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To: Dr. R.S. Lane 15th June, 1988.

Yield of F.VIII:C from 8Y in building 27

This review addresses the questions:

Why is the F.VIII:C yield of 8Y from B27 so low, and to what extent are there prospects for improvement?

If PFC's Z8 gives a much higher yield, why does BPL not adopt manufacture of Z8?

In what follows, yields of F.VIII:C in iu/kg plasma (or % stage yield) will be considered in three sections.

(1) Primary recovery of F.VIII:C in redissolved cryoprecipitate (/4 in BPL/PFL jargon). This is the first reliable point of assessment incorporating losses in collection and separation of blood; delays in freezing; losses during inadequate freezing or temperature cycling in transit; warming before stripping; "conditioning" proper after stripping; crushing; thawing proper; centrifugal collection of cryoprecipitate and resolution.

(2) Processing to formulation and pre-filtration (/5).

(3) Finishing, incorporating losses on sterile filtration, dispensing, freeze-drying, dry heating (/8).

(1) Primary recovery of F.VIII:C in redissolved cryoprecipitate

Some relevant data are presented in Table 2. There are two main hypotheses that might explain why B27 cryo yields are so low.

(a) Changes in plasma collection or complicated FFP storage and transfer patterns over the last year or two have resulted in a stock of FFP which on average has lost much of its total or cryoprecipitable F.VIII:C.

or

(b) The "average stock" of FFP has preserved recoverable F.VIII:C, but losses are occurring somewhere in the stripping and thawing chain in B27.

Present evidence, considered in more detail in Appendix 1, favours (a) but leaves open the possibility that (b) is contributing.

(2) Processing to pre-filtered /5

The yield through heparin precipitation, glycine/NaCl precipitation and de-salting is now within a few points of 70% in B27, at PFL, and on any scale; this was also the settled yield in B25. The reasons for any occasional excursions from this performance are largely understood.

(3) Finishing

Arriving at sterile filtration at a process yield of 70% x 320 iu/kg

≈ 224 iu/kg, further losses are incurred in several ways.

Sterile filtration: current losses average 20%, mostly because

(a) any losses on freezing the pool, still a frequent occurrence, are taken here

(b) current high protein levels, since moving to 3300 kg, leading to multiple filters, blocked filters and waste.

Dispensing: substantial volumes, though small as a percentage of litres, are lost or unaccounted for.

Freeze-drying and heating: combined recovery of 85% has reached established performance in building 25.

Comparison with PFC's Z8

Comparisons are not simple. All PFC data quoted here are based on one-stage assays, which are particularly likely to differ from two-stage assays at the plasma, cryo and cryosuper stages of most interest. Even PFC's own two-stage assay currently gives lower absolute potencies for concentrates than their one-stage assay. PFC do not sample for yield assessment at precisely the same stages as PFL and BPL. Furthermore, PFC are currently getting a lower cryoprecipitate and final yield than in the earlier experience quoted e.g. at the 1987 ISTH; this is attributed to improvisations in plasma conditioning while their custom-built conditioning unit is out of commission.

I have therefore interpolated some missing points on conservative assumptions. In Table 1, the following simplifications are used:

(a) PFL, B25 and B27 process yields (cryo /4 to pre-filter /5) are nearly constant at 70%.

(b) PFL and B25 finishing yield (/5 to heated product /8) amalgamates to 77%, with small and ever-varying differences in loss distribution. B27 finishing yield is currently less than 70%, largely because of filtration losses which must be avoidable; a supplementary column shows the effect of improving /4 and /6 yield to levels regularly achieved before the move to B27.

(c) I have assumed a filtration and dispensing yield of 90% for PFC's Z8, important only for the assessment of partial yields.

From simplified Table 1A, it emerges that

(1) Primary recovery of F.VIII:C in redissolved cryoprecipitate

PFC obtain a higher total yield of F.VIII:C from their FFP, which is probably of no higher specification than BPL or PFL's corresponding plasma. PFC's apparent cryo yield from the best recovered FFP currently exceeds by 10% PFL's performance on all but the best machine apheresis plasma.

(2) Processing to pre-filtered solution, 8Y v Z8

On the face of it, "8Y processing" recovers 70% of the cryo F.VIII:C, whereas "Z8 processing" currently recovers 60% (formerly approximately 68%). In absolute terms, PFC are still getting higher manufacturing yields than B27, because of the better starting cryo yield. PFL's yield from best apheresis plasma is currently higher than PFC's, but still ~10% less than PFC's best former performance.

(3) Finishing

There is no significant difference between B25, PFL, current PFC and former PFC performance. B27 loses 5-10% more than any of these at "sterile filtration", incorporating losses due to delays, re-filtration, freezing and thawing, and mechanical losses in scaled up dispensing apparatus. Freeze-drying and heating losses are acceptable. Current PFC performance pertains to heating at 75° only, whereas 80° was used formerly.

