservative.

ISE Detergent Diluent contains 140 mmol Sodium; 4 mmol Potassium and 44 mmol Sodium azide.

After opening, these three reagents are stable for thirty (30) days at room temperature.

ISE Calibrator Serum Low contains 120 mmol Sodium; 2.0 mmol Potassium and a stabilizer.

ISE Calibrator Serum High contains 160 mol Sodium; 8.0 mmol Potassium and a stabilizer.

These two reagents are used for calibration of the machine.

ISE Detergent contains Surfactant.

After opening, the ISE Calibrator Serum High and Low are stable for twenty-four (24) hours at 2 - 10°C. A working ISE Detergent preparation is made with 12.0 ml of detergent diluent added to 12 ml of ISE detergent. The blend is mixed gently by inversion until completely dissolved.

(ii) **Chloride**

DCL Chloride reagent contains 0.8 mmol Mercuric nitrate; 1.8 mmol Potassium thiocyanate; 35 mmol Ferric nitrate and 108 mmol Nitric acid. No stabilizers or preservatives are added. After opening, this reagent remains stable for thirty (30) days in the refrigerated compartment of the Technicon Dax system. The Technicon

Dax system requires programming for this reagent. The following parameters are entered on the Colour Reagent Screen:-

Test Name	Cl
Type	End-up
Wavelengths	476/572 nm
M.point	3
Type	Standard
Sample Volume	4.0 μl
Reagent 1 volume	CL 400 μl

3.5.7.3 *ASSAY PROCEDURE*

(i) Dilution of samples for these assays were not required. The frozen samples were at room temperature before using.

(ii) Those samples which appeared turbid, were centrifuged prior to insertion into the analyzer.

(iii) The samples and reagents were placed into the various allocated holders and channels in the analyzer, and the relevant assay programmes selected.

(iv) The readings were sent via the interface to the printer. They were calculated in the computer, and a printout containing all the assay results per sample, were printed on a separate page per sample.

(v) Reported values were calculated from the Nernst Equation automatically. The software contains a series of detectors and limits, to assure that the sample had been aspirated correctly, diluted accurately and that the electronic modules were balanced according to assigned specifications. As results were reported, abnormal values were flagged.

Expected Values:

Sodium	**136 - 144 mEq/l**
Potassium	**3.2 - 4.8 mEq/l**
Chloride	**99 - 106 mEq/l**

3.6 STATISTICAL ANALYSIS

The data from all the assays were extrapolated from the various worksheets into a Lotus 123 spreadsheet. The results are displayed in Appendix II for each of the assays in the following order: FVIII, Fibrinogen, von Willebrand Factor, Fibrin Degradation Products, pH determination, Magnesium, Total Protein, Albumin, Calcium, Alanine aminotransferase, Sodium, Potassium and Chloride. The results were entered into columns according to the run number, against their production run number reflected in the rows. Those results falling outside the assay specification ranges are termed outliers, and are displayed with a * next to the data result.

The information was supplied on computer disk to the Department for Biostatistics of the Medical Research Council, for analysis. The statistical method applied to the data was:- *Analysis of Variance (ANOVA)*. The **Fractional Factorial Analysis** involved the estimation and testing for significance of all main effects of the variables and all two-factor interactions. The results obtained, are shown in Appendix III.

CHAPTER 4

RESULTS

The results of the assays, determine whether donor and production variables play a role in contributing to an improved final FVIII yield, were analyzed by the method of Analysis of Variance. This was applied to all the measurements in each assay category of the variables and their factors considered.

A total of 128 blood donors were used for the study, 32 were required for each complete reproduction of the design (Materials & Methods, Table 3.1 Fractional Factorial Design), which was repeated four times to obtain reliable estimates of factor means. Healthy blood donors between the ages of 18 - 65 years, who fell within the bleeding criteria, were used. The selection of the volunteer blood donors was on a random basis, in that as they presented themselves at the selected bleeding venue and met with the acceptance donation criteria, were used for the study.

The statistical data based on the results of all assays was analyzed by the Institute for Biostatistics of the MRC. The results were supplied on disc, via the computer programme Lotus 123 spreadsheet. The results of all the assays performed are presented in Appendix II.

A complete exposition of all the statistical results for the study is presented in

Appendix III. A significance level of 0.05 was used in the statistical analysis. Samples were drawn at three stages, from the time the donor was bled, until the cryoprecipitate was complete. The first sample was taken from the donor prior to donation and was labelled the PRE SAMPLE. The second was taken after the plasma and red blood cells were separated (V6), and labelled POST SAMPLE . The third sample was the cryoprecipitate itself, which was the final finished product and was labelled PRODUCTION SAMPLE.

Unfortunately due to choice of the factorial design, design numbers V6 and V14 were identical (refer to Table 3.1, page 44). Problems were experienced in the interpretation of certain of the results. When applied to the data, V6 and V14 gave the same value and as a consequence the effect for V6 is always identical to the effect of V14. The factors are said to be confounded. As a result it was not always known whether the value obtained, represents the effect of V6 or V14, or the effect of both. Some of the variables and the interactions, in the production phase are confounded with the variables and interactions from the pre - post phase. This would not have caused any problems if one could have assumed that there were no carry-over effects. In other words, if the variables in the pre-post phase had no influence on the data in the production phase.

The levels of the variables are indicated by (+ 1) and (-1) , coinciding with high and low factors reflected in Table 3.2 (page 45). In the analysis the (+) and (-) factor levels indicate which data are added and which data are subtracted, to produce a measure of effect of a factor. Effect is the influence of that variable on the factor

level (+1) or factor level (-1), being measured. Due to the nature of the design, in order to recognize significant effects, the results are expressed as a table of means with a standard error (SE) of marginal means and SE of body of table means for each variable. A table of means for the interactions between all the variables for FVIII, Fibrinogen and Von Willebrand Factor assays, are displayed. For the pH determination, plasma proteins and electrolytes, tables for only the significant variables are displayed. Graphs were not used to illustrate the results, since the factors have only two levels, and thus not very illuminating.

In the production cycle, for all the assays performed, variables V7, V9 and V11 are free of confounding. The same applies to interactions V7 by V13, V9 by V13 and V11 by V13.

For the purpose of the statistical analysis, all the PRE results were used to set the baseline levels, in each assay performed. The POST results were then analyzed against the PRE results and the outcome displayed in Appendix III as "POST - PRE" data. For the analysis of the PRODUCTION results, the POST results were used as the baseline levels and the PRODUCTION results analyzed against them. The outcome of these results are shown in Appendix III, as "PRODUCTION" data. For clarity in the discussion of the results to follow, the "POST - PRE" data will be referred to as STAGE A and the "PRODUCTION" data as STAGE B.

A key for the variables used in the tables is given for easy reference during this chapter.

POST - PRE ANALYSIS STAGE A		PRODUCTION ANALYSIS STAGE B	
V1	Donor Exercise	V7	Thawing Temperature
V2	Blood Group	V8	Freezing Rate
V3	Bleeding Time	V9	Freezing Temperature
V4	Anticoagulant	V10	Freezing Technique
V5	Pre-process Delay	V11	Centrifugation
V6	Pre-process Storage	V12	Holding Time
		V13	Thawing Technique
		V14	Precipitation

4.1 FACTOR VIII

Using the data from Appendix III, under Factor VIII - Post/Pre, the standard errors (SE) for the means in Tables 4.1 to 4.15 in Stage A are as follows:

SE of marginal means : 0.027
SE of body of table means : 0.039

Least significant difference (5%) between body of table means: 0.108

The table of means for each of the interactions for variables V1 to V6 (Stage A) are summarized below in Tables 4.1 to 4.15. The interpretation of the significant variables and two-factor interactions are addressed at the end of Stage A.

		V2		
		(-1)	(+1)	Means
V1	(-1)	0.081	0.069	0.075
	(+1)	0.071	0.045	0.058
Means		0.076	0.058	0.067

Table 4.1 *Donor exercise levels (V1) vs Donor blood group (V2)*

		V3		
		(-1)	(+1)	Means
V1	(-1)	0.058	0.092	0.075
	(+1)	0.044	0.072	0.058
Means		0.051	0.082	0.067

Table 4.2 *Donor exercise levels (V1) vs Bleeding time (V3)*

		V3		
		(-1)	(+1)	Means
V2	(-1)	0.101	0.051	0.076
	(+1)	0.001	0.113	0.057
Means		0.051	0.082	0.067

Table 4.3 *Donor Blood group (V2) vs Bleeding time (V3)*

		V4		
		(-1)	(+1)	Means
V1	(-1)	-0.054	0.204	0.075
	(+1)	0.026	0.090	0.058
Means		-0.014	0.147	0.067

Table 4.4 *Donor exercise levels (V1) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V2	(-1)	-0.028	0.179	0.076
	(+1)	-0.000	0.115	0.057
Means		-0.014	0.147	0.066

Table 4.5 *Donor blood group (V2) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V3	(-1)	-0.025	0.128	0.051
	(+1)	-0.003	0.167	0.082
Means		-0.014	0.147	0.067

Table 4.6 *Bleeding time (V3) vs Anticoagulant (V4)*

		V5		
		(-1)	(+1)	Means
V1	(-1)	-0.060	0.210	0.075
	(+1)	-0.053	0.169	0.058
Means		-0.056	0.189	0.067

Table 4.7 *Donor exercise levels (V1) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V2	(-1)	-0.039	0.191	0.076
	(+1)	-0.073	0.187	0.057
Means		-0.056	0.189	0.067

Table 4.8 *Donor blood group (V2) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V3	(-1)	-0.060	0.163	0.051
	(+1)	-0.052	0.216	0.082
Means		-0.056	0.189	0.067

Table 4.9 *Bleeding time (V3) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V4	(-1)	-0.133	0.105	-0.014
	(+1)	0.021	0.273	0.147
Means		-0.056	0.189	0.067

Table 4.10 *Anticoagulant (V4) vs Pre-process delay (V5)*

		V6		
		(-1)	(+1)	Means
V1	(-1)	0.140	0.010	0.075
	(+1)	0.089	0.067	0.058
Means		0.115	0.018	0.067

Table 4.11 *Donor exercise levels (V1) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V2	(-1)	0.140	0.012	0.076
	(+1)	0.090	0.025	0.058
Means		0.115	0.018	0.067

Table 4.12 *Donor blood group (V2) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V3	(-1)	0.072	0.031	0.051
	(+1)	0.158	0.006	0.082
Means		0.115	0.018	0.067

Table 4.13 *Bleeding time (V3) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V4	(-1)	0.017	-0.045	-0.014
	(+1)	0.212	0.082	0.147
Means		0.115	0.018	0.067

Table 4.14 *Anticoagulant (V4) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V5	(-1)	0.024	-0.136	-0.056
	(+1)	0.206	0.173	0.189
Means		0.115	0.018	0.067

Table 4.15 *Pre-process delay (V5) vs Pre-process storage (V6)*

The results showed that the significant variables in the pre - post phase in the preparation of FVIII were variables V4 (p = 0.0001), being the type of anticoagulant used, V5 (p = 0.0001) being the pre-process time delay and V6 (p = 0.0142) being the pre-process storage temperature.

V4 was the type of anticoagulant, which yielded a higher value for factor level (+1), which was the anticoagulant CPD, than over factor level (-1), being heparin.

V5 was the variable controlling the pre-processing time delay and here once again, the factor level (+1) being a processing time under 6 hours, gave a higher value than the factor level (-1) which was a processing delay of 12 hours. V6 showed the opposite effect in which the factor level (-1), being a storage temperature of 20°C prior to processing, gave a significantly higher value than the factor level (+1), which was a storage temperature of 4°C. In addition there were two significant interactions, that in Table 4.3 and Table 4.4.

Table 4.3 reflects a significant interaction between the donor blood group (V2) and the bleeding time (V3) ($p = 0.0376$). A significant interaction indicates that the difference between the levels of one factor, is not the same at each level of the second factor. For example, in Table 4.3 the difference between V3 at factor level (-1) of V2 were 0.051 - 0.101 = -0.050, whilst at factor level (+1) of V2, the difference was 0.113 - 0.001 = 0.112. This is a difference which is to be compared with the value 0.108. The analysis indicated that the difference of these differences is 0.112 - (-0.050) = 0.162, which was significant and measures an interaction. It can be seen that the effect of variable V3 was not consistent over the levels of variable V2, when we measured the effect of a factor by the difference in the variable levels. Equivalently, it could be said that the effect of variable V2 was not consistent over the factor levels of V3, and the difference in the two-factor levels for variable V2 was different for each factor level of variable V3. In practical terms, if a high measurement of FVIII was to be achieved, then as far as variables V2 and V3 were concerned, both variables at (-1) factor level or both at (+1) factor level were not a viable option. The ideal combination would be

one of two options: Group A donors bled in a bleeding time of longer than 8 minutes or other blood groups, other than A, bled in under 8 minutes.

The only other significant interaction in this phase was the association of donor exercise levels (V1) with the type of anticoagulant used (V4), presented in Table 4.4 ($p = 0.0136$). This table shows that at level (-1) of V1, level (+1) of V4 was significantly greater than level (-1) of V4. At level (+1) of V1 there was no significant difference between the factor levels of V4. Clearly the combination of (-1) of V4, CPD anticoagulant and level (-1) of V1 with no degree of donor exercise, produced the highest value of FVIII.

Using the data from Appendix III, under Factor VIII - Production, the standard errors SE for the means in Tables 4.16 to 4.28 in Stage B are as follows:-

SE of marginal means : 1.069
SE of body of table means : 1.511

Least significant difference (5%) between body of table means: 5.92

The tables for Stage B, the production phase, were first transcribed and thereafter the interpretation of the data was outlined.

		V11		
		(-1)	(+1)	Means
V7	(-1)	10.85	10.22	10.53
	(+1)	11.51	13.00	12.26
Means		11.18	11.61	11.40

Table 4.16 *Thawing temperature (V7) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V8	(-1)	11.76	10.38	11.07
	(+1)	10.59	12.89	11.72
Means		11.18	11.61	11.40

Table 4.17 *Freezing rate (V8) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V9	(-1)	8.61	10.63	9.62
	(+1)	13.75	12.59	13.17
Means		11.18	11.61	11.40

Table 4.18 *Freezing temperature (V9) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V10	(-1)	10.27	9.70	9.99
	(+1)	12.09	13.53	12.81
Means		11.18	11.61	11.40

Table 4.19 *Freezing technique (V10) vs Centrifugation (V11)*

		V12		
		(-1)	(+1)	Means
V11	(-1)	10.77	11.59	11.18
	(+1)	11.56	11.66	11.61
Means		11.16	11.63	11.40

Table 4.20 *Centrifugation (V11) vs Holding time (V12)*

		V13		
		(-1)	(+1)	Means
V7	(-1)	9.59	11.48	10.53
	(+1)	12.50	12.01	12.26
Means		11.05	11.75	11.40

Table 4.21 *Thawing temperature (V7) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V8	(-1)	10.80	11.35	11.07
	(+1)	11.29	12.15	11.72
Means		11.05	11.76	11.40

Table 4.22 *Freezing rate (V8) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V9	(-1)	9.71	9.53	9.62
	(+1)	12.38	13.96	13.17
Means		11.05	11.75	11.40

Table 4.23 *Freezing Temperature (V9) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V10	(-1)	9.38	10.60	9.99
	(+1)	12.72	12.90	12.81
Means		11.05	11.75	11.40

Table 4.24 *Freezing technique (V10) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V11	(-1)	11.28	11.08	11.18
	(+1)	10.81	12.41	11.61
Means		11.05	11.75	11.04

Table 4.25 *Centrifugation (V11) vs Thawing Technique (V13)*

		V13		
		(-1)	(+1)	Means
V12	(-1)	13.98	8.35	11.16
	(+1)	8.11	15.14	11.63
Means		11.05	11.75	11.40

Table 4.26 *Holding Time (V12) vs Thawing technique (V13)*

		V14		
		(-1)	(+1)	Means
V11	(-1)	9.38	12.97	11.18
	(+1)	11.24	11.98	11.61
Means		10.31	12.48	11.40

Table 4.27 *Centrifugation (V11) vs Precipitation (V14)*

		V14		
		(-1)	(+1)	Means
V13	(-1)	9.46	12.63	11.05
	(+1)	11.17	12.32	11.75
Means		10.31	12.48	11.40

Table 4.28 *Thawing technique (V13) vs Precipitation (V14)*

Variable V9, that of freezing temperature, proved to be significant in this FVIII assay ($p = 0.0207$). The level (+1) produced a significantly higher value of 13.17 than level (-1) with a value of 9.62.

In the assay results, a large outlier of 51.09 U/ml had an influence on the results of variable V10, freezing technique. There was some indication of a difference between the levels of V10 ($p = 0.0696$), freezing in either an alcohol/dry ice bath, or freezing in the vapour of a liquid nitrogen tank. The variable was confounded, so the significance is uncertain, but at the same time the difference was less marked when the outlier was omitted ($p = 0.1159$).

There was a confounded interaction evident in Table 4.26 with the interaction of holding time (V12) versus thawing technique (V13). Due to the confounding by V4, the type of anticoagulant used, the effect was uncertain but the significance was possibly due to the type of thawing technique used ($p = 0.0001$).

4.2 FIBRINOGEN

Using the data from Appendix III, under Fibrinogen Post/Pre, the standard errors (SE) for the means in Tables 4.29 to 4.43 in Stage A are as follows:-

SE of marginal means : 0.050
SE of body of table means : 0.071
Least significant difference (5%) between body of table means: 0.199

The table of means for variables V1 to V6 for the fibrinogen assay are given in Tables 4.29 to 4.43. Significant variables and their two-factor interactions are explained at the end of the section.

		V2		
		(-1)	(+1)	Means
V1	(-1)	-0.195	-0.069	-0.132
	(+1)	-0.098	-0.053	-0.075
Means		-0.147	0.060	-0.103

Table 4.29 *Donor exercise levels (V1) vs Donor blood group (V2)*

		V3		
		(-1)	(+1)	Means
V1	(-1)	-0.183	-0.080	-0.131
	(+1)	-0.094	-0.056	-0.075
Means		-0.139	-0.068	-0.103

Table 4.30 *Donor exercise levels (V1) vs Bleeding time (V3)*

		V3		
		(-1)	(+1)	Means
V2	(-1)	-0.185	-0.108	-0.147
	(+1)	-0.093	-0.028	-0.060
Means		-0.138	-0.068	-0.103

Table 4.31 *Donor blood group (V2) vs Bleeding time (V3)*

		V4		
		(-1)	(+1)	Means
V1	(-1)	-0.184	-0.080	-0.132
	(+1)	0.008	-0.158	-0.075
Means		-0.088	-0.119	-0.103

Table 4.32 *Donor exercise levels (V1) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V2	(-1)	-0.154	-0.139	-0.147
	(+1)	-0.023	-0.098	-0.060
Means		-0.088	-0.119	-0.103

Table 4.33 *Donor blood group (V2) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V3	(-1)	-0.132	-0.146	-0.139
	(+1)	-0.045	-0.091	-0.068
Means		-0.088	-0.119	-0.103

Table 4.34 *Bleeding time (V3) vs Anticoagulant (V4)*

		V5		
		(-1)	(+1)	Means
V1	(-1)	-0.155	-0.108	-0.132
	(+1)	-0.071	-0.079	-0.075
Means		-0.113	-0.094	-0.103

Table 4.35 *Donor exercise levels (V1) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V2	(-1)	-0.196	-0.098	-0.147
	(+1)	-0.030	-0.091	-0.060
Means		-0.113	-0.094	-0.103

Table 4.36 *Donor blood group (V2) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V3	(-1)	-0.113	-0.165	-0.139
	(+1)	-0.113	-0.023	-0.068
Means		-0.113	-0.094	-0.103

Table 4.37 *Bleeding time (V3) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V4	(-1)	0.024	-0.200	-0.088
	(+1)	-0.250	0.012	-0.119
Means		-0.113	-0.094	-0.103

Table 4.38 *Anticoagulant (V4) vs Pre-process delay (V5)*

		V6		
		(-1)	(+1)	Means
V1	(-1)	-0.002	-0.261	-0.131
	(+1)	-0.058	-0.093	-0.075
Means		-0.030	-0.177	-0.103

Table 4.39 *Donor exercise levels (V1) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V2	(-1)	-0.031	-0.262	-0.147
	(+1)	-0.029	-0.092	-0.060
Means		-0.030	-0.177	-0.103

Table 4.40 *Donor blood group (V2) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V3	(-1)	-0.031	-0.247	-0.139
	(+1)	-0.028	-0.108	-0.068
Means		-0.030	-0.177	-0.103

Table 4.41 *Bleeding time (V3) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V4	(-1)	0.005	-0.181	-0.088
	(+1)	-0.064	-0.173	-0.119
Means		-0.030	-0.177	-0.103

Table 4.42 *Anticoagulant (V4) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V5	(-1)	-0.038	-0.188	-0.113
	(+1)	-0.022	-0.166	-0.094
Means		-0.030	-0.177	-0.103

Table 4.43 *Pre-process delay (V5) vs Pre-process storage (V6)*

Only one variable, V6 was significant independently of the other variables in this phase, that being the storage temperature prior to the separation of the red cells and plasma ($p = 0.0402$). The factor (+1) level, the temperature of 4°C produced a slightly lower value than the factor level (-1) with a temperature of 20°C.

Only one interaction, that in Table 4.38, was significant ($p = 0.009$) and another in Table 4.32 was close to significance ($p = 0.060$).

n Table 4.38, the highest value was produced when the factors were at the same evels, and the lowest value when they were at different factor levels. The significant interaction indicated in this table was the combination of the type of anticoagulant (V4) in conjunction with a time delay prior to processing (V5).

n Table 4.32, the interaction between donor exercise levels (V1) and the type of anticoagulant used (V4), was close to significance. In the table of means for this nteraction, the reverse was observed. The lowest value was produced when the factors were at the same levels, and the highest value when the factors were at different levels. Thus the lowest level was obtained when the CPD anticoagulant was used with exercised donors, and heparin with non exercised blood donors. The high level resulted when CPD was used on non exercised donors and heparin on exercised blood donors.

Using the data from Appendix III, under Fibrinogen Production, the standard errors SE) for the means in Tables 4.44 to 4.56 for Stage B are as follows:-

SE of marginal means : 0.54
SE of body of table means : 0.77

Least significant difference (5%) between body of table means: 2.16

nteractions and significant variables are discussed at the completion of the tabular display. Of importance in this assay is that there are no carry-over effects from the pre - post variables in Stage A to the production Stage B.

		V11		
		(-1)	(+1)	Means
V7	(-1)	6.88	8.48	7.68
	(+1)	9.30	9.66	9.48
Means		8.09	9.07	8.58

Table 4.44 *Thawing temperature (V7) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V8	(-1)	7.17	8.91	8.04
	(+1)	9.00	9.23	9.12
Means		8.09	9.07	8.58

Table 4.45 *Freezing rate (V8) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V9	(-1)	6.47	8.30	7.39
	(+1)	9.70	9.84	9.77
Means		8.09	9.07	8.58

Table 4.46 *Freezing temperature (V9) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V10	(-1)	7.34	7.53	7.44
	(+1)	8.83	10.61	9.72
Means		8.09	9.07	8.58

Table 4.47 *Freezing technique (V10) vs Centrifugation (V11)*

		V12		
		(-1)	(+1)	Means
V11	(-1)	7.42	8.76	8.09
	(+1)	8.85	9.29	9.07
Means		8.13	9.02	8.58

Table 4.48 *Centrifugation (V11) vs Holding time (V12)*

		V13		
		(-1)	(+1)	Means
V7	(-1)	7.78	7.57	7.68
	(+1)	9.79	9.17	9.48
Means		8.79	8.37	8.58

Table 4.49 *Thawing temperature (V7) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V8	(-1)	8.19	7.88	8.04
	(+1)	9.38	8.86	9.12
Means		8.79	8.37	8.58

Table 4.50 *Freezing rate (V8) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V9	(-1)	6.38	8.39	7.39
	(+1)	11.19	8.35	9.77
Means		8.79	8.37	8.58

Table 4.51 *Freezing Temperature (V9) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V10	(-1)	7.39	7.48	7.44
	(+1)	10.18	9.26	9.72
Means		8.79	8.37	8.58

Table 4.52 *Freezing technique (V10) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V11	(-1)	8.87	7.31	8.09
	(+1)	8.71	9.44	9.07
Means		8.79	8.37	8.58

Table 4.53 *Centrifugation (V11) vs Thawing Technique (V13)*

		V13		
		(-1)	(+1)	Means
V12	(-1)	8.27	8.00	8.13
	(+1)	9.31	8.74	9.02
Means		8.79	8.37	8.58

Table 4.54 *Holding Time (V12) vs Thawing technique (V13)*

		V14		
		(-1)	(+1)	Means
V11	(-1)	6.59	9.58	8.09
	(+1)	8.82	9.32	9.07
Means		7.71	9.45	8.58

Table 4.55 *Centrifugation (V11) vs Precipitation (V14)*

		V14		
		(-1)	(+1)	Means
V13	(-1)	7.41	10.16	8.79
	(+1)	8.00	8.74	8.37
Means		7.71	9.45	8.58

Table 4.56 *Thawing technique (V13) vs Precipitation (V14)*

The variables V7 (thawing temperature p = 0.0211), V9 (freezing temperature p = 0.0025)), V10 (freezing technique p = 0.0038) and V14 (precipitation p = 0.0255), were all significant. Nevertheless, V10 and V14 were confounded. In each case, level (+1) had a significantly higher value than level (-1). To explain further, the thawing temperature at 4°C, the freezing temperature at >-60°C, the freezing technique in alcohol/dry ice and precipitation by means of a second spin, all produced a higher value, but due to the confounding effect of V10 and V14, is difficult to comment on their relevance.

The interaction between the freezing temperature (V9) and the thawing technique (V13) in Table 4.51, was significant and was not confounded (p = 0.0021). Although the freezing temperature was significant when used in combination with the thawing technique, it is only at the factor level (-1) of V13 that V9 showed significance. The factor level (-1) of the thawing technique was that of the plasma packs placed on a moving rack in a water bath of constant water. Therefore the freezing temperature only became relevant under this thawing condition, to produce notable fibrinogen results.

4.3 VON WILLERBRAND FACTOR

Using the data from Appendix III, under vWF Post/Pre, the standard errors (SE) for the means in Tables 4.57 to 4.71 in Stage A are as follows:

SE of marginal means : 5.07
SE of body of table means : 7.18

Least significant difference (5%) between body of table means: 20.13

The tables of means for variables V1 to V6 for vWF are shown in the Tables 4.57 to 4.71. The significant variables and their two-factor interactions are discussed at the end of the section.

		V2		
		(-1)	(+1)	Means
V1	(-1)	4.03	-11.17	-3.57
	(+1)	-5.14	1.20	-1.97
Means		-0.55	- 4.98	-2.77

Table 4.57 *Donor exercise levels (V1) vs Donor blood group (V2)*

		V3		
		(-1)	(+1)	Means
V1	(-1)	-15.38	8.23	-3.57
	(+1)	- 2.17	-1.77	-1.97
Means		-8.77	3.23	-2.77

Table 4.58 *Donor exercise levels (V1) vs Bleeding time (V3)*

		V3		
		(-1)	(+1)	Means
V2	(-1)	-8.92	7.81	-0.55
	(+1)	-8.63	-1.34	-4.98
Means		-8.77	3.23	-2.77

Table 4.59 *Donor blood group (V2) vs Bleeding time (V3)*

		V4		
		(-1)	(+1)	Means
V1	(-1)	1.17	-8.31	-3.57
	(+1)	-7.55	3.61	-1.97
Means		-3.19	-2.35	-2.77

Table 4.60 *Donor exercise levels (V1) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V2	(-1)	-1.23	0.13	-0.55
	(+1)	-5.14	-4.83	-4.98
Means		-3.19	-2.35	-2.77

Table 4.61 *Donor blood group (V2) vs Anticoagulant (V4)*

		V4		
		(-1)	(+1)	Means
V3	(-1)	-9.64	-7.91	-8.77
	(+1)	3.27	3.20	3.23
Means		-3.19	-2.35	-2.77

Table 4.62 *Bleeding time (V3) vs Anticoagulant (V4)*

		V5		
		(-1)	(+1)	Means
V1	(-1)	-2.66	-4.48	-3.57
	(+1)	-8.33	4.39	-1.97
Means		-5.49	-0.05	-2.77

Table 4.63 *Donor exercise levels (V1) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V2	(-1)	-9.74	8.63	-0.55
	(+1)	-1.25	-8.72	-4.98
Means		-5.49	-0.05	-2.77

Table 4.64 *Donor blood group (V2) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V3	(-1)	-19.52	1.97	-8.77
	(+1)	8.53	-2.06	3.23
Means		-5.49	-0.05	-2.77

Table 4.65 *Bleeding time (V3) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V4	(-1)	-0.133	0.105	-3.19
	(+1)	0.021	0.273	-2.35
Means		-5.49	-0.05	-2.77

Table 4.66 *Anticoagulant (V4) vs Pre-process delay (V5)*

		V6		
		(-1)	(+1)	Means
V1	(-1)	4.50	-11.64	-3.57
	(+1)	-8.88	4.94	-1.97
Means		-2.19	-3.35	-2.77

Table 4.67 *Donor exercise levels (V1) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V2	(-1)	1.84	-2.95	-0.55
	(+1)	-6.22	-3.75	-4.98
Means		-2.19	-3.35	-2.77

Table 4.68 *Donor blood group (V2) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V3	(-1)	-10.97	-6.58	-8.77
	(+1)	6.59	-0.13	3.23
Means		-2.19	-3.35	-2.77

Table 4.69 *Bleeding time (V3) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V4	(-1)	0.188	-6.56	-3.19
	(+1)	-4.56	-0.14	-2.35
Means		-2.19	-3.35	-2.77

Table 4.70 *Anticoagulant (V4) vs Pre-process storage (V6)*

		V6		
		(-1)	(+1)	Means
V5	(-1)	-3.47	-7.52	-5.49
	(+1)	-0.91	0.81	-0.05
Means		-2.19	-3.35	-2.77

Table 4.71 *Pre-process delay (V5) vs Pre-process storage (V6)*

None of the variables V1 to V6 in Stage A, Tables 4.57 - 4.71, in this assay were significant when viewed individually. This then indicated that either the factor level (-1) or (+1) of each variable, may have been chosen. However, two interactions were significant, one in Table 4.65 and the other in Table 4.67. The interaction in Table 4.64 was close to significance ($p = 0.0749$).

In the table of means for Table 4.65, which involves the variables relating to bleeding time (V3) and pre-process delay (V5), the interaction effect was clear ($p = 0.0276$). If one were looking for the factor level of each variable separately which produced the highest value, then the level (+1) for each variable would have been selected. However, Table 4.65 indicated that the level (-1) should be taken for V5, if V3 was taken at level (+1). In other words a pre-process delay for 12 hours in combination with a bleeding time of less than 8 minutes was significant.

In the table of means for Table 4.67, the interaction between donor exercise levels (V1) and pre-process storage temperatures (V6) was also significant ($p = 0.0394$). There was very little difference between the factor levels of each variable when

considered separately, but in combination, a high value was obtained when the factors were at the same level (-1) for V1 and V6, or level (+1) factor for V1 and V6. This implies that, non exercised blood donors produced a high value when their blood was stored at 20°C prior to processing, or exercised blood donors produced a high value when their blood was stored at 4°C.

There was an indication of an interaction in Table 4.64 between the donor blood group (V2) *and the pre-process time delay prior to processing* (V5), *in that it* approached significance ($p = 0.0749$). In the table of means the marginal mean (5.07) suggested that the lowest value was obtained with factor level (-1) of V5 and factor level (+1) of V2, but the table indicated that a low level value was obtained when the variables were at the same factor levels. The interpretation being that Group A blood donors, in conjunction with a pre-process delay of less than 6 hours, was meaningful as well as when other blood groups (other than Group A) were linked with a pre-process delay of 12 hours.

Using the data from Appendix III, under von Willebrand Factor Production, the standard errors (SE) for the means in Tables 4.72 to 4.84 in Stage B are as follows:-

SE of marginal means : 5.42
SE of body of table means : 7.66

Least significant difference (5%) between body of table means: 21.23

The tables are first displayed and then explanation of the significant variables and their two-factor interactions are addressed.

