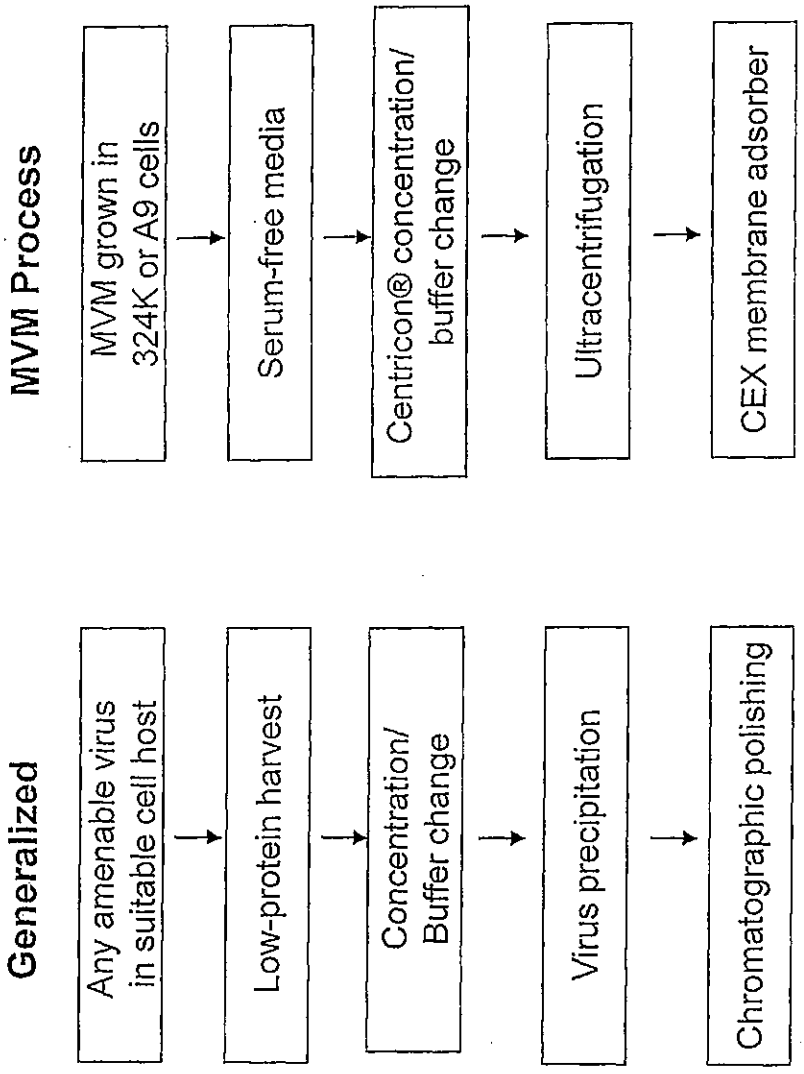
MVM Process
MVM grown in 324K or A9 cells
Serum-free media
Centricon® concentration/ buffer change
Ultracentrifugation
CEX membrane adsorber
Generalized
Any amenable virus in suitable cell host
Low-protein harvest
Concentration/ Buffer change
Virus precipitation
Chromatographic polishing

