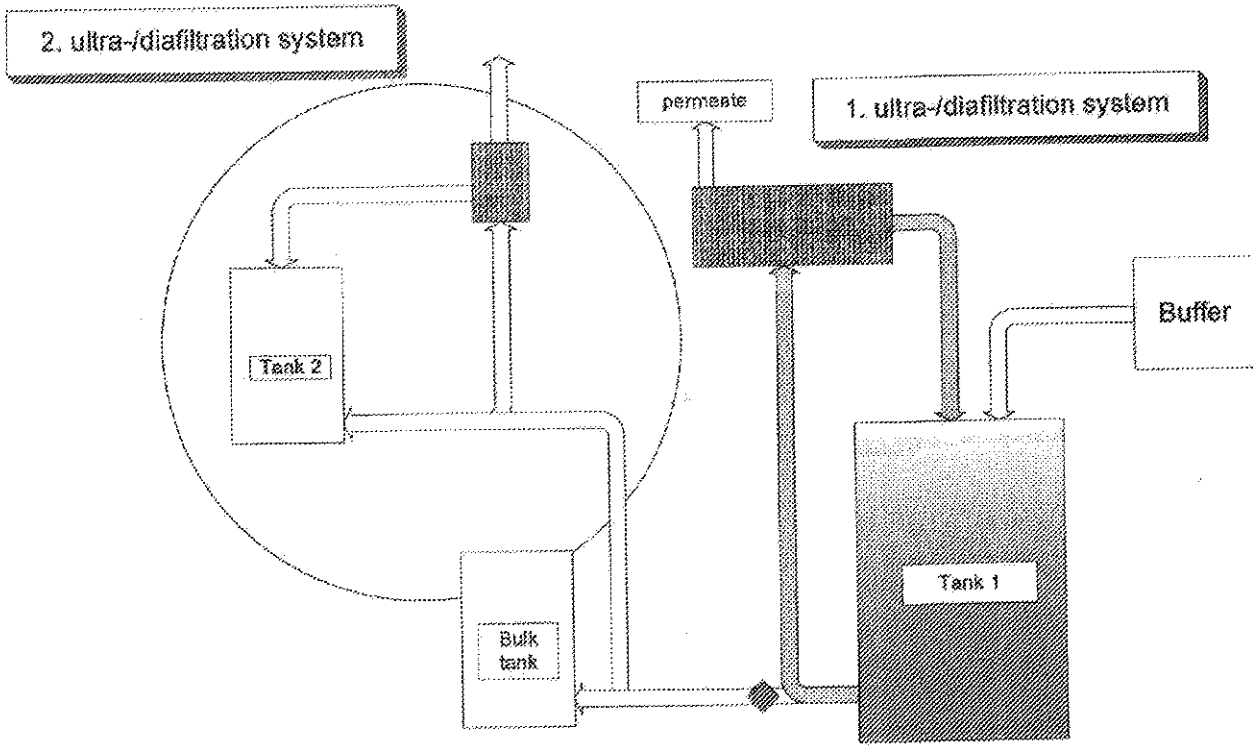
Graphic Illustration of Ultrafiltration/Diafiltration System

1. Step: Concentration of the nanofiltrate, diafiltration of the first concentrate and final concentration of the bulk solution to a protein value of >22%.

The ultra-/diafiltration system is equipped with a membrane specifically designed to concentrate protein solutions to high protein values (e.g. Millipore V-screen).



As the final volume of the concentrated solution in tank 1 of a Sub Q NG 20% batch is only half of a GAMMAGARD® LIQUID batch tank 1 has to be designed to handle low volumes ideally by a conic bottom.