		V11		
		(-1)	(+1)	Means
V7	(-1)	-38.03	-17.27	-27.65
	(+1)	-27.21	-15.42	-21.32
Means		-32.62	-16.35	-24.49

Table 4.72 *Thawing temperature (V7) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V8	(-1)	-35.94	-14.33	-25.14
	(+1)	-29.30	-18.36	-23.83
Means		-32.62	-16.35	-24.49

Table 4.73 *Freezing rate (V8) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V9	(-1)	-46.38	-28.55	-37.47
	(+1)	-18.86	- 4.14	-11.50
Means		-32.62	-16.35	-24.49

Table 4.74 *Freezing temperature (V9) vs Centrifugation (V11)*

		V11		
		(-1)	(+1)	Means
V10	(-1)	-40.11	-30.79	-35.45
	(+1)	-25.13	- 1.91	-13.52
Means		-32.62	-16.35	-24.49

Table 4.75 *Freezing technique (V10) vs Centrifugation (V11)*

		V12		
		(-1)	(+1)	Means
V11	(-1)	-40.91	-24.33	-32.62
	(+1)	-15.80	-16.89	-16.35
Means		-28.36	-20.61	-24.49

Table 4.76 *Centrifugation (V11) vs Holding time (V12)*

		V13		
		(-1)	(+1)	Means
V7	(-1)	-28.44	-26.86	-27.65
	(+1)	-19.22	-23.41	-21.32
Means		-23.83	-25.14	-24.49

Table 4.77 *Thawing temperature (V7) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V8	(-1)	-27.51	-22.77	-25.14
	(+1)	-20.16	-27.51	-23.83
Means		-23.83	-25.14	-24.49

Table 4.78 *Freezing rate (V8) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V9	(-1)	-33.82	-41.12	-37.47
	(+1)	-13.84	- 9.16	-11.50
Means		-23.83	-25.14	-24.49

Table 4.79 *Freezing Temperature (V9) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V10	(-1)	-35.00	-35.89	-35.45
	(+1)	-12.66	-14.38	-13.52
Means		-23.83	-25.14	-24.49

Table 4.80 *Freezing technique (V10) vs Thawing technique (V13)*

		V13		
		(-1)	(+1)	Means
V11	(-1)	-27.97	-37.27	-32.62
	(+1)	-19.69	-13.00	-16.35
Means		-23.83	-25.14	-24.49

Table 4.81 *Centrifugation (V11) vs Thawing Technique (V13)*

		V13		
		(-1)	(+1)	Means
V12	(-1)	-18.85	-37.87	-28.36
	(+1)	-28.81	-12.41	-20.61
Means		-23.83	-25.14	-24.49

Table 4.82 *Holding Time (V12) vs Thawing technique (V13)*

		V14		
		(-1)	(+1)	Means
V11	(-1)	-37.08	-28.16	-32.62
	(+1)	-23.04	- 9.66	-16.35
Means		-30.06	-18.91	-24.49

Table 4.83 *Centrifugation (V11) vs Precipitation (V14)*

		V14		
		(-1)	(+1)	Means
V13	(-1)	-30.38	-17.28	-23.83
	(+1)	-29.73	-20.54	-25.14
Means		-30.06	-18.91	-24.49

Table 4.84 *Thawing technique (V13) vs Precipitation (V14)*

In the production phase, variables V9 (p = 0.0010), V10 (p = 0.0051) and V11 (p = 0.0361) were significant. Both V9 and V11 were free of confounding, but V10 was confounded in that it experienced a carry over effect V3/V13. In each example of these variables, the level (+1) had a significantly higher value than the level (-1). As a result, a freezing temperature of >-60°C, a rapid centrifugation of 4733 X g for 25 minutes and a freezing technique in alcohol/dry ice, were identified as production criteria which positively influenced the vWF assay.

The interaction of the holding time (V12) and thawing technique (V13) in Table 4.82, was significant (p = 0.0228), but was confounded with the carry over effect of the anticoagulant V4.

4.4 FIBRIN DEGRADATION PRODUCTS (FDP)

These results were not be analyzed by statistical means as all the samples were tested by the qualitative screening test. Of the 128 samples analyzed, 7 were found to be reactive by means of an agglutination technique. The results are shown

in Appendix II, 6 of the positive results were found in the pre sample taken from the blood donor prior to donation, indicating the presence of abnormal levels of XL-FDP in the plasma and that fibrinolysis was in progress. No semiqualitative tests were carried out on the positive samples because the degree of fibrinolysis was of no relevance at the time.

Of significance was the fact that Design Number 11 whose blood donors were Group A and not exercised, yielded a positive result with Run 1 and 3. In Run 3 Design 11, a positive result was also detected in the post sample (the sample taken prior to the separation of the red cells and plasma), but a negative result obtained in the final product. Despite the evidence of fibrinolysis, the FVIII yield was 18.66 U/ml, however heparin was the anticoagulant used, which could have accounted for the excellent FVIII result. The positive result in the pre sample taken from Design 21 was also from a Group A non exercised donor. The donors were bled over a timeframe of eight weeks, however care was taken to ensure that personnel remained constant, in order to avoid differences in phlebotomy techniques. The positive results obtained, had no influence on the vWF and fibrinogen assay results.

4.5 pH

The pH results ranged between 7.25 - 8.10, indicating that the myriad production variables, did not cause significant changes in pH fluctuations. The statistical assessment demonstrated that during Stage A, prior to separation of the red cells

om the plasma, the storage temperature (V6) had a significant influence on the h ($p = 0.0095$). The interaction of donor exercise levels (V1) and pre-process storage temperature (V6) approached significance ($p = 0.0649$), and the table of means is as follows:

SE of marginal means : 0.026
SE of body of table means : 0.037

Least significant difference (5%) between body of table means: 0.105

		V6		
		(-1)	(+1)	Means
V1	(-1)	0.099	0.267	0.183
	(+1)	0.121	0.150	0.136
Means		0.110	0.208	0.159

Table 4.85 *Donor exercise levels (V1) vs Pre-process storage (V6)*

Variable V6 was significant at level (-1) of V1. There was no significant difference at level (+1). In other words V6, being the pre-process storage temperature, was significant at the (-1) level. This involved a storage temperature of 20°C prior to separation, but not at the (+1) level which was a temperature of 4°C, when joined with V1, which were exercised blood donors.

When examining the data from the final samples, variables V7 (thawing temperature $p = 0.0247$), V8 (freezing rate $p = 0.0032$), V10 (freezing technique $p = 0.0373$) and V14 (precipitation $p = 0.0461$) were all found to be significant as individual variables. However V8, V10 and V14 were confounded. The interaction of the holding time (V12) by the thawing technique (V13), was

significant, but also confounded V4, the type of anticoagulant used (p = 0.0003). The interaction of the freezing technique (V9) by the thawing technique (V13) was significant and free from confounding (p = 0.0079). The table of means for these variables is displayed in Table 4.86 below.

		V13		
		(-1)	(+1)	Means
V9	(-1)	-0.146	-0.062	-0.104
	(+1)	-0.077	-0.123	-0.100
Means		-0.112	-0.092	-0.102

Table 4.86 *Freezing temperature (V9) vs Thawing technique (V13)*

SE of marginal means : 0.017
SE of body of table means : 0.024

Least significant difference (5%) between body of table means: 0.067

The interpretation of this interaction was that the freezing temperature at either -35°C or >-60°C, was meaningful in unification with the thawing technique in which it made no difference whether the water in the thawing bath was circulating or whether it was stationary.

4.6 EVALUATION OF PLASMA PROTEINS AND ELECTROLYTES

The following plasma proteins and electrolytes were measured and evaluated. Each assay was also analyzed by statistical means.

- Magnesium (Mg)
- Total Protein (TP)
- Albumin (ALB)
- Calcium (Ca)
- Alanine Aminotransferase (ALT)
- Sodium (Na)
- Potassium (K)
- Chloride (Cl)

4.6.1 MAGNESIUM

The statistical analysis demonstrated that during Stage A, prior to separation of the red cells from the plasma, that the type of anticoagulant used (V4), had a close to significant ($p = 0.0518$) influence on the magnesium determination. Level (+1), using CPD, produced a higher value than level (-1) which was heparin. The interaction of donor exercise levels (V1) and donor blood group (V2), and the pre-processing time delay (V5) and pre-process storage temperature (V6), were significant ($p = 0.0373$ and 0.0294) and the table of means is as follows:

SE of marginal means : 0.016
SE of body of table means : 0.022

Least significant difference (5%) between body of table means: 0.062

		V2		
		(-1)	(+1)	Means
V1	(-1)	-0.166	-0.093	-0.130
	(+1)	-0.083	-0.103	-0.093
Means		-0.124	-0.098	-0.111

Table 4.87 *Donor exercise levels (V1) vs Donor blood group (V2)*

		V6		
		(-1)	(+1)	Means
V5	(-1)	-0.096	-0.119	-0.107
	(+1)	-0.152	-0.078	-0.115
Means		-0.124	-0.099	-0.111

Table 4.88 *Pre-process delay (V5) vs Pre-process storage (V6)*

In Table 4.87, the smallest value was produced when both the variables were at level (-1). In summary, this occurred when blood donors were not exercised and their blood group was other than Blood Group A. There was no difference between other combinations of the variables.

Table 4.88 demonstrates that the smallest value was created when V5 was at level (-1) and V6 was at level (-1). To expand further, when a pre-processing delay of <6 hours was used in conjunction with a pre-storage temperature of 20°C, the interaction was of notable importance. There was no difference between other associations of the variables.

When the data of the production phase was analyzed for this assay, no effects were found to be significant. The interaction V12 and V13, which was confounded, approached significance (p = 0.0653).

4.6.2 TOTAL PROTEIN

The review of the data for this assay in Stage A of the manufacturing process, brought to light the significance of variables V1, donor exercise levels (p = 0.0358) and V4, type of anticoagulant used (p = 0.0075). In both instances the level (+1) resulted in a higher value than the level (-1), i.e. these were exercised blood donors who were bled into donation packs containing CPD as the anticoagulant.

There was some indication of an interaction between V1 and V2 (p = 0.0903). The data for these factors were skewed and on transformation to normality, the interaction became significant ((p = 0.0410). The table of means for the original data is as follows:

		V2		
		(-1)	(+1)	Means
V1	(-1)	-10.00	-4.38	-7.19
	(+1)	- 2.81	-3.60	-3.20
Means		- 6.41	-3.98	-5.201

Table 4.89 *Donor exercise levels (V1) vs Donor blood group (V2)*

SE of marginal means : 1.32
SE of body of table means : 1.87

Least significant difference (5%) between body of table means: 3.72

In Stage B, one of the results was an outlier in comparison to the other results seen in Appendix II, Total Protein Production Run 4, Sample 15, with a reading of 9.0 g/L. The analysis, with and without this value, produced no significant results, although with the outlier there was some indication of an interaction between V10 and V13, but was also confounded. In the analysis of carry-over effects, there was some indication of V5 having a significant carry over effect. This variable was not confounded.

4.6.3 ALBUMIN

There was evidence of non-normality with the assay results for the data in Stage A of the manufacturing process. Analysis of the transformed data yielded comparable results. Variables V1 ($p = 0.0167$) and V4 ($p = 0.0133$) were significant, being donor exercise levels and type of anticoagulant used. In each case the level (+1) produced a significantly higher value than the (-1) level. Explained in ordinary terms, this involved exercised blood donors being bled into blood packs containing CPD. The same trend was evident for V6, which approached significance ($p = 0.0699$).

The interaction of V3 and V6 also approached significance ($p = 0.0657$) and the table of means is as follows:

		V6		
		(-1)	(+1)	Means
V3	(-1)	-4.12	-4.16	-4.14
	(+1)	-7.25	-3.22	-5.23
Means		-5.69	-3.69	-4.69

Table 4.90 *Bleeding time (V3) vs Pre-process storage (V6)*

SE of marginal means : 0.77
SE of body of table means : 1.09

Least significant difference (5%) between body of table means: 3.06

From the analysis of all the results in the production phase, the data reflected V10 (p = 0.0601) and V12 (p = 0.0558) being close to significance, however both variables were confounded. Four outlier results were recorded. The interaction of V10 and V13 although confounded, was significant (p = 0.0301). In the absence of the 4 outliers, V10 and V12 were no longer near significance, the interaction between V10 and V13 moved to only close to significance. The interaction between V12 and V13, although confounded, then became significant.

4.6.4 CALCIUM

In Stage A of the calcium assay, only the type of anticoagulant used (V4), had a significant effect (p = 0.0023). The level (-1) produced a higher value than the value for level (+1). The level (-1) of V4, involved use of heparin as the anticoagulant. Calcium chloride had to be added to certain of the plasma in which heparin had been added. The indication was that this had an influence on the data.

In Stage B of the manufacturing process, the presence of outliers was once again observed. No reasonable explanation for the cause was able to be established. With the omission of the outliers V10, freezing technique and V14, the precipitation method (confounded) became significant. It was also logical to accept that the interaction between V10 and V13 represented a real effect and was significant. These two variables represent the freezing and thawing techniques.

4.6.5 ALANINE AMINOTRANSFERASE

V4, anticoagulant in Stage A was close to significance (p = 0.0587), with factor level (-1) producing the larger value than (+1) factor level. The (-1) factor level for this factor was heparin as the anticoagulant. Three interactions were significant, V1 by V3, V3 by V5 and V4 by V5.

The table of means for the three interactions is as follows:

SE of marginal means : 1.66
SE of body of table means : 2.35

Least significant difference (5%) between body of table means: 6.58

		V3		
		(-1)	(+1)	Means
V1	(-1)	5.28	1.47	3.38
	(+1)	-0.44	5.09	2.33
Means		2.24	3.28	2.85

Table 4.91 *Donor exercise levels (V1) vs Bleeding time (V3)*

		V5		
		(-1)	(+1)	Means
V3	(-1)	6.81	-1.97	2.42
	(+1)	1.97	4.59	3.28
Means		4.39	1.31	2.85

Table 4.92 *Bleeding time (V3) vs Pre-process delay (V5)*

		V5		
		(-1)	(+1)	Means
V4	(-1)	9.16	1.03	5.09
	(+1)	-0.38	1.59	0.61
Means		4.39	1.31	2.85

Table 4.93 *Anticoagulant (V4) vs Pre-process delay (V5)*

The data for this set of assay results contained four influential outliers. The influence of the outliers is discussed after the dialogue of the data analysis.

The largest value was obtained in Table 4.91 when the variables were on the same (-1) or (+1) factor levels. In other words, exercised blood donors bled within 8 minutes, or alternately non exercised blood donors who were bled in a bleeding time of longer than 8 minutes. The scenario in Table 4.92 was similar to the one above, in that V3 by V5 obtained the highest value when similar levels were observed. A unit of blood bled within 8 minutes, when processed within 6 hours, produced high yields. The reverse, being a bleeding time of longer than 8 minutes processed at 12 hours, also showed favourable results. At factor level (-1) of V4 in Table 4.93, there was a significant difference between the levels of V5, but no

significant difference at level (+1) of V4, being heparin with a time delay before processing of up to 12 hours.

The outliers were found in Appendix II, under alanine aminotransferase Pre Run 4, Number 10; Post Run 1, Number 21; Post Run 2, Number 18 and Post Run 4, Number 14. If these are omitted from the analysis the results change as follows. Variable V4 and interaction V4 by V5 are no longer significant. The interaction of V3 by V5 remains the same, while that of V1 by V3 moves close to significance. V5 pre-process time delay by V6, pre-process storage temperature becomes significant.

The analysis of all the data for Stage B, showed V12 (confounded), V14 (confounded) and the interaction V8 by V13 (confounded), to be significant ($p = 0.0249$). In the absence of the outliers, V14 and the interaction V8 by V13, remained significant. V12, holding time was no longer important. However, V7, thawing temperature which was not confounded by other variables, became significant.

4.6.6 SODIUM

Variables V4 and V6 in Stage A of this assay were both significant ($p = 0.0001$ and $p = 0.0253$). The factor level (+1) of V4, i.e. the use of CPD as the anticoagulant, produced a higher value than the factor level (-1). Similarly for V6,

the pre-process storage temperature of 4°C, had a higher level (+1), than level (-1).

One interaction was significant, that between V1 by V2, with the table of means as follows:

SE of marginal means : 1.78
SE of body of table means : 2.51

Least significant difference (5%) between body of table means: 7.1

		V2		
		(-1)	(+1)	Means
V1	(-1)	31.0	38.9	35.0
	(+1)	40.3	37.5	38.9
Means		35.7	38.2	36.9

Table 4.94 *Donor exercise levels (V1) vs Donor Blood Group (V2)*

A significantly lower value was evident in Table 4.94, when both variables were at level (-1) factors. This implies that blood donors who did not exercise prior to donation, and were of any blood group type (other than Group A) when bled, and their plasma processed into cryoprecipitate, provided a good FVIII yield. No significant difference in values were produced by any of the other combinations. The production data produced no significant effect, although the holding time in V12, which was confounded, was close to significance ($p = 0.0626$). There were a number of outlying values present which could have had an influence on the results, but as there was no justification for their omission, they were included.

4.6.7 **POTASSIUM**

Once again there was evidence of four outlying values. The decision was made to analyze the data with these excluded.

In Stage A, the assay results, when analyzed, indicated three variables as being significant, all independent of each other. They were V3 (p = 0.082), V4 (p = 0.0352) and V5 (p = 0.0001). V3, the factor level (+1) was a bleeding time under 8 minutes, having a greater factor level than (-1). For V4, anticoagulant and V5, pre-process time delay level (-1) was significantly greater than level (+1), meaning that heparin as the choice of anticoagulant used in conjunction with a pre-process delay of 12 hours, proved a suitable combination. The interaction of V5 by V6 was significant (p = 0.0112) and the table of means is as follows:

SE of marginal means (approximately): 0.164
SE of body of table means (approx): 0.236

Least significant difference (5%) between body of table means (approx): 0.662

		V6		
		(-1)	(+1)	Means
V5	(-1)	-0.675	0.124	-0.276
	(+1)	-1.106	-1.503	-1.305
Means		-0.891	-0.690	-0.802

Table 4.95 *Pre-process delay (V5) vs Pre-process storage (V6)*

In Table 4.95, it was observed that the difference in levels of V5 at factor level

-1) of V6, was more than three times the magnitude of the difference at factor level (-1) of V6.

In the examination of the data for Stage B, once again outliers were demonstrated (total of 8), but as with the data of Stage A, these were omitted. No effects were found to be of any importance and no significant interactions were noted. Variables V10 and V14 were confounded, but were of significance, only when the outliers were omitted (p =0, 0314).

4.6.8 CHLORIDE

The data for Stage A for this assay was skewed and required a transformation to normality. The results of the analysis of the transformed data were virtually the same, except the transformation brought some interactions closer to significance. V4, the type of anticoagulant used, was significant (p = 0.0292). V1, V3 and V6 were close to significance. The interactions of V1 by V3 and V3 by V4 were close to significance and the table of means for them is as follows:

SE of marginal means : 1.57
SE of body of table means : 2.26
Least significant difference (5%) between body of table means: 6.2

		V3		
		(-1)	(+1)	Means
V1	(-1)	-23.0	-22.5	-22.8
	(+1)	-14.8	-22.6	-18.7
Means		-18.9	-22.6	2.85

Table 4.96 *Donor exercise levels (V1) vs Bleeding time (V3)*

		V4		
		(-1)	(+1)	Means
V3	(-1)	-19.5	-18.3	-18.9
	(+1)	-26.9	-18.2	-22.6
Means		-23.2	-18.3	-20.7

Table 4.97 *Bleeding time (V3) vs Anticoagulant (V4)*

Table 4.96 reflected a small decrease by the combination of factor level (+1) for V1, and factor level (-1) for V3. No other significant difference between the other combinations were seen. This meant that exercised donors bled into heparin anticoagulant, yielded an important effect. Table 4.97 shows that the greatest decrease was produced by the association of factor level (+1) for V3 and factor level (-1) for V4. No other significant differences between the other combinations were observed. In this case, of relevance, was a bleeding time of under 8 minutes combined with heparin as the anticoagulant.

In the production stage, a number of outliers (11 in total) were noted and the decision was made to omit them. Interactions V8 by V13 (confounded) and V10 by V13, were found to be significant. The freezing rate and freezing technique reflected strong interactions with the technique utilized for the thawing of the cryoprecipitate.

CHAPTER 5

DISCUSSION

Several studies on the activity and stability of FVIII in blood and plasma have been made, in order to obtain a maximum yield of this labile factor in either plasma or cryoprecipitates (Carlebjork *et al.*, 1983). The aim of this study was to determine which variables actively had an effect on the FVIII yield. Blood collection and separation procedures all involve variables which have the potential of activating the coagulation system, thus affecting the yield of FVIII.

During the analysis of the FVIII data, it was found that variable V4, the type of anticoagulant used, was significant. The level of factor (+1) gave the highest value, which was the use of CPD as the primary anticoagulant, rather than adding heparin /calcium chloride combination after the donation. Although the opinion is that anticoagulants with lower levels of citrate are more beneficial in the generation of FVIII (Rock *et al.*, 1988) and that half strength citrate solutions should be utilised (Prowse *et al.*, 1987), in South Africa the registered anticoagulant is CPD, with a citrate strength of 23 mMol. In 1996, a further anticoagulant viz. SAG-M (Sodium; adenine; glucose and mannitol) was registered in South Africa. It was not used for our study, as at the time of registration all the technical work had been completed. Previous studies by Rock *et al.* (1979); Krachmalnikoff and Thomas (1983); Mikaelsson *et al.* (1983) and Cumming *et al.* (1987), all confirmed the beneficial effect of heparin rather than CPD on the stability of plasma FVIII. This effect is due

to the presence of physiological ionised calcium levels in heparinised plasma. In all these studies heparin was the primary anticoagulant, which was not the case in our study due to regulatory implications by the Medicines Control Council (MCC). De Wit *et al.* (1986) on the other hand, found that the use of CPD as the primary anticoagulant was superior than heparin, which correlated well with the findings of our study. The FVIII assay statistical findings in our study indicated better FVIII yields when using CPD rather than heparin.

Variable V5, the pre-processing time delay of < 6 hours and V6 the pre-process storage temperature of 20°C, were both highlighted as significant variables according to the analysis, affecting the FVIII yield in the cryoprecipitate in a positive manner. Rock and Tittley (1979) found FVIII levels were significantly higher when whole blood was stored between 6 and 24 hours at 22°C, than when it was stored at 4°C before separation, Spivey *et al.* (1992) reported similar findings. Hughes *et al.* (1988) found FVIII to be better preserved over a 24 hour period when stored in whole blood at 22°C, than when stored in plasma at 22°C or 4°C, or in whole blood at 4°C, but at the same time, major losses of FVIII occurred after a 6 hour processing delay and were dependent on temperature. FVIII recovery was 100% for 6 hour old plasma and 79% for 18 hour old plasma. Vermeer *et al.* (1976b) demonstrated that the temperature of stored whole blood should not decrease below 10°C, while Allersma *et al.* (1996) reported no significant difference in a processing delay of either 3 hours or 12 - 15 hours provided the temperature was 20°C. The investigation by Pietersz *et al.* (1989) concluded that the effect of rapid cooling to 20 -24°C of whole blood immediately

after collection, using "cooling units" with butane-1,4-diol, prolonged storage for up to 24 hours with a 80% FVIII recovery of the initial value, and provided optimal conditions for blood component preparation. The findings in our study correlate well with the findings of the above authors, and is evident that these two variables, i.e. processing whole blood within six hours which has been stored at 20 - 24°C, are critical variables to which precise attention must be given.

The variable relating to blood groups was not found to be a significant variable on its own. Previous reports by Preston and Barr (1964) and Jeremic *et al.* (1976), found Blood Group A to have a greater influence on the FVIII yield, although they were only investigating ABO blood group status at the time. However, in this study, blood groups (V2), showed a significance with the interaction of bleeding time (V3), specifically using Blood Group A donors, bled in a donation time of longer than 8 minutes. This correlates well with a study by Reiss and Katz (1976), in which they found no difference in platelet concentrate or FVIII quality from blood collections lasting between 8 and 12 minutes. It is suggested that the eight minute bleeding time is restrictive, and that blood collected from non-traumatic and free-flowing phlebotomies of up to 12 minutes be acceptable. The possibility of selecting high value donors such as Group A donors, along with the extended bleeding time, clearly are notable variables for the enhancement of FVIII quality. The interaction donor exercise levels (V1) and type of anticoagulant used (V4), was significant. The interaction showed that when using CPD as the anticoagulant, highest yields of FVIII were achieved when the blood donors were not exercised, which is contrary to the findings in the study by Van Gastel *et al.* (1973) and

Strand *et al.* (1974), who found that the FVIII activity was increased by 40%, when blood donors were exercised. Burka *et al.* (1975), proved exercise will double the measurable FVIII level in the donor's blood. The increase in activity is for a relatively short period, following strenuous muscular exercise, and it could well be that in many instances other variables call for a time delay before processing of the blood, which could affect the outcome of the FVIII yield, because the levels had already decreased. If there were some guarantee that all whole blood which was to be processed into cryoprecipitate could be processed within 2 - 3 hours, it would then be feasible to recommend that blood donors be exercised prior to their blood donation. This would also drastically reduce the number of units of blood available for FVIII production, as the majority of the whole blood is collected by mobile units. Transportation of donated blood at mobile clinics to the processing laboratory can be delayed, and not reach the processing laboratory within the deadline required to obtain the high FVIII level that was present on donation. Failure to meet this requirement makes the exercise stage of the donation procedure for the donors, which needs extra time, a worthless recommendation.

The freezing temperature (V9), proved to be significant in Stage B of the production cycle i.e. those variables involved in the process after the separation of the red cells and plasma. The freezing technique (V10), whether to use alcohol / dry ice baths or liquid nitrogen vapour, showed some indication of significance, but there was the presence of a large outlier (51,09 U/ml), which had an influence on the results, and in addition, the variable was confounded, so the significance is uncertain and for this reason no further consideration was given to its importance.

The interaction of the holding time, V12 (the delay before the final centrifugation) and thawing technique, V13 was significant, but due to confounding the effect was uncertain. Due to the nature of the variables, it was thought the effect was due to the thawing technique used, that of using a thawing water bath in which the water circulates at a fixed temperature, or that of the plasma fixed to moving arms of the water bath, in unmoving water. The freezing temperature was reached in two ways, a fast freeze at >-60°C or a slow freeze at -35°C. This study found that the fast freeze gave results that had higher FVIII yields, which compares favourably with a review by Farrugia and Prowse (1985), who also found the fast freeze was better for optimal yields of FVIII.In addition to the fast freeze, the plasma to be frozen must be of the same volume and compressed between metal plates, to make a thin plasma slab with even density, and placed in ethanol baths where energy transport is most effective (Carlebjork *et al.*, 1986b). Kasper *et al.* (1975) along with Rock and Tittley (1979) found that very low freezing temperatures were unnecessary, however recommendations in later studies, yielded a far superior cryoprecipitate (Carlebjork *et al.*, 1986b).

Many different FVIII assay methods have been used to date. However, the chromogenic Coatest^{R} Factor VIII assay technique is easy to perform, with high precision, and is routinely used in the quality control of cryoprecipitate in many laboratories (Carlebjork *et al.*, 1987), replacing the formerly used one-stage clotting method. It is also in routine use at Western Province Blood Transfusion Service for all quality control FVIII assays on cryoprecipitates and concentrates.

Quantitatively, fibrinogen is the major protein constituent in cryoprecipitate and is an extremely stable plasma protein compared to FVIII (Howard *et al.*, 1991). Factor VIII and fibrinogen are classified together, because of biologic similarities and because their activity is destroyed during the coagulation process (Ness and Perkins, 1980). Despite these similar characteristics, the levels of fibrinogen and FVIII in a healthy blood donor are not necessarily correlated, i.e. a donor with an elevated level of FVIII may have a relatively low level of fibrinogen. The wide range of values cited in the literature, reflects the number of variables that can influence the yield of fibrinogen in cryoprecipitates. Hoffman *et al.* (1987) obtained an average of 100 mg of fibrinogen in their study. Higher values of 250 mg were acquired by Ness and Perkins (1980). Our result of 10.93 g/l correlated better with that of Smit Sibinga *et al.* (1988), who obtained a value of 15 g/l.

The storage temperature (V6) prior to separation of the red cells and plasma at a temperature of 20°C, showed to have a significant effect on the fibrinogen results in this study. In an earlier study by Hoffman *et al.* (1987), fibrinogen was found to be stable during 4 hours of storage at room temperature, whereas Saxena *et al.* (1991) found the fibrinogen content to decrease when whole blood was stored at 1 - 6°C, prior to processing. The type of anticoagulant, especially heparin, played a role in the determination of the higher fibrinogen content in the cryoprecipitate as well as the delay before processing. Individual variables which demonstrated an effect on fibrinogen levels during the production cycle were: a thawing temperature (V7) at 4°C, the freezing temperature (V9) at >-60°C, and a freezing technique (V10) using a combination of alcohol and dry ice and precipitation (V14), by means

of a second centrifugation spin. The interaction between the freezing temperature (V9) and the thawing technique (V13) was significant, with the emphasis on using a thawing water bath in which the packs were attached to a moving arm, and moved through the water which remained constant. As long as the plasma was frozen, the temperature at which this was carried out was immaterial.

The influence of the variables on the data for von Willebrand Factor, could not be referenced to any work done previously, as nobody has looked at the influence of the production variables on vWF. Variables which influence the outcome of the Von Willebrand Factor assay, prior to the separation of the red cells and plasma are: the bleeding time of the donor, the exercise level of the donor, the delay before separation, and the temperature at which whole blood was stored at before the removal of plasma. These variables were not of importance individually, but only became relevant when they interacted with each other. A donation time (V3) of under 8 minutes was consequential, only if the whole blood was reserved for 12 hours (V5) before processing. Hughes *et al.* (1988), found that the ratios of FVIII to vWF were 1.0 in both plasma and cryoprecipitate, stored for 6 hours prior to further processing, and were 0.7 : 0.5 in 18-hour old plasma.

If blood donors are not requested to exercise (V1) prior to donation, this would only be significant if their blood were stored at 20°C (V6) before separation. Conversely, the opposite is also important. If whole blood is to be cooled to 4°C prior to separation, the blood donors should exercise prior to the donation. Most cryoprecipitate production units usually do not request their donors to exercise

before donation, but attempt to cool the whole blood as much as possible before the plasma is removed, two procedures which seem acceptable on their own, but according to our study data, do not result in adequate vWF yields. The interaction between the blood group of the donor and the delay before separation of the plasma and red cells, was only close to significance ($p = 0.0749$), and for this reason did not warrant any relevance to the outcome of the study. In the next phase of the production cycle three individual variables became critical. The centrifugation speed to separate the red cells and plasma at 4733 x g (V11), followed by freezing the plasma (V9) at >-60°C in alcohol/dry ice freezing bath (V1), were of particular significance on the vWF data. The freezing technique experienced a confounded effect and could not be considered as relevant. The interaction of the thawing technique (V13) and time delay (V12), before finally snap-freezing the cryoprecipitate, was significant, but was confounded by the carry over effect of the type of anticoagulant used, and it was difficult to derive a final conclusion on these two variables.

The plasma concentration of cross-linked fibrin degradation products (FDP containing the D-dimer domain) in normal donors is usually less than 0.25 μg/ml, so the pre starting sample from donors prior to their donation is expected to give a negative result. Concentrations of 0.25 μg/ml FDP or more, will cause agglutination with the Dimertest^{R} Latex assay. Elevated D-dimer levels indicate the degree of coagulation and fibrinolytic activation (Sato *et al.*, 1995). A plasma screening test for FDP is of diagnostic value in disseminated intravascular coagulation (DIC), acute vascular diseases and other thrombotic episodes. The

amount of FDP detected in a sample will depend on several factors, such as the degree of coagulation, the rate of cross-linked fibrin formation, and time elapsed prior to testing. The D-dimer latex assay provides a sensitive tool to study the events leading to the production of fibrin from fibrinogen and activation of FVIII. This test, when performed on donor plasma, can provide insight into the variation of haemostatic parameters on individual donors prior to donation. The results obtained in our study indicate that in most instances no activation of FVIII occurs, during blood donation. If activation were to transpire, it would occur initially as a result of improper insertion of the needle into the vein, or when the blood is first drawn through the tubing into the pack (Carlebjork *et al.*, 1983). Of further importance is the initial mixing of the blood and anticoagulant, and personnel should be instructed about optimal mixing procedures and its influence on the FVIII present in the plasma. Care in stripping the donation line is also a critical part of the donation procedure, failure in doing so could cause small clots to form, an indication that fibrinolysis is under way and once again affect the quality of FVIII yield.

6 Of the 7 positive results were identified on pre donation samples taken prior to donating the unit of blood. The reason for this could have been caused by inferior phlebotomy techniques, an unstable haemostatic condition present in the donor which had passed undiagnosed, or inadequate mixing with the anticoagulant after the sample had been drawn. As the same phlebotomist was used throughout the study, it would be fair to eliminate a reason as being inferior phlebotomy technique. The unstable haemostatic condition is unlikely given the good health of the blood

donors, however FDP levels can sometimes be elevated during menstruation (Carlebjork *et al.*, 1986a). If this test was introduced as a routine test for all donors prior to donation, those donors presenting a positive test result due to a haemostatic condition, could be referred for further treatment and eliminated from donation. In this way, quality plasma for cryoprecipitate production could be sourced.