FIG. 1

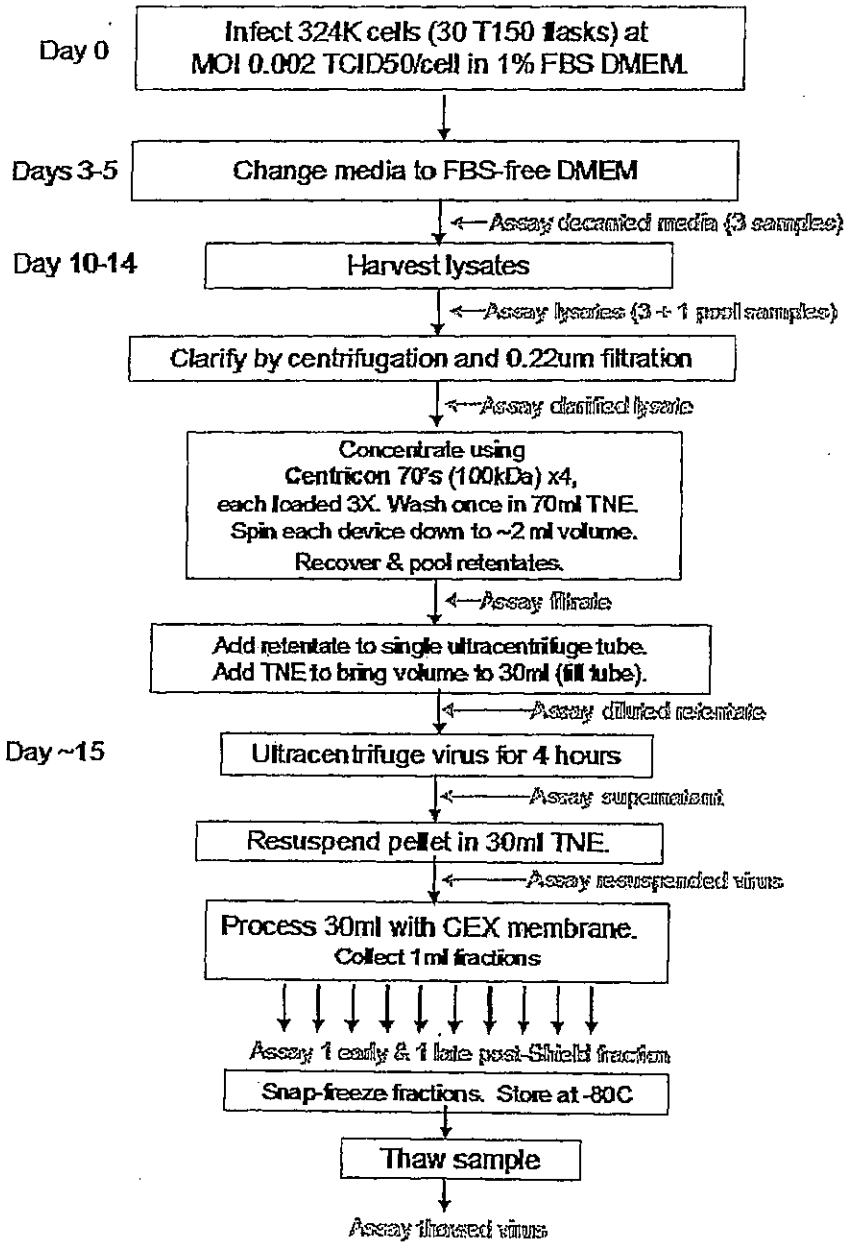
Day 0
Infect 324K cells (30 T150 flasks) at MOI 0.002 TCID50/cell in 1% FBS DMEM
Days 3-5
Change media to FBS-free DMEM
Assay decanted media (3 samples)
Day 10-14
Harvest lysates
Assay lysates (3 + 1 pool samples)
Clarify by centrifugation and 0.22um filtration
Assay clarified lysate
Concentrate using Centricon 70's (100kDa) x4, each loaded 3X. Wash once in 70ml TNE. Spin each device down to ~2 ml volume. Recover & pool retentates.
Assay filtrate
Add retentate to single ultracentrifuge tube. Add TNE to bring volume to 30ml (fill tube).
Assay diluted retentate
Day ~15
Ultracentrifuge virus for 4 hours
Assay supernatant
Resuspend pellet in 30ml TNE.
Assay resuspended virus
Process 30ml with CEX membrane. Collect 1ml fractions
Assay 1 early & 1 late post-Shield fraction
Snap-freeze fractions. Store at -80C
Thaw sample
Assay thawed virus

