ISSN 0261-1597

BIOTEST FIFTH SYMPOSIUM Vol 1 No 2
Edinburgh, 26 September 1981

Aspects of Blood Component Production in the Regional Transfusion Centre

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NORTH WESTERN REGIONAL HEALTH AUTHORITY

NATIONAL BLOOD TRANSFUSION SERVICE

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WTD/ 5032

NHBT0027395_008_0001

THE FOLLOWING INFORMATION HAS BEEN KINDLY GIVEN TO US BY

DR. R.T. WENSLEY FROM THE BIOTEST FIFTH SYMPOSIUM VOL 1

NO. 2, EDINBURGH, 26 SEPTEMBER 1981.

ASPECTS OF BLOOD COMPONENT PRODUCTION IN THE REGIONAL TRANSFUSION CENTRE.

INTRODUCTION.

Dr. Hogman.

Ladies and gentlement, we now come to the next paper which will be given by Dr. Wensley from Manchester who is going to speak on cryoprecipitate production.

Biotest Bulletin 2: 106-108 (1982).

CRYOPRECIPITATE

R.T. Wensley

Manchester Blood Transfusion Centre.

The use of cryoprecipitate in the treatment of Haemophillia A (Classical Haemophillia) is declining in England and Wales in favour of lyophilised Factor V111 concentrate. However cryo-precipitate is the first stage raw material from which lyophilised Factor V111 is eventually prepared. Improvements in cryoprecipitate Factor V111 yield discovered during small scale manufacture can sometimes be incorporated in practice into the large scale manufacturing process.

Cryoprecipitate is simple and economical to produce. What is it being used for at present?

TABLE 1 Indications for Cryoprecipitate.

VON WILLIBRAND DISEASE.
MILD HAEMOPHILIA.
HAEMOPHILIA CARRIERS.
CONGENITAL AFIBRINOGENAEMIA.
FIBRONECTIN DEFICIENCY.
SOURCE FOR FREEZE-DRYING.

Freeze dried cryoprecipitate is presently being produced at the Glasgow and Dublin Blood Transfusion Centres.

What conditions are necessary to obtain the best yield of Factor V111 clotting activity in cryoprecipitate? Table 11 shows a number of 'variables' which may be incorporated during the production of cryoprecipitate and which will enhance the yield.

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NHBT0027395_008_0002

TABLE 11 High V111 Yield Cryoprecipitate.

GROUPS A.B. A.B.O.
C.P.D. A.C.D.
HEPARIN[2]
CONTINUOUS MIXING[3]
IMMEDIATE PROCESSING 1
RAPID FREEZING TO BELOW -25°C4
RAPID THAW 1
PROMPT HARVESTING[3]
THAW SIPHON 6

In Manchester we find that the demand for cryoprecipitate is decreasing as more haemophiliacs are converted from hospital out patient cryoprecipiate Factor V111 therapy and join our home treatment programme. This employs lyophillised Factor V111 concentrate for which the demand continues to rise and there is a reciprocal decline in cryoprecipitate usage.

Table 111 illustrates the Factor V111 cryoprecipitate yields in Manchester cryoprecipitate.

TABLE 111

MEAN YIELD - 84 1.U/bag
ASSUME 1 1.U/ML OF DONOR PLASMA
% YIELD = $\frac{84}{180}$ X 100 = 47%
YIELD PER LITRE STARTING PLASMA = 470 1.U.

We process 180 mls of Citrate Phosphate Dextrose anticoagulated plasma which is accurately weighted by employing plasma which is accurately weighed by employing simple tip over balances in a published survey of Factor V111 yields in which equal number of Group O and Group A donations were examined, 1 the final product contained an average of 84 1.U. activity. If we arbitratily assign a value of 180 1.U. Factor V111 to the starting plasma we achieve a yield of 47% or expressed somewhat differently a yield of 470 1.U. of Factor V111 per litre of plasma processed into cryoprecipitate. This yield compares favourably with lyophillised intermediate Factor V111 concentrate produced at the Blood Products Laboratory at Elstree which at best achieves a yield of 260 1.U. Factor V111 per litre of plasma processed.

Our cryoprecipitate is re-suspended in a small volume of plasma (mean 7 mls) and a bag rinse out technique is required to remove all the Factor V111 activity from the pack. With such a small volume of plasma in each bag, patient reactions to the material are very infrequent.

As we remove a constant 180 mls of plasma from every donation processed for cryoprecipitate the haematocrit of the residual red cells averages 63% ± so we hardly ever issue a plasma reduced unit with a haematocrit exceeding 70%

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NHBT0027395_008_0003

Cryoprecipitate production employs four essential stages plasma separation, plasma freezing, thawing of the plasma and final centrifugation to harvest the cryoprecipitate. We have automated the two middle stages of freezing and thawing of the plasma and have devised an automatic freeze-thaw cabinet, the FACTOR EIGHTOR.

This freeze thaw cabinet has a capacity for 90 x 180ml plasma satellite packs which are automatically frozen and thawed while they remain attached to an empty transfer pack. The plasma reduced red cells have been sealed off and removed previously. They are not included in the cabinet because of the extremes of temperature which are employed.

Plasma freezing is carried out at -70°C conventional mechanical refrigeration is used in the cabinet. When fully frozen the thaw process is initiated automatically using a pre-set timing devise and the plasma is thawed in approximately two hours. The bags are now taken out of the cabinet for final cryoprecipitate harvesting by centrifugation. Using this automatic freeze thaw cabinet it has been possible for the same number of Medical laboratory Scientific staff to process about twice the number of donations for cryoprecipitate compared with previously. We expect that the production gains (increase in donations processed and in Factor V111 yield) will off-set the capital cost of the equipment within a relatively short time.