Factor VIII is stable in a narrow pH range around 7.0, thereafter it dissociates reversibly in the presence of high salt concentrations (International Forum, 1983). Pool in 1967 showed that there was no difference in FVIII recoveries in cryoprecipitate prepared from plasma with pH values between 6.0 and 8.0. The pH results in our study, using either plasma collected into CPD plus heparin, or into CPD alone, ranged between pH 7.25 and 8.10 and correlated well with the outcome reported by Pool (1967). The design samples whose plasma had heparin added, had a pH range of pH 7.9 to 8.10 whereas the plasma containing only CPD had a range of pH 7.25 to 7.8. There has been no reported significance in the relationship between the pH of the pre donor plasma and the pH during the production cycle (Shanberge *et al.*, 1972). The pre storage temperature (V6), had a significant influence on the pH determination.

The interaction of the pre storage temperature (V6), in particular that of 20°C, had some influence on the pH determination, when blood donors who had been exercised (V1) were used. This relates well to the FVIII analysis, in which the 20°C pre-storage temperature showed FVIII to be more stable at 20°C than at 4°C.

Similar findings were experienced by Vermeer *et al.* (1976b). During the production cycle, those variables showing significance, independently of each other, were the thawing temperature (V7), freezing rate (V8), freezing technique (V10) and the precipitation step (V14). Unfortunately the freezing rate, freezing technique and precipitation were confounded and their significance difficult to comment on. The thawing temperature experienced no carry over effects from Stage A phase, and was free from confounding by any of the other variables. The thawing temperature of between 4°C and 8°C can play an important role on the final outcome in the pH determination. The interaction of the freezing temperature (V9), and the manner in which the plasma was thawed (V13), was also found to be of relevance. The pH determination is relevant when a freezing temperature range, of between >-60°C or -35°C is used together with a thawing technique involving either circulating water or by moving the packs. No other variables or interactions were significant when determining the pH.

Trace amounts of plasma proteins are present in cryoprecipitate, but their concentrations vary (Smit Sibinga and Das, 1984). It was decided to examine the effect of the variables on the important plasma proteins (total protein and albumin), and others not mentioned in the literature to date. Of the plasma proteins, fibrinogen was analyzed separately and has already been discussed. It was decided not to assay fibronectin due to the cost factor and complications involved in the assay technique (Boughton and Simpson, 1985). The presence of fibronectin in cryoprecipitates has been well documented in the literature by Garelli *et al.* (1986), Horowitz *et al.* (1984) and Amrani *et al.* (1982). The levels of the immunoglobulin

determination (IgG, IgA, IgM, and IgE) and antithrombin-III are often undetectable, insignificant, and costly (Palmer *et al.*, 1990). It was for these reasons that the mentioned assays were excluded. An oversight at the time of planning the study, was the exclusion of Fibrinopeptide A (FpA) assay, which would have correlated well with the FDP results, and detects early activation in the coagulation system (Carlebjork *et al.*, 1983). Similarities in the results of certain plasma proteins and electrolytes were found, and will therefore be discussed as one entity. Individual variables rather than interactions were more prominent between the variables, with the majority of variables having more influence during Stage A (prior to production), rather than in Stage B (during production cycle). Variables and interactions that were close to significance, were eliminated. Confounding in many of Stage B interactions were evident, making it difficult to comment on the relevance of the association. The reason being, these plasma proteins and electrolytes are only present in trace amounts and if definite significance was not evident, they would then have little bearing on the outcome of the entire Fractional Factorial Design.

During Stage A, the following variables and interactions played a role on the outcome of the plasma proteins. Exercised blood donors (V1) affected the albumin and total protein results, while a bleed in under 8 minutes (V3) had an influence on the potassium results. Anticoagulant played a role in all the plasma proteins and electrolytes, with CPD influencing the magnesium, total protein, albumin and sodium data, as compared to heparin and calcium chloride influencing the potassium, chloride and calcium data. This was evident by the higher assay results being obtained in those instances when heparin and calcium chloride were added

to the plasma, prior to processing. It would be acceptable to presume that the calcium results that were elevated, were caused whenever heparin and calcium chloride had been added to the plasma. Whole blood stored for 12 hours (V5) would have raised the potassium, which is known to increase on storage. Sodium levels rose when the whole blood was cooled to 4°C, prior to separation of the red cells and plasma. Magnesium, sodium and total protein analysis reflected the same outcome when the blood donors were not requested to exercise, and blood groups other than Group A were used. The reason for this is not clear. Whole blood that has been stored at a temperature of 20°C and then had the red cells separated from the plasma within 6 hours, constructively influenced the magnesium, ALT and potassium results. A possible explanation for this occurrence could be that those plasma proteins are less unstable at a temperature of 20°C. A whole blood donation with a bleeding time of less than 8 minutes, processed within 6 hours, had a beneficial control over the ALT results.

In Stage B of the production phase, magnesium, total protein and sodium were not biased by any of the variables, although evident in total protein data, was a carry over effect from V5, the pre-process delay variable. V14, the precipitation technique demonstrated a connection with the results obtained from the calcium and ALT assays, but were confounded, and as a consequence, reservation about its significance is expressed. The same applies to the interaction V8 by V13 (freezing rate and thawing technique) for ALT and chloride, V12 by V13 (holding time and thawing technique) for albumin and V10 by V14 (freezing technique and precipitation) for potassium. It is suggested that these plasma proteins and

electrolytes are more sensitive to the freezing and thawing techniques than other plasma proteins and electrolytes.

Significant variables were the freezing technique (V10) for calcium and the thawing temperature (V7) for ALT. The reason for these variables showing significance, is uncertain and further studies on the plasma proteins would be required, if the answer is to be determined. The freezing technique (V10), in conjunction with the thawing technique (V13), play a critical part in the calcium and chloride determinations. Better insight into the plasma proteins and electrolytes and the effect of the many variables on them, has been gained by our study, however only play a limited role in the final yield of FVIII.

CHAPTER 6

CONCLUSIONS

Statistically designed experiments have been used in industrial experimentation for approximately the last 20 years, and more recently in the medical field. Sophisticated designs and methods of analysis have appeared since digital computers and their statistical programmes became available in the mid-1960s (Hendrix, 1979). Statistical designs such as the one used in our study, are valuable instruments, when countless variables need to be assessed at any one given time.

In this study, our results and statistical analysis thereof, highlight which individual variables or combination of variables and their factors are significant to optimize the preparation of cryoprecipitate for FVIII.

In Stage A, of the production cycle ie. from the time of blood donation, to prior to separation of the red cells and plasma, variables V4 (anticcagulant), V5 (pre-process delay prior to separation of red cells and plasma) and V6 (pre-process storage temperature), all play an important role in FVIII yield when examined as independent variables. Less importance was placed on variables V1 (donor exercise levels), V2 (donor blood group), and V3 (time taken to bleed the unit of blood) as no significance was evident for these variables when evaluated as independent variables. Donor blood group was significant when interacted with other variables.

CPD as the primary anticoagulant (V4) influenced the FVIII levels by producing superior yields when compared to other anticoagulants used. CPD influenced the pH, fibrinogen, vWF assay results, and majority of the plasma proteins levels, by showing, either no change in the outcome, or an improvement of FVIII yields. Heparin appeared to be more suited to the electrolytes, however, this does not appear to be of any significant value, due to the small concentrations present in the plasma.

The pre-process time delay of less than 6 hours before separation was of vital importance to the FVIII levels, due to the fact that a longer delay resulted in a decrease of FVIII activity. The shorter pre-process time seemed to produce better results in most of the plasma proteins, with the longer delay showing an increase in potassium levels.

Variable V6, that of the pre-storage temperature prior to processing, of 20°C is of prime importance. Improved results in the FVIII, pH, fibrinogen, plasma protein and electrolyte assays were detected. Only sodium showed preference for the colder temperature of 4°C. Currently, in our present production cycle, not much attention is paid to variable V6. The temperature of the incoming blood, from mobile clinics, ranges between 4 - 30°C. This same variable (V6) showed an interaction with donor exercise levels (V1) for vWF and pH determination.

Two other interactions for the FVIII statistical analysis of data results, were significant,

- Group A blood donors (V2) bled within 12 minutes (V3)
- non exercised donors bled into packs containing CPD

n Stage B of the manufacturing process, variable V8 (freezing rate) was of no significance, with the freezing technique V10 (which was confounded in most instances), the holding time V12, the precipitation step V14 showing only intermittent significance, in relation to fibrinogen and pH. Variable V11, being that of centrifugation, demonstrated relevance only with vWF data.

Variables V7, that of the thawing temperature below 8°C and V9, freezing temperature >-60°C was the most significant in relation to the FVIII analysis. The fast freeze at >-60°C showed significance with the FVIII, fibrinogen and vWF results with increased yields, whereas the thawing temperature affected the pH and fibrinogen the most. The freezing temperature (V9), displayed interactions for the pH and fibrinogen analysis with the thawing technique (V13). Here either of the two factors of the variables could be relevant to either variable. A slow freezing rate results in a cryoprecipitate which is about twice the weight of a cryoprecipitate obtained by a fast freezing rate. A heavier cryoprecipitate contains FVIII with a lower specific activity.

It is important that FVIII in the cryoprecipitate be in as natural a state as possible. Activation of FVIII by traces of thrombin will have adverse effects on the quality of the product. It has been shown that FVIII which has been activated by thrombin, loses activity rapidly (Rick and Hoyer, 1978). The amount of FpA in a sample is

proportional to the level of thrombin formation and an indication of the extent of activation of FVIII. FpA is influenced by the method of blood collection, and its assay content is an indication of FVIII activation. Plasma with a low FpA content, is processed more easily and the product remains stable (Pflugshaupt and Kurt, 1983). Care in stripping the donation line is also an important part of the donation procedure, in ensuring low plasma FpA levels ((Prowse *et al.*, 1984). FpA content also indicates the importance of blood flow through the collecting tube devoid of anticoagulants (Skjonsberg *et al.*, 1986). Fibrinopeptide A determination is a valuable test, which is able to give indications of the status of the blood donation prior to the commencement of processing and should therefore be incorporated as part of the routine in bleeding procedure.

The positive results of the FDP D-dimer latex assay in this study, were more likely to be that of a haemostatic condition in the donor possibly caused by menstruation (Carlebjork *et al.*, 1986a), than poor phlebotomy techniques. Rather than pursue this assay in future studies, we would recommend all donations be tested by FpA determination which is a far more practical and sensitive assay method . Detection of a positive result should exclude the unit of donated blood from being batched for processing into cryoprecipitate.

To summarize, the findings of our study indicate that there are 17 simple steps in the FVIII production process, to which attention to detail should be paid to optimize FVIII yields :

* the venipuncture should be a first time clean entry into a vein

* strenuous exercise is not required
* Group A blood donors should be used for cryoprecipitate production
* the primary anticoagulant should be CPD
* blood should be mixed with anticoagulant during phlebotomy
* cryoprecipitate must be prepared from plasma within 6 hours of collection
* whole blood temperature must be maintained at 20 - 24°C prior to processing
* FpA assay should be performed on all donations prior to processing
* separation of red cells / plasma should be by means of a rapid centrifugation at 4733 X g
* plasma must be frozen using a rapid freeze technique with a temperature of greater than -60°C
* plasma bags should be placed in a polyethylene over-wrap, immersed in a bath of 95% ethanol and dry ice as a recommendation for freezing
* plasma must be of constant volume
* plasma must be compressed between metal plates to ensure uniform density
* thawing of the frozen plasma should be below 8°C, in a water environment in which agitation occurs either by movement of the water or plasma packs
* precipitation must be carried out by means of centrifugation
* after drainage cryoprecipitate should be refrozen rapidly with dry ice

* cryoprecipitate should be stored at below -30°C

Of the 17 steps that have been highlighted above, it is important to communicate that 12 are already being applied during the production process at WPBTS.

Further attention must be given to the following areas (in addition to the 12 already used), in order to achieve the optimal quality of cryoprecipitate, containing high yields of FVIII:

* only bleeding of Group A donors;
* ensuring that the blood reaches a temperature of 20°C prior to separation;
* performing a FpA assay prior to processing;
* fast freezing rate of >-60°C;
* constant starting plasma volume.

Of the above five parameters, those most difficult to implement in a large scale routine production laboratory, would be:

* only bleeding Group A donors for cryoprecipitate production, which would require a much larger donor base, but plasmapheresis could be an option and would require further investigation of this route;
* monitoring of the whole blood temperature at 20°C;
* introduction of routine FpA testing, as it would increase the cost of production.

However, with modifications to certain procedures, the above could be achieved.

These findings further indicate that fibrinogen is present in therapeutically useful quantities in the cryoprecipitate. Unfortunately fibronectin was not included in the study, but is a recommendation for future work on the production of cryoprecipitate. The plasma proteins and electrolyte assays involve considerable time and cost for relatively inconsequential information, and we advocate to exclude them hereafter in further studies relating to cryoprecipitate.

As additional suggestion, what is required in the future, is that the Fractional Factorial design should be selected so that carry-over effects of the pre-production factors are not confounded with the production factors. This would include altering the design of the two variables that were identical, to avoid future confounding of the factors. When all the corrections have been made, we would repeat the study, excluding the plasma protein and electrolyte assays,but including the fibronectin assay. The FDP D-dimer assay would be exchanged for the FpA assay. assay.

In conclusion, we wish to report that we were able to achieve our objective, which was to identify those variables and interactions of factors which optimize the production of Factor VIII in cryoprecipitate. Our recommendations are feasible for large scale cryoprecipitate production. Furthermore, we have gained additional knowledge of those assays which are relevant when determining FVIII yields and stability.

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Appendix 1
BLOOD DONOR DESIGN PROTOCOL

Design Number	
Date Bled	

A. DONOR INFORMATION

Donor Number	
Date Last Bled	
Sex	
Age	
Blood Pressure	
Pulse	
Medical History	
Iron Level	
Pack Weight (g)	
Plasma Weight (g)	

B. DETAILS OF SPECIFIC DESIGN TYPE

Variable No.	Variable Category	High (+)/ Low (-)	Explanation & Comments
V1	Donor Exercise		
V2	Blood Group		
V3	Anticoagulant		
V4	Bleeding Time		
V5	Pre-Process Delay		
V6	Pre-Process Storage		
V7	Thawing Temperature		
V8	Freezing Rate		
V9	Freezing Temperature		
V10	Freezing Technique		
V11	Centrifugation		
V12	Holding Time		
V13	Thawing Technique		
V14	Precipitation		

Donor Samples Taken (X 2): YES / NO
Cell Samples Taken (X 2) : YES / NO

* indicates an outlier result

Factor VIII (Normal Range 0.5 - 2.00 U/ml; Cryo Normal Range +/- 10.00 U/ml)

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	0.88	0.66	1.09	1.36	0.38	0.86	1.45	1.80	2.03	8.44	4.44	12.63
2	0.60	0.83	0.83	0.84	0.36	0.87	1.06	0.70	8.07	16.81	13.03	9.20
3	0.41	0.81	1.02	0.87	0.77	1.11	1.10	1.34	2.98	12.46	3.15	15.02
4	0.58	0.44	1.10	1.05	0.78	0.51	0.76	1.00	12.13	12.15	7.21	29.37
5	0.79	1.23	1.05	0.74	0.66	1.39	0.80	0.61	14.45	24.00	6.42	21.86
6	0.80	1.16	0.54	1.31	1.06	1.71	0.74	1.33	28.33	29.86	3.88	28.00
7	0.50	1.18	1.11	0.86	0.64	1.42	0.77	1.07	14.55	25.43	14.36	39.14
8	0.93	0.37	1.32	1.26	1.24	0.65	1.33	1.34	21.92	1.31	4.76	3.55
9	0.73	0.74	0.64	0.51	0.56	0.37	0.36	0.57	7.84	4.98	5.95	10.23
10	1.04	0.53	0.53	0.64	0.91	0.64	0.49	0.71	1.43	0.59	0.57	2.00
11	0.47	0.66	0.98	0.84	0.42	0.65	0.92	0.80	4.37	7.21	18.66	8.77
12	0.30	0.71	0.78	1.06	0.47	0.52	0.58	1.02	6.90	5.70	8.06	9.81
13	0.64	0.34	0.82	0.47	0.44	0.19	0.51	0.42	7.25	3.21	12.14	2.85
14	1.15	0.62	0.90	0.55	1.25	0.69	0.77	1.00	41.36	9.27	7.01	18.93
15	0.46	0.95	0.74	0.72	0.59	0.87	0.58	0.81	12.04	8.82	9.43	4.71
16	0.96	0.90	0.65	0.77	0.42	0.56	0.48	0.46	5.80	8.21	11.49	5.30
17	0.71	0.65	0.78	0.54	0.83	0.92	0.94	0.76	9.47	9.73	18.91	8.04
18	0.65	0.55	0.55	0.44	0.40	0.60	0.77	0.56	4.27	10.80	14.76	3.34
19	0.82	0.66	0.46	0.57	0.75	0.72	0.41	0.57	12.18	11.46	3.36	35.56
20	0.73	0.66	0.54	1.10	1.00	1.25	0.38	1.15	12.22	19.73	7.31	27.87
21	0.83	0.49	0.59	1.16	0.76	0.33	0.27	0.61	7.72	4.39	4.21	7.49
22	0.71	0.58	1.24	0.98	0.64	0.90	0.86	0.87	8.58	9.04	15.33	10.55
23	0.62	0.68	0.49	1.12	0.49	0.82	0.77	1.84	8.58	5.05	4.57	12.24
24	0.62	0.79	0.78	0.82	1.00	0.67	0.65	0.79	14.16	8.11	51.09 *	25.35
25	0.30	0.32	1.05	0.53	0.55	0.48	1.09	0.76	10.61	10.77	23.62	10.60
26	0.69	0.73	1.08	0.88	0.78	0.82	1.12	0.80	10.17	25.44	8.88	4.21
27	0.49	1.15	0.57	0.90	0.09	1.33	0.60	1.12	1.74	4.88	1.07	1.30
28	0.60	0.52	0.66	0.71	0.79	1.46	1.13	1.07	11.46	12.59	15.76	17.23
29	0.62	0.74	0.54	0.61	0.49	0.91	0.67	0.98	13.03	24.76	6.22	7.61
30	0.66	0.63	0.81	0.68	0.71	0.96	1.21	0.74	16.83	13.72	27.59	13.33
31	1.06	0.70	1.36	1.11	1.15	1.17	1.22	1.04	15.33	14.64	21.51	39.21
32	0.70	0.87	0.97	0.97	1.16	1.26	2.02	1.35	9.34	7.43	15.03	21.35

Fibrinogen (Normal Range 2.00 - 4.00 g/l)

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	1.80	3.26	2.00	2.40	1.50	3.00	2.24	2.58	2.00	9.60	2.58	4.60
2	2.40	2.58	1.55	1.90	1.78	2.49	1.55	1.72	10.32	13.80	5.20	8.20
3	1.72	3.26	2.75	3.00	2.00	2.58	2.49	3.44	10.32	9.60	8.00	7.20
4	2.58	2.00	2.58	2.40	2.58	1.70	2.58	2.49	13.76	10.00	13.00	13.00
5	3.00	2.49	3.60	2.58	2.40	2.06	3.60	2.49	14.40	9.30	19.60	18.00
6	2.40	3.26	2.24	2.58	2.32	3.26	1.78	2.24	17.20	10.30	6.88	10.00
7	2.40	3.00	1.80	2.32	3.44	2.49	1.72	1.90	13.76	6.00	14.40	26.10
8	2.32	2.75	2.58	2.58	2.32	2.58	2.40	2.24	16.00	4.10	3.00	2.58
9	3.26	2.30	2.58	2.00	2.49	2.00	2.58	2.40	17.20	8.60	13.80	19.20
10	3.26	4.00	2.58	2.75	2.49	3.00	2.58	3.26	3.00	3.60	3.44	4.10
11	2.40	2.32	2.40	2.24	2.49	2.00	2.06	1.80	6.88	8.60	12.00	9.30
12	1.46	2.24	2.49	2.75	1.60	2.32	2.40	2.75	7.20	10.30	9.00	14.40
13	1.80	2.32	2.06	1.72	2.24	2.49	1.80	2.58	13.04	19.60	10.30	13.00
14	1.80	1.90	2.06	2.06	1.72	1.80	2.06	1.80	11.00	7.20	9.30	9.00
15	1.90	2.06	2.58	2.24	1.78	1.80	2.75	2.00	6.88	8.60	16.00	2.40
16	2.58	1.80	2.40	3.44	2.24	1.72	2.32	2.00	10.30	12.00	13.00	9.30
17	2.24	2.58	2.06	2.58	1.70	2.40	2.32	2.32	9.60	8.00	9.60	14.40
18	2.75	2.06	1.38	3.26	3.00	2.06	1.55	3.44	8.00	9.60	8.00	13.00
19	4.00	4.30	2.00	2.49	3.44	3.26	2.32	1.80	18.00	19.60	9.00	11.00
20	2.49	2.58	1.80	2.75	2.40	2.58	1.80	2.06	19.60	19.60	9.00	17.20
21	1.50	2.00	1.90	2.40	1.70	2.00	1.80	2.24	6.88	12.00	9.60	13.00
22	2.75	3.44	3.00	2.58	3.00	3.44	3.00	3.26	18.00	13.00	20.40	13.00
23	2.32	2.24	2.40	3.26	2.24	2.32	2.49	2.75	13.04	8.60	12.00	8.60
24	2.40	2.00	1.80	3.26	2.49	1.70	1.78	2.24	6.00	6.88	11.00	13.00
25	2.58	2.00	2.75	3.44	2.49	2.00	2.75	2.75	13.04	13.80	17.20	16.00
26	2.75	2 75	2.58	2.58	3.44	2.40	2.49	2.58	6.88	19.60	9.60	6.00
27	1.90	2.40	1.78	2.58	2.24	2.49	2.00	2.49	2.49	3.00	2.24	4.30
28	2.32	1.46	1.90	1.72	2.40	1.80	2.24	1.40	18.00	8.00	9.00	5.16
29	4.00	2.58	3.26	4.00	3.00	2.40	2.75	3.00	18.00	19.60	13.80	18.00
30	1.80	2.24	2.40	2.00	2.00	2.40	3.00	2.06	11.00	18.00	13.80	6.00
31	2.06	2.40	4.30	2.49	2.32	2.58	3.00	2.40	14.40	16.00	13.80	16.00
32	2 40	3.00	1.46	0 95	3.26	2.32	2.24	1.12	5.50	8.00	5.20	4.00

von Willebrand Factor (Normal Range 100%)

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	110.0	120.5	110.0	190.0	40.0	160.0	170.0	85.0	5.0	65.0	20.0	95.0
2	85.0	110.5	75.0	30.0	22.5	180.5	120.0	90.5	42.5	90.0	60.5	65.0
3	70.5	80.5	95.0	130.0	40.0	140.0	150.0	85.0	75.0	55.0	42.5	47.5
4	110.0	75.0	80.5	110.0	80.0	95.0	190.0	130.5	85.0	55.0	100.0	150.0
5	110.0	110.0	90.0	60.5	85.0	180.0	95.0	50.5	120.0	100.0	55.5	95.0
6	120.0	170.0	120.0	120.0	40.0	320.0	120.0	160.0	120.5	120.0	70.0	170.0
7	90.0	190.0	120.5	90.5	50.0	210.0	100.5	95.0	45.0	120.5	150.0	200.0
8	180.0	95.0	280.0	180.0	110.0	100.5	180.0	140.0	140.0	30.0	32.5	20.0
9	170.0	120.5	80.0	40.0	75.5	110.0	47.5	45.0	80.5	60.0	30.0	85.0
10	95.5	60.0	40.0	60.5	15.0	80.5	80.0	90.5	11.3	10.0	9.4	8.1
11	60.0	75.0	160.0	60.5	75.5	90.0	110	42.5	27.5	40.0	90.0	47.5
12	85.0	95.5	75.0	170.0	37.5	90.0	95.5	90.0	60.0	80.0	40.0	95.0
13	75.5	75.0	170.0	55.0	42.5	120.5	110.0	47.5	50.0	18.8	70.0	32.5
14	130.0	60.5	90.0	65.0	85.0	42.5	130.0	85.0	170.5	70.0	65.0	95.0
15	60.0	90.0	90.0	100.5	25.0	160.5	95.0	70.0	27.5	90.0	70.0	25.0
16	110.0	100.0	60.0	55.0	42.5	180.0	80.0	60.0	45.5	90.0	50.0	55.5
17	90.5	95.5	75.5	60.0	110.0	190.0	100.5	75.0	75.5	85.5	80.0	80.0
18	85.0	47.5	85.0	85.0	65.0	100.0	85.0	80.5	37.5	55.0	75.5	55.0
19	100.0	160.5	100.5	50.5	50.5	95.0	55.5	80.5	70.5	120.5	40.0	85.0
20	100.0	95.5	60.5	110.0	70.0	190.5	50.5	110.0	110.0	160.5	40.0	170.0
21	120.0	120.0	40.5	120.0	90.0	42.5	42.5	55.0	60.0	30.0	22.5	90.0
22	65.5	140.0	120.5	70.0	42.5	160.5	180.0	120.5	85.0	47.5	140.0	85.0
23	120.0	170.0	70.5	130.0	35.0	170.0	95.5	150.0	60.0	37.5	70.0	100.5
24	120.0	45.0	65.0	70.5	75.0	120.5	80.0	75.0	85.0	140.0	85.0	100.0
25	90.0	50.0	180.5	90.5	55.0	35.0	200.0	110.0	47.5	42.5	170.0	110.0
26	140.5	95.0	90.0	170.0	50.0	90.0	160.0	190.0	50.0	85.5	47.5	45.0
27	60.0	220.0	47.5	90.0	22.5	190.5	60.0	100.0	7.5	15.0	12.5	12.5
28	60.0	160.0	75.5	70.0	27.5	190.0	130.0	60.0	60.5	170.5	100.0	75.5
29	80.0	60.0	55.0	85.0	42.5	110.5	100.0	70.0	65.0	90.0	90.0	50.0
30	140.0	95.0	80.0	70.5	30.0	160.5	160.5	47.5	100.0	140.0	170.0	42.5
31	160.0	260.0	150.0	70.5	85.0	190.0	120.5	80.0	120.5	190.5	120.0	80.0
32	160.5	120.5	170.5	150.0	90.0	160.0	90.0	160.0	42.5	110.0	65.0	95.5

Fibrin Degradation Products (Normal Range "-")

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-
11	+	-	+	-	-	-	+	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	+	-	-	-	-	-	-	-	-
21	-	-	-	+	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-		-	-	-
24	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-
26	+	-	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-	-	-

pH (Normal Range +/- 7.43)

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	7.88	7.73	7:72	7.57	8.10	7.91	7.95	7.98	7.83	7.66	7.80	7.76
2	7.80	7.84	7.82	7.72	7.77	7.94	7.53	7.44	7.83	7.75	7.68	7.61
3	7.33	7.92	7.91	7.58	7.48	7.75	7.92	7.93	7.90	7.75	7.82	7.71
4	7.25	7.66	7.74	7.28	7.80	7.66	7.85	7.76	7.81	7.75	7.89	7.74
5	7.66	7.56	7.87	7.84	7.83	7.94	7.98	7.82	7.72	7.87	7.64	7.62
6	7.70	7.86	7.93	7.85	7.97	7.94	7.88	7.87	7.65	7.85	7.71	7.64
7	7.74	7.88	7.71	7.41	7.76	7.76	7.73	7.83	7.71	7.70	7.82	7.71
8	7.56	7.83	7.64	7.73	7.91	7.73	7.73	7.72	7.75	7.57	7.75	7.66
9	7.77	7.56	7.73	7.70	7.89	7.92	7.70	7.87	7.65	7.76	7.68	7.76
10	7.56	7.52	7.62	7.32	7.75	7.74	7.55	7.52	7.57	7.67	7.52	7.59
11	7.51	7.54	7.77	7.62	7.98	7.74	7.94	7.97	7.77	7.65	7.75	7.77
12	7.56	7.76	7.91	7.79	7.86	8.02	7.62	7.82	7.63	7.77	7.67	7.55
13	7.90	7.97	8.06	7.62	7.85	7.88	7.72	7.87	7.76	7.88	7.77	7.75
14	7.33	7.69	7.88	7.56	7.78	7.80	7.60	7.76	7.84	7.77	7.76	7.66
15	7.95	7.40	7.53	7.35	7.77	7.49	7.63	7.52	7.51	7.81	7.58	7.56
16	7.54	7.96	7.93	7.76	8.12	8.04	7.83	8.06	7.61	7.65	7.86	7.90
17	7.63	7.88	7.77	7.65	7.95	8.04	7.80	7.94	7.76	7.74	7.70	7.65
18	7.87	7.47	7.60	7.56	7.80	8.05	7.73	7.68	7.57	7.75	7.58	7.65
19	7.59	7.63	7.65	7.41	7.73	7.79	7.49	7.55	7.55	7.68	7.70	7.65
20	7.45	7.65	7.64	7.67	7.70	7.71	7.57	7.62	7.66	7.48	7.75	7.83
21	7.50	7.38	7.86	7.40	7.91	8.11	8.12	7.85	7.62	7.72	7.74	7.76
22	7.63	7.55	7.86	7.46	7.58	7.93	7.54	7.75	7.65	7.69	7.71	7.84
23	7.33	7.85	7.82	7.39	7.89	7.90	7.87	7.97	7.55	7.72	7.71	7.59
24	7.67	7.54	7.87	7.70	8.05	8.11	7.86	7.75	7.62	7.80	7.85	7.65
25	7.41	7.59	7.40	7.37	7.95	7.93	7.92	7.84	7.62	7.76	7.67	7.82
26	7.84	7.62	7.72	7.67	7.70	7.85	7.91	7.84	7.68	7.71	7.70	7.75
27	7.51	7.52	7.87	7.60	7.61	7.82	7.93	7.81	7.63	7.59	7.73	7.65
28	7.65	7.50	7.40	7.31	7.74	7.85	7.70	7.71	7.61	7.68	7.60	7.71
29	7.24	7.46	7.86	7.57	7.70	7.85	7.71	7.81	7.72	7.75	7.74	7.74
30	7.54	7.40	7.32	7.24	7.84	7.57	7.84	7.68	7.72	7.64	7.67	7.86
31	7.74	7.78	7.89	7.72	7.75	7.83	7.77	7.69	7.67	7.76	7.69	7.70
32	7.25	7.71	7.74	7.77	7.52	7.58	7.75	7.61	7.49	7.52	7.50	7.58

Magnesium (Normal Range 0.7 - 1.1 mmol/l)

* indicates outlier

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	0.83	0.74	0.60	0.92	0.69	0.66	0.66	0.73	0.57	0.55	0.63	0.73
2	0.88	0.94	0.99	0.91	0.67	0.71	0.65	0.71	0.65	0.76	0.70	0.70
3	0.87	0.63	0.89	0.92	0.60	0.66	0.66	0.72	0.83	0.64	0.63	0.71
4	1.03	0.51	0.67	0.67	0.63	0.67	0.65	0.71	0.58	0.58	0.67	0.73
5	0.89	0.64	0.91	0.60	0.70	0.61	0.70	0.76	0.67	0.47	0.67	0.74
6	0.83	0.78	0.85	0.56	0.64	0.62	0.71	0.73	0.63	0.41	0.68	0.70
7	0.84	0.74	0.68	1.00	0.67	0.67	0.63	0.72	0.41	0.44	0.68	0.69
8	0.77	0.71	0.69	0.59	0.64	0.75	0.60	0.64	0.68	0.64	0.49	0.60
9	0.86	0.51	0.83	0.88	0.67	0.66	0.68	0.65	0.59	0.57	0.62	0.64
10	0.92	0.73	1.11	0.85	0.69	0.81	0.77	0.80	0.82	0.71	0.58	0.96
11	0.58	0.72	0.90	0.81	0.58	0.63	0.67	0.67	0.65	0.48	0.58	0.63
12	0.91	0.59	0.86	0.55	0.72	0.67	0.66	0.76	0.58	0.58	0.62	0.77
13	0.88	0.70	0.85	1.05	0.67	0.61	0.65	0.76	0.66	0.57	0.52	0.78
14	0.50	0.69	0.89	0.62	0.71	0.72	0.69	0.71	0.67	0.55	0.57	0.74
15	0.97	0.82	1.00	0.68	0.81	0.75	0.71	0.70	0.74	0.61	0.60	0.22 *
16	0.90	0.87	0.89	0.91	0.61	0.72	0.65	0.72	0.47	0.63	0.65	0.67
17	0.87	0.72	0.52	0.89	0.60	0.71	0.58	0.71	0.56	0.61	0.54	0.67
18	0.89	0.82	0.87	1.00	0.76	0.80	0.63	0.78	0.66	0.68	0.35	0.85
19	0.91	0.79	0.83	0.76	0.68	0.71	0.69	0.72	0.66	0.60	0.71	0.56
20	0.88	0.76	0.83	0.55	0.67	0.74	0.64	0.67	0.70	0.39 *	0.58	0.65
21	0.98	0.87	0.59	0.78	0.70	0.74	0.68	0.68	0.64	0.58	0.57	0.68
22	0.94	0.94	0.86	0.72	0.76	0.90	0.63	0.70	0.72	0.76	0.68	0.72
23	0.85	0.76	0.90	0.88	0.51	0.68	0.71	0.68	0.51	0.71	0.73	0.67
24	0.90	0.87	0.72	0.75	0.77	0.75	0.66	0.74	0.67	0.62	0.64	0.66
25	0.95	0.58	0.66	0.76	0.66	0.65	0.64	0.63	0.56	0.53	0.64	0.55
26	0.58	0.61	0.80	0.82	0.56	0.77	0.53	0.74	0.54	0.62	0.56	0.81
27	0.86	0.83	0.95	0.87	0.61	0.71	0.73	0.72	0.50	0.51	0.33 *	0.82
28	0.80	0.86	0.97	0.80	0.64	0.63	0.67	0.63	0.55	0.60	0.59	0.70
29	0.98	0.88	0.50	0.91	0.76	0.64	0.69	0.67	0.58	0.55	0.65	0.65
30	0.93	0.72	0.97	0.69	0.64	0.68	0.70	0.73	0.65	0.56	0.69	0.76
31	0.73	0.62	0.56	0.71	0.70	0.75	0.67	0.70	0.59	0.66	0.68	0.68
32	0.83	0.93	0.59	0.66	0.63	0.60	0.65	0.71	0.61	0.63	0.92	0.43