FIG. 2

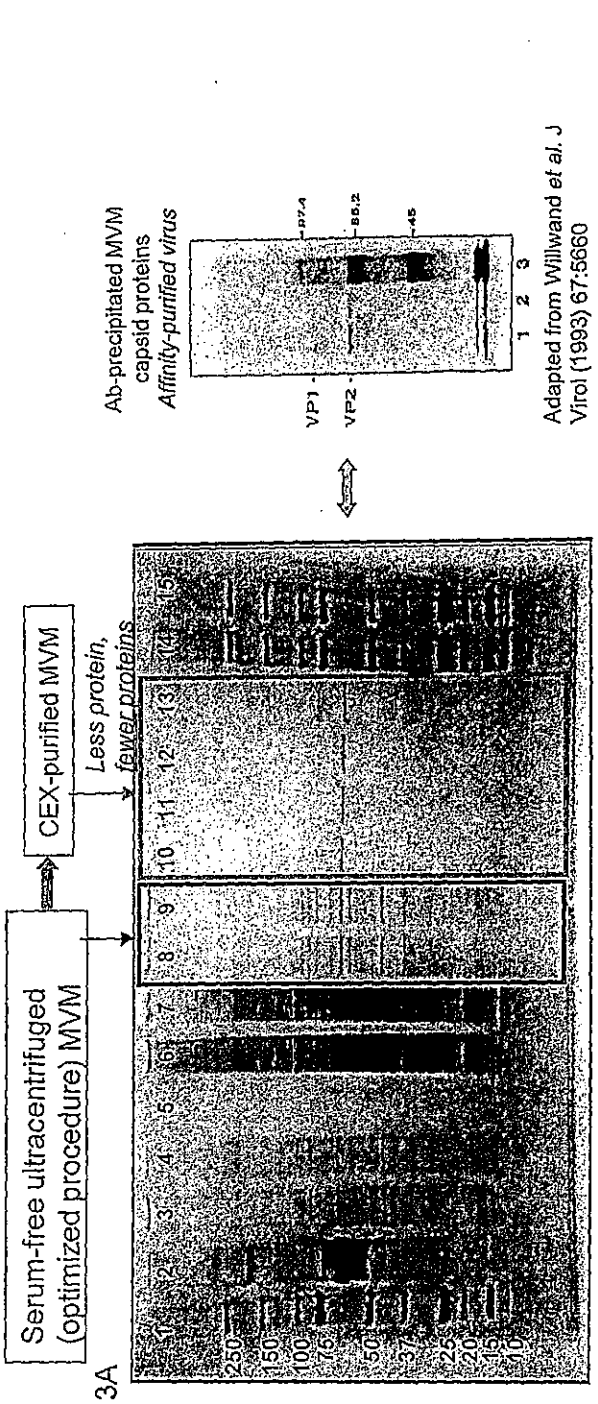

FIG. 3A

FIG. 3B

Lane		2	3	4	5	6	7	8	9	10	11	12	13
Evaluation Run		1	1	1	1	1	1	1	1	1	1	2	2
Desc	Day 3 media change	Lysate pool	Clarified Lysate	Centricon filtrate	Diluted Centricon retentate	Ultracent supernatant	Ultracent pellet	Ultracent pellet .22um filtered	CEX early fraction	CEX late fraction	CEX early fraction	CEX late fraction
Titer (log TCID50/ml)	6.3	7.5	7.8	4.7	8.8	7.6	9.0	8.9	8.6	8.6	8.8	8.9
Protein (ug/ml)					483.49	450.92	35.54	39.18	3.36	12.58	2.74	12.86
Protein (fg/TCID50)					860	10571	36	52	9	35	4	17
DNA (ug/ml)	1.8	2.7	1.7	lod	21.8	16.1	4.2	5.9	0.7	3.3	0.9	5.1
DNA (fg/TCID50)	1012	85	26		39	377	4	8	2	9	1	7