In conclusion cryoprecipitate can be produced simply and cost effectively and in its freeze dried form should be suitable for self-administration in home treatment programmes. The yield of cryoprecipitate Factor V111 from a given amount of starting plasma is about twice that of lyophilised intermediate Factor V111 concentrate. I believe that countries which are planning for complete self sufficiency in Factor V111 supplies should consider the advantages of employing cryoprecipitate manufactured by the automated system described above.

Manufactured now by J. De Roma (PLR) Limited (Cryo-King Ultra Low), Newstet Road, Kirkby Industrial Estate, Liverpool L33 7TJ Merseyside.

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NHBT0027395_008_0004

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DISCUSSIONS ON DR. WENSLEY'S AND DR. MYLLYLA'S PAPERS.

DR. SMIT SIBINGA.

Would Dr. Wensley please remind us of the air temperature used for actual thawing? You also mentioned Dr. Wensley that the plasma volume was approximately 180ml. This is an extraordinarily small amount of plasma from a donation.

DR. WENSLEY

180mls is the standard volume of fresh plasma taken from most donations in the United Kingdom. We have found that the best air temperature for thawing is one of approximately 24°C. We have 12 KW of heat in the unit and we blow this at a controlled temperature of +24°C over the bags. There is a control bag in the cabinet and as soon as all the ice has disappeared in this bag then the air temperature is reduced automatically from +24°C down to +3°C.

DR. SMIT SIBINGA

Is your standard donation 450mls of blood?

DR. WENSLEY.

No, it is approximately 425mls.

DR. HOGMAN.

I would like to ask Dr. Wensley whether he has studied losses during the refreezing of the cryoprecipitate?

DR. WENSLEY.

No, but we have looked at the losses in cryoprecipitate during thawing. We thaw in a water bath at 37°C and aspirate immediately after the bag is washed out with 5mls of saline. We have noticed that without the salina wash technique there is as much as 35% of the Factor V111 activity left behind in the bag.

DR. GANSHIRT (FRANKFURT).

I imagine the thickness of the plasma bag will influence the freezing and the length of thawing time. I suspect that the thinner the plasma bag is the better the yield of Factor V111 will be. Have you studied this problem in order to ascertain the optimal thickness that your frozen plasma bag should be?

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NHBT0027395_008_0005

DR. WENSLEY.

The 180 mls of plasma in the bag fits into a stainless steel former and is a fairly tight fit. It is wedge shaped, however so that is can readily be put in and out of the machine. We have not studied the effect of various thicknesses of plasma bags.

DR. CASH.

Dr. Wensley I appreciate that you are rather short of time and I would therefore be most grateful if you could go over once again, perhaps giving a little bit more detail, the actual practical procedures involved as you use this machine.

DR. WENSLEY.

The bags of blood come off the centrifuge in batches of 6 and this is the reason why each stainless steel rack contains 6 formers. We have a mechanism of selecting the bar that we want to place the rack on by simply pressing a button on the outside of the cabinet. The selected bar halts behind the door, the door is opened and the rack is placed on the bar. The door is closed, the button is pressed and the rack circulates in the air. In order to get equal heat and refrigeration distribution we have designed a rotisserie system within the cabinet itself. It takes approximately 30 to 40 minutes to get down to a core temperature of approximately -23°C. This time of course, depends somewhat on the degree of loading of the cabinet but even when fully loaded we would anticipate the temperature will have reached -23°C by 40 minutes. We often start loading the machine at 2 p.m. and go on loading until 7 p.m. depending on the supplies of blood. During this time the cabinet will be at -70°C for the whole period. Unloading is completed either by using a manual switch or an automatic one and sequences change so that the temperature in the cabinet will be maintained at -30°C. By introducing this sytem the cabinet can now act as an ordinary conventional deep freeze, although the bags still rotate within it for as long as we wish. Depending on the time sequence set thawing starts by increasing the temperature to +24°C and then reducing it to +3°C. The thaw is complete in about 2 hours.

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CRYOPRECIPITATE FACTOR VIII

There are four essential stages in the production of cryoprecipitate: plasma separation, plasma freezing, thawing of the plasma and final centrifugation to harvest the cyroprecipitate.

The two middle stages of freezing and thawing of the plasma have now been automated - by use of the 'Factor Eightor', produced by J. De Roma (PLR) Ltd. of Newstet Road, Kirkby Industrial Estate, Liverpool L33 7TJ, Merseyside. Telephone 051-546-7240.

This new freeze-thaw cabinet has a capacity for 90 x 180 ml plasma satellite packs which are automatically frozen and thawed while they remain attached to an empty transfer pack. The plasma free red cells have been sealed off and removed previously because of the extremes of temperature which are employed.

Plasma freezing is carried out at -70c. When fully frozen the thaw process is initiated by using a pre-set timing device and the plasma is thawed in approximately two hours. The bags are now taken out of the cabinet for final cryoprecipitate harvesting by centrifugation.

In practice at Manchester, medical laboratory staff are able to process about double the number of donations for cryoprecipitate compared with previously. Using this system a yield of 470 i.u. of Factor VIII per litre of plasma processed into cryoprecipitate has been achieved. This yield compares favourably with lyophilised intermediate Factor VIII concentrate achieving perhaps a yield of up to 260 i.u. per litre of plasma.

Demand for cryoprecipitate is increasing on a worldwide basis - the advantages of the 'Factor Eightor' are therefore clear and fuller information can be obtained from J. De Roma (PLR) Ltd. at the address given above.

WTD/ 5038