Total Protein (Normal Range 64 - 88 g/l)

* indicates an outlier result

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	73.0	55.0	50.0	61.0	58.0	57.0	59.0	57.0	58.0	55.0	54.0	54.0
2	71.0	74.0	76.0	72.0	57.0	58.0	62.0	56.0	55.0	70.0	66.0	59.0
3	71.0	48.0	70.0	71.0	61.0	58.0	60.0	60.0	86.0	46.0	65.0	57.0
4	76.0	31.0	57.0	55.0	60.0	55.0	58.0	58.0	59.0	52.0	62.0	60.0
5	66.0	47.0	73.0	41.0	62.0	58.0	65.0	63.0	65.0	42.0	74.0	62.0
6	71.0	59.0	73.0	46.0	59.0	58.0	57.0	57.0	68.0	33.0	55.0	57.0
7	74.0	53.0	50.0	70.0	58.0	62.0	53.0	59.0	37.0	50.0	58.0	56.0
8	56.0	59.0	54.0	45.0	54.0	54.0	54.0	58.0	55.0	50.0	51.0	50.0
9	63.0	30.0	65.0	79.0	49.0	52.0	54.0	66.0	50.0	48.0	51.0	61.0
10	64.0	56.0	70.0	74.0	54.0	62.0	55.0	70.0	67.0	57.0	42.0	74.0
11	46.0	60.0	65.0	70.0	53.0	60.0	56.0	53.0	54.0	54.0	53.0	52.0
12	65.0	38.0	72.0	51.0	56.0	60.0	59.0	62.0	46.0	55.0	59.0	62.0
13	68.0	47.0	73.0	74.0	59.0	57.0	56.0	57.0	59.0	54.0	45.0	55.0
14	42.0	54.0	73.0	53.0	64.0	58.0	55.0	60.0	69.0	49.0	52.0	73.0
15	74.0	75.0	76.0	55.0	61.0	55.0	66.0	59.0	64.0	51.0	55.0	9.0 *
16	70.0	69.0	73.0	71.0	53.0	57.0	60.0	57.0	56.0	52.0	55.0	57.0
17	76.0	51.0	52.0	66.0	56.0	50.0	56.0	54.0	53.0	52.0	56.0	55.0
18	70.0	70.0	68.0	71.0	58.0	63.0	53.0	60.0	57.0	57.0	26.0	60.0
19	70.0	68.0	74.0	56.0	59.0	60.0	61.0	61.0	63.0	56.0	57.0	44.0
20	71.0	66.0	72.0	42.0	58.0	64.0	57.0	64.0	65.0	44.0	51.0	66.0
21	66.0	69.0	47.0	73.0	53.0	57.0	51.0	61.0	52.0	50.0	48.0	62.0
22	68.0	77.0	71.0	65.0	55.0	59.0	56.0	60.0	58.0	56.0	65.0	68.0
23	70.0	71.0	70.0	74.0	55.0	61.0	55.0	61.0	58.0	66.0	61.0	60.0
24	73.0	61.0	50.0	53.0	62.0	59.0	57.0	59.0	56.0	54.0	61.0	47.0
25	79.0	39.0	51.0	63.0	59.0	58.0	59.0	58.0	54.0	47.0	59.0	58.0
26	46.0	50.0	73.0	70.0	55.0	57.0	58.0	51.0	50.0	54.0	64.0	57.0
27	69.0	72.0	74.0	74.0	52.0	58.0	55.0	60.0	48.0	41.0	28.0	71.0
28	70.0	75.0	68.0	62.0	60.0	57.0	54.0	53.0	51.0	56.0	51.0	56.0
29	69.0	69.0	38.0	75.0	57.0	55.0	57.0	58.0	59.0	58.0	56.0	60.0
30	70.0	63.0	71.0	59.0	60.0	63.0	55.0	55.0	53.0	70.0	56.0	57.0
31	70.0	44.0	40.0	66.0	63.0	59.0	56.0	57.0	59.0	56.0	58.0	51.0
32	67.0	74.0	49.0	51.0	60.0	65.0	54.0	56.0	60.0	65.0	76.0	39.0

Albumin (Normal Range 30 - 55 g/l)

* indicates outlier results

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	46.0	35.0	33.0	40.0	37.0	32.0	36.0	33.0	39.0	32.0	36.0	33.0
2	43.0	43.0	50.0	45.0	33.0	32.0	38.0	35.0	31.0	38.0	40.0	35.0
3	42.0	30.0	41.0	45.0	37.0	34.0	35.0	37.0	49.0	28.0	36.0	35.0
4	48.0	19.0	36.0	32.0	36.0	34.0	38.0	32.0	35.0	33.0	37.0	32.0
5	38.0	28.0	43.0	28.0	33.0	34.0	36.0	39.0	34.0	24.0	37.0	36.0
6	42.0	37.0	46.0	31.0	33.0	33.0	34.0	34.0	31.0	18.0 *	33.0	32.0
7	46.0	34.0	28.0	41.0	37.0	33.0	36.0	34.0	21.0	27.0	36.0	31.0
8	36.0	37.0	33.0	24.0	32.0	33.0	32.0	28.0	33.0	33.0	30.0	27.0
9	42.0	21.0	37.0	43.0	32.0	30.0	30.0	34.0	31.0	29.0	29.0	32.0
10	41.0	36.0	45.0	48.0	31.0	38.0	34.0	35.0	40.0	36.0	24.0	42.0
11	29.0	35.0	40.0	43.0	30.0	33.0	32.0	31.0	32.0	31.0	30.0	31.0
12	39.0	27.0	41.0	29.0	34.0	36.0	38.0	36.0	27.0	34.0	38.0	35.0
13	44.0	31.0	45.0	47.0	36.0	35.0	36.0	38.0	36.0	33.0	29.0	34.0
14	27.0	32.0	44.0	33.0	38.0	35.0	35.0	35.0	39.0	30.0	33.0	43.0
15	46.0	48.0	46.0	33.0	37.0	34.0	36.0	35.0	37.0	30.0	30.0	6.0 *
16	44.0	44.0	47.0	46.0	34.0	34.0	36.0	36.0	33.0	32.0	33.0	36.0
17	49.0	34.0	33.0	39.0	34.0	32.0	33.0	32.0	33.0	33.0	33.0	33.0
18	39.0	44.0	44.0	46.0	35.0	35.0	32.0	35.0	34.0	35.0	15.0 *	37.0
19	45.0	42.0	46.0	36.0	38.0	35.0	35.0	35.0	36.0	33.0	33.0	26.0
20	42.0	41.0	45.0	27.0	32.0	34.0	35.0	34.0	35.0	23.0	30.0	35.0
21	43.0	42.0	30.0	45.0	34.0	33.0	32.0	36.0	33.0	30.0	31.0	34.0
22	45.0	41.0	37.0	39.0	35.0	33.0	27.0	33.0	34.0	34.0	32.0	37.0
23	41.0	44.0	46.0	44.0	31.0	38.0	35.0	33.0	34.0	40.0	38.0	31.0
24	47.0	36.0	37.0	30.0	35.0	37.0	35.0	35.0	32.0	34.0	36.0	29.0
25	49.0	24.0	34.0	35.0	35.0	34.0	32.0	33.0	33.0	29.0	32.0	32.0
26	28.0	29.0	45.0	40.0	34.0	31.0	33.0	33.0	29.0	30.0	36.0	37.0
27	47.0	44.0	44.0	46.0	33.0	36.0	34.0	37.0	27.0	26.0	18.0 *	43.0
28	46.0	43.0	44.0	37.0	37.0	35.0	35.0	34.0	33.0	33.0	32.0	36.0
29	44.0	43.0	24.0	45.0	33.0	35.0	35.0	34.0	34.0	34.0	33.0	33.0
30	43.0	36.0	43.0	34.0	34.0	36.0	33.0	33.0	30.0	37.0	33.0	35.0
31	44.0	25.0	26.0	41.0	37.0	33.0	32.0	36.0	31.0	30.0	32.0	32.0
32	45.0	45.0	30.0	33.0	35.0	38.0	31.0	35.0	36.0	38.0	44.0	23.0

Calcium (Normal Range 2.3 - 2.7 mmol/l)

* indicates an outlier result

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	2.49	1.80	1.73	2.26	2.05	1.40	2.12	1.42	2.16	1.43	2.10	1.48
2	2.35	2.32	2.43	2.30	2.06	1.92	2.16	1.42	2.00	2.13	2.24	1.50
3	2.21	1.72	2.29	2.44	1.42	1.33	1.44	1.46	1.86	1.31	1.53	1.59
4	2.47	1.18	1.85	1.93	1.44	1.32	1.45	1.40	1.44	1.35	1.49	1.47
5	2.32	1.51	2.45	1.47	1.36	1.32	1.51	1.42	1.39	1.19	1.52	1.48
6	2.32	1.96	2.30	1.69	1.37	1.38	1.35	1.34	1.44	1.08	1.29	1.45
7	2.39	1.92	1.52	2.20	1.37	1.38	1.35	1.34	0.93 *	1.21	1.42	1.39
8	1.95	2.09	1.85	1.52	1.27	1.32	1.34	1.34	1.31	1.32	1.29	1.37
9	2.27	1.10	2.19	2.52	1.41	1.33	2.00	1.43	1.41	1.30	1.99	1.47
10	2.25	1.86	2.39	2.41	1.31	1.48	1.36	1.40	1.62	1.46	1.03	1.71
11	1.60	1.93	2.29	2.42	1.37	1.38	1.87	1.44	1.46	1.36	1.80	1.52
12	2.21	1.52	2.39	1.10	1.46	1.41	2.19	1.51	1.21	1.39	2.22	1.61
13	2.46	1.65	2.40	2.45	2.22	1.35	2.00	1.48	2.20	1.35	1.74	1.51
14	1.40	1.66	2.39	1.70	1.39	1.34	1.32	1.35	1.44	1.22	1.29	1.55
15	2.38	2.30	2.32	1.94	1.42	1.24	1.35	1.41	1.47	1.19	1.21	0.60
16	2.31	2.30	2.41	2.29	2.06	1.40	2.03	1.47	2.06	1.40	1.93	1.55
17	2.50	1.81	1.64	2.12	2.02	1.39	1.98	1.36	1.96	1.44	1.97	1.45
18	2.38	2.45	2.33	2.48	2.21	1.47	1.98	1.47	2.22	1.50	1.11	1.57
19	2.38	2.23	2.39	1.88	1.45	1.37	1.37	1.29	1.48	1.38	1.35	1.18
20	2.34	1.98	2.31	1.53	1.31	1.31	1.33	1.41	1.41	1.01	1.24	1.51
21	2.25	2.31	1.62	2.24	2.14	1.45	1.95	1.41	2.09	1.38	1.83	1.43
22	2.34	2.43	2.41	2.18	2.09	1.49	1.70	1.34	2.11	1.51	1.90	1.55
23	2.29	2.24	2.39	2.40	1.34	1.48	2.34	1.47	1.51	1.67	2.62	1.50
24	2.50	1.90	2.01	1.79	1.36	1.40	1.40	1.37	1.31	1.36	1.46	1.28
25	2.44	1.43	1.88	2.02	1.39	1.42	2.00	1.41	1.33	1.30	2.02	1.44
26	1.68	1.72	2.35	2.32	2.04	1.41	1.98	1.39	1.89	1.37	2.20	1.62
27	2.35	2.48	2.44	2.55	2.03	1.45	2.00	1.44	1.11	1.20	1.10	1.73
28	2.38	2.33	2.47	1.84	1.41	1.30	1.36	1.31	2.01	1.35	1.31	1.49
29	2.35	2.25	1.29	2.40	1.98	1.95	2.05	1.36	1.35	1.91	2.03	1.45
30	2.29	1.93	2.35	2.02	1.33	1.40	1.33	1.28	1.86	1.47	1.39	1.40
31	2.28	1.45	1.46	2.16	1.37	1.38	1.39	1.36	1.30	1.37	1.47	1.44
32	2.46	2.46	1.82	1.75	1.36	1.44	1.30	1.40	1.42	1.54	1.75	1.03

Alanine Aminotransferase (Normal Range 5 - 43 g/l @ 37)

* indicates outlier results

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	12.0	13.0	11.0	10.0	17.0	10.0	8.0	22.0	16.0	9.0	11.0	19.0
2	7.0	28.0	9.0	4.0	15.0	32.0	6.0	6.0	13.0	22.0	10.0	6.0
3	10.0	16.0	5.0	11.0	20.0	10.0	4.0	12.0	31.0	15.0	5.0	14.0
4	9.0	0.0 *	21.0	6.0	12.0	-3.0 *	10.0	9.0	7.0	0.0	11.0	7.0
5	17.0	9.0	6.0	8.0	12.0	11.0	11.0	12.0	14.0	5.0	13.0	8.0
6	9.0	7.0	15.0	6.0	9.0	7.0	11.0	9.0	8.0	4.0	11.0	9.0
7	21.0	27.0	18.0	12.0	7.0	10.0	10.0	11.0	3.0	7.0	8.0	11.0
8	11.0	18.0	8.0	10.0	10.0	9.0	9.0	26.0	9.0	8.0	11.0	19.0
9	5.0	3.0	7.0	6.0	17.0	6.0	9.0	5.0	14.0	5.0	10.0	7.0
10	10.0	5.0	10.0	48.0	12.0	18.0	8.0	9.0	17.0	21.0	8.0	10.0
11	5.0	8.0	16.0	37.0	12.0	16.0	3.0	15.0	14.0	16.0	11.0	12.0
12	10.0	10.0	18.0	14.0	19.0	18.0	7.0	26.0	14.0	18.0	8.0	22.0
13	14.0	18.0	17.0	9.0	13.0	15.0	18.0	26.0	11.0	14.0	14.0	19.0
14	8.0	6.0	13.0	9.0	17.0	10.0	24.0	57.0	16.0	7.0	19.0	61.0
15	12.0	5.0	12.0	6.0	15.0	11.0	26.0	10.0	15.0	9.0	18.0	2.0
16	9.0	13.0	8.0	21.0	12.0	9.0	8.0	27.0	10.0	6.0	8.0	21.0
17	6.0	33.0	11.0	10.0	10.0	11.0	8.0	11.0	10.0	11.0	13.0	11.0
18	7.0	11.0	14.0	10.0	11.0	73.0	24.0	12.0	10.0	74.0	11.0	12.0
19	6.0	24.0	8.0	7.0	9.0	5.0	19.0	10.0	9.0	7.0	13.0	7.0
20	53.0 *	9.0	15.0	11.0	42.0	13.0	7.0	12.0	41.0	8.0	6.0	11.0
21	19.0	12.0	18.0	20.0	112.0 *	23.0	38.0	26.0	100.0 *	19.0	33.0	23.0
22	9.0	8.0	6.0	18.0	14.0	4.0	7.0	27.0	10.0	4.0	9.0	28.0
23	6.0	15.0	9.0	34.0	8.0	18.0	11.0	31.0	8.0	15.0	13.0	27.0
24	25.0	26.0	15.0	10.0	8.0	28.0	24.0	5.0	8.0	20.0	24.0	7.0
25	25.0	7.0	5.0	13.0	17.0	11.0	16.0	22.0	13.0	9.0	17.0	17.0
26	7.0	7.0	12.0	7.0	11.0	10.0	9.0	8.0	10.0	9.0	15.0	7.0
27	24.0	21.0	12.0	22.0	26.0	15.0	21.0	38.0	19.0	10.0	10.0	39.0
28	20.0	8.0	9.0	20.0	12.0	5.0	8.0	12.0	23.0	7.0	9.0	12.0
29	8.0	18.0	8.0	14.0	20.0	25.0	15.0	14.0	10.0	21.0	19.0	12.0
30	20.0	24.0	23.0	7.0	49.0	16.0	25.0	8.0	14.0	13.0	26.0	9.0
31	14.0	6.0	12.0	21.0	21.0	14.0	18.0	13.0	45.0	11.0	19.0	12.0
32	10.0	25.0	10.0	11.0	12.0	32.0	3.0	14.0	10.0	32.0	9.0	9.0

Sodium (Normal Range 136 - 144 mmol/l)

* indicates outlier results

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	142.0	116.0	113.0	138.0	166.0	157.0	161.0	163.0	171.0	154.0	153.0	164.0
2	141.0	140.0	141.0	142.0	167.0	157.0	162.0	160.0	159.0	156.0	167.0	161.0
3	142.0	106.0	140.0	140.0	172.0	167.0	164.0	166.0	223.0 *	160.0	171.0	177.0
4	140.0	88.0	118.0	119.0	167.0	162.0	170.0	165.0	158.0	159.0	169.0	165.0
5	141.0	102.0	139.0	96.0	171.0	172.0	166.0	167.0	165.0	146.0	163.0	165.0
6	139.0	113.0	135.0	101.0	167.0	169.0	169.0	164.0	166.0	139.0	169.0	166.0
7	137.0	123.0	102.0	132.0	171.0	164.0	166.0	162.0	110.0	134.0	167.0	162.0
8	124.0	126.0	115.0	105.0	174.0	167.0	160.0	164.0	166.0	166.0	155.0	163.0
9	133.0	82.0	140.0	143.0	167.0	167.0	156.0	163.0	160.0	156.0	154.0	159.0
10	135.0	109.0	138.0	140.0	173.0	162.0	168.0	169.0	197.0 *	154.0	127.0	204.0
11	104.0	125.0	140.0	135.0	158.0	159.0	165.0	164.0	160.0	156.0	156.0	167.0
12	135.0	107.0	140.0	182.0	170.0	167.0	165.0	165.0	136.0	156.0	165.0	170.0
13	143.0	114.0	140.0	142.0	167.0	169.0	159.0	165.0	160.0	161.0	144.0	166.0
14	99.0	118.0	142.0	101.0	168.0	171.0	170.0	164.0	163.0	151.0	163.0	175.0
15	138.0	137.0	141.0	115.0	175.0	173.0	170.0	164.0	175.0	162.0	149.0	41.0
16	140.0	143.0	139.0	139.0	169.0	166.0	159.0	166.0	163.0	158.0	148.0	169.0
17	142.0	119.0	108.0	131.0	161.0	165.0	161.0	165.0	151.0	166.0	153.0	166.0
18	140.0	140.0	138.0	142.0	169.0	168.0	167.0	169.0	167.0	165.0	102.0	174.0
19	138.0	136.0	138.0	121.0	167.0	168.0	164.0	168.0	160.0	163.0	158.0	158.0
20	137.0	118.0	138.0	107.0	174.0	175.0	159.0	167.0	171.0	128.0	146.0	171.0
21	140.0	141.0	110.0	135.0	168.0	166.0	162.0	165.0	161.0	156.0	153.0	159.0
22	141.0	141.0	140.0	138.0	166.0	166.0	160.0	171.0	159.0	168.0	174.0	187.0
23	136.0	133.0	141.0	139.0	149.0	165.0	163.0	164.0	165.0	180.0	179.0	166.0
24	142.0	120.0	121.0	110.0	170.0	169.0	167.0	165.0	159.0	159.0	164.0	152.0
25	138.0	102.0	117.0	119.0	162.0	165.0	156.0	163.0	152.0	151.0	159.0	162.0
26	113.0	115.0	138.0	138.0	169.0	163.0	163.0	166.0	154.0	154.0	183.0	192.0 *
27	144.0	140.0	140.0	141.0	157.0	169.0	164.0	163.0	129.0	134.0	89.0 *	192.0
28	138.0	140.0	140.0	126.0	173.0	171.0	170.0	166.0	155.0	169.0	167.0	179.0
29	138.0	142.0	95.0	140.0	165.0	165.0	162.0	167.0	158.0	152.0	163.0	173.0
30	141.0	125.0	137.0	127.0	167.0	164.0	167.0	166.0	148.0	162.0	170.0	167.0
31	136.0	100.0	107.0	130.0	174 0	168.0	170.0	168.0	154.0	156.0	178.0	179.0
32	140.0	138.0	111.0	109.0	169.0	165.0	169.0	167.0	162.0	173.0	227.0 *	112.0

Potassium (Normal Range 3.2 - 4.8 mmol/l)

* indicates outlier results

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	4.5	3.3	3.6	4.2	3.3	3.2	3.5	3.5	3.3	3.3	3.3	3.5
2	3.7	3.6	3.9	4.1	3.2	3.0	3.3	3.2	3.0	3.0	3.4	3.2
3	3.5	5.2	3.8	4.6	3.6	3.7	3.2	3.4	4.7	3.6	3.3	3.6
4	5.0	4.2	3.6	5.6	5.1	4.2	4.2	4.3	4.8	4.2	4.2	4.3
5	4.1	4.9	4.8	14.5 *	4.6	4.9	5.4	4.7	4.5	4.1	5.3	4.6
6	3.8	7.6	9.1	5.6	3.2	3.4	3.2	3.0	3.2	2.8	3.2	3.0
7	6.2	3.4	9.3	4.1	3.4	2.9	3.3	3.1	2.2	2.4	3.3	3.1
8	4.0	6.6	3.9	3.4	3.2	3.1	3.3	3.1	3.1	3.2	3.2	3.1
9	8.5	4.2	3.9	5.4	4.9	5.0	3.9	4.1	4.6	4.7	3.9	4.0
10	3.5	6.2	8.0	4.2	3.9	3.6	3.9	4.1	4.4	3.4	2.9	4.8
11	3.3	3.9	4.0	3.9	3.0	3.0	3.1	3.2	3.0	3.0	2.9	3.3
12	4.3	3.3	4.1	2.9	3.9	3.5	3.8	3.7	3.1	3.2	3.8	3.8
13	4.3	3.0	4.0	4.3	4.5	5.4	4.2	4.7	4.4	5.1	3.9	4.6
14	2.8	3.3	4.9	17.0 *	3.6	3.1	3.5	3.2	3.6	2.8	3.3	3.3
15	4.1	6.7	3.9	7.1	4.0	3.9	3.5	3.2	4.0	3.7	3.1	0.8 *
16	4.2	3.9	4.4	3.5	4.2	4.5	4.3	4.9	4.0	4.3	4.1	5.0
17	5.0	6.0	5.7	5.5	3.5	3.4	3.4	3.7	3.3	3.5	3.2	3.7
18	4.6	4.1	3.9	4.3	4.3	3.5	3.9	4.1	4.2	3.5	2.5	4.2
19	4.3	5.8	4.1	3.6	4.2	5.1	4.5	5.0	4.0	4.9	4.3	4.8
20	4.0	5.0	4.4	2.7	3.9	3.5	3.8	3.9	3.9	2.6	3.5	4.0
21	4.2	3.9	3.1	3.8	4.3	4.7	4.1	4.3	4.1	4.4	3.9	4.1
22	4.2	4.3	5.0	3.8	4.2	3.7	3.4	3.9	4.0	3.7	3.7	4.2
23	3.5	3.9	4.1	4.4	3.2	3.2	3.6	3.1	3.5	3.5	4.0	3.1
24	3.9	13.8 *	4.2	7.3	4.8	4.8	4.9	4.3	4.5	4.6	4.9	4.0
25	4.3	5.6	5.3	12.6 *	3.4	3.2	3.2	3.2	3.2	2.9	3.4	3.2
26	3.4	5.0	3.8	3.7	3.2	3.0	3.1	3.9	3.0	2.9	3.5	4.5
27	4.0	5.1	4.6	4.8	3.9	3.7	4.5	3.7	3.1	3.0	2.4	4.4
28	3.7	3.7	4.1	6.3	3.2	3.0	2.8	3.2	3.8	3.0	2.8	3.4
29	3.9	3.7	5.3	4.8	3.3	3.1	3.0	3.3	3.0	2.8	3.0	3.4
30	4.0	3.5	7.6	4.0	3.0	3.2	3.4	3.5	2.9	3.1	3.5	3.5
31	6.5	3.4	3.5	3.6	4.3	3.9	3.8	3.6	2.8	3.6	4.0	3.8
32	3.8	4.5	3.5	7.4	3.4	3.4	3.6	3.4	3.3	3.6	4.8	2.2

Chloride (Normal Range 99 -106 mmol/l)

* indicates outlier results

	PRE				POST				FINAL			
NO	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4	RUN 1	RUN 2	RUN 3	RUN 4
1	105.0	86.0	82.0	105.0	74.0	71.0	73.0	74.0	73.0	67.0	60.0	69.0
2	109.0	109.0	103.0	106.0	80.0	73.0	70.0	78.0	73.0	70.0	68.0	73.0
3	106.0	80.0	108.0	107.0	76.0	75.0	77.0	79.0	106.0	71.0	76.0	83.0
4	105.0	62.0	92.0	92.0	79.0	71.0	76.0	78.0	66.0	71.0	68.0	73.0
5	110.0	73.0	110.0	77.0	84.0	73.0	83.0	80.0	73.0	56.0	77.0	74.0
6	104.0	80.0	105.0	72.0	77.0	68.0	77.0	74.0	73.0	54.0	72.0	70.0
7	105.0	91.0	81.0	99.0	78.0	78.0	77.0	71.0	28.0	52.0	72.0	71.0
8	92.0	97.0	85.0	79.0	74.0	71.0	79.0	85.0	65.0	68.0	69.0	78.0
9	101.0	54.0	106.0	107.0	74.0	77.0	76.0	81.0	65.0	67.0	69.0	73.0
10	99.0	83.0	106.0	106.0	81.0	76.0	76.0	82.0	86.0	67.0	42.0 *	92.0
11	78.0	92.0	109.0	98.0	75.0	73.0	71.0	72.0	75.0	68.0	61.0	68.0
12	102.0	76.0	107.0	54.0	72.0	71.0	77.0	78.0	39.0 *	61.0	70.0	76.0
13	107.0	85.0	109.0	106.0	76.0	67.0	70.0	72.0	67.0	60.0	56.0	68.0
14	72.0	88.0	106.0	83.0	83.0	75.0	74.0	70.0	71.0	57.0	65.0	66.0
15	100.0	106.0	109.0	88.0	80.0	74.0	78.0	77.0	73.0	69.0	55.0	1.0 *
16	106.0	109.0	105.0	103.0	79.0	79.0	82.0	71.0	71.0	71.0	70.0	66.0
17	104.0	89.0	80.0	99.0	80.0	78.0	74.0	75.0	67.0	78.0	62.0	72.0
18	106.0	106.0	107.0	104.0	80.0	71.0	75.0	73.0	74.0	68.0	29.0 *	71.0
19	106.0	107.0	105.0	94.0	80.0	78.0	79.0	76.0	72.0	68.0	70.0	68.0
20	103.0	89.0	105.0	77.0	76.0	79.0	68.0	77.0	70.0	38.0 *	56.0	74.0
21	105.0	106.0	81.0	101.0	74.0	77.0	73.0	77.0	63.0	66.0	65.0	66.0
22	113.0	103.0	108.0	103.0	74.0	78.0	74.0	74.0	69.0	77.0	81.0	77.0
23	101.0	103.0	106.0	103.0	66.0	77.0	74.0	74.0	71.0	89.0	83.0	69.0
24	105.0	96.0	90.0	86.0	81.0	74.0	76.0	72.0	71.0	65.0	70.0	59.0
25	103.0	50.0	89.0	94.0	67.0	77.0	77.0	73.0	57.0	69.0	73.0	66.0
26	85.0	85.0	103.0	106.0	77.0	79.0	72.0	80.0	65.0	70.0	81.0	92.0
27	105.0	103.0	101.0	105.0	68.0	74.0	79.0	75.0	36.0	48.0	13.0 *	36.0
28	108.0	107.0	102.0	99.0	78.0	76.0	73.0	77.0	60.0	72.0	69.0	80.0
29	105.0	111.0	72.0	103.0	78.0	77.0	76.0	70.0	63.0	67.0	69.0	68.0
30	104.0	99.0	107.0	99.0	77.0	78.0	75.0	74.0	65.0	75.0	74.0	70.0
31	104.0	71.0	79.0	99.0	75.0	76.0	74.0	76.0	66.0	67.0	73.0	82.0
32	102.0	103.0	81.0	81.0	76.0	78.0	74.0	78.0	72.0	79.0	106.0	29.0

FACTOR VIII : POST PRE

General Linear Models Procedure * denotes significance

Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	4.50442500	0.18768437	3.94	0.0001
Error	103	4.90266250	0.04759867		
Corrected Total	127	9.40708750			

R-Square	C.V.	Root MSE	Y Mean
0.478833	327.7689	0.21817118	0.06656250

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	0.43823125	0.14607708	3.07	0.0312	
V1	1	0.00945312	0.00945312	0.20	0.6568	
V2	1	0.01125000	0.01125000	0.24	0.6279	
V1 * V2	1	0.00165312	0.00165312	0.03	0.8525	
V3	1	0.03001250	0.03001250	0.63	0.4290	
V1 * V3	1	0.00025312	0.00025312	0.01	0.9420	
V2 * V3	1	0.21125000	0.21125000	4.44	0.0376	*
V4	1	0.82882812	0.82882812	17.41	0.0001	*
V1 * V4	1	0.30031250	0.30031250	6.31	0.0136	*
V2 * V4	1	0.06752812	0.06752812	1.42	0.2364	
V3 * V4	1	0.00227812	0.00227812	0.05	0.8273	
V5	1	0.93061250	0.93061250	40.56	0.0001	*
V1 * V5	1	0.01757813	0.01757813	0.37	0.5447	
V2 * V5	1	0.00720000	0.00720000	0.15	0.6981	
V3 * V5	1	0.01531250	0.01531250	0.32	0.5718	
V4 * V5	1	0.00165312	0.00165312	0.03	0.8525	
V6	1	0.29645000	0.29645000	6.23	0.0142	*
V1 * V6	1	0.03712812	0.03712812	0.78	0.3792	
V2 * V6	1	0.03125000	0.03125000	0.66	0.4197	
V3 * V6	1	0.09901250	0.09901250	2.08	0.1523	
V4 * V6	1	0.03712813	0.03712813	0.78	0.3792	
V5 * V6	1	0.13005000	0.13005000	2.73	0.1014	

FACTOR VIII : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.05796875	0.02727140	0.0359
-1	0.07515625	0.02727140	0.0069

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.05718750	0.02727140	0.0384
-1	0.07593750	0.02727140	0.0064

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.04500000	0.03856758	0.2460
1	-1	0.07093750	0.03856758	0.0688
-1	1	0.06937500	0.03856758	0.0750
-1	-1	0.08093750	0.03856758	0.0383

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.08187500	0.02727140	0.0034
-1	0.05125000	0.02727140	0.0630

FACTOR VIII : POST PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.07187500	0.03856758	0.0652
1	-1	0.04406250	0.03856758	0.2559
-1	1	0.09187500	0.03856758	0.0190
-1	-1	0.05843750	0.03856758	0.1328

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.11312500	0.03856758	0.0041
1	-1	0.00125000	0.03856758	0.9742
-1	1	0.05062500	0.03856758	0.1922
-1	-1	0.10125000	0.03856758	0.0100

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.14703125	0.02727140	0.0001
-1	-0.01390625	0.02727140	0.6112

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.09000000	0.03856758	0.0216
1	-1	0.02593750	0.03856758	0.5028
-1	1	0.20406250	0.03856758	0.0001
-1	-1	-0.05375000	0.03856758	0.1664

FACTOR VIII : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.11468750	0.03856758	0.0037
1	-1	-0.00031250	0.03856758	0.9936
-1	1	0.17937500	0.03856758	0.0001
-1	-1	-0.02750000	0.03856758	0.4774

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.16656250	0.03856758	0.0001
1	-1	0.00281250	0.03856758	0.9420
-1	1	0.12750000	0.03856758	0.0013
-1	-1	-0.02500000	0.03856758	0.5183