FIG. 3C

Protocol Cellhost Format Eval run #		324K T150 x 30 AVERAGE	STD DEV	PROC-004 324K T150 x 30 1	PROC-005 324K T150 x 30 2	PROC-006 324K T150 x 30 3	PROC-007 324K T150 x 30 4
Clar lysate	Titer (log TCID50/ml)	7.71	±0.06	7.70	7.63	7.75	7.75
Centricon filtrate (discarded)	Titer (log TCID50/ml)	4.52	±0.26	4.44	4.69	4.75	4.19
Centricon retentate (diluted to 30ml)	Titer (log TCID50/ml)	8.85	±0.24	8.75	8.81	9.19	8.63
	Protein (ug/ml)	631.31	±209.04	483.49		779.12	
	DNA (ug/ml)	30.18	±11.85	21.80		38.56	
U/C Sup (discarded)	Titer (log TCID50/ml)	7.27	±0.33	7.63	7.44	7.13	6.88
	Protein (ug/ml)	561.68	±156.64	450.92		672.44	
	DNA (ug/ml)	20.95	±6.85	16.10		25.79	
	Virus Loss	3%		8%	4%	1%	2%
U/C Pellet	Titer (log TCID50/ml)	8.75	±0.20	9.00	8.81	8.63	8.56
	Protein (ug/ml)	43.11	±10.70	35.54		50.67	
	DNA (ug/ml)	6.41	±3.12	4.20		8.61	
CEX early fract	Titer (log TCID50/ml)	8.66	±0.12	8.56	8.81	8.69	8.56
	Protein (ug/ml)	8.27	±6.03	3.36	2.74	13.66	13.30
	Protein/Virus (fg/TCID50)	19.50	±15.29	9.25	4.24	27.89	36.63
	DNA (ug/ml)	0.94	±0.43	0.70	0.90	1.55	0.59
	DNA (fg/TCID50)	2.03	±0.79	1.93	1.39	3.16	1.62
CEX late fract	Titer (log TCID50/ml)	8.82	±0.07	8.73	8.88	8.88	8.81
	Protein (ug/ml)	21.23	±10.59	12.58	12.86	24.90	34.58
	Protein/virus (fg/TCID50)	31.74	±15.94	23.61	16.95	32.82	53.56
	DNA (ug/ml)	6.15	±2.70	3.30	5.10	6.52	9.68
	DNA (fg/TCID50)	9.13	±4.04	6.19	6.72	8.60	14.99
Total Virus Harvest	log TCID50	10.46	±0.06	10.45	10.38	10.50	10.50
Total Virus Yield	log TCID50	10.22	±0.09	10.12	10.32	10.26	10.16
Purification Loss	log TCID50	0.24	±0.13	0.33	0.06	0.24	0.34

FIG. 4

Lane	1	2	3	4	5	6	7	8	9	10	11
Desc		Media change	Lysate pool	Clarified lysate	Clarified lysate	Centricon filtrate	Centricon retentate (diluted)	Ultracent Supernatant	Resusp pellet	Scrubbed fract 3	Scrubbed fract 27
Titer (log tCID50/ml)	6.7		9.8	9.9	9.9	6.7	10.3	9.2	10.6	10.6	10.3
Protein (ug/ml)			0	0	0	0	9856	9366	135	94	105
Protein (fg/TCID50)	0		0	0	0	0	483	6047	4	2	5