Conclusions

Differences in cryo yield between BPL and PFC may be affected by a one-stage/two-stage differential; by consistent QC campaigning on plasma quality; or by the efficiency of plasma stripping, conditioning and thawing. No crucial single experiment can be devised to determine which of these influences are most important. It is quite laborious to learn even what is changing with time at BPL, or between BPL and PFL.

There is no evidence that the heart of the Z8 process gives a better yield than the heart of the 8Y process. In particular, current Z8 processing appears to be no more efficient in retaining F.VIII:C than is 8Y in B27. Imminent refinements in the Z8 process, including a return to 80° heating, are unlikely to improve yield.

B27 finishing efficiency might reasonably be expected to improve by 10% as scale-up/commissioning attains earlier levels of performance. PFC do not appear to have a significant advantage at this stage.

Appendix 1. Assessment of current cryo yield in B27

Current performance in B27 at 3300 kg scale is 320 iu/kg. Selection of plasma packs at random from nominally the same plasma stock, and thawing manually in 20-donation pools, gives 329 iu/kg for recovered plasma and 443 iu/kg for Haemonetics plasma. In the latter experiments, approximately the same % recovery is achieved from the F.VIII:C in the FFP as determined by a rapid 37°C thaw of a portion of cryo-suspension. This manual thawing from BPL Haemonetics stock agrees closely with PFL models on Haemonetics FFP (452 iu/kg) and PFL 300 kg batches of Haemonetics FFP (460 iu/kg). These data suggest that both model experiments and manufacturing are recovering the usual proportion of F.VIII:C from the plasma, but that the average stock of recovered FFP at BPL contains a low level of F.VIII:C.

It must be emphasised that this drop in F.VIII:C content of the plasma stock appears to have occurred while CF were still in B25. The multi-factorial yields from B27's first commissioning runs tend to conceal this. The first 100 batches of 8Y in B25 yielded 361 iu/kg at /4 - probably not too far from mean yield at /4 in the old HL (but far short of the 500 iu/kg being achieved in 1983 with the best recovered plasma). The next 21 batches in B25 continued at 372 iu/kg, but cryo yield then suddenly dropped to 323 iu/kg at 8Y 3540, the level now seen in B27 after all the associated changes in stripping and thawing equipment and procedures. It is believed that the drop in cryo yield coincided with a scale-down from 1500 to 1200 kg batches in CF. There might have been e.g. a catastrophic melt at Salvesens.

There are other indications that this is not the whole story, and that B27 may be failing to recover F.VIII:C from plasma of conventional quality. The difficulty in discriminating these explanations arises, of course, from the impossibility of getting a representative "sample" of FFP from 2000-3300 kg pools of single donations.

(1) 4 x 300 kg batches of 5L packs from BPL stock gave a cryo yield of 403 iu/kg at PFL, similar to recent performance at PFL on occasional batches of recovered donations in PVC packs (404 iu/kg). Of course, if temperature cycling during transport or borderline storage conditions were involved, 5L packs would always fare better than loose SDs. However, a single 300 kg batch of SAG-M FFP stripped in B25 last year, also gave 409 iu/kg at PFL; this FFP was special in that it stayed at BPL only for a few days while being stripped, and did not go to Salvesen.

(2) The first four models run at PFL on packs taken at random from CF's intended batches before stripping yielded 391 iu/kg, while the B27 batches were running at 320 iu/kg. Two pools run at PFL after B27 stripping and conditioning gave 433 iu/kg. Unless there was gross uni-directional sampling bias, these data suggest that there are losses during B27's processes after stripping and conditioning.

(3) The next series of 13 models run by R & D at BPL from CF's routine stripped and conditioned stock gave 357 iu/kg, while manufacturing pools were giving 322 iu/kg. 15 models from CF's conditioned and crushed FFP gave 332 iu/kg, while manufacturing pools were giving 316 iu/kg. These differences could only be confirmed as significant by very many repetitions, since the errors in sampling and assay are of comparable magnitude.

Because of limitations on both pack strippers in B27, FFP may spend the best part of a week in the region -5 to -15° before entering full thawing. That holding period is very variable and is totally outside the guidelines for plasma conditioning developed at PFL last year. It

is quite likely that this floating period harms F.VIII:C, or its recoverability, and there may be additional cross-influences on F.VIII and cryo composition from the total storage history at BPL and Salvesen.

Any deleterious effect of B27 crushing methods seems to be less than 8% and would take a very large number of experiments to establish.

Some pointers may be gained by special selection of plasma for B27's prospective routine batches and by extension of PFL's fractionation of routine stocks stripped at BPL. However, convincing explanations and cures may not be forthcoming until B27 can open 3300 kg of FFP at much lower temperature than the present -12° or so.