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.18937500	0.02727140	0.0001
-1	-0.05625000	0.02727140	0.0417

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.16906250	0.03856758	0.0001
1	-1	-0.05312500	0.03856758	0.1714
-1	1	0.20968750	0.03856758	0.0001
-1	-1	-0.05937500	0.03856758	0.1267

FACTOR VIII : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.18750000	0.03856758	0.0037
1	-1	-0.07312500	0.03856758	0.0608
-1	1	0.19125000	0.03856758	0.0001
-1	-1	-0.03937500	0.03856758	0.3097

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.21562500	0.03856758	0.0001
1	-1	-0.05187500	0.03856758	0.1816
-1	1	0.16312500	0.03856758	0.0001
-1	-1	-0.06062500	0.03856758	0.1190

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.27343750	0.03856758	0.0001
1	-1	0.02062500	0.03856758	0.5940
-1	1	0.10531250	0.03856758	0.0074
-1	-1	-0.13312500	0.03856758	0.0008

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.01843750	0.02727140	0.5005
-1	0.11468750	0.02727140	0.0001

FACTOR VIII : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.02687500	0.03856758	0.4875
1	-1	0.08906250	0.03856758	0.0229
-1	1	0.01000000	0.03856758	0.7959
-1	-1	0.14031250	0.03856758	0.0004

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.02468750	0.03856758	0.5235
1	-1	0.08968750	0.03856758	0.0220
-1	1	0.01218750	0.03856758	0.7526
-1	-1	0.13968750	0.03856758	0.0005

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.00593750	0.03856758	0.8779
1	-1	0.15781250	0.03856758	0.0001
-1	1	0.03093750	0.03856758	0.4243
-1	-1	0.07156250	0.03856758	0.0664

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.08187500	0.03856758	0.0362
1	-1	0.21218750	0.03856758	0.0001
-1	1	-0.04500000	0.03856758	0.2460
-1	-1	0.01718750	0.03856758	0.6568

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.17312500	0.03856758	0.0001
1	-1	0.20562500	0.03856758	0.0001
-1	1	-0.13625000	0.03856758	0.0006
-1	-1	0.02375000	0.03856758	0.5394

FACTOR VIII : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	2908.75898125	121.19829089	1.66	0.0431
Error	103	7530.04179922	73.10720193		
Corrected Total	127	10438.80078047			

R-Square	C.V.	Root MSE	Y Mean
0.278649	75.03275	8.55027496	11.39539062

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	217.79180859	72.59726953	0.99	0.3992
V7	1	94.78923828	94.78923828	1.30	0.2575
V8	1	13.44859453	13.44859453	0.18	0.6689
V9	1	403.74163203	403.74163203	5.52	0.0207 *
V10	1	254.50500078	254.50500078	3.48	0.0649
V11	1	6.04215703	6.04215703	0.08	0.7743
V7 * V11	1	36.36978828	36.36978828	0.59	0.4822
V8 * V11	1	106.01500078	106.01500078	1.45	0.2313
V9 * V11	1	81.32906953	81.32906953	1.11	0.2940
V10 * V11	1	32.37106953	32.37106953	0.44	0.5073
V12	1	6.82189453	6.82189453	0.09	0.7606
V11 * V12	1	4.12203828	4.12203828	0.06	0.8128
V13	1	15.71501953	15.71501953	0.21	0.6439
V7 * V13	1	45.47003203	45.47003203	0.62	0.4321
V8 * V13	1	0.74572578	0.74572578	0.01	0.9197
V9 * V13	1	24.36892578	24.36892578	0.33	0.5650
V10 * V13	1	8.64760078	8.64760078	0.12	0.7316
V11 * V13	1	25.82109453	25.82109453	0.35	0.5536
V12 * V13	1	1283.66111328	1283.66111328	17.56	0.0001 *
V14	1	149.62337578	149.62337578	2.05	0.1556
V11 * V14	1	65.10831328	65.10831328	0.89	0.3475
V13 * V14	1	32.25048828	65.25048828	0.44	0.5081

FACTOR V111 : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	12.2559375	1.0687844	0.0001
-1	10.5348437	1.0687844	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	11.7195312	1.0687844	0.0001
-1	11.0712500	1.0687844	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	13.1714062	1.0687844	0.0001
-1	9.6193750	1.0687844	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	12.8054687	1.0687844	0.0001
-1	9.9853125	1.0687844	0.0001

FACTOR V11 : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	11.6126562	1.0687844	0.0001
-1	11.1781250	1.0687844	0.0001

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	13.0062500	1.5114894	0.0001
1	-1	11.5056250	1.5114894	0.0001
-1	1	10.2190625	1.5114894	0.0001
-1	-1	10.8506250	1.5114894	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	11.8468750	1.5114894	0.0001
1	-1	11.5921875	1.5114894	0.0001
-1	1	10.3784375	1.5114894	0.0001
-1	-1	11.7640625	1.5114894	0.0001

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.5915625	1.5114894	0.0001
1	-1	13.7512500	1.5114894	0.0001
-1	1	10.6337500	1.5114894	0.0001
-1	-1	8.6050000	1.5114894	0.0001

FACTOR VIII : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	13.5256250	1.5114894	0.0001
1	-1	12.0853125	1.5114894	0.0001
-1	1	9.6996875	1.5114894	0.0001
-1	-1	10.2709375	1.5114894	0.0001

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	11.6262500	1.0687844	0.0001
-1	11.1645312	1.0687844	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	11.6640625	1.5114894	0.0001
1	-1	11.5612500	1.5114894	0.0001
-1	1	11.5884375	1.5114894	0.0001
-1	-1	10.7678125	1.5114894	0.0001

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	11.7457812	1.0687844	0.0001
-1	11.0450000	1.0687844	0.0001

FACTOR VIII : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.0103125	1.5114894	0.0001
1	-1	12.5015625	1.5114894	0.0001
-1	1	11.4812500	1.5114894	0.0001
-1	-1	9.5884375	1.5114894	0.0001

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.1462500	1.5114894	0.0001
1	-1	11.2928125	1.5114894	0.0001
-1	1	11.3453125	1.5114894	0.0001
-1	-1	10.7971875	1.5114894	0.0001

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	13.9581250	1.5114894	0.0001
1	-1	12.3846875	1.5114894	0.0001
-1	1	9.5334375	1.5114894	0.0001
-1	-1	9.7053125	1.5114894	0.0001

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.8959375	1.5114894	0.0001
1	-1	12.7150000	1.5114894	0.0001
-1	1	10.5956250	1.5114894	0.0001
-1	-1	9.3750000	1.5114894	0.0001

FACTOR V111 : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.4121875	1.5114894	0.0001
1	-1	10.8131250	1.5114894	0.0001
-1	1	11.0793750	1.5114894	0.0001
-1	-1	11.2768750	1.5114894	0.0001

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	15.1434375	1.5114894	0.0001
1	-1	8.1090625	1.5114894	0.0001
-1	1	8.3481250	1.5114894	0.0001
-1	-1	13.9809375	1.5114894	0.0001

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	12.4765625	1.0687844	0.0001
-1	10.3142187	1.0687844	0.0001

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	11.9806250	1.5114894	0.0001
1	-1	11.2446875	1.5114894	0.0001
-1	1	12.9725000	1.5114894	0.0001
-1	-1	9.3837500	1.5114894	0.0001

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.3250000	1.5114894	0.0001
1	-1	11.1665625	1.5114894	0.0001
-1	1	12.6281250	1.5114894	0.0001
-1	-1	9.4618750	1.5114894	0.0001

FIBRINOGEN : POST - PRE

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	5.92653125	0.24693880	1.54	0.0717
Error	103	16.52995625	0.16048501		
Corrected Total	127	22.45648750			

R-Square	C.V.	Root MSE	Y Mean
0.263912	-327.2926	0.40060581	-0.10343750

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	0.85211875	0.28403958	1.77	0.1576	
V1	1	0.10237812	0.10237812	0.64	0.4263	
V2	1	0.23805000	0.23805000	1.48	0.2260	
V1 * V2	1	0.05362812	0.05362812	0.33	0.5645	
V3	1	0.16102813	0.16102813	1.00	0.3188	
V1 * V3	1	0.03380000	0.03380000	0.21	0.6473	
V2 * V3	1	0.00090312	0.00090312	0.01	0.9403	
V4	1	0.03001250	0.03001250	0.19	0.6663	
V1 * V4	1	0.58050312	0.58050312	3.63	0.0600	
V2 * V4	1	0.06480000	0.06480000	0.40	0.5266	
V3 * V4	1	0.00812813	0.00812813	0.05	0.8224	
V5	1	0.01162813	0.01162813	0.07	0.7883	
V1 * V5	1	0.02420000	0.02420000	0.15	0.6986	
V2 * V5	1	0.20320313	0.20320313	1.27	0.2631	
V3 * V5	1	0.15961250	0.15961250	0.99	0.3210	
V4 * V5	1	1.88665312	1.88665312	11.76	0.0009	*
V6	1	0.69325312	0.69325312	4.32	0.0402	*
V1 * V6	1	0.40051250	0.40051250	2.50	0.1172	
V2 * V6	1	0.22612813	0.22612813	1.41	0.2379	
V3 * V6	1	0.14851250	0.14851250	0.93	0.3383	
V4 * V6	1	0.04727812	0.04727812	0.29	0.5885	
V5 * V6	1	0.00020000	0.00020000	0.00	0.9719	

FIBRINOGEN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.07515625	0.05007573	0.1365
-1	-0.13171875	0.05007573	0.0098

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.06031250	0.05007573	0.2312
-1	-0.14656250	0.05007573	0.0042

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05250000	0.07081777	0.4602
1	-1	-0.09781250	0.07081777	0.1702
-1	1	-0.06812500	0.07081777	0.3383
1	-1	-0.19531250	0.07081777	0.0069

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.06796875	0.05007573	0.1776
-1	-0.13890625	0.05007573	0.0066

FIBRINOGEN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05593750	0.07081777	0.4314
1	-1	-0.09437500	0.07081777	0.1856
-1	1	-0.08000000	0.07081777	0.2612
-1	-1	-0.18343750	0.07081777	0.0110

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.02750000	0.07081777	0.6986
1	-1	0.09312500	0.07081777	0.1914
-1	1	-0.10843750	0.07081777	0.1288
-1	-1	-0.18468750	0.07081777	0.0105

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.11875000	0.05007573	0.0001
-1	-0.08812500	0.05007573	0.0814

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.15781250	0.07081777	0.6986
1	-1	0.00750000	0.07081777	0.9159
-1	1	-0.07968750	0.07081777	0.2631
-1	-1	-0.18375000	0.07081777	0.0108

FIBRINOGEN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09812500	0.07081777	0.1689
1	-1	-0.02250000	0.07081777	0.7513
-1	1	-0.13937500	0.07081777	0.0517
-1	-1	-0.15375000	0.07081777	0.0322

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09125000	0.07081777	0.2005
1	-1	-0.04468750	0.07081777	0.5294
-1	1	-0.14625000	0.07081777	0.0414
-1	-1	-0.13156250	0.07081777	0.0661

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09390625	0.05007573	0.0636
-1	-0.11296875	0.05007573	0.0262

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07937500	0.07081777	0.2650
1	-1	-0.07093750	0.07081777	0.3188
-1	1	-0.10843750	0.07081777	0.1288
-1	-1	-0.15500000	0.07081777	0.0309

FIBRINOGEN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09062500	0.07081777	0.2035
1	-1	-0.03000000	0.07081777	0.6727
-1	1	-0.09718750	0.07081777	0.1729
-1	-1	-0.19593750	0.07081777	0.0067

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.02312500	0.07081777	0.7447
1	-1	-0.11281250	0.07081777	0.1142
-1	1	-0.16468750	0.07081777	0.0220
-1	-1	-0.11312500	0.07081777	0.1132

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.01218750	0.07081777	0.8637
1	-1	-0.24968750	0.07081777	0.0006
-1	1	-0.20000000	0.07081777	0.0057
-1	-1	0.02375000	0.07081777	0.7380

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.17703125	0.05007573	0.0006
-1	-0.02984375	0.05007573	0.5525

FIBRINOGEN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09281250	0.07081777	0.1929
1	-1	-0.05750000	0.07081777	0.4187
-1	1	-0.26125000	0.07081777	0.0004
-1	-1	-0.00218750	0.07081777	0.9754

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09187500	0.07081777	0.1974
1	-1	-0.02875000	0.07081777	0.6856
-1	1	-0.26218750	0.07081777	0.0003
-1	-1	-0.03093750	0.07081777	0.6631

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.10750000	0.07081777	0.1321
1	-1	-0.02843750	0.07081777	0.6888
-1	1	-0.24656250	0.07081777	0.0007
-1	-1	-0.03125000	0.07081777	0.6599

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.17312500	0.07081777	0.0162
1	-1	-0.06437500	0.07081777	0.3655
-1	1	-0.18093750	0.07081777	0.0121
-1	-1	0.00468750	0.07081777	0.9474

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.16625000	0.07081777	0.0208
1	-1	-0.02156250	0.07081777	0.7614
-1	1	-0.18781250	0.07081777	0.0093
-1	-1	-0.03812500	0.07081777	0.5915

FIBRINOGEN : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1065.46106250	44.39421094	2.34	0.0017
Error	103	1955.06312500	18.98119539		
Corrected Total	127	3020.524187507			

R-Square	C.V.	Root MSE	Y Mean
0.352740	50.79082	4.35674137	8.57781250

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	13.90491250	4.63497083	0.24	0.8653	
V7	1	104.04031250	104.04031250	5.48	0.0211	*
V8	1	37.41125000	37.41125000	1.97	0.1634	
V9	1	181.59415313	181.59415313	9.57	0.0025	*
V10	1	166.75945313	166.75945313	8.79	0.0038	*
V11	1	31.00781250	31.00781250	1.63	0.2041	
V7 * V11	1	12.12781250	12.12781250	0.64	0.4259	
V8 * V11	1	18.12020000	18.12020000	0.95	0.3308	
V9 * V11	1	22.93337812	22.93337812	1.21	0.2742	
V10 * V11	1	20.36815313	20.36815313	1.07	0.3027	
V12	1	25.40062813	25.40062813	1.34	0.2500	
V11 * V12	1	6.41715312	6.41715312	0.34	0.5622	
V13	1	5.49461250	5.49461250	0.29	0.5917	
V7 * V13	1	1.33661250	1.33661250	0.07	0.7913	
V8 * V13	1	0.36551250	0.36551250	0.02	0.8899	
V9 * V13	1	188.51965312	188.51965312	9.93	0.0021	*
V10 * V13	1	8.03002812	8.03002812	0.42	0.5169	
V11 * V13	1	41.90701250	41.90701250	2.21	0.1404	
V12 * V13	1	0.71102812	0.71102812	0.04	0.8469	
V14	1	97.47570313	97.47570313	5.14	0.0255	*
V11 * V14	1	49.67552812	49.67552812	2.62	0.1088	
V13 * V14	1	31.86015312	31.86015312	1.68	0.1980	

FIBRINOGEN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.47937500	0.54459267	0.0001
-1	7.67625000	0.54459267	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.11843750	0.54459267	0.0001
-1	8.03718750	0.54459267	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.76890625	0.54459267	0.0001
-1	7.38671875	0.54459267	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.71921875	0.54459267	0.0001
-1	7.43640625	0.54459267	0.0001

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.07000000	0.54459267	0.0001
-1	8.08562500	0.54459267	0.0001

FIBRINOGEN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.66375000	0.77017034	0.0001
1	-1	9.29500000	0.77017034	0.0001
-1	1	8.47625000	0.77017034	0.0001
-1	-1	6.87625000	0.77017034	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.23437500	0.77017034	0.0001
1	-1	9.00250000	0.77017034	0.0001
-1	1	8.90562500	0.77017034	0.0001
-1	-1	7.16875000	0.77017034	0.0001

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.83781250	0.77017034	0.0001
1	-1	9.70000000	0.77017034	0.0001
-1	1	8.30218750	0.77017034	0.0001
-1	-1	6.47125000	0.77017034	0.0001

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	10.6103125	0.77017034	0.0001
1	-1	8.8281250	0.77017034	0.0001
-1	1	7.5296875	0.77017034	0.0001
-1	-1	7.3431250	0.77017034	0.0001

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.02328125	0.54459267	0.0001
-1	8.13234375	0.54459267	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.29156250	0.77017034	0.0001
1	-1	8.84843750	0.77017034	0.0001
-1	1	8.75500000	0.77017034	0.0001
-1	-1	7.41625000	0.77017034	0.0001

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	8.37062500	0.54459267	0.0001
-1	8.78500000	0.54459267	0.0001

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.17000000	0.77017034	0.0001
1	-1	9.78875000	0.77017034	0.0001
-1	1	7.57125000	0.77017034	0.0001
-1	-1	7.78125000	0.77017034	0.0001

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	8.85781250	0.77017034	0.0001
1	-1	9.37906250	0.77017034	0.0001
-1	1	7.88343750	0.77017034	0.0001
-1	-1	8.19093750	0.77017034	0.0001

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	8.34812500	0.77017034	0.0001
1	-1	11.18968750	0.77017034	0.0001
-1	1	8.39312500	0.77017034	0.0001
-1	-1	6.38031250	0.77017034	0.0001

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.2615625	0.77017034	0.0001
1	-1	10.1768750	0.77017034	0.0001
-1	1	7.4796875	0.77017034	0.0001
-1	-1	7.3931250	0.77017034	0.0001

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.43500000	0.77017034	0.0001
1	-1	8.70500000	0.77017034	0.0001
-1	1	7.30625000	0.77017034	0.0001
-1	-1	8.86500000	0.77017034	0.0001

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	8.74156250	0.77017034	0.0001
1	-1	9.30500000	0.77017034	0.0001
-1	1	7.99968750	0.77017034	0.0001
-1	-1	8.26500000	0.77017034	0.0001

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	9.45046875	0.54459267	0.0001
-1	7.70515625	0.54459267	0.0001

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.31968750	0.77017034	0.0001
1	-1	8.82031250	0.77017034	0.0001
-1	1	9.58125000	0.77017034	0.0001
-1	-1	6.59000000	0.77017034	0.0001

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	8.74437500	0.77017034	0.0001
1	-1	7.99687500	0.77017034	0.0001
-1	1	10.15656250	0.77017034	0.0001
-1	-1	7.41343750	0.77017034	0.0001

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	150292.93750000	6262.20572917	3.80	0.0001
Error	103	169731.01367188	1647.87391914		
Corrected Total	127	320023.95117188			

R-Square	C.V.	Root MSE	Y Mean
0.469630	-1465.736	40.59401334	-2.76953125

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	103759.83398437	34586.11322812	20.99	0.0001	
V1	1	82.08007813	82.08007814	0.05	0.2838	
V2	1	627.90820313	627.90820313	0.38	0.5384	
V1 * V2	1	3714.14257813	3714.14257813	0.25	0.1363	
V3	1	4614.00195313	4614.00195313	2.80	0.0973	
V1 * V3	1	4307.08007813	4307.08007813	2.61	0.1090	
V2 * V3	1	714.89257813	714.89257813	0.43	0.5116	
V4	1	22.36132813	22.36132813	0.01	0.9075	
V1 * V4	1	3408.28320313	3408.28320313	2.07	0.1534	
V2 * V4	1	8.76757813	8.76757813	0.01	0.9420	
V3 * V4	1	25.83007813	25.83007813	0.02	0.9006	
V5	1	948.84570313	948.84570313	0.58	0.4497	
V1 * V5	1	1692.89257813	1692.89257813	1.03	0.3132	
V2 * V5	1	5336.73632813	5336.73632813	3.24	0.0749	
V3 * V5	1	8232.04882813	8232.04882813	5.00	0.0276	*
V4 * V5	1	2902.26757813	2902.26757813	1.76	0.1874	
V6	1	43.36132813	43.36132813	0.03	0.8715	
V1 * V6	1	7177.51757813	7177.51757813	4.36	0.0394	*
V2 * V6	1	422.31445313	422.31445313	0.26	0.6138	
V3 * V6	1	987.34570313	987.34570313	0.60	0.4407	
V4 * V6	1	998.48632813	998.48632813	0.61	0.4381	
V5 * V6	1	265.93945313	265.93945313	0.16	0.6887	

VON WILLEBRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.96875000	5.07425167	0.6988
-1	-3.57031250	5.07425167	0.4833

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-4.98437500	5.07425167	0.3283
-1	-0.55468750	5.07425167	0.9132

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	1.2031250	7.17607558	0.8672
1	1	-5.1406250	7.17607558	0.4754
-1	1	11.1718750	7.17607558	0.1226
-1	1	4.0312500	7.17607558	0.5755

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	3.23437500	5.07425167	0.5253
1	-8.77343750	5.07425167	0.0868

VON WILLIBRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.7656250	7.1760755	0.8061
1	-1	2.1718750	7.1760755	0.7628
-1	1	8.2343750	7.1760755	0.2538
-1	-1	-15.3750000	7.1760755	0.0345

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.34375000	7.1760755	0.8518
1	1	-8.62500000	7.1760755	0.2322
-1	1	7.81250000	7.1760755	0.2788
-1	-1	-8.91218750	7.1760755	0.2166

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.35156250	5.07425167	0.6440
-1	-3.18750000	5.07425167	0.5313

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	3.60937500	7.1760755	0.6161
1	-1	-7.54687500	7.1760755	0.2954
-1	1	-8.31250000	7.1760755	0.2494
-1	1	1.17187500	7.1760755	0.8706

VON WILLEBRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.82812500	7.1760755	0.5026
1	-1	-5.14062500	7.1760755	0.4754
-1	1	0.12500000	7.1760755	0.9861
-1	-1	1.23437500	7.1760755	0.8638

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	3.20312500	7.1760755	0.6563
1	-1	3.26562500	7.1760755	0.6500
-1	1	-7.90625000	7.1760755	0.2731
-1	-1	-9.64062500	7.1760755	0.1821

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.04687500	5.07425167	0.9926
-1	5.49218750	5.07425167	0.2816

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.39062500	7.1760755	0.5420
1	-1	-8.32812500	7.1760755	0.2485
-1	1	-4.48437500	7.1760755	0.5334
-1	-1	-2.65625000	7.1760755	0.7120

VON WILLEBRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-8.71875000	7.1760755	0.2272
1	-1	-1.25000000	7.1760755	0.8621
-1	1	8.62500000	7.1760755	0.2322
-1	-1	-9.73437500	7.1760755	0.1779

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.0625000	7.1760755	0.7744
1	-1	8.5312500	7.1760755	0.2372
-1	1	1.9687500	7.1760755	0.7844
-1	-1	19.5156250	7.1760755	0.0077

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.3906250	7.1760755	0.5420
1	-1	-0.3125000	7.1760755	0.9653
-1	1	4.2968750	7.1760755	0.5506
-1	-1	-10.6718750	7.1760755	0.1400

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.35156250	5.07425167	0.5104
-1	-2.18750000	5.07425167	0.6673

VON WILLEBRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.9375000	7.1760755	0.4930
1	-1	-8.8750000	7.1760755	0.2190
-1	1	-11.6406250	7.1760755	0.1078
-1	-1	4.5000000	7.1760755	0.5320

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.75000000	7.1760755	0.6024
1	-1	-6.21875000	7.1760755	0.3882
-1	1	-2.95312500	7.1760755	0.6815
-1	-1	1.84375000	7.1760755	0.7977

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.1250000	7.1760755	0.9861
1	-1	6.5937500	7.1760755	0.3603
-1	1	-6.5781250	7.1760755	0.3615
-1	-1	-10.9687500	7.1760755	0.1294

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.14062500	7.1760755	0.9844
1	-1	-4.56250000	7.1760755	0.5263
-1	1	-6.56250000	7.1760755	0.3626
-1	-1	0.18750000	7.1760755	0.9792

BRAND FACTOR : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.81250000	7.1760755	0.9101
1	-1	0.90625000	7.1760755	0.8998
-1	1	-7.51562500	7.1760755	0.2974
-1	-1	-8.46875000	7.1760755	0.6299

VON WILLEBRAND FACTOR : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	164085.03437500	6836.87643229	3.64	0.0001
Error	103	193626.78117187	1879.87166186		
Corrected Total	127	357711.81554688			

R-Square	C.V.	Root MSE	Y Mean
0.458707	-177.0879	43.35748680	-24.48359375

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	90719.55148437	30239.85049479	16.09	0.0001
V7	1	1284.61132812	1284.61132812	0.68	0.4103
V8	1	54.47070312	54.47070312	0.03	0.8652
V9	1	21577.43445312	21577.43445312	11.48	0.0010 *
V10	1	15389.15820312	15389.15820312	8.19	0.0051 *
V11	1	8474.39257812	8474.39257812	4.51	0.0361 *
V7 * V11	1	643.95632812	643.95632812	0.34	0.5596
V8 * V11	1	908.97820312	908.97820312	0.48	0.4884
V9 * V11	1	77.34570312	77.34570312	0.04	0.8397
V10 * V11	1	1546.37507813	1546.37507813	0.82	0.3665
V12	1	1921.22507812	1921.22507812	1.02	0.3144
V11 * V12	1	2498.36132812	2498.36132812	1.33	0.2517
V13	1	54.47070313	54.47070313	0.03	0.8652
V7 * V13	1	267.09382812	267.09382812	0.14	0.7070
V8 * V13	1	1169.46570312	1169.46570312	0.62	0.4321
V9 * V13	1	1149.00195313	1149.00195313	0.61	0.4361
V10 * V13	1	5.65320312	5.65320312	0.00	0.5964
V11 * V13	1	2047.20007813	2047.20007813	1.09	0.2991
V12 * V13	1	10037.67382813	10037.67382813	5.34	0.0228*
V14	1	3977.20507812	3977.20507812	2.12	0.1488
V11 * V14	1	159.53445313	159.53445313	0.08	0.7714
V13 * V14	1	121.87507812	121.87507812	0.06	0.7995

VON WILLEBRAND FACTOR : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-21.3156250	5.4196859	0.0002
-1	-27.65156257	5.1496859	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-23.8312500	5.4196859	0.0001
-1	-25.1359375	5.1496859	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-11.5000000	5.4196859	0.0362
-1	-37.4671875	5.1496859	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	13.5187500	5.4196859	0.0142
-1	-35.4484375	5.1496859	0.0001

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-16.3468750	5.4196859	0.0032
1	-32.6203125	5.1496859	0.0001

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-15.4218750	7.6645932	0.0468
1	-1	-27.2093750	7.6645932	0.0006
-1	1	-17.2718750	7.6645932	0.0263
1	-1	-38.0312500	7.6645932	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-18.3593750	7.6645932	0.0184
1	-1	-29.3031250	7.6645932	0.0002
-1	1	-14.3343750	7.6645932	0.0463
-1	-1	-35.9375000	7.6645932	0.0001

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.1406250	7.6645932	0.5902
1	-1	-18.8593750	7.6645932	0.0155
1	1	-28.5531250	7.6645932	0.0003
1	-1	-46.3812500	7.6645932	0.0001

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.9062500	7.6645932	0.8041
1	-1	-25.1312500	7.6645932	0.0014
-1	1	-30.7875000	7.6645932	0.0001
-1	-1	-40.1093750	7.6645932	0.0001

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-20.6093750	5.4196859	0.0002
-1	-28.3578125	5.1496859	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.8906250	7.6645932	0.0298
1	-1	-15.8031250	7.6645932	0.0417
-1	1	-24.3281250	7.6645932	0.0020
-1	-1	-40.9125000	7.6645932	0.0001

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-25.1359375	5.4196859	0.0142
-1	-28.8312500	5.1496859	0.0001

VON WILLEBRAND FACTOR : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-23.4125000	7.6645932	0.0029
1	-1	-19.2187500	7.6645932	0.0137
-1	1	-26.8593750	7.6645932	0.0007
-1	-1	-28.4437500	7.6645932	0.0003

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-27.5062500	7.6645932	0.0005
1	-1	-20.1562500	7.6645932	0.0099
-1	1	-22.7656250	7.6645932	0.0037
-1	-1	-27.5062500	7.6645932	0.0005

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-9.1562500	7.6645932	0.2350
1	-1	-13.8437500	7.6645932	0.0738
-1	1	-41.1156250	7.6645932	0.0001
-1	-1	-33.8187500	7.6645932	0.0001

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-14.3812500	7.6645932	0.0634
1	-1	-12.6562500	7.6645932	0.1017
-1	1	-35.8906250	7.6645932	0.0001
-1	-1	-35.0062500	7.6645932	0.0001

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-13.0000000	7.6645932	0.0929
1	-1	-19.6937500	7.6645932	0.0116
-1	1	-37.2718750	7.6645932	0.0001
-1	-1	-27.9687500	7.6645932	0.0004

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	12.4062500	7.6645932	0.1068
1	-1	-28.8125000	7.6645932	0.0003
-1	1	-37.8656250	7.6645932	0.0001
-1	-1	-18.8500000	7.6645932	0.0156

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-18.9093750	5.4196859	0.0007
-1	-30.0578125	5.1496859	0.0001

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	9.6562500	7.6645932	0.2106
1	-1	-23.0375000	7.6645932	0.0033
-1	1	-28.1625000	7.6645932	0.0004
-1	-1	37.0781250	7.6645932	0.0001

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-20.5375000	7.6645932	0.0086
1	-1	-29.7343750	7.6645932	0.0002
-1	1	-17.2812500	7.6645932	0.0263
-1	-1	-30.3812500	7.6645932	0.0001

pH : POST - PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1.85393125	0.07724714	1.73	0.0311
Error	103	4.59661875	0.04462737		
Corrected Total	127	6.45055000			

R-Square	C.V.	Root MSE	Y Mean
0.287407	132.5502	0.21125190	0.15937500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.85446250	0.85446250	6.38	0.0005
V1	1	0.07125312	0.07125312	1.60	0.2092
V2	1	0.03850313	0.03850313	0.86	0.3551
V1 * V2	1	0.01051250	0.01051250	0.24	0.6285
V3	1	0.00211250	0.00211250	0.05	0.8282
V1 * V3	1	0.04425312	0.04425312	0.99	0.3217
V2 * V3	1	0.01487812	0.01487812	0.33	0.5649
V4	1	0.12005000	0.12005000	2.69	0.1040
V1 * V4	1	0.01487812	0.01487812	0.33	0.5649
V2 * V4	1	0.05865313	0.05865313	1.31	0.2543
V3 * V4	1	0.04651250	0.04651250	1.04	0.3097
V5	1	0.04500000	0.04500000	1.01	0.3177
V1 * V5	1	0.00052812	0.00052812	0.01	0.9136
V2 * V5	1	0.00002813	0.00002813	0.00	0.9800
V3 * V5	1	0.01280000	0.0128000C	0.29	0.5934
V4 * V5	1	0.01531250	0.01531250	0.34	0.5593
V6	1	0.31205000	0.31205000	6.99	0.0095 *
V1 * V6	1	0.15540313	0.15540313	3.48	0.0649
V2 * V6	1	0.00000313	0.00000313	0.00	0.9933
V3 * V6	1	0.03511250	0.03511250	0.79	0.3771
V4 * V6	1	0.00101250	0.00101250	0.02	0.8806
V5 * V6	1	0.00061250	0.00061250	0.01	0.9070

pH : POST PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.13578125	0.02640649	0.0001
-1	0.18296875	0.02640649	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.17671875	0.02640649	0.0001
-1	0.14203125	0.02640649	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.14406250	0.03734441	0.0002
1	-1	0.12750000	0.03734441	0.0009
-1	1	0.20937500	0.03734441	0.0001
-1	-1	0.15656250	0.03734441	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.15531250	0.02640649	0.0001
-1	0.16343750	0.02640649	0.0001

pH : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.15031250	0.03734441	0.0001
1	-1	0.12125000	0.03734441	0.0016
-1	1	0.16031250	0.03734441	0.0001
-1	-1	0.2-562500	0.03734441	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.16187500	0.03734441	0.0001
1	-1	0.19156250	0.03734441	0.0001
-1	1	0.14875000	0.03734441	0.0001
-1	-1	0.13531250	0.03734441	0.0005

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.15531250	0.02640649	0.0001
-1	0.16343750	0.02640649	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.11593750	0.03734441	0.0025
1	-1	0.15562500	0.03734441	0.0001
-1	1	0.14156250	0.03734441	0.0003
-1	1	0.22437500	0.03734441	0.0001

pH : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.12468750	0.03734441	0.0012
1	-1	0.22875000	0.03734441	0.0001
-1	1	0.13281250	0.03734441	0.0006
-1	-1	0.15125000	0.03734441	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.14375000	0.03734441	0.0002
1	-1	0.16687500	0.03734441	0.0001
-1	1	0.11375000	0.03734441	0.0029
-1	-1	0.21312500	0.03734441	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.17812500	0.02640649	0.0001
-1	0.14062500	0.02640649	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.15656250	0.03734441	0.0001
1	-1	0.11500000	0.03734441	0.0027
-1	1	0.19968750	0.03734441	0.0001
-1	-1	0.16625000	0.03734441	0.0001

pH : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.19500000	0.03734441	0.0001
1	-1	0.15843750	0.03734441	0.0001
-1	1	0.16125000	0.03734441	0.0001
-1	-1	0.12281250	0.03734441	0.0014