FIG. 5A

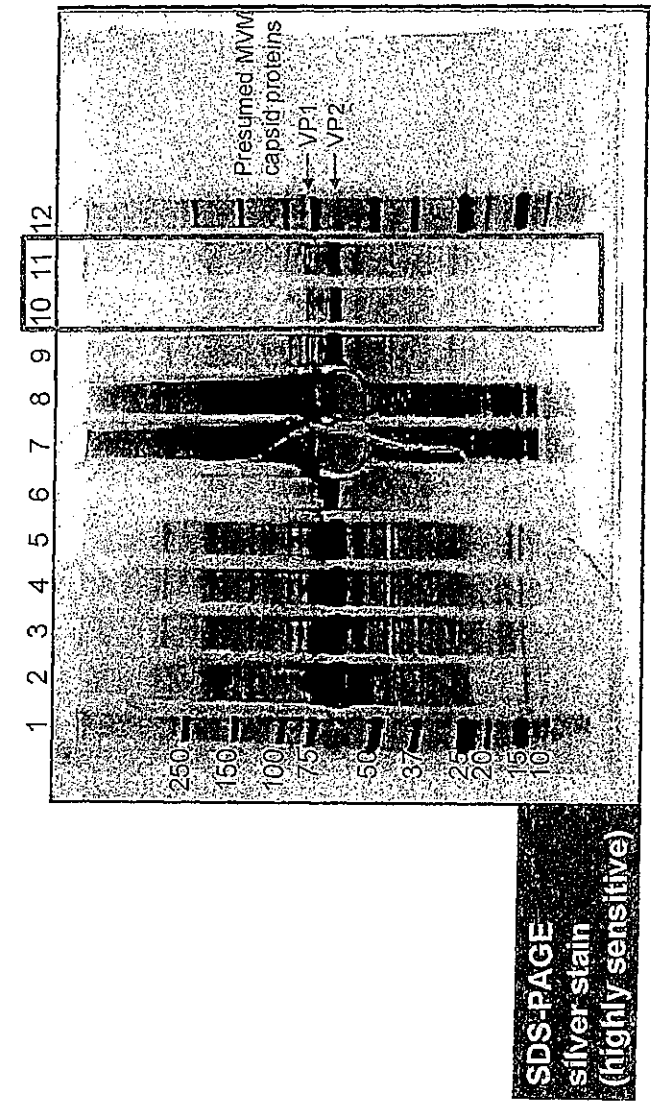

FIG. 5B

		Protocol Cellhost Format **Eval run #**	PROC-010 A9 T150 x 30 5
Clar lysate	Titer (log TCID50/ml)		9.88
Centricon filtrate (discarded)	Titer (log TCID50/ml)		6.69
Centricon retentate (diluted to 30ml)	Titer (log TCID50/ml) Protein (ug/ml)		10.31 9856.00
U/C Sup (discarded)	Titer (log TCID50/ml) Protein (ug/ml) Virus Loss		9.19 9366.00 8%
U/C Pellet	Titer (log TCID50/ml) Protein (ug/ml)		10.56 134.56
CEX early fract	Titer (log TCID50/ml) Protein (ug/ml) Protein/virus (fg/TCID50)		10.63 93.63 2.19
CEX late fract.	Titer (log TCID50/ml) Protein (ug/ml) Protein/virus (fg/TCID50)		10.31 104.90 5.14
Total Virus Harvest Total Virus Yield Purification Loss	log TCID50 log TCID50 log TCID50		12.63 11.98 0.65

