Zinc Prep. 21.11.85

METHOD SHEET FOR PASTEURISED FVIII PILOT-SCALE PRODUCTION TEST

BATCH ZHT

DAY 1

1. Tris extract (pH7.0) 830 ml ℓ *

2. Titrate slowly from 7.0 to 6.7 6.7 with 0.05N Hcl
   Use E₂ vibromixer
   Volume used ~~/~~ ML's
   Total volume 830 ML's (V_1)

3. Zinc/heparin precipitation, pH6.7
   Use E₂ vibromixer
   ~~0.04ML, Heparin/ℓ(V_1) _____ ML's~~
   333.3 ML's, 4mM ZnAc/ℓ(V_1) 277 ML's add slowly.
   Final pH, 6.7

4. Equilibrate 10 minutes R.T. with gentle mixing.

5. Centrifuge 10 minutes R.T. 4000 rpm.
   Post-centrifugation, zinc-supernatant 980 mℓs(V_2)*

6. Mixture of citrate and calcium chloride to 0.02M and ~~5.5~~ 2.5 mM
   a) 41.7 MLS/ℓ(V_2) = 41.7 x 980 mℓ(V_2) = 40.9 MLS 0.5M Citrate
   b) 11.1 MLS/ℓ V_2) = ~~11.1~~ 5.05 x 980 mℓ(V_2) = 4.9 MLS 0.5M Calcium Chloride.
   Mix 40.9 MLS 0.5M $CaCℓ_2$ with 4.9 MLS 0.5M Citrate
   Add slowly to (V_2) use E₂ vibromixer
   Final volume 1030 ℓ *

Adjust to pH 7.4 and 0.45 filter 2x20ml aliquots the rest in 1 lump.

7. ~~Clarify through a NBP cartridge _____ ℓ*(V_3)~~ 800 ml

~~8. Addition of glycine and sorbitol stabilisers~~

   a) 50 g/ℓ(V_3) = 50 g x ____ ℓ(V_3) = _____g glycine.
   Add slowly mix with E_2 vibromixer.
   Keep pH above 7.0 during glycine addition with 1N NaOH and adjust to final pH 7.5.

   b) 1850 g/ℓ(V_3) = 1850 g x _____ℓ(V_3) = _____g sorbitol
   Add over 30 minutes with stirrer at 70 rpm. INCREASE temperature GRADUALLY from +20°C to +40°C.
   Fill glycine/sorbitol VIII (G+S) into MSE bottles
   _____ x _____ML'S

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	Vol mL's.	1-ST FVIII u/mL	u	2-ST FVIII u/mL	u.	TP g/L	g	2-ST % VIII Recovery	1-ST. % VIII Recovery
TBS pH7.0	830	8.96	7437	7.04	5843	34.8 (29) (28884)	20.5 (17)		
TBS pH 6.7	830	9.14	7586	7.23	6001	35.3 (29)	17.9		
Z-S	980	5.94	5821	4.97	4871	16.5 (16)	4.9	77	81
Z-S +CIT/Ca.	1030	5.79	5964	5.26	5418	17.3 (17.8)	7.2	79	90
Post 1.2µ	-	5.70		5.43		16.7	7.0		
" 0.8µ	-	5.34		4.64		16.6	7.0		
" 0.45µ	860	5.33	4584	4.88	4145	16.8 (14.5)	7.1 (6.1)	60	70

% loss TP. 50

% TP 64

METHOD SHEET FOR PASTEURISED FVIII PILOT-SCALE PRODUCTION TEST

BATCH ZHT BY5069 2.12.85

DAY 1

1. Tris extract (pH7.0) 525 mls ℓ *

2. Titrate slowly from 7.1 to 6.7 6.7 with 0.05N Hcl
   Use E₂ vibromixer
   Volumeused _____ ML's
   Total volume 500 ML's (V_1)

3. Zinc/heparin precipitation, pH6.7
   Use E₂ vibromixer
   0.04ML, Heparin/ℓ(V_1) — ML's
   333.3 ML's, 4mM ZnAc/ℓ(V_1) 167 ML's add slowly.
   Final pH, 6.65

4. Equilibrate 10 minutes R.T. with gentle mixing.

5. Centrifuge 10 minutes R.T. 4000 rpm.
   Post-centrifugation, zinc-supernatant 640 ℓ(V_2)* (620

6. Mixture of citrate and calcium chloride to 0.02M and ~~5.5~~ 7.5 mM
   a) 41.7 MLS/ℓ(V_2) = 41.7 x ___ℓ(V_2) = 25.9 MLS 0.5M Citrate
   b) ~~11.1~~ 5.5 MLS/ℓ V_2) = ~~11.1~~ 5.5 x ___ (V_2) = 3.4 MLS 0.5M Calcium Chloride.
   Mix 3.4 MLS 0.5M $CaCl_2$ with 26.0 MLS 0.5M Citrate
   Add slowly to (V_2) use E₂ vibromixer adjust pH to 7.4
   Final volume 670 ℓ *

7. Clarify through a NBP cartridge _____ ℓ*(V_3)
   filter through 1.2µ + 0.8µ and 0.45µ, 0.8 just-filtered 630ml
   Filtered no problem

8. Addition of glycine and sorbitol stabilisers
   a) 50 g/ℓ(V_3) = 50 g x ___ ℓ(V_3) = _____g glycine.
   Add slowly mix with E₂ vibromixer.
   Keep pH above 7.0 during glycine addition with 1N NaOH and adjust to final pH 7.5.
   b) 1850 g/ℓ(V_3) = 1850 g x _____ℓ(V_3) = _____g sorbitol
   Add over 30 minutes with stirrer at 70 rpm. INCREASE temperature GRADUALLY from +20°C to +40°C.
   Fill glycine/sorbitol VIII (G+S) into MSE bottles
   _____ x _____ML'S

NY5069. 7.12.85.

	Vol	1ST FVIII u/ml	u	2ST FVIII u/ml	u	TP g/l	g	TΦ g/l	g	%Recovery 2-ST VIII	%Recovery 1-ST VIII
TB1 pH7.0 (SRS)		7.8	4095	7.1	3728	29.7	156	14.7	7.7		
TB1 pH6.7 (SRS)		7.1	3728	7.2	3780	28.1	147	11.2	5.9		
Z-S	(690)	4.4	2816	4.5	2880	11.3	7.2	2.5	1.6		
Z-S+CIT+Ca	(670)	4.0	2680	4.4	2948	11.2	7.5	3.1	2.1		
pH7.4 "	(670)	4.9	3283	4.7	3149	11.1	7.4	2.9	1.9	88.0	83.0
POST-0.8μ FILT.		4.0		4.1		6.4		2.5			
POST-0.45M FILT.	(630)	3.72	2344	3.7	2331	9.8	6.2	2.8	1.8	63.0	62.0

	%Recovery 2-ST VIII	%Recovery 1-ST VIII	%Loss TP	%Loss TΦ.
POST Z-S+CIT+Ca	88	83	41	68
POST-0.45M FILT.	63	62	51	69