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.18406250	0.03734441	0.0001
1	-1	0.12656250	0.03734441	0.0010
-1	1	0.17218750	0.03734441	0.0001
-1	-1	0.15468750	0.03734441	0.0001

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.13656250	0.03734441	0.0004
1	-1	0.12093750	0.03734441	0.0016
-1	1	0.21968750	0.03734441	0.0001
-1	-1	0.16031250	0.03734441	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.20875000	0.02640649	0.0001
-1	0.11000000	0.02640649	0.0001

pH : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.15031250	0.03734441	0.0001
1	-1	0.12125000	0.03734441	0.0016
-1	1	0.26718750	0.03734441	0.0001
-1	-1	0.09875000	0.03734441	0.0095

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.22593750	0.03734441	0.0001
1	-1	0.12750000	0.03734441	0.0009
-1	1	0.19156250	0.03734441	0.0001
-1	-1	0.09250000	0.03734441	0.0149

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.22125000	0.03734441	0.0001
1	-1	0.08937500	0.03734441	0.0185
-1	1	0.19625000	0.03734441	0.0001
-1	-1	0.13062500	0.03734441	0.0007

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.17531250	0.03734441	0.0001
1	-1	0.08218750	0.03734441	0.0300
-1	1	0.24218750	0.03734441	0.0001
-1	-1	0.13781250	0.03734441	0.0004

pH : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.22531250	0.03734441	0.0001
1	-1	0.13093750	0.03734441	0.0007
-1	1	0.19218750	0.03734441	0.0001
-1	-1	0.08906250	0.03734441	0.0189

pH : PRODUCTION

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1.24002188	0.05166758	2.80	0.0002
Error	103	1.89785000	0.01842573		
Corrected Total	127	3.13787187			

R-Square	C.V.	Root MSE	Y Mean
0.395179	-133.0390	0.13574140	-0.10203125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.15230313	0.05076771	2.76	0.0462
V7	1	0.09570313	0.09570313	5.19	0.0247 *
V8	1	0.16820000	0.16820000	9.13	0.0032 *
V9	1	0.00052812	0.00052812	0.03	0.8659
V10	1	0.08201250	0.08201250	4.45	0.0373 *
V11	1	0.03001250	0.03001250	1.63	0.2047
V7 * V11	1	0.02205000	0.02205000	1.20	0.2765
V8 * V11	1	0.00015313	0.00015313	0.01	0.9275
V9 * V11	1	0.00080000	0.00080000	0.04	0.8354
V10 * V11	1	0.05200312	0.05200312	2.82	0.0960
V12	1	0.00125000	0.00125000	0.07	0.7950
V11 * V12	1	0.04277813	0.04277813	2.32	0.1306
V13	1	0.01201250	0.01201250	0.65	0.4213
V7 * V13	1	0.00211250	0.00211250	0.11	0.7356
V8 * V13	1	0.01320312	0.01320313	0.72	0.3993
V9 * V13	1	0.13520000	0.13520000	7.34	0.0079 *
V10 * V13	1	0.00300313	0.00300313	0.16	0.6873
V11 * V13	1	0.06037812	0.06037812	3.28	0.0732
V12 * V13	1	0.26462813	0.26462813	14.36	0.0003 *
V14	1	0.07507813	0.07507813	4.07	0.0461 *
V11 * V14	1	0.02000000	0.02000000	1.09	0.2999
V13 * V14	1	0.00661250	0.00661250	0.36	0.5504

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.07468750	0.01696768	0.0001
-1	-0.12937500	0.01696768	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.06578125	0.01696768	0.0001
-1	-0.13828125	0.01696768	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.10000000	0.01696768	0.0001
-1	0.10406250	0.01696768	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.07671875	0.01696768	0.0001
-1	-0.12734375	0.01696768	0.0001

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.08671875	0.01696768	0.0001
1	-0.11734375	0.01696768	0.0001

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07250000	0.02399592	0.0032
1	-1	-0.07687500	0.02399592	0.0018
-1	1	-0.10093750	0.02399592	0.0001
-1	-1	-0.15781250	0.02399592	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04937500	0.02399592	0.0422
1	-1	-0.08218750	0.02399592	0.0009
-1	1	-0.12406250	0.02399592	0.0001
-1	-1	-0.15250000	0.02399592	0.0001

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08718750	0.02399592	0.0004
1	-1	-0.11281250	0.02399592	0.0001
1	1	-0.08625000	0.02399592	0.0005
1	-1	-0.12187500	0.02399592	0.0001

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08156250	0.02399592	0.0010
1	-1	-0.07187500	0.02399592	0.0034
-1	1	-0.09187500	0.02399592	0.0002
-1	-1	-0.16281250	0.02399592	0.0001

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09890625	0.01696768	0.0001
-1	-0.10515625	0.01696768	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06531250	0.02399592	0.0076
1	-1	-0.10812500	0.02399592	0.0001
-1	1	-0.13250000	0.02399592	0.0001
-1	-1	-0.10218750	0.02399592	0.0001

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09234375	0.01696768	0.0001
-1	-0.11171875	0.01696768	0.0001

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06093750	0.02399592	0.0126
1	-1	-0.08843750	0.02399592	0.0004
-1	1	-0.12375000	0.02399592	0.0001
-1	-1	-0.13500000	0.02399592	0.0001

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06625000	0.02399592	0.0068
1	-1	-0.06531250	0.02399592	0.0076
-1	1	-0.11843750	0.02399592	0.0001
-1	-1	-0.15812500	0.02399592	0.0001

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12281250	0.02399592	0.0001
1	-1	-0.07718750	0.02399592	0.0017
-1	1	-0.06187500	0.02399592	0.0113
-1	-1	-0.14625000	0.02399592	0.0001

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06218750	0.02399592	0.0109
1	-1	-0.09125000	0.02399592	0.0002
-1	1	-0.12250000	0.02399592	0.0001
-1	-1	-0.13218750	0.02399592	0.0001

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09875000	0.02399592	0.0001
1	-1	-0.07468750	0.02399592	0.0024
-1	1	-0.08593750	0.02399592	0.0005
-1	-1	-0.14875000	0.02399592	0.0001

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04375000	0.02399592	0.0712
1	-1	-0.15406250	0.02399592	0.0001
-1	1	-0.14093750	0.02399592	0.0001
-1	-1	-0.06937500	0.02399592	0.0047

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.12625000	0.01696768	0.0001
-1	-0.07781250	0.01696768	0.0001

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09843750	0.02399592	0.0001
1	-1	-0.07500000	0.02399592	0.0023
-1	1	-0.15406250	0.02399592	0.0001
1	-1	-0.08062500	0.02399592	0.0011

pH : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12375000	0.02399592	0.0001
1	-1	-0.06093750	0.02399592	0.0126
-1	1	-0.12875000	0.02399592	0.0001
-1	-1	-0.09468750	0.02399592	0.0001

MAGNESIUM : POST - PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	0.84258125	0.03510755	2.27	0.0025
Error	103	1.59489297	0.01548440		
Corrected Total	127	2.43747422			

R-Square	C.V.	Root MSE	Y Mean
0.345678	-111.7744	0.12443632	-0.11132812

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.40160859	0.13386953	8.65	0.0001
V1	1	0.04314453	0.04314453	2.79	0.0981
V2	1	0.02178828	0.02178828	1.41	0.2383
V1 * V2	1	0.06891328	0.06891328	4.45	0.0373 *
V3	1	0.02970703	0.02970703	1.92	0.1690
V1 * V3	1	0.02503203	0.02503203	1.62	0.2064
V2 * V3	1	0.00253828	0.00253828	0.16	0.6864
V4	1	0.05994453	0.05994453	3.87	0.0518 *
V1 * V4	1	0.00006328	0.00006328	0.00	0.9492
V2 * V4	1	0.00003828	0.00003828	0.00	0.9604
V3 * V4	1	0.03219453	0.03219453	2.08	0.1524
V5	1	0.00219453	0.00219453	0.14	0.7073
V1 * V5	1	0.00106953	0.00106953	0.07	0.7932
V2 * V5	1	0.01509453	0.01509453	0.97	0.3258
V3 * V5	1	0.00796953	0.00796953	0.51	0.4747
V4 * V5	1	0.01106328	0.01106328	0.71	0.3999
V6	1	0.02075703	0.02075703	1.34	0.2496
V1 * V6	1	0.00657038	0.00657038	0.04	0.8372
V2 * V6	1	0.00591328	0.00591328	0.38	0.5380
V3 * V6	1	0.00735078	0.00735078	0.47	0.4924
V4 * V6	1	0.00997578	0.00997578	0.64	0.4240
V5 * V6	1	0.07556328	0.07556328	4.88	0.0294 *

MAGNESIUM : **POST - PRE**

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09296875	0.01555454	0.0001
-1	-0.12968750	0.01555454	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09828125	0.01555454	0.0001
-1	-0.12437500	0.01555454	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.10312500	0.02199744	0.0001
1	-1	-0.08281250	0.02199744	0.0003
-1	1	0.09343750	0.02199744	0.0001
-1	-1	-0.16593750	0.02199744	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.12656250	0.01555454	0.0001
-1	-0.09609375	0.01555454	0.0001

MAGNESIUM : ***POST - PRE***

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12218750	0.02199744	0.0001
1	-1	-0.06375000	0.02199744	0.0046
-1	1	-0.13093750	0.02199744	0.0001
-1	-1	-0.12843750	0.02199744	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.10906250	0.02199744	0.0001
1	-1	-0.08750000	0.02199744	0.0001
-1	1	-0.14406250	0.02199744	0.0001
-1	-1	-0.10468750	0.02199744	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.08968750	0.01555454	0.0001
-1	-0.13296875	0.01555454	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07062500	0.02199744	0.0018
1	-1	-0.11531250	0.02199744	0.0001
-1	1	-0.10875000	0.02199744	0.0001
-1	-1	-0.15062500	0.02199744	0.0001

MAGNESIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07718750	0.02199744	0.0007
1	-1	-0.11937500	0.02199744	0.0001
-1	1	-0.10218750	0.02199744	0.0001
-1	-1	-0.14656250	0.02199744	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08906250	0.02199744	0.0001
1	-1	-0.16406250	0.02199744	0.0001
-1	1	-0.09031250	0.02199744	0.0001
-1	-1	-0.10187500	0.02199744	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.11546875	0.01555454	0.0001
-1	-0.10718750	0.01555454	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.10000000	0.02199744	0.0001
1	-1	-0.08593750	0.02199744	0.0002
-1	1	-0.13093750	0.02199744	0.0001
-1	-1	-0.12843750	0.02199744	0.0001

MAGNESIUM : ***POST - PRE***

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09156250	0.02199744	0.0001
1	-1	-0.10500000	0.02199744	0.0001
-1	1	-0.13937500	0.02199744	0.0001
-1	-1	-0.10937500	0.02199744	0.0001

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12281250	0.02199744	0.0001
1	-1	-0.13031250	0.02199744	0.0001
-1	1	-0.10812500	0.02199744	0.0001
-1	-1	-0.08406250	0.02199744	0.0002

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.10312500	0.02199744	0.0001
1	-1	-0.07625000	0.02199744	0.0008
-1	1	-0.12781250	0.02199744	0.0001
-1	-1	-0.13812500	0.02199744	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09859375	0.01555454	0.0001
-1	-0.12406250	0.01555454	0.0001

MAGNESIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08250000	0.02199744	0.0003
1	-1	-0.10343750	0.02199744	0.0001
-1	1	-0.11468750	0.02199744	0.0001
-1	-1	-0.14468750	0.02199744	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07875000	0.02199744	0.0005
1	-1	-0.11781250	0.02199744	0.0001
-1	1	-0.11843750	0.02199744	0.0001
-1	-1	-0.13031250	0.02199744	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.10625000	0.02199744	0.0001
1	-1	-0.14687500	0.02199744	0.0001
-1	1	-0.09093750	0.02199744	0.0001
-1	-1	-0.10125000	0.02199744	0.0001

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06812500	0.02199744	0.0025
1	-1	-0.11125000	0.02199744	0.0001
-1	1	-0.12906250	0.02199744	0.0001
-1	-1	-0.13687500	0.02199744	0.0001

MAGNESIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.07843750	0.02199744	0.0006
1	-1	-0.15250000	0.02199744	0.0001
-1	1	-0.11875000	0.02199744	0.0001
-1	-1	-0.09562500	0.02199744	0.0001

MAGNESIUM : ***PRODUCTION***

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	0.28127500	0.01171979	1.13	0.3298
Error	103	1.07177422	0.01040557		
Corrected Total	127	1.35304922			

R-Square	C.V.	Root MSE	Y Mean
0.207882	-177.6461	0.10200772	-0.05742187

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.13507109	0.04502370	4.33	0.0065
V7	1	0.00056953	0.00056953	0.05	0.8155
V8	1	0.00219453	0.00219453	0.21	0.6470
V9	1	0.00564453	0.00564453	0.54	0.4631
V10	1	0.02338203	0.02338203	2.25	0.1369
V11	1	0.00003828	0.00003828	0.00	0.9518
V7 * V11	1	0.02231328	0.02231338	2.14	0.1461
V8 * V11	1	0.00253828	0.00253828	0.24	0.6224
V9 * V11	1	0.00013203	0.00013203	0.01	0.9105
V10 * V11	1	0.01381953	0.01381953	1.33	0.2518
V12	1	0.00796593	0.00796593	0.77	0.3835
V11 * V12	1	0.00271953	0.00271953	0.26	0.6103
V13	1	0.00034453	0.00034453	0.03	0.8560
V7 * V13	1	0.00118828	0.00118828	0.11	0.7361
V8 * V13	1	0.01381953	0.01381953	1.33	0.2518
V9 * V13	1	0.00172578	0.00172578	0.17	0.6847
V10 * V13	1	0.03611328	0.03611328	3.47	0.0653
V11 * V13	1	0.00828828	0.00828828	0.80	0.3742
V12 * V13	1	0.00001953	0.00001953	0.00	0.9655
V14	1	0.00253203	0.00253203	0.20	0.6595
V11 * V14	1	0.00106953	0.00106953	0.10	0.7492
V13 * V14	1	0.00028203	0.00028203	0.03	0.8696

MAGNESIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.05531250	0.01275096	0.0001
-1	-0.05953125	0.01275096	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.05328125	0.01275096	0.0001
-1	-0.06156250	0.01275096	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.06406250	0.01275096	0.0001
-1	-0.05078125	0.01275096	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.07093750	0.01275096	0.0001
-1	-0.04396250	0.01275096	0.0008

MAGNESIUM : ***PRODUCTION***

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.05796875	0.01275096	0.0001
-1	-0.05687500	0.01275096	0.0008

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06906250	0.01803259	0.0002
1	-1	-0.04156250	0.01803259	0.0232
-1	1	-0.04687500	0.01803259	0.0107
-1	-1	-0.07218750	0.01803259	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04937500	0.01803259	0.0073
1	-1	-0.05718750	0.01803259	0.0020
-1	1	-0.06656250	0.01803259	0.0004
-1	-1	-0.05656250	0.01803259	0.0022

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06562500	0.01803259	0.0004
1	-1	-0.06250000	0.01803259	0.0008
-1	1	-0.05031250	0.01803259	0.0063
-1	-1	-0.05125000	0.01803259	0.0054

MAGNESIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08187500	0.01803259	0.0001
1	-1	-0.06000000	0.01803259	0.0012
-1	1	-0.03406250	0.01803259	0.0617
-1	-1	-0.05375000	0.01803259	0.0036

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.04953125	0.01275096	0.0002
-1	-0.06531250	0.01275096	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05468750	0.01803259	0.0031
1	-1	-0.06125000	0.01803259	0.0010
-1	1	-0.04437500	0.01803259	0.0155
-1	-1	-0.06937500	0.01803259	0.0002

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.05578125	0.01275096	0.0001
-1	-0.05906250	0.01275096	0.0001

MAGNESIUM : *PRODUCTION*

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05062500	0.01803259	0.0060
1	-1	-0.06000000	0.01803259	0.0012
-1	1	-0.06093750	0.01803259	0.0010
-1	-1	-0.05812500	0.01803259	0.0017

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04125000	0.01803259	0.0242
1	-1	-0.06531250	0.01803259	0.0005
-1	1	-0.07031250	0.01803259	0.0002
-1	-1	-0.05281280	0.01803259	0.0042

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05875000	0.01803259	0.0015
1	-1	-0.06937500	0.01803259	0.0002
-1	1	-0.05281250	0.01803259	0.0042
-1	-1	-0.04875000	0.01803259	0.0080

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05250000	0.01803259	0.0044
1	-1	-0.08937500	0.01803259	0.0001
-1	1	-0.05906250	0.01803259	0.0014
-1	-1	-0.02875000	0.01803259	0.1139

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06437500	0.01803259	0.0005
1	-1	-0.05156250	0.01803259	0.0051
-1	1	-0.04718750	0.01803259	0.0102
-1	-1	-0.06656250	0.01803259	0.0004

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04750000	0.01803259	0.0097
1	-1	-0.05156250	0.01803259	0.0051
-1	1	-0.06406250	0.01803259	0.0006
-1	-1	-0.06656250	0.01803259	0.0004

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.06140625	0.01275096	0.0001
-1	-0.05343750	0.01275096	0.0001

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.05906250	0.01803259	0.0014
1	-1	-0.05687500	0.01803259	0.0021
-1	1	-0.06375000	0.01803259	0.0006
-1	-1	-0.05000000	0.01803259	0.0066

MAGNESIUM : *PRODUCTION*

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06125000	0.01803259	0.0010
1	-1	-0.05031250	0.01803259	0.0063
-1	1	0.06156250	0.01803259	0.0009
-1	1	-0.05656250	0.01803259	0.0022

TOTAL PROTEIN : POST PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	5044.43750000	210.18489583	1.87	0.0164
Error	103	11569.67968750	112.32698726		
Corrected Total	127	16614.11718750			

R-Square	C.V.	Root MSE	Y Mean
0.303624	-204.0001	10.598844268	-5.19531250

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	1584.77343750	528.25781250	4.70	0.0041	
V1	1	508.00781250	508.00781250	4.52	0.0358	*
V2	1	187.69531250	187.69531250	1.67	0.1990	
V1 * V2	1	328.32031250	328.32031250	2.92	0.0903	
V3	1	17.25781250	17.25781250	0.15	0.6959	
V1 * V3	1	134.07031250	134.07031250	1.19	0.2772	
V2 * V3	1	11.88281250	11.88281250	0.11	0.7457	
V4	1	835.38281250	835.38281250	7.44	0.0075	*
V1 * V4	1	5.69531250	5.69531250	0.05	0.8223	
V2 * V4	1	2.25781250	2.25781250	0.02	0.8875	
V3 * V4	1	244.75781250	244.75781250	2.18	0.1430	
V5	1	0.38281250	0.38281250	0.00	0.9536	
V1 * V5	1	267.38281250	267.38281250	2.38	0.1259	
V2 * V5	1	89.44531250	89.44531250	0.80	0.3743	
V3 * V5	1	29.07031250	29.07031250	0.26	0.6120	
V4 * V5	1	173.44531250	173.44531250	1.54	0.2168	
V6	1	409.69531250	409.69531250	3.65	0.0589	*
V1 * V6	1	14.44531250	14.44531250	3.13	0.7206	
V2 * V6	1	13.13281250	13.13281250	0.12	0.7331	
V3 * V6	1	126.00781250	126.00781250	1.12	0.2920	
V4 * V6	1	4.88281250	4.88281250	0.04	0.8353	
V5 * V6	1	56.44531250	56.44531250	0.50	0.4800	

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.20312500	1.32480534	0.0174
-1	-7.18750000	1.32480534	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.98437500	1.32480534	0.0033
-1	-6.40625000	1.32480534	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.59375000	1.87355767	0.0579
1	-1	-2.8125000	1.87355767	0.1364
-1	1	-4.3750000	1.87355767	0.0215
-1	-1	-10.0000000	1.87355767	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.56250000	1.32480534	0.0001
-1	-4.82812500	1.32480534	0.0004

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.59375000	1.87355767	0.0159
1	-1	-1.81250000	1.87355767	0.3356
-1	1	-6.53125000	1.87355767	0.0007
-1	-1	-7.84375000	1.87355767	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.65625000	1.87355767	0.0146
1	-1	-3.31250000	1.87355767	0.0800
-1	1	-6.46875000	1.87355767	0.0008
-1	-1	-6.34375000	1.87355767	0.0010

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.64062500	1.32480534	0.0489
-1	-7.75000000	1.32480534	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.43750000	1.87355767	0.8158
1	-1	-5.96875000	1.87355767	0.0019
-1	1	-4.84375000	1.87355767	0.0111
-1	-1	-9.53125000	1.87355767	0.0001

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.56250000	1.87355767	0.4062
1	-1	-6.40625000	1.87355767	0.0009
-1	1	-3.71875000	1.87355767	0.0498
-1	-1	-9.09375000	1.87355767	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.62500000	1.87355767	0.3878
1	-1	-9.50000000	1.87355767	0.0001
-1	1	-3.65625000	1.87355767	0.0537
-1	-1	-6.00000000	1.87355767	0.0018

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.14062500	1.32480534	0.0002
-1	-5.25000000	1.32480534	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.59375000	1.87355767	0.0159
1	-1	-1.81250000	1.87355767	0.3356
-1	1	-5.68750000	1.87355767	0.0030
-1	-1	-8.68750000	1.87355767	0.0001

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.09375000	1.87355767	0.1017
1	-1	-4.87500000	1.87355767	0.0106
-1	1	-7.18750000	1.87355767	0.0002
-1	-1	-5.62500000	1.87355767	0.0034

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.03125000	1.87355767	0.0084
1	-1	-6.09375000	1.87355767	0.0015
-1	1	-5.25000000	1.87355767	0.0061
-1	-1	-4.40625000	1.87355767	0.0206

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.75000000	1.87355767	0.0480
1	-1	-1.53125000	1.87355767	0.4156
-1	1	-6.53125000	1.87355767	0.0007
-1	-1	-8.96875000	1.87355767	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.40625000	1.32480534	0.0116
-1	-6.98437500	1.32480534	0.0001

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.75000000	1.87355767	0.3525
1	-1	-4.65625000	1.87355767	0.0146
-1	1	-5.06250000	1.87355767	0.0081
-1	-1	-9.31250000	1.87355767	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.87500000	1.87355767	0.3193
1	-1	-6.09375000	1.87355767	0.0015
-1	1	-4.93750000	1.87355767	0.0097
-1	-1	-7.87500000	1.87355767	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.78125000	1.87355767	0.1407
1	-1	-8.34375000	1.87355767	0.0001
-1	1	-4.03125000	1.87355767	0.0338
-1	-1	-5.62500000	1.87355767	0.0034

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.65625000	1.87355767	0.7269
1	-1	4.62500000	1.87355767	0.0152
-1	1	-6.15625000	1.87355767	0.0014
-1	-1	-9.34375000	1.87355767	0.0001

TOTAL PROTEIN : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.68750000	1.87355767	0.1545
1	-1	-7.59375000	1.87355767	0.0001
-1	1	-4.12500000	1.87355767	0.0299
-1	-1	-6.37500000	1.87355767	0.0010

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1512.65625000	63.02734375	0.72	0.8169
Error	103	8970.31250000	87.09041262		
Corrected Total	127	10482.96875000			

R-Square	C.V.	Root MSE	Y Mean
0.144297	-417.6660	9.33222442	-.23437500

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	467.90625000	155.96875000	1.79	0.1536
V7	1	72.00000000	72.00000000	0.83	0.3653
V8	1	52.53125000	52.53125000	0.60	0.4391
V9	1	4.50000000	4.50000000	0.05	0.8206
V10	1	210.12500000	210.12500000	2.41	0.1234
V11	1	0.78125000	0.78125000	0.01	0.9247
V7 * V11	1	36.12500000	36.12500000	0.41	0.5210
V8 * V11	1	34.03125000	34.03125000	0.39	0.5333
V9 * V11	1	6.12500000	6.12500000	0.07	0.7914
V10 * V11	1	66.12500000	66.12500000	0.76	0.3856
V12	1	195.03125000	195.03125000	2.24	0.1376
V11 * V12	1	0.78125000	0.78125000	0.01	0.9247
V13	1	0.28125000	0.28125000	0.00	0.9548
V7 * V13	1	3.12500000	3.12500000	0.04	0.8501
V8 * V13	1	30.03125000	30.03125000	0.34	0.5583
V9 * V13	1	1.12500000	1.12500000	0.01	0.9097
V10 * V13	1	276.12500000	276.12500000	3.17	0.0779
V11 * V13	1	7.03125000	7.03125000	0.08	0.7769
V12 * V13	1	38.28125000	38.28125000	0.44	0.5088
V14	1	5.28125000	5.28125000	0.06	0.8060
V11 * V14	1	3.78125000	3.78125000	0.04	0.8354
V13 * V14	1	1.53125000	1.53125000	0.02	0.8948

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.48437500	1.6652805	0.2061
-1	-2.98437500	1.6652805	0.0120

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.59375000	1.6652805	0.1748
-1	-2.87500000	1.6652805	0.0154

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.04687500	1.6652805	0.0823
-1	-2.42187500	1.6652805	0.0404

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.51562500	1.6652805	0.0032
-1	-0.95312500	1.6652805	0.4158

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.15625000	1.6652805	0.0674
-1	-2.31250000	1.6652805	0.0501

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.93750000	1.64971979	0.2429
1	-1	-1.03125000	1.64971979	0.5333
-1	1	-2.37500000	1.64971979	0.1530
-1	-1	-3.59375000	1.64971979	0.0317

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.00000000	1.64971979	0.5457
1	-1	-2.18750000	1.64971979	0.1878
-1	1	-3.31250000	1.64971979	0.0473
-1	-1	-2.43750000	1.64971979	0.1426

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.18750000	1.64971979	0.1878
1	-1	-1.90625000	1.64971979	0.2506
-1	1	-2.12500000	1.64971979	0.2006
-1	-1	-2.71875000	1.64971979	0.1024

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.15625000	1.64971979	0.0133
1	-1	-2.87500000	1.64971979	0.0844
-1	1	-0.15625000	1.64971979	0.9247
-1	-1	-1.75000000	1.64971979	0.2913

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.00000000	1.6652805	0.3933
-1	-3.46875000	1.6652805	0.0037

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.00000000	1.64971979	0.5457
1	-1	-3.31250000	1.64971979	0.0473
-1	1	-1.00000000	1.64971979	0.5457
-1	-1	-3.62500000	1.64971979	0.0302

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.18750000	1.6652805	0.0636
-1	-2.28125000	1.6652805	0.0532

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.28125000	1.64971979	0.4391
1	-1	-1.68750000	1.64971979	0.3088
-1	1	-3.09375000	1.64971979	0.0636
-1	-1	-2.87500000	1.64971979	0.0844

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.06250000	1.64971979	0.5210
1	-1	-2.12500000	1.64971979	0.2006
-1	1	-3.31250000	1.64971979	0.0473
-1	-1	-2.43750000	1.64971979	0.1426

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.90625000	1.64971979	0.2506
1	-1	-2.18750000	1.64971979	0.1878
-1	1	-2.46875000	1.64971979	0.1376
-1	-1	-2.37500000	1.64971979	0.1530

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.00000000	1.64971979	0.2282
1	-1	-5.03125000	1.64971979	0.0029
-1	1	-2.37500000	1.64971979	0.1530
-1	-1	-0.46875000	1.64971979	0.7769

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.34375000	1.64971979	0.1584
1	-1	-1.96875000	1.64971979	0.2355
-1	1	-2.03125000	1.64971979	0.2210
-1	-1	-2.59375000	1.64971979	0.1190

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.50000000	1.64971979	0.3653
1	-1	-0.50000000	1.64971979	0.7624
-1	1	-2.87500000	1.64971979	0.0844
-1	-1	-4.06250000	1.64971979	0.0155

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.03125000	1.6652805	0.0846
-1	-2.43750000	1.6652805	0.0391

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.78125000	1.64971979	0.2828
1	-1	-2.53125000	1.64971979	0.1280
-1	1	-2.28125000	1.64971979	0.1697
-1	-1	-2.34375000	1.64971979	0.1584

TOTAL PROTEIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.09375000	1.64971979	0.2072
1	-1	-2.28125000	1.64971979	0.1697
-1	1	-1.96875000	1.64971979	0.2355
-1	-1	-2.59375000	1.64971979	0.1190

ALBUMIN : POST - PRE

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1858.43750000	77.43489583	2.03	0.0078
Error	103	3929.06250000	38.14623786		
Corrected Total	127	5787.50000000			

R-Square	C.V.	Root MSE	Y Mean
0.321112	-131.7603	6.17626407	-4.68750000

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	616.81250000	205.60416667	5.39	0.0017	
V1	1	225.78125000	225.78125000	5.92	0.0167	*
V2	1	75.03125000	75.03125000	1.97	0.1638	
V1 * V2	1	60.50000000	60.50000000	1.59	0.2107	
V3	1	38.28125000	38.28125000	1.00	0.3188	
V1 * V3	1	60.50000000	60.50000000	1.59	0.2107	
V2 * V3	1	4.50000000	4.50000000	0.12	0.7319	
V4	1	242.00000000	242.00000000	6.34	0.0133	*
V1 * V4	1	2.53125000	2.53125000	0.07	0.7972	
V2 * V4	1	3.78125000	3.78125000	0.10	0.7535	
V3 * V4	1	87.78125000	87.78125000	2.30	0.1323	
V5	1	5.28125000	5.28125000	0.14	0.7106	
V1 * V5	1	91.12500000	91.12500000	2.39	0.1253	
V2 * V5	1	6.12500000	6.12500000	0.16	0.6895	
V3 * V5	1	24.50000000	24.50000000	0.64	0.4247	
V4 * V5	1	7.03125000	7.03125000	0.18	0.6686	
V6	1	128.00000000	128.00000000	3.36	0.0699	*
V1 * V6	1	9.03125000	9.03125000	0.24	0.6276	
V2 * V6	1	22.78125000	22.78125000	0.60	0.4414	
V3 * V6	1	132.03125000	132.03125000	3.46	0.0657	*
V4 * V6	1	12.50000000	12.50000000	0.33	0.5683	
V5 * V6	1	2.53125000	2.53125000	0.07	0.7972	

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.35937500	0.77203301	0.0001
-1	-6.01562500	0.77203301	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.92187500	0.77203301	0.0001
-1	-5.45312500	0.77203301	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.28125000	1.09181955	0.0033
1	-1	-3.43750000	1.09181955	0.0021
-1	1	-4.56250000	1.09181955	0.0001
-1	-1	-7.46875000	1.09181955	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.23437500	0.77203301	0.0001
-1	-4.14062500	0.77203301	0.0001

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.59375000	1.09181955	0.0001
1	-1	-2.12500000	1.09181955	0.0543
-1	1	-5.87500000	1.09181955	0.0001
-1	-1	-6.15625000	1.09181955	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.65625000	1.09181955	0.0001
1	-1	-3.18750000	1.09181955	0.0043
-1	1	-5.81250000	1.09181955	0.0001
-1	-1	-5.09375000	1.09181955	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.31250000	0.77203301	0.0001
-1	-6.06250000	0.77203301	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.84375000	1.09181955	0.0943
1	-1	-4.87500000	1.09181955	0.0001
-1	1	-4.78125000	1.09181955	0.0001
-1	-1	-7.25000000	1.09181955	0.0001

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.71875000	1.09181955	0.0144
1	-1	-5.12500000	1.09181955	0.0001
-1	1	-3.90625000	1.09181955	0.0005
-1	-1	-7.00000000	1.09181955	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.03125000	1.09181955	0.0065
1	-1	-7.43750000	1.09181955	0.0001
-1	1	-3.59375000	1.09181955	0.0014
-1	-1	-4.68750000	1.09181955	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-4.48437500	0.77203301	0.0001
-1	-4.89062500	0.77203301	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.00000000	1.09181955	0.0004
1	-1	-2.71875000	1.09181955	0.0144
-1	1	-4.96875000	1.09181955	0.0001
-1	-1	-7.06250000	1.09181955	0.0001

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.50000000	1.09181955	0.0018
1	-1	-4.34375000	1.09181955	0.0001
-1	1	-5.46875000	1.09181955	0.0001
-1	-1	-5.43750000	1.09181955	0.0001

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.59375000	1.09181955	0.0001
1	-1	-5.87500000	1.09181955	0.0001
-1	1	-4.37500000	1.09181955	0.0001
-1	-1	-3.09625000	1.09181955	0.0005

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.34375000	1.09181955	0.0028
1	-1	-3.28125000	1.09181955	0.0033
-1	1	-5.62500000	1.09181955	0.0001
-1	-1	-6.50000000	1.09181955	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-3.68750000	0.77203301	0.0001
-1	-5.68750000	0.77203301	0.0001