FIG. 6

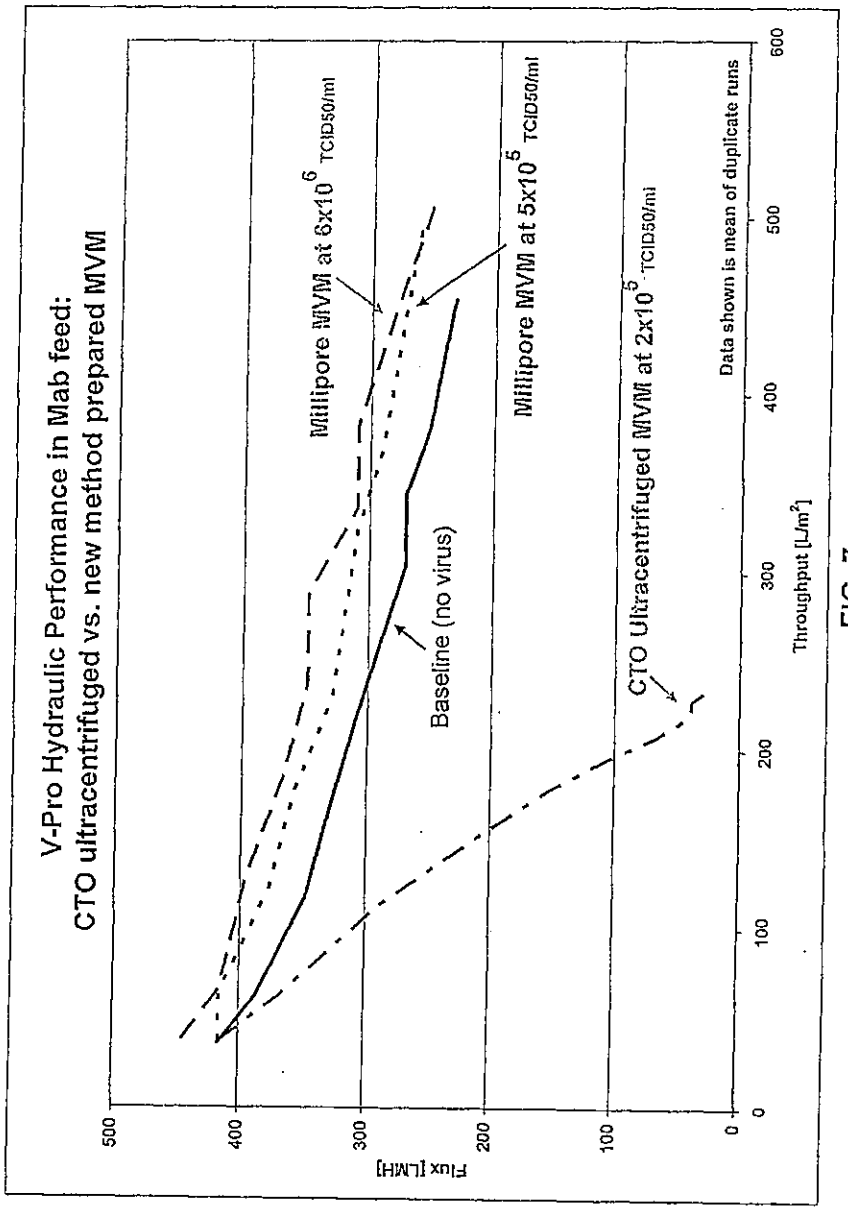
V-Pro Hydraulic Performance in Mab feed:
CTO ultracentrifuged vs. new method prepared MVM
Millipore MVM at 6x10^6 TCID50/ml
Millipore MVM at 5x10^5 TCID50/ml
Baseline (no virus)
CTO Ultracentrifuged MVM at 2x10^5 TCID50/ml
Data shown is mean of duplicate runs
Flux [LMH]
Throughput [L/m²]
0
100
200
300
400
500
600