Fig. 1A

2. Step: Emptying of the ultra-diafiltration system

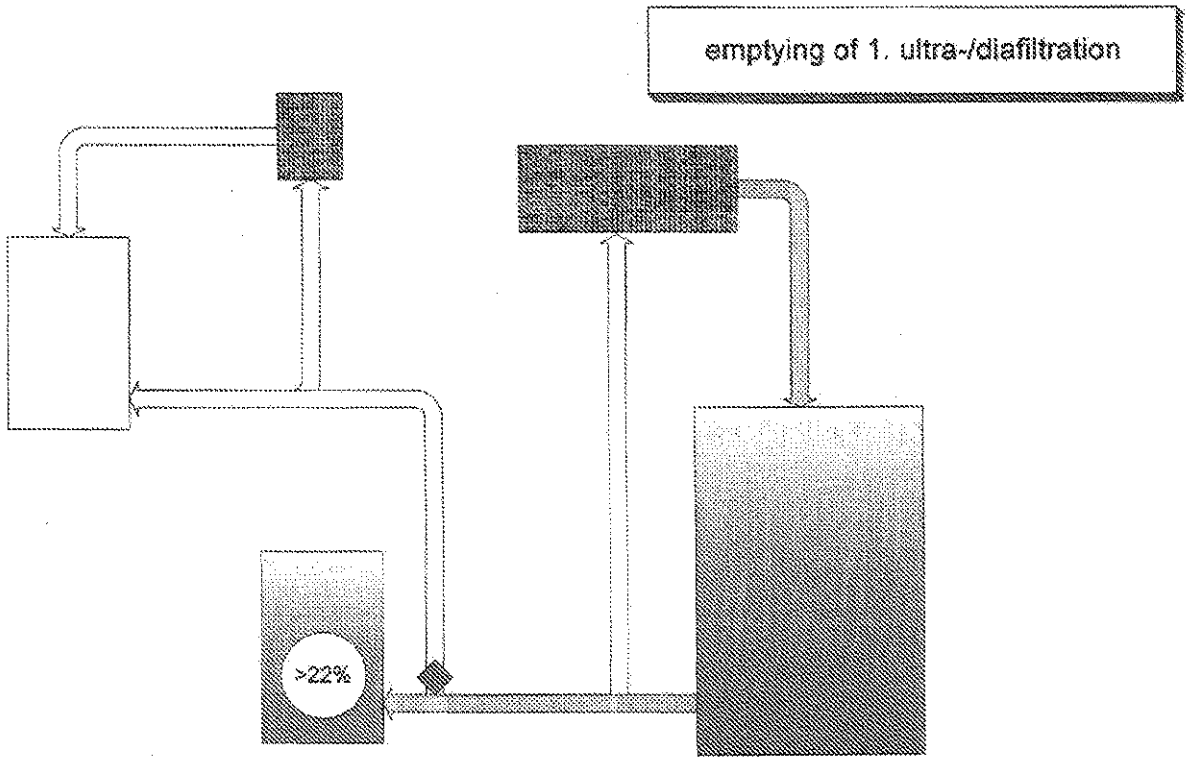


Fig. 1B

3. Step: Post-wash of the first ultra-/diafiltration device including re-circulation of post-wash buffer in system 1 and transfer into system 2

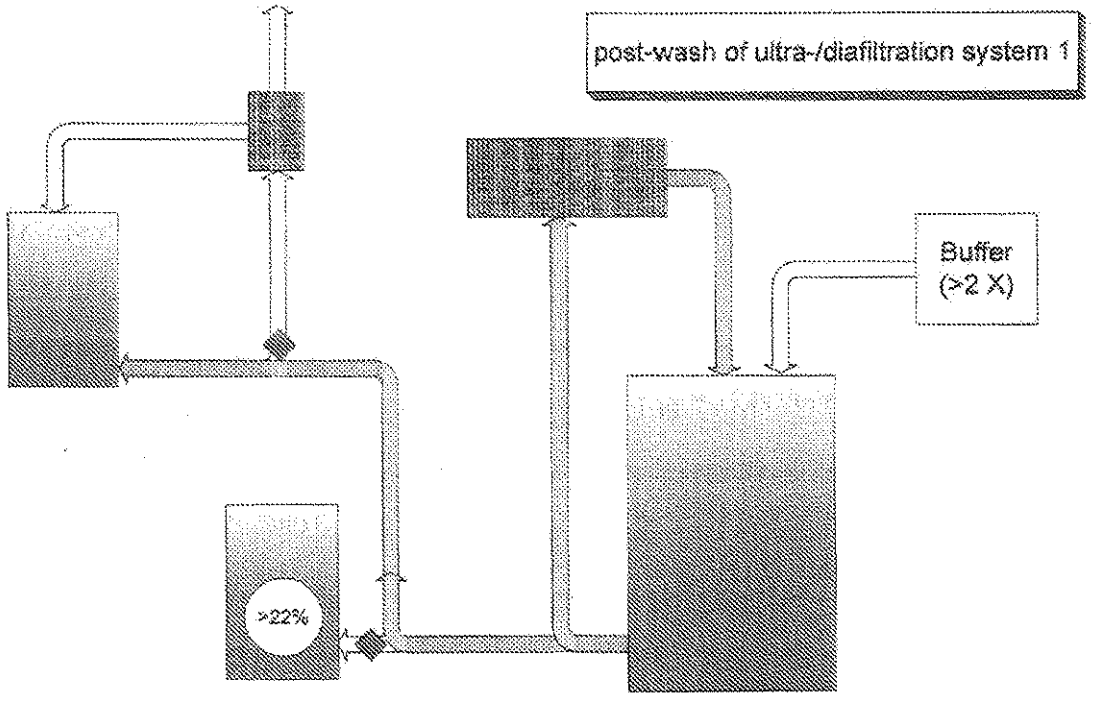


Fig. 1C

4. Step: Concentration of the post-wash by the second smaller ultrafiltration device equipped with the same type of membranes as the big ultrafiltration system