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.62500000	1.09181955	0.0180
1	-1	-4.09375000	1.09181955	0.0003
-1	1	-4.75000000	1.09181955	0.0001
-1	-1	-7.28125000	1.09181955	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.50005000	1.09181955	0.0241
1	-1	-5.34375000	1.09181955	0.0001
-1	1	-4.87500000	1.09181955	0.0001
-1	-1	-6.03125000	1.09181955	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.21875000	1.09181955	0.0040
1	-1	-7.25000000	1.09181955	0.0001
-1	1	-4.15625000	1.09181955	0.0002
-1	-1	-4.12500000	1.09181955	0.0003

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.00000000	1.09181955	0.0699
1	-1	-4.62500000	1.09181955	0.0001
-1	1	-5.37500000	1.09181955	0.0001
-1	-1	-6.75000000	1.09181955	0.0001

ALBUMIN : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.34375000	1.09181955	0.0028
1	-1	-5.62500000	1.09181955	0.0001
-1	1	-4.03125000	1.09181955	0.0004
-1	-1	-5.75000000	1.09181955	0.0001

ALBUMIN : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	583.37500000	24.30729167	0.85	0.6655
Error	103	2943.74218750	28.58002124		
Corrected Total	127	3527.11718750			

R-Square	C.V.	Root MSE	Y Mean
0.165397	-315.3418	5.34602855	-1.69531250

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	55.77343750	18.59114583	0.65	0.5844	
V7	1	11.88281250	11.88281250	0.42	0.5205	
V8	1	9.57031250	9.57031250	0.33	0.5641	
V9	1	0.94531250	0.94531250	0.03	0.8560	
V10	1	103.32031250	103.32031250	3.62	0.0601	*
V11	1	20.32031250	20.32031250	0.71	0.4011	
V7 * V11	1	21.94531250	21.94531250	0.77	0.3829	
V8 * V11	1	7.50781250	7.50781250	0.26	0.6094	
V9 * V11	1	4.13281250	4.13281250	0.14	0.7045	
V10 * V11	1	14.44531250	14.44531250	0.51	0.4787	
V12	1	106.94531250	106.94531250	3.74	0.0558	*
V11 * V12	1	1.75781250	1.75781250	0.06	0.8046	
V13	1	0.94531250	0.94531250	0.03	0.8560	
V7 * V13	1	1.75781250	1.75781250	0.06	0.8046	
V8 * V13	1	5.69531250	5.69531250	0.20	0.6562	
V9 * V13	1	10.69531250	10.69531250	0.37	0.5421	
V10 * V13	1	138.19531250	138.19531250	4.84	0.0301	*
V11 * V13	1	15.82031250	15.82031250	0.55	0.4586	
V12 * V13	1	43.94531250	43.94531250	1.54	0.2178	
V14	1	2.25781250	2.25781250	0.08	0.7792	
V11 * V14	1	0.63281250	0.63281250	0.02	0.8820	
V13 * V14	1	4.88281250	4.88281250	0.17	0.6802	

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.39062500	0.66825357	0.0399
-1	-2.98437500	0.66825357	0.0035

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.42187500	0.66825357	0.0357
-1	-1.96875000	0.66825357	0.0040

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.78125000	0.66825357	0.0089
-1	-1.60937500	0.66825357	0.0178

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.59375000	0.66825357	0.0002
-1	-0.79687500	0.66825357	0.2358

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.29687500	0.66825357	0.0550
-1	-2.09375000	0.66825357	0.0023

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.40625000	0.94505326	0.1398
1	-1	-1.37500000	0.94505326	0.1487
-1	1	-1.18750000	0.94505326	0.2118
-1	-1	-2.81250000	0.94505326	0.0036

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.78125000	0.94505326	0.4103
1	-1	-2.06250000	0.94505326	0.0314
-1	1	-1.81250000	0.94505326	0.0579
-1	-1	-2.12500000	0.94505326	0.0267

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.56250000	0.94505326	0.1013
1	-1	-2.00000000	0.94505326	0.0367
-1	1	-1.03125000	0.94505326	0.2777
-1	-1	-2.18750000	0.94505326	0.0226

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.53125000	0.94505326	0.0086
1	-1	-2.65625000	0.94505326	0.0059
-1	1	-0.06250000	0.94505326	0.9474
-1	-1	-1.53125000	0.94505326	0.1082

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.78125000	0.66825357	0.2451
-1	-2.60937500	0.66825357	0.0002

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.50000000	0.94505326	0.5979
1	-1	-2.09375000	0.94505326	0.0289
-1	1	-1.06250000	0.94505326	0.2635
-1	-1	-3.12500000	0.94505326	0.0013

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.60937500	0.66825357	0.0178
-1	-1.78125000	0.66825357	0.0089

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.18750000	0.94505326	0.2118
1	-1	-1.59375000	0.94505326	0.0947
-1	1	-2.03125000	0.94505326	0.0339
-1	-1	-1.96875000	0.94505326	0.0397

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.12500000	0.94505326	0.2366
1	-1	-1.71875000	0.94505326	0.0719
-1	1	-2.09375000	0.94505326	0.0289
-1	-1	-1.84375000	0.94505326	0.0538

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.40625000	0.94505326	0.1398
1	-1	-2.15625000	0.94505326	0.0246
-1	1	-1.81250000	0.94505326	0.0579
-1	-1	-1.40625000	0.94505326	0.1398

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.46875000	0.94505326	0.1232
1	-1	-3.18750000	0.94505326	0.0002
-1	1	-1.75000000	0.94505326	0.0669
-1	-1	0.15625000	0.94505326	0.8690

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.56250000	0.94505326	0.1013
1	-1	-1.03125000	0.94505326	0.2777
-1	1	-1.65625000	0.94505326	0.0827
-1	-1	-2.53125000	0.94505326	0.0086

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.28125000	0.94505326	0.1781
1	-1	-0.28125000	0.94505326	0.7666
-1	1	-1.93750000	0.94505326	0.0429
-1	-1	-3.28125000	0.94505326	0.0008

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.56250000	0.66825357	0.0213
-1	1.82812500	0.66825357	0.0073

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.09375000	0.94505326	0.2498
1	-1	-1.50000000	0.94505326	0.1155
-1	1	-2.03125000	0.94505326	0.0339
1	-1	-2.15625000	0.94505326	0.0246

ALBUMIN : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.28125000	0.94505326	0.1781
1	-1	-1.93750000	0.94505326	0.0429
-1	1	-1.84375000	0.94505326	0.0538
-1	-1	-1.71875000	0.94505326	0.0719

CALCIUM : POST - PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	4.89423750	0.203926563	1.27	0.2027
Error	103	15.51191797	0.16030988		
Corrected Total	127	21.40615547			

R-Square	C.V.	Root MSE	Y Mean
0.228637	-70.04176	0.40038717	-0.57164062

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	1.14738359	0.38246120	2.39	0.0734	
V1	1	0.45243828	0.45243828	2.82	0.0960	
V2	1	0.04766328	0.04766328	0.30	0.5867	
V1 * V2	1	0.01260078	0.01260078	0.08	0.7798	
V3	1	0.16031953	0.16031953	1.00	0.3196	
V1 * V3	1	0.16173828	0.16173828	1.01	0.3175	
V2 * V3	1	0.09084453	0.09084453	0.57	0.4533	
V4	1	1.56423828	1.56423828	9.76	0.0023	*
V1 * V4	1	0.05737578	0.05737578	0.36	0.5510	
V2 * V4	1	0.01734453	0.01734453	0.11	0.7429	
V3 * V4	1	0.12313203	0.12313203	0.77	0.3828	
V5	1	0.06256953	0.06256953	0.39	0.5335	
V1 * V5	1	0.11221953	0.11221953	0.70	0.4047	
V2 * V5	1	0.00735078	0.00735078	0.05	0.8309	
V3 * V5	1	0.01509453	0.01509453	0.09	0.7596	
V4 * V5	1	0.11943828	0.11943828	0.75	0.3901	
V6	1	0.48388203	0.48388203	3.02	0.0853	
V1 * V6	1	0.02503203	0.02503203	0.16	0.6935	
V2 * V6	1	0.14512578	0.14512578	0.91	0.3436	
V3 * V6	1	0.03746953	0.03746953	0.23	0.6298	
V4 * V6	1	0.00017578	0.00017578	0.00	0.9736	
V5 * V6	1	0.05080078	0.05080078	0.32	0.5747	

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.51218750	0.05004840	0.0001
-1	-0.63109375	0.05004840	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.55234375	0.05004840	0.0001
-1	0.59093750	0.05004840	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.50281250	0.07077912	0.0001
1	-1	-0.52156250	0.07077912	0.0001
-1	1	0.60187500	0.07077912	0.0001
-1	-1	-0.66031250	0.07077912	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.60703125	0.05004840	0.0001
-1	-0.53625000	0.05004840	0.0001

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.58312500	0.07077912	0.0001
1	-1	-0.44125000	0.07077912	0.0001
-1	1	-0.63093750	0.07077912	0.0001
-1	-1	-0.63125000	0.07077912	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.61437500	0.07077912	0.0001
1	-1	-0.49031250	0.07077912	0.0001
-1	1	-0.59968750	0.07077912	0.0001
-1	-1	-0.58218750	0.07077912	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.68218750	0.05004840	0.0001
-1	-0.46109375	0.05004840	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.60156250	0.07077912	0.0001
1	-1	-0.42281250	0.07077912	0.0001
-1	1	-0.76281250	0.07077912	0.0001
-1	-1	-0.49937500	0.07077912	0.0001

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.65125000	0.07077912	0.0001
1	-1	-0.45343750	0.07077912	0.0001
-1	1	-0.71312500	0.07077912	0.0001
-1	-1	-0.46875000	0.07077912	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.68656250	0.07077912	0.0001
1	-1	-0.52750000	0.07077912	0.0001
-1	1	-0.67781250	0.07077912	0.0001
-1	-1	-0.39468750	0.07077912	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.54953125	0.05004840	0.0001
-1	-0.59375000	0.05004840	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.51968750	0.07077912	0.0001
1	1	-0.50468750	0.07077912	0.0001
-1	1	-0.57937500	0.07077912	0.0001
-1	-1	-0.68281250	0.07077912	0.0001

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.53781250	0.07077912	0.0001
1	-1	-0.56687500	0.07077912	0.0001
-1	1	-0.56125000	0.07077912	0.0001
-1	-1	-0.62062500	0.07077912	0.0001

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.57406250	0.07077912	0.0001
1	-1	-0.64000000	0.07077912	0.0001
-1	1	-0.52500000	0.07077912	0.0001
-1	-1	-0.54750000	0.07077912	0.0001

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.69062500	0.07077912	0.0001
1	-1	-0.67375000	0.07077912	0.0001
-1	1	-0.40843750	0.07077912	0.0001
-1	-1	-0.51375000	0.07077912	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.51015625	0.05004840	0.0001
-1	-0.63312500	0.05004840	0.0001

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.46468750	0.07077912	0.0001
1	-1	-0.55968750	0.07077912	0.0001
-1	1	-0.55562500	0.07077912	0.0001
-1	-1	-0.70656250	0.07077912	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.45718750	0.07077912	0.0001
1	-1	-0.64750000	0.07077912	0.0001
-1	1	-0.56312500	0.07077912	0.0001
-1	-1	-0.61875000	0.07077912	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.52843750	0.07077912	0.0001
1	-1	-0.68562500	0.07077912	0.0001
-1	1	-0.49187500	0.07077912	0.0001
-1	-1	-0.58062500	0.07077912	0.0001

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.62187500	0.07077912	0.0001
1	-1	-0.74250000	0.07077912	0.0001
-1	1	-0.39843750	0.07077912	0.0001
-1	-1	-0.52375000	0.07077912	0.0001

CALCIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.46812500	0.07077912	0.0001
1	-1	-0.63093750	0.07077912	0.0001
-1	1	-0.55218750	0.07077912	0.0001
-1	-1	-0.63531250	0.07077912	0.0001

CALCIUM : PRODUCTION

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	1.20776875	0.05032370	0.98	0.4978
Error	103	5.28668672	0.05132706		
Corrected Total	127	6.49445547			

R-Square	C.V.	Root MSE	Y Mean
0.185969	-3411.648	0.22655475	-0.00664062

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.17185859	0.05728620	1.12	0.3461
V7	1	0.00009453	0.00009453	0.00	0.9659
V8	1	0.01220703	0.01220703	0.24	0.6268
V9	1	0.00861328	0.00861328	0.17	0.6829
V10	1	0.16747578	0.16747578	3.26	0.0738
V11	1	0.08150703	0.08150703	1.59	0.2105
V7 * V11	1	0.04843828	0.04843828	0.94	0.3336
V8 * V11	1	0.00085078	0.00085078	0.02	0.8978
V9 * V11	1	0.00439453	0.00439453	0.09	0.7704
V10 * V11	1	0.09845703	0.09845703	1.92	0.1690
V12	1	0.19453203	0.19453203	3.79	0.0543 *
V11 * V12	1	0.01143828	0.01143828	0.22	0.6379
V13	1	0.01106328	0.01106328	0.22	0.6434
V7 * V13	1	0.04388203	0.04388203	0.85	0.3573
V8 * V13	1	0.03219453	0.03219453	0.63	0.4302
V9 * V13	1	0.03347578	0.03347578	0.65	0.4212
V10 * V13	1	0.21043828	0.21043828	4.10	0.0455 *
V11 * V13	1	0.01033203	0.01033203	0.20	0.6546
V12 * V13	1	0.06345703	0.06345703	1.24	0.2688
V14	1	0.00017578	0.00017578	0.00	0.9534
V11 * V14	1	0.00203203	0.00203203	0.04	0.8427
V13 * V14	1	0.00085078	0.00085078	0.02	0.8978

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.00578125	0.02831934	0.8386
-1	-0.00750000	0.02831934	0.7917

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.00312500	0.02831934	0.9123
-1	-0.01640625	0.02831934	0.5636

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.00156250	0.02831934	0.9561
-1	-0.01484375	0.02831934	0.6013

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.04281250	0.66825357	0.1337
-1	0.02953125	0.66825357	0.2995

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.01859375	0.02831934	0.5129
-1	-0.03187500	0.02831934	0.2630

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.00000000	0.04004960	1.0000
1	-1	-0.01156250	0.04004960	0.7734
-1	1	0.03718750	0.04004960	0.3553
-1	-1	-0.05118750	0.04004960	0.1955

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.03093750	0.04004960	0.4416
1	-1	-0.02468750	0.04004960	0.5390
-1	1	0.00625000	0.04004960	0.8763
-1	-1	-0.03906250	0.04004960	0.3317

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.02093750	0.04004960	0.6022
1	-1	-0.01781250	0.04004960	0.6574
-1	1	0.01625000	0.04004960	0.6858
-1	-1	-0.04593750	0.04004960	0.2540

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04531250	0.04004960	0.2605
1	-1	-0.04031250	0.04004960	0.3165
-1	1	0.08250000	0.04004960	0.0419
-1	-1	-0.02343750	0.04004960	0.5597

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.03234375	0.02831934	0.2561
-1	-0.04562500	0.02831934	0.1102

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.04812500	0.04004960	0.2323
1	-1	-0.01093750	0.04004960	0.7853
-1	1	0.01656250	0.04004960	0.6801
-1	-1	-0.08031250	0.04004960	0.0476

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.01593750	0.02831934	0.5748
-1	0.00265625	0.02831934	0.9255

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.00343750	0.04004960	0.9318
1	-1	-0.01500000	0.04004960	0.7088
-1	1	-0.03531250	0.04004960	0.3800
-1	-1	0.02031250	0.04004960	0.6131

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.00968750	0.04004960	0.8093
1	-1	-0.00343750	0.04004960	0.9318
-1	1	-0.04156250	0.04004960	0.3018
-1	-1	0.00875000	0.04004960	0.8275

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.00843750	0.04004960	0.8336
1	-1	-0.00531250	0.04004960	0.8947
-1	1	-0.04031250	0.04004960	0.3165
-1	-1	0.01062500	0.04004960	0.7913

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.01156250	0.04004960	0.7734
1	-1	-0.07406250	0.04004960	0.0673
-1	1	-0.02031250	0.04004960	0.6131
-1	-1	0.07937500	0.04004960	0.0502

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.00031250	0.04004960	0.9938
1	-1	0.03687500	0.04004960	0.3593
-1	1	-0.03218750	0.04004960	0.4234
-1	-1	-0.03156250	0.04004960	0.4325

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.04531250	0.04004960	0.2605
1	-1	0.01937500	0.04004960	0.6296
-1	1	-0.07718750	0.04004960	0.0567
-1	-1	-0.01406250	0.04004960	0.7262

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.00546875	0.02831934	0.8473
-1	-0.00781250	0.02831934	0.7832

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.02375000	0.04004960	0.5545
1	-1	0.01343750	0.04004960	0.7379
-1	1	-0.03468750	0.04004960	0.3884
-1	-1	-0.02906250	0.04004960	0.4697

CALCIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.01218750	0.04004960	0.7615
1	-1	-0.01968750	0.04004960	0.6241
-1	1	0.00125000	0.04004960	0.9752
-1	-1	0.00406250	0.04004960	0.9194

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	5792.00000000	241.33333333	1.37	0.1411
Error	103	18144.21875000	176.15746359		
Corrected Total	127	23936.21875000			

R-Square	C.V.	Root MSE	Y Mean
0.241976	469.3015	13.27243247	2.82812500

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	326.78125000	108.92708333	0.62	0.6047	
V1	1	38.28125000	38.28125000	0.22	0.6421	
V2	1	0.12500000	0.12500000	0.00	0.9788	
V1 * V2	1	420.50000000	420.50000000	2.39	0.1254	
V3	1	26.28125000	26.28125000	0.15	0.7001	
V1 * V3	1	712.53125000	712.53125000	4.04	0.0469	*
V2 * V3	1	45.12500000	45.12500000	0.26	0.6139	
V4	1	657.03125000	657.03125000	3.73	0.0562	*
V1 * V4	1	47.53125000	47.53125000	0.27	0.6046	
V2 * V4	1	10.12500000	10.12500000	0.06	0.8110	
V3 * V4	1	94.53125000	94.53125000	0.54	0.4655	
V5	1	294.03125000	294.03125000	1.67	0.1993	
V1 * V5	1	13.78125000	13.78125000	0.08	0.7803	
V2 * V5	1	36.12500000	36.12500000	0.21	0.6516	
V3 * V5	1	1023.78125000	1023.78125000	5.81	0.0177	*
V4 * V5	1	830.28125000	830.28125000	4.71	0.0322	*
V6	1	338.00000000	338.00000000	1.92	0.1690	
V1 * V6	1	153.12500000	153.12500000	0.87	0.3533	
V2 * V6	1	427.78125000	427.78125000	2.43	0.1222	
V3 * V6	1	50.00000000	50.00000000	0.28	0.5953	
V4 * V6	1	36.12500000	36.12500000	0.21	0.6516	
V5 * V6	1	210.12500000	210.12500000	1.19	0.2773	

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	2.28125000	1.65905406	0.1721
-1	3.37500000	1.65905406	0.0445

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	2.79687500	1.65905406	0.0949
-1	2.85937500	1.65905406	0.0878

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.43750000	2.34625675	0.8524
1	-1	4.12500000	2.34625675	0.0817
-1	1	5.15625000	2.34625675	0.0302
-1	-1	1.59375000	2.34625675	0.4985

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	3.28125000	1.65905406	0.0506
-1	2.37500000	1.65905406	0.1553

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	5.09375000	2.34625675	0.0322
1	-1	-0.53125000	2.34625675	0.8213
-1	1	1.46875000	2.34625675	0.5327
-1	-1	5.28125000	2.34625675	0.0265

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	2.65625000	2.34625675	0.2602
1	-1	2.93750000	2.34625675	0.2134
-1	1	3.90625000	2.34625675	0.0990
-1	-1	1.81250000	2.34625675	0.4416

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	0.56250000	1.65905406	0.7353
-1	5.09375000	1.65905406	0.0027

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.62500000	2.34625675	0.7905
1	-1	3.93750000	2.34625675	0.0963
-1	1	0.50000000	2.34625675	0.8317
-1	-1	6.25000000	2.34625675	0.0090

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.81250000	2.34625675	0.7298
1	-1	4.78125000	2.34625675	0.0441
-1	1	0.31250000	2.34625675	0.8943
-1	-1	5.40625000	2.34625675	0.0232

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	1.87500000	2.34625675	0.4260
1	-1	4.68750000	2.34625675	0.0484
-1	1	-0.75000000	2.34625675	0.7499
-1	-1	5.50000000	2.34625675	0.0210

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1.31250000	1.65905406	0.4307
-1	4.34375000	1.65905406	0.0102

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	1.09375000	2.34625675	0.6421
1	-1	3.46875000	2.34625675	0.1423
-1	1	1.53125000	2.34625675	0.5154
-1	-1	5.21875000	2.34625675	0.0283

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.75000000	2.34625675	0.7499
1	-1	4.84375000	2.34625675	0.0415
-1	1	1.87500000	2.34625675	0.4260
-1	-1	3.84375000	2.34625675	0.1044

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.59375000	2.34625675	0.0529
1	-1	1.96875000	2.34625675	0.4034
-1	1	-1.96875000	2.34625675	0.4034
-1	-1	6.71875000	2.34625675	0.0051

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	1.59375000	2.34625675	0.4985
1	-1	-0.46875000	2.34625675	0.8420
-1	1	1.03125000	2.34625675	0.6612
-1	-1	9.15625000	2.34625675	0.0002

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	4.45312500	1.65905406	0.0085
-1	1.20312500	1.65905406	0.4700

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	2.81250000	2.34625675	0.2334
1	-1	1.75000000	2.34625675	0.4574
-1	1	6.09375000	2.34625675	0.0108
-1	-1	0.65625000	2.34625675	0.7803

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	6.25000000	2.34625675	0.0090
1	-1	-0.65625000	2.34625675	0.7803
-1	1	2.65625000	2.34625675	0.2602
-1	-1	3.06250000	2.34625675	0.1947

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.28125000	2.34625675	0.0709
1	-1	2.28125000	2.34625675	0.3332
-1	1	4.62500000	2.34625675	0.0514
-1	-1	0.12500000	2.34625675	0.9576

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	2.71875000	2.34625675	0.2492
1	-1	-1.59375000	2.34625675	0.4985
-1	1	6.18750000	2.34625675	0.0097
-1	-1	4.00000000	2.34625675	0.0921

ALANINE AMINOTRANSFERASE : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	4.21875000	2.34625675	0.0751
1	-1	-1.59375000	2.34625675	0.4985
-1	1	4.68750000	2.34625675	0.0484
-1	-1	4.00000000	2.34625675	0.0912

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	847.56250000	35.31510417	1.25	0.2211
Error	103	2915.86718750	28.30939017		
Corrected Total	127	3763.42968750			

R-Square	C.V.	Root MSE	Y Mean
0.225210	-417.8185	5.32065693	-1.27343750

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	60.27343750	20.09114583	0.71	0.5484	
V7	1	1.32031250	1.32031250	0.05	0.8294	
V8	1	7.50781250	7.50781250	0.27	0.6077	
V9	1	15.82031250	15.82031250	0.56	0.4564	
V10	1	7.50781250	7.50781250	0.27	0.6077	
V11	1	0.63281250	0.63281250	0.02	0.8814	
V7 * V11	1	5.69531250	5.69531250	0.20	0.6547	
V8 * V11	1	48.75781250	48.75781250	1.72	0.1923	
V9 * V11	1	53.82031250	53.82031250	1.90	0.1709	
V10 * V11	1	2.82031250	2.82031250	0.10	0.7529	
V12	1	168.82031250	163.82031250	5.96	0.0163	*
V11 * V12	1	6.57031250	6.57031250	0.23	0.6310	
V13	1	17.25781250	17.25781250	0.61	0.4367	
V7 * V13	1	11.88281250	11.88281250	0.42	0.5185	
V8 * V13	1	146.63281250	146.63281250	5.18	0.0249	*
V9 * V13	1	73.50781250	73.50781250	2.60	0.1102	
V10 * V13	1	8.50781250	8.50781250	0.30	0.5847	
V11 * V13	1	27.19531250	27.19531250	0.96	0.3293	
V12 * V13	1	37.19531250	37.19531250	1.31	0.2543	
V14	1	118.19531250	118.19531250	4.18	0.0436	*
V11 * V14	1	25.38281250	25.38281250	0.90	0.3459	
V13 * V14	1	2.25781250	2.25781250	0.08	0.7782	

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.37500000	0.66508212	0.0412
-1	-1.17187500	0.66508212	0.0812

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.03125000	0.66508212	0.1241
-1	-1.51562500	0.66508212	0.0247

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.62500000	0.66508212	0.0163
-1	-0.92187500	0.66508212	0.1687

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.51562500	0.66508212	0.0247
-1	-1.03125000	0.66508212	0.1241

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.34375000	0.66508212	0.0459
-1	-1.20312500	0.66508212	0.0734

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.65625000	0.94056815	0.0812
1	-1	-1.09375000	0.94056815	0.2476
-1	1	-1.03125000	0.94056815	0.2755
-1	-1	-1.31250000	0.94056815	0.1659

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.71875000	0.94056815	0.0705
1	-1	-0.34375000	0.94056815	0.7155
-1	1	-0.96875000	0.94056815	0.3054
-1	-1	-2.06250000	0.94056815	0.0306

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.34375000	0.94056815	0.0143
1	-1	-0.90625000	0.94056815	0.3375
-1	1	-0.34375000	0.94056815	0.7155
-1	-1	-1.50000000	0.94056815	0.1138

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.43750000	0.94056815	0.1295
1	-1	-1.59375000	0.94056815	0.0932
-1	1	-1.25000000	0.94056815	0.1868
-1	-1	-0.81250000	0.94056815	0.3897

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.12500000	0.66508212	0.8513
-1	-2.42187500	0.66508212	0.0004

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.03125000	0.94056815	0.9736
1	-1	-2.71875000	0.94056815	0.0047
-1	1	-0.28125000	0.94056815	0.7655
-1	-1	-2.12500000	0.94056815	0.0260

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.90625000	0.66508212	0.1760
-1	-1.64062500	0.66508212	0.0153

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.31250000	0.94056815	0.1659
1	-1	-1.43750000	0.94056815	0.1295
-1	1	-0.50000000	0.94056815	0.5962
-1	-1	-1.84375000	0.94056815	0.0527

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.40625000	0.94056815	0.6667
1	-1	-2.46875000	0.94056815	0.0100
-1	1	-2.21875000	0.94056815	0.0202
-1	-1	-0.81250000	0.94056815	0.3897

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.50000000	0.94056815	0.5962
1	-1	-2.75000000	0.94056815	0.0043
-1	1	-1.31250000	0.94056815	0.1659
-1	-1	-0.53125000	0.94056815	0.5734

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.40625000	0.94056815	0.1379
1	-1	-1.62500000	0.94056815	0.0870
-1	1	-0.40625000	0.94056815	0.6667
-1	-1	-1.65625000	0.94056815	0.0812

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.43750000	0.94056815	0.1295
1	-1	-1.25000000	0.94056815	0.1868
-1	1	-0.37500000	0.94056815	0.6909
-1	-1	-2.03125000	0.94056815	0.0331

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	0.78125000	0.94056815	0.4081
1	-1	-1.03125000	0.94056815	0.2755
-1	1	-2.59375000	0.94056815	0.0069
-1	-1	-2.25000000	0.94056815	0.0186

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.23437500	0.66508212	0.0011
-1	-0.31250000	0.66508212	0.6394

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.75000000	0.94056815	0.0043
1	-1	0.06250000	0.94056815	0.9471
-1	1	-1.71875000	0.94056815	0.0705
-1	-1	-0.68750000	0.94056815	0.4665

ALANINE AMINOTRANSFERASE : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.00000000	0.94056815	0.0359
1	-1	0.18750000	0.94056815	0.8424
-1	1	-2.46875000	0.94056815	0.0100
-1	-1	-0.81250000	0.94056815	0.3897

SODIUM : POST - PRE

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	12004.12500000	500.17187500	2.48	0.0009
Error	103	20812.24218750	202.06060376		
Corrected Total	127	32816.36718750			

R-Square	C.V.	Root MSE	Y Mean
0.365797	38.49153	14.21480228	36.92968750

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	2729.52343750	909.84114583	4.50	0.0052	
V1	1	492.19531250	492.19531250	2.44	0.1217	
V2	1	207.57031250	207.57031250	1.03	0.3132	
V1 * V2	1	929.88281250	929.88281250	4.60	0.0343	*
V3	1	223.13281250	223.13281250	1.10	0.2958	
V1 * V3	1	164.25781250	164.25781250	0.81	0.3694	
V2 * V3	1	43.94531250	43.94531250	0.22	0.6419	
V4	1	4266.57031250	4266.57031250	21.12	0.0001	*
V1 * V4	1	103.32031250	103.32031250	0.51	0.4762	
V2 * V4	1	48.75781250	48.75781250	0.24	0.6243	
V3 * V4	1	168.82031250	168.82031250	0.84	0.3628	
V5	1	92.82031250	92.82031250	0.46	0.4994	
V1 * V5	1	103.32031250	103.32031250	0.51	0.4762	
V2 * V5	1	381.57031250	381.57031250	1.89	0.1724	
V3 * V5	1	76.57031250	76.57031250	0.38	0.5395	
V4 * V5	1	347.82031250	347.82031250	1.72	0.1924	
V6	1	1040.82031250	1040.82031250	5.15	0.0253	*
V1 * V6	1	56.44531250	56.44531250	0.28	0.5983	
V2 * V6	1	303.19531250	303.19531250	1.50	0.2234	
V3 * V6	1	51.25781250	51.25781250	0.25	0.6156	
V4 * V6	1	82.88281250	82.88281250	0.41	0.5233	
V5 * V6	1	89.44531250	89.44531250	0.44	0.5073	

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	38.8906250	1.7768503	0.0001
-1	34.9687500	1.7768503	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	38.2031250	1.7768503	0.0001
-1	34.9687500	1.7768503	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	37.4687500	2.51228458	0.0001
1	-1	40.3125000	2.51228458	0.0001
-1	1	38.9375000	2.51228458	0.0001
-1	-1	31.0000000	2.51228458	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	35.6093750	1.7768503	0.0001
-1	38.2500000	1.7768503	0.0001

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	36.4375000	2.51228458	0.0001
1	-1	41.3437500	2.51228458	0.0001
-1	1	34.7812500	2.51228458	0.0001
-1	-1	35.1562500	2.51228458	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	37.4687500	2.51228458	0.0001
1	-1	38.9375000	2.51228458	0.0001
-1	1	33.7500000	2.51228458	0.0001
-1	-1	37.5625000	2.51228458	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	42.7031250	1.7768503	0.0001
-1	31.1562500	1.7768503	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	45.5625000	2.51228458	0.0001
1	-1	32.2187500	2.51228458	0.0001
-1	1	39.8437500	2.51228458	0.0001
-1	-1	30.0937500	2.51228458	0.0001

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	44.5937500	2.51228458	0.0001
1	-1	31.8125000	2.51228458	0.0001
-1	1	40.8125000	2.51228458	0.0001
-1	-1	30.5000000	2.51228458	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	42.5312500	2.51228458	0.0001
1	-1	28.6875000	2.51228458	0.0001
-1	1	42.8750000	2.51228458	0.0001
-1	-1	33.6250000	2.51228458	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	37.7812500	1.7768503	0.0001
-1	36.0781250	1.7768503	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	38.8437500	2.51228458	0.0001
1	-1	38.9375000	2.51228458	0.0001
-1	1	36.7187500	2.51228458	0.0001
-1	-1	33.2187500	2.51228458	0.0001

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	40.7812500	2.51228458	0.0001
1	-1	35.6250000	2.51228458	0.0001
-1	1	34.7812500	2.51228458	0.0001
-1	-1	36.5312500	2.51228458	0.0001

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	35.6875000	2.51228458	0.0001
1	-1	35.5312500	2.51228458	0.0001
-1	1	39.8750000	2.51228458	0.0001
-1	-1	36.6250000	2.51228458	0.0001

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	41.9062500	2.51228458	0.0001
1	-1	43.5000000	2.51228458	0.0001
-1	1	33.6562500	2.51228458	0.0001
-1	-1	28.6562500	2.51228458	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	39.7812500	1.7768503	0.0001
-1	34.0781250	1.7768503	0.0001

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	42.4062500	2.51228458	0.0001
1	-1	35.3750000	2.51228458	0.0001
-1	1	37.1562500	2.51228458	0.0001
-1	-1	32.7812500	2.51228458	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	42.5937500	2.51228458	0.0001
1	-1	33.8125000	2.51228458	0.0001
-1	1	36.9687500	2.51228458	0.0001
-1	-1	34.3437500	2.51228458	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	39.0937500	2.51228458	0.0001
1	-1	32.1250000	2.51228458	0.0001
-1	1	40.4687500	2.51228458	0.0001
-1	-1	36.0312500	2.51228458	0.0001