FIG. 7

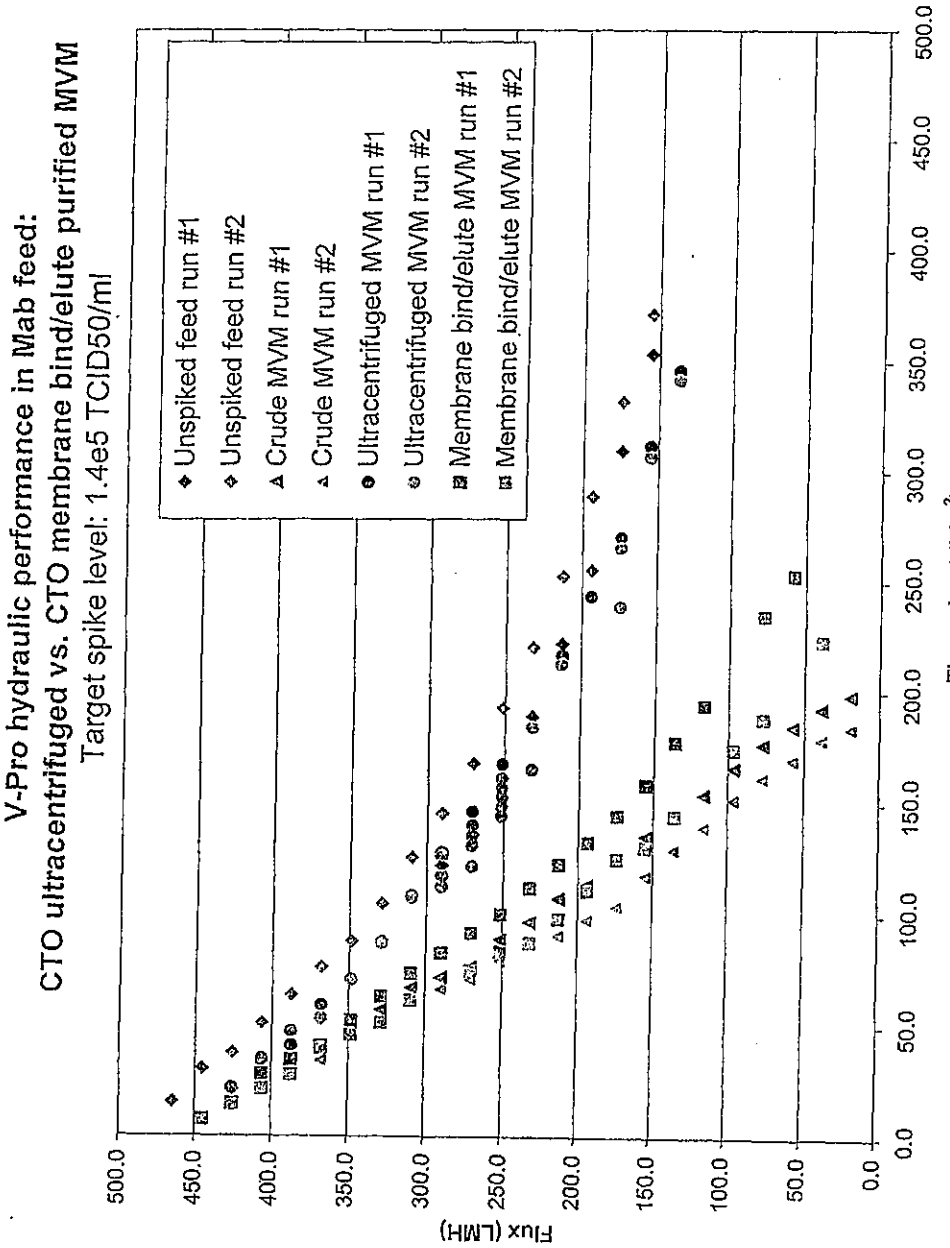
V-Pro hydraulic performance in Mab feed:
CTO ultracentrifuged vs. CTO membrane bind/elute purified MVM
Target spike level: 1.4e5 TCID50/ml
Unspiked feed run #1
Unspiked feed run #2
Crude MVM run #1
Crude MVM run #2
Ultracentrifuged MVM run #1
Ultracentrifuged MVM run #2
Membrane bind/elute MVM run #1
Membrane bind/elute MVM run #2
Flux (LMH)
Throughput (L/m²)
0.0
50.0
100.0
150.0
200.0
250.0
300.0
350.0
400.0
450.0
500.0

FIG. 8

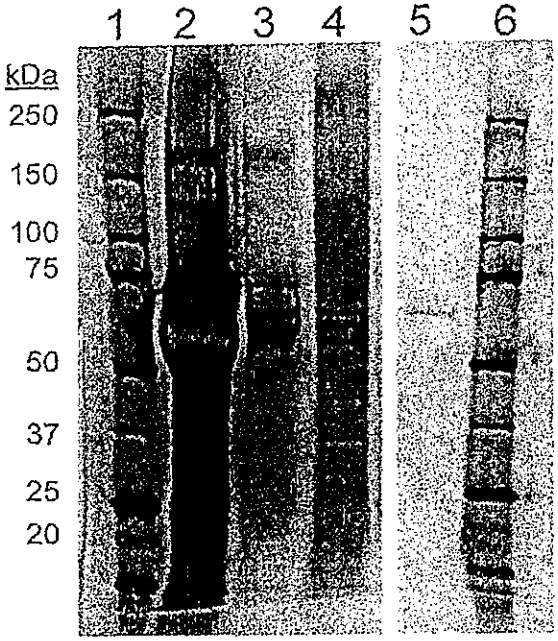

FIG. 9