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	44.7500000	2.51228458	0.0001
1	-1	40.6562500	2.51228458	0.0001
-1	1	34.8125000	2.51228458	0.0001
-1	-1	27.5000000	2.51228458	0.0001

SODIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	41.4687500	2.51228458	0.0001
1	-1	34.0937500	2.51228458	0.0001
-1	1	38.0937500	2.51228458	0.0001
-1	-1	34.0625000	2.51228458	0.0001

SODIUM : PRODUCTION

General Linear Models Procedure
Dependent Variable : Y

* denotes significance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	8043.56250000	335.14843750	0.74	0.7986
Error	103	46612.86718750	452.55210862		
Corrected Total	127	54656.42968750			

R-Square	C.V.	Root MSE	Y Mean
0.147166	-368.4680	21.27327217	-5.77343750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	1372.77343750	457.59114583	1.01	0.3910
V7	1	39.38281250	30.38281250	0.09	0.7686
V8	1	217.88281250	217.88281250	0.48	0.4893
V9	1	250.32031250	250.32031250	0.55	0.4587
V10	1	1134.07031250	1134.07031250	2.51	0.1165
V11	1	35.07031250	35.07031250	0.08	0.7813
V7 * V11	1	178.13281250	178.13281250	0.39	0.5318
V8 * V11	1	61.88281250	61.88281250	0.14	0.7123
V9 * V11	1	56.44531250	56.44531250	0.12	0.7247
V10 * V11	1	347.82031250	347.82031250	0.77	0.3827
V12	1	1603.19531250	1603.19531250	3.54	0.0626 *
V11 * V12	1	438.82031250	438.82031250	0.97	0.3271
V13	1	2.82031250	2.82031250	0.01	0.9372
V7 * V13	1	18.75781250	18.75781250	0.04	0.8391
V8 * V13	1	532.19531250	532.19531250	1.18	0.2807
V9 * V13	1	96.25781250	96.25781250	0.21	0.6456
V10 * V13	1	1281.44531250	1281.44531250	2.83	0.0955
V11 * V13	1	168.82031250	168.82031250	0.37	0.5427
V12 * V13	1	192.57031250	192.57031250	0.43	0.5156
V14	1	2.82031250	2.82031250	0.01	0.9372
V11 * V14	1	0.19531250	0.19531250	0.00	0.9835
V13 * V14	1	11.88281250	11.88281250	0.03	0.8716

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.21875000	2.65915902	0.0524
-1	-6.32812500	2.65915902	0.0192

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-4.46875000	2.65915902	0.0959
-1	-7.07812500	2.65915902	0.0090

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-7.17187500	2.65915902	0.0082
-1	-4.37500000	2.65915902	0.1030

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.75000000	2.65915902	0.0014
-1	-2.79687500	2.65915902	0.2954

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.25000000	2.65915902	0.0510
-1	-6.29687500	2.65915902	0.0198

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.87500000	3.76061875	0.1213
1	-1	-4.56250000	3.76061875	0.2278
-1	1	-4.62500000	3.76061875	0.2216
-1	-1	-8.03125000	3.76061875	0.0351

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.25000000	3.76061875	0.3895
1	-1	-5.68750000	3.76061875	0.1335
-1	1	-7.25000000	3.76061875	0.0566
-1	-1	-6.90625000	3.76061875	0.0692

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-7.31250000	3.76061875	0.0546
1	-1	-7.03125000	3.76061875	0.0644
-1	1	-3.18750000	3.76061875	0.3986
-1	-1	-5.56250000	3.76061875	0.1422

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-9.87500000	3.76061875	0.0100
1	-1	-7.62500000	3.76061875	0.0452
-1	1	-0.62500000	3.76061875	0.8683
-1	-1	-4.96975000	3.76061875	0.1893

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-2.23437500	2.65915902	0.4027
-1	-9.31250000	2.65915902	0.0007

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.5625000	3.76061875	0.3457
1	-1	-6.9375000	3.76061875	0.0679
-1	1	-0.9062500	3.76061875	0.8100
-1	-1	-11.6875000	3.76061875	0.0024

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.62500000	2.65915902	0.0368
-1	-5.92187500	2.65915902	0.0281

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-4.68750000	3.76061875	0.2154
1	-1	-5.75000000	3.76061875	0.1293
-1	1	-6.56250000	3.76061875	0.0840
-1	-1	-6.09375000	3.76061875	0.1082

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-2.28125000	3.76061875	0.5454
1	-1	-6.65625000	3.76061875	0.0797
-1	1	-8.96875000	3.76061875	0.0189
-1	-1	-5.18750000	3.76061875	0.1708

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-6.15625000	3.76061875	0.1047
1	-1	-8.18750000	3.76061875	0.0318
-1	1	-5.09375000	3.76061875	0.1785
-1	-1	-3.65625000	3.76061875	0.3332

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.4375000	3.76061875	0.1512
1	-1	-12.0625000	3.76061875	0.0018
-1	1	-5.8125000	3.76061875	0.1253
-1	-1	0.2187500	3.76061875	0.9537

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-6.25000000	3.76061875	0.0996
1	-1	-4.25000000	3.76061875	0.2610
-1	1	-5.00000000	3.76061875	0.1866
-1	-1	-7.59375000	3.76061875	0.0461

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-3.3125000	3.76061875	0.3805
1	-1	-1.1562500	3.76061875	0.7591
-1	1	-7.9375000	3.76061875	0.0372
-1	-1	-10.6875000	3.76061875	0.0054

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.62500000	2.65915902	0.0368
1	-5.92187500	2.65915902	0.0281

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.06250000	3.76061875	0.1812
1	-1	-5.43750000	3.76061875	0.1512
-1	1	-6.18750000	3.76061875	0.1029
-1	-1	-6.40625000	3.76061875	0.0915

SODIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.78125000	3.76061875	0.1273
1	-1	-5.46875000	3.76061875	0.1489
-1	1	-5.46875000	3.76061875	0.1489
-1	-1	-6.37500000	3.76061875	0.0931

POTASSIUM : POST - PRE

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	84.66077332	3.52753222	2.14	0.0048
Error	99	163.24850087	1.64897476		
Corrected Total	127	247.90927419			

R-Square	C.V.	Root MSE	Y Mean
0.341499	-160.0315	1.28412412	-0.80241935

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	4.90697477	1.63565826	0.99	0.3999
V1	1	0.19249006	0.19249006	0.12	0.7333
V2	1	0.53396202	0.53396202	0.32	0.5706
V1 * V2	1	0.44176415	0.44176415	0.27	0.6059
V3	1	6.59583190	6.59583190	4.00	0.0482 *
V1 * V3	1	4.83121733	4.83121733	2.93	0.0901
V2 * V3	1	1.23717575	1.23717575	0.75	0.3885
V4	1	7.52170621	7.52170621	4.56	0.0352 *
V1 * V4	1	0.15722329	0.15722329	0.10	0.7581
V2 * V4	1	1.17876265	1.17876265	0.71	0.3999
V3 * V4	1	0.47808908	0.47808908	0.29	0.5915
V5	1	32.62942012	32.62942012	19.79	0.0001 *
V1 * V5	1	0.28113683	0.28113683	0.17	0.6806
V2 * V5	1	0.08803141	0.08803141	0.05	0.8178
V3 * V5	1	1.86417603	1.86417603	1.13	0.2903
V4 * V5	1	0.19901383	0.19901383	0.12	0.7290
V6	1	1.23951670	1.23951670	0.75	0.3880
V1 * V6	1	0.56397458	0.56397458	0.34	0.5600
V2 * V6	1	4.46733357	4.46733357	2.71	0.1029
V3 * V6	1	1.48171515	1.48171515	0.90	0.3455
V4 * V6	1	2.01602177	2.01602177	1.22	0.2715
V5 * V6	1	11.01134347	11.01134347	6.68	0.0112 *

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.82970677	0.16376500	0.0001
-1	-0.75066977	0.16363869	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.85602049	0.16534957	0.0001
-1	-0.72435605	0.16207046	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.83570144	0.23139024	0.0005
1	-1	-0.82371210	0.23138038	0.0006
-1	1	-0.87633954	0.23575416	0.0003
-1	-1	-0.62500000	0.22700322	0.0070

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.55850154	0.16707500	0.0012
-1	-1.02187500	0.16051552	0.0001

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.40003855	0.23610471	0.0933
1	-1	-1.25937500	0.22700322	0.0001
-1	1	-0.71696454	0.23575416	0.0030
-1	-1	-0.78437500	0.22700322	0.0008

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.11312500	0.24048174	0.0033
1	-1	-0.00125000	0.22700322	0.0001
-1	1	-0.05062500	0.23138038	0.0930
-1	-1	-0.10125000	0.22700322	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.03727049	0.16534957	0.0001
-1	-0.54310605	0.16207046	0.0011

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.11253855	0.23610471	0.0001
1	-1	-0.54687500	0.22700322	0.0178
-1	1	-0.96200243	0.23137038	0.0001
-1	-1	-0.53933710	0.23138038	0.0218

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.20082888	0.23576709	0.0001
1	-1	-0.51121210	0.23138038	0.0295
-1	1	-0.87371210	0.23138038	0.0003
-1	-1	-0.57500000	0.22700322	0.0129

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.74329098	0.24048174	0.0026
1	-1	-0.37371210	0.23138038	0.1095
-1	1	-1.33125000	0.22700322	0.0001
-1	-1	-0.71250000	0.22700322	0.0022

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-1.30470677	0.16376500	0.0001
-1	-0.27566977	0.16363869	0.0952

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.39195141	0.23139024	0.0001
1	-1	-0.26746210	0.23138038	0.2505
-1	1	-1.21746210	0.23138038	0.0001
-1	-1	-0.28387743	0.23137038	0.2228

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.34378855	0.23610471	0.0001
1	-1	-0.36825243	0.23137038	0.1147
-1	1	-1.26562500	0.22700322	0.0001
-1	-1	-0.18308710	0.23138038	0.4307

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.95003855	0.23610471	0.0001
1	-1	-0.16696454	0.23575416	0.4805
-1	1	-0.65937500	0.22700322	0.0001
-1	-1	-0.38437500	0.22700322	0.0936

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.59195144	0.23139024	0.0001
1	-1	-0.48258954	0.23575416	0.0433
-1	1	-1.01746210	0.23138038	0.0001
-1	-1	-0.06875000	0.22700322	0.7626

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.68975154	0.16707500	0.0001
-1	-0.89062500	0.16051552	0.0001

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.79691355	0.23610471	0.0011
1	-1	-0.86250000	0.22700322	0.0003
-1	1	-0.58258954	0.23575416	0.0152
-1	-1	-0.91875000	0.22700322	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.56516598	0.24048174	0.0208
1	-1	-1.14687500	0.22700322	0.0001
-1	1	-0.81433710	0.23138038	0.0007
-1	-1	-0.63437500	0.22700322	0.0062

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.34825308	0.24520555	0.1587
1	-1	-0.76875000	0.22700322	0.0010
-1	1	-1.03125000	0.22700322	0.0001
-1	-1	-1.01250000	0.22700322	0.0001

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.80891598	0.24048174	0.0011
1	-1	-1.26562500	0.22700322	0.0001
-1	1	-0.57058710	0.23138038	0.0154
-1	-1	-0.51562500	0.22700322	0.0253

POTASSIUM : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-1.50316355	0.23610471	0.0001
1	-1	-1.10625000	0.22700322	0.0001
-1	1	0.12366046	0.23575416	0.6011
-1	-1	-0.67500000	0.22700322	0.0037

POTASSIUM : PPRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	3.31687500	0.13820313	0.57	0.9400
Error	103	24.77429687	0.24052715		
Corrected Total	127	28.09117187			

R-Square	C.V.	Root MSE	Y Mean
0.118075	-375.9028	0.49043568	-0.13046875

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	0.50273438	0.16757813	0.70	0.5561
V7	1	0.00632813	0.00632813	0.03	0.8715
V8	1	0.04882812	0.04882812	0.20	0.6533
V9	1	0.10695313	0.10695313	0.44	0.5064
V10	1	0.41632813	0.41632813	1.73	0.1912
V11	1	0.17257812	0.17257812	0.72	0.3989
V7 * V11	1	0.05695312	0.05695312	0.24	0.6276
V8 * V11	1	0.01757813	0.01757813	0.07	0.7874
V9 * V11	1	0.00070312	0.00070312	0.00	0.9570
V10 * V11	1	0.35070312	0.35070312	1.46	0.2300
V12	1	0.73507812	0.73507812	3.06	0.0834
V11 * V12	1	0.10695312	0.10695312	0.44	0.5064
V13	1	0.00382812	0.00382812	0.02	0.8999
V7 * V13	1	0.00195313	0.00195313	0.01	0.9284
V8 * V13	1	0.07507818	0.07507813	0.31	0.5776
V9 * V13	1	0.02257813	0.02257813	0.09	0.7599
V10 * V13	1	0.61882813	0.61882813	2.57	0.1118
V11 * V13	1	0.00070312	0.00070312	0.00	0.9570
V12 * V13	1	0.02257812	0.02257812	0.09	0.7599
V14	1	0.00007812	0.00007812	0.00	0.9857
V11 * V14	1	0.04882812	0.04882812	0.02	0.6533
V13 * V14	1	0.00070313	0.00070313	0.00	0.9570

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.13750000	0.06130446	0.0270
-1	-0.12343750	0.06130446	0.0467

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.11093750	0.06130446	0.0733
-1	-0.15000000	0.06130446	0.0161

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.15937500	0.06130446	0.0107
-1	-0.10156250	0.06130446	0.1006

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.18750000	0.06130446	0.0028
-1	-0.07343750	0.06130446	0.2337

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.09375000	0.06130446	0.1293
-1	-0.16718750	0.06130446	0.0075

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12187500	0.08669760	0.1628
1	-1	-0.15312500	0.08669760	0.0803
-1	1	-0.06562500	0.08669760	0.4508
-1	-1	-0.18125000	0.08669760	0.0390

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06250000	0.08669760	0.4726
1	-1	-0.15937500	0.08669760	0.0689
-1	1	-0.12500000	0.08669760	0.1524
-1	-1	-0.17500000	0.08669760	0.0461

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12500000	0.08669760	0.1524
1	-1	-0.19375000	0.08669760	0.0276
-1	1	-0.06250000	0.08669760	0.4726
-1	-1	-0.14062500	0.08669760	0.1079

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.20312500	0.08669760	0.0211
1	-1	-0.17187500	0.08669760	0.0501
-1	1	-0.01562500	0.08669760	0.8573
-1	-1	-0.16250000	0.08669760	0.0637

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.05468750	0.06130446	0.3744
-1	-0.20625000	0.06130446	0.0011

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.04687500	0.08669760	0.5899
1	-1	-0.14062500	0.08669760	0.1079
-1	1	-0.06250000	0.08669760	0.4726
-1	-1	-0.27187500	0.08669760	0.0022

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.12500000	0.06130446	0.0440
-1	-0.13593750	0.06130446	0.0288

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12812500	0.08669760	0.1425
1	-1	-0.14687500	0.08669760	0.0933
-1	1	-0.12187500	0.08669760	0.1628
-1	-1	-0.12500000	0.08669760	0.1524

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.08125000	0.08669760	0.3509
1	-1	-0.14062500	0.08669760	0.1079
-1	1	-0.16875000	0.08669760	0.0543
-1	-1	-0.13125000	0.08669760	0.1331

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.14062500	0.08669760	0.1079
1	-1	-0.17812500	0.08669760	0.0425
-1	1	-0.10937500	0.08669760	0.2100
-1	-1	-0.09375000	0.08669760	0.2821

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.11250000	0.08669760	0.1973
1	-1	-0.26250000	0.08669760	0.0031
-1	1	0.13750000	0.08669760	0.1158
-1	-1	-0.00937500	0.08669760	0.9141

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.09062500	0.08669760	0.2983
1	-1	-0.09687500	0.08669760	0.2664
-1	1	-0.15937500	0.08669760	0.0689
-1	-1	-0.17500000	0.08669760	0.0461

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.06250000	0.08669760	0.4726
1	-1	-0.04687500	0.08669760	0.5899
-1	1	-0.18750000	0.08669760	0.0329
-1	-1	-0.22500000	0.08669760	0.0108

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-0.12968750	0.06130446	0.0368
-1	-0.13125000	0.06130446	0.0346

V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.11250000	0.08669760	0.1973
1	-1	-0.07500000	0.08669760	0.3890
-1	1	-0.14687500	0.08669760	0.0933
-1	-1	-0.18750000	0.08669760	0.0329

POTASSIUM : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-0.12187500	0.08669760	0.1628
1	-1	-0.12812500	0.08669760	0.1425
-1	1	-0.13750000	0.08669760	0.1158
-1	-1	-0.13437500	0.08669760	0.1242

CHLORIDE : POST - PRE

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	6782.62500000	282.60937500	1.78	0.0246
Error	103	16326.80468750	158.51266687		
Corrected Total	127	23109.42968750			

R-Square	C.V.	Root MSE	Y Mean
0.293500	-60.74418	12.59018137	-20.72656250

Source	DF	Type III SS	Mean Square	F Value	Pr > F	
R	3	1736.33593750	578.77864583	3.65	0.0150	
V1	1	524.07031250	524.07031250	3.31	0.0719	
V2	1	388.50781250	388.50781250	2.45	0.1205	
V1 * V2	1	341.25781250	341.25781250	2.15	0.1454	
V3	1	438.82031250	438.82031250	2.77	0.0992	
V1 * V3	1	548.63281250	548.63281250	3.46	0.0657	
V2 * V3	1	6.57031250	6.57031250	0.04	0.8391	
V4	1	775.19531250	775.19531250	4.89	0.0292	*
V1 * V4	1	23.63281250	23.63281250	0.15	0.7002	
V2 * V4	1	14.44531250	14.44531250	0.09	0.7634	
V3 * V4	1	446.25781250	446.25781250	2.82	0.0964	
V5	1	46.32031250	46.32031250	0.29	0.5900	
V1 * V5	1	288.44531250	288.44531250	1.44	0.2327	
V2 * V5	1	303.19531250	303.19531250	1.91	0.1696	
V3 * V5	1	51.25781250	51.25781250	0.32	0.5708	
V4 * V5	1	150.94531250	150.94531250	0.95	0.3314	
V6	1	500.07031250	500.07031250	3.15	0.0787	
V1 * V6	1	5.69531250	5.69531250	0.04	0.8500	
V2 * V6	1	15.82031250	15.82031250	0.10	0.7527	
V3 * V6	1	56.44531250	56.44531250	0.36	0.5520	
V4 * V6	1	11.88281250	11.88281250	0.07	0.7848	
V5 * V6	1	168.82031250	168.82031250	1.07	0.3045	

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-18.7031250	1.5737727	0.0001
-1	-22.7500000	1.5737727	0.0001

V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-18.9843750	1.5737727	0.0001
-1	-22.4687500	1.5737727	0.0001

V1	V2	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-18.5937500	2.2256507	0.0001
1	-1	-18.8125000	2.2256507	0.0001
-1	1	-19.3750000	2.2256507	0.0001
-1	-1	-26.1250000	2.2256507	0.0001

V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-22.5781250	1.5737727	0.0001
-1	-22.8750000	1.5737727	0.0001

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-22.6250000	2.2256507	0.0001
1	-1	-14.7812500	2.2256507	0.0001
-1	1	-22.5312500	2.2256507	0.0001
-1	-1	-22.9687500	2.2256507	0.0001

V2	V3	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-21.0625000	2.2256507	0.0001
1	-1	-19.9062500	2.2256507	0.0001
-1	1	-24.0937500	2.2256507	0.0001
-1	-1	-20.8437500	2.2256507	0.0001

V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-18.2656250	1.5737727	0.0001
-1	-23.1875000	1.5737727	0.0001

V1	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-15.8125000	2.2256507	0.0001
1	-1	-21.5937500	2.2256507	0.0001
-1	1	-20.7187500	2.2256507	0.0001
-1	-1	-24.7812500	2.2256507	0.0001

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.1875000	2.2256507	0.0001
1	-1	-21.7812500	2.2256507	0.0001
-1	1	-20.3437500	2.2256507	0.0001
-1	-1	-24.5937500	2.2256507	0.0001

V3	V4	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-18.2500000	2.2256507	0.0001
1	-1	-26.9062500	2.2256507	0.0001
-1	1	-18.2812500	2.2256507	0.0001
-1	-1	-19.4687500	2.2256507	0.0001

V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-20.1250000	1.5737727	0.0001
-1	-21.3281250	1.5737727	0.0001

V1	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-19.4375000	2.2256507	0.0001
1	-1	-17.9687500	2.2256507	0.0001
-1	1	-20.8125000	2.2256507	0.0001
-1	-1	-24.6875000	2.2256507	0.0001

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V2	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.8437500	2.2256507	0.0001
1	-1	-21.1250000	2.2256507	0.0001
-1	1	-23.4062500	2.2256507	0.0001
-1	-1	-21.5312500	2.2256507	0.0001

V3	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-21.3437500	2.2256507	0.0001
1	-1	-23.8125000	2.2256507	0.0001
-1	1	-18.9062500	2.2256507	0.0001
-1	-1	-18.8437500	2.2256507	0.0001

V4	V5	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-18.7500000	2.2256507	0.0001
1	-1	-17.7812500	2.2256507	0.0001
-1	1	-21.5000000	2.2256507	0.0001
-1	-1	-24.8750000	2.2256507	0.0001

V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-18.7500000	1.5737727	0.0001
-1	-22.7031250	1.5737727	0.0001

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V1	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.9375000	2.2256507	0.0001
1	-1	-20.4687500	2.2256507	0.0001
-1	1	-20.5625000	2.2256507	0.0001
-1	1	-24.9375000	2.2256507	0.0001

V2	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.6562500	2.2256507	0.0001
1	-1	-21.3125000	2.2256507	0.0001
-1	1	-20.8437500	2.2256507	0.0001
-1	-1	-24.0937500	2.2256507	0.0001

V3	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-19.9375000	2.2256507	0.0001
1	-1	-25.2187500	2.2256507	0.0001
-1	1	-17.5625000	2.2256507	0.0001
-1	-1	-20.1875000	2.2256507	0.0001

V4	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-16.5937500	2.2256507	0.0001
1	-1	-19.9375000	2.2256507	0.0001
-1	1	-20.9062500	2.2256507	0.0001
-1	-1	-25.4687500	2.2256507	0.0001

CHLORIDE : POST - PRE

General Linear Models Procedure
Least Square Means

V5	V6	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-17.0000000	2.2256507	0.0001
1	-1	-23.2500000	2.2256507	0.0001
-1	1	-20.5000000	2.2256507	0.0001
-1	-1	-22.1562500	2.2256507	0.0001

CHLORIDE : PRODUCTION

General Linear Models Procedure * denotes significance
Dependent Variable : Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	4512.43750000	188.01822917	0.93	0.5658
Error	103	20882.74218750	202.74506978		
Corrected Total	127	25395.17968750			

R-Square	C.V.	Root MSE	Y Mean
0.177689	-164.6408	14.23885774	-8.6483750

Source	DF	Type III SS	Mean Square	F Value	Pr > F
R	3	61.02343750	20.34114583	0.10	0.9597
V7	1	33.00781250	33.00781250	0.16	0.6874
V8	1	402.57031250	402.57031250	1.99	0.1618
V9	1	0.19531250	0.19531250	0.00	0.9753
V10	1	255.94531250	255.94531250	1.26	0.2638
V11	1	41.63281250	41.63281250	0.21	0.6514
V7 * V11	1	187.69531250	187.69531250	0.93	0.3382
V8 * V11	1	4.13281250	4.13281250	0.02	0.8867
V9 * V11	1	155.32031250	155.32031250	0.77	0.3835
V10 * V11	1	395.50781250	395.50781250	1.95	0.1655
V12	1	995.69531250	995.69531250	4.91	0.0289 *
V11 * V12	1	402.57031250	402.57031250	1.99	0.1618
V13	1	13.13281250	13.13281250	0.06	0.7996
V7 * V13	1	126.00781250	126.00781250	0.62	0.4323
V8 * V13	1	424.13281250	424.13281250	2.09	0.1511
V9 * V13	1	99.75781250	99.75781250	0.49	0.4846
V10 * V13	1	825.19531250	825.19531250	4.07	0.0462 *
V11 * V13	1	18.75781250	18.75781250	0.09	0.7616
V12 * V13	1	6.57031250	6.57031250	0.03	0.8575
V14	1	46.32031250	46.32031250	0.23	0.6337
V11 * V14	1	17.25781250	17.25781250	0.09	0.7711
V13 * V14	1	0.00781250	0.00781250	0.00	0.9951

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.14062500	1.77985722	0.0001
-1	-9.15625000	1.77985722	0.0001

V8	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-6.8750000	1.77985722	0.0002
-1	-10.4218750	1.77985722	0.0001

V9	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.68750000	1.77985722	0.0001
-1	-8.60937500	1.77985722	0.0001

V10	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-10.0625000	1.77985722	0.0001
-1	-7.2343750	1.77985722	0.0001

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.07812500	1.77985722	0.0001
-1	-9.21875000	1.77985722	0.0001

V7	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-8.7812500	2.5170982	0.0007
1	-1	-7.5000000	2.5170982	0.0036
-1	1	-7.3750000	2.5170982	0.0042
-1	-1	-10.9375000	2.5170982	0.0001

V8	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-6.1250000	2.5170982	0.0167
1	-1	-7.6250000	2.5170982	0.0031
-1	1	-10.0312500	2.5170982	0.0001
-1	-1	-10.8125000	2.5170982	0.0001

V9	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-9.2187500	2.5170982	0.0004
1	-1	-8.1562500	2.5170982	0.0016
-1	1	-6.9375000	2.5170982	0.0069
-1	-1	-10.8125000	2.5170982	0.0001

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V10	V11	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-11.2500000	2.5170982	0.0001
1	-1	-8.8750000	2.5170982	0.0006
-1	1	-4.9062500	2.5170982	0.0540
-1	-1	-9.5625000	2.5170982	0.0002

V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-5.8593750	1.77985722	0.0014
-1	-11.4375000	1.77985722	0.0001

V11	V12	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-7.0625000	2.5170982	0.0060
1	-1	-9.0937500	2.5170982	0.0005
-1	1	-4.6562500	2.5170982	0.0672
-1	-1	-13.7812500	2.5170982	0.0001

V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.96875000	1.77985722	0.0001
-1	-8.32812500	1.77985722	0.0001

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V7	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-7.4687500	2.5170982	0.0037
1	-1	-8.8125000	2.5170982	0.0007
-1	1	-10.4687500	2.5170982	0.0001
-1	-1	-7.8437500	2.5170982	0.0024

V8	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-5.3750000	2.5170982	0.0351
1	-1	-8.3750000	2.5170982	0.0012
-1	1	-12.5625000	2.5170982	0.0001
-1	-1	-8.2812500	2.5170982	0.0014

V9	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-8.12500000	2.5170982	0.0017
1	-1	-9.25000000	2.5170982	0.0004
-1	1	-9.81250000	2.5170982	0.0002
-1	-1	-7.40625000	2.5170982	0.0040

V10	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-7.8437500	2.5170982	0.0024
1	-1	-12.2812500	2.5170982	0.0001
-1	1	-10.0937500	2.5170982	0.0001
-1	-1	-4.3750000	2.5170982	0.0852

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V11	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-8.78125000	2.5170982	0.0007
1	-1	-7.37500000	2.5170982	0.0042
-1	1	-9.15625000	2.5170982	0.0004
-1	-1	-9.28125000	2.5170982	0.0004

V12	V13	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-6.4062500	2.5170982	0.0124
1	-1	-5.3125000	2.5170982	0.0372
-1	1	-11.5312500	2.5170982	0.0001
-1	-1	-11.3437500	2.5170982	0.0001

V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	-8.04687500	1.77985722	0.0001
-1	-9.25000000	1.77985722	0.0001

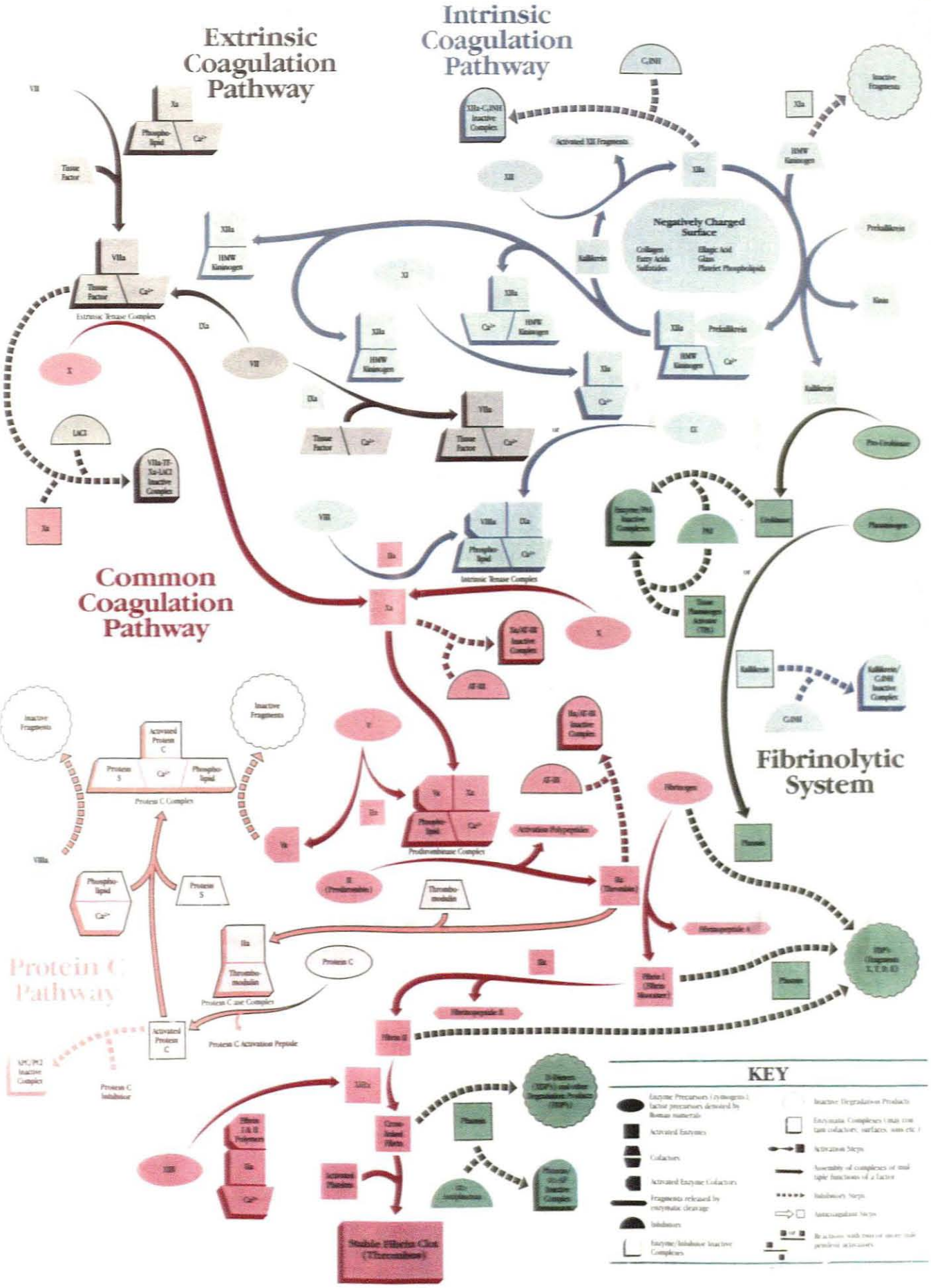
V11	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-7.8437500	2.5170982	0.0024
1	-1	-8.3125000	2.5170982	0.0013
-1	1	-8.2500000	2.5170982	0.0014
-1	-1	10.1875000	2.5170982	0.0001

CHLORIDE : PRODUCTION

General Linear Models Procedure
Least Square Means

V13	V14	Y LSMEAN	Std Err LSMEAN	Pr > ITI HO: LSMEAN = 0
1	1	-8.37500000	2.5170982	0.0012
1	-1	-9.56250000	2.5170982	0.0002
-1	1	-7.71875000	2.5170982	0.0028
-1	-1	-8.93750000	2.5170982	0.0006

Appendix IV


Extrinsic Coagulation Pathway
Intrinsic Coagulation Pathway
Common Coagulation Pathway
Fibrinolytic System
Protein C Pathway
Negatively Charged Surface
Collagen
Fatty Acids
Sulfatides
Ellagic Acid
Glass
Platelet Phospholipids
Extrinsic Tenase Complex
Intrinsic Tenase Complex
Prothrombinase Complex
Protein C Complex
Protein C Activation Peptide
Activation Polypeptide
Stable Fibrin Clot (Thrombin)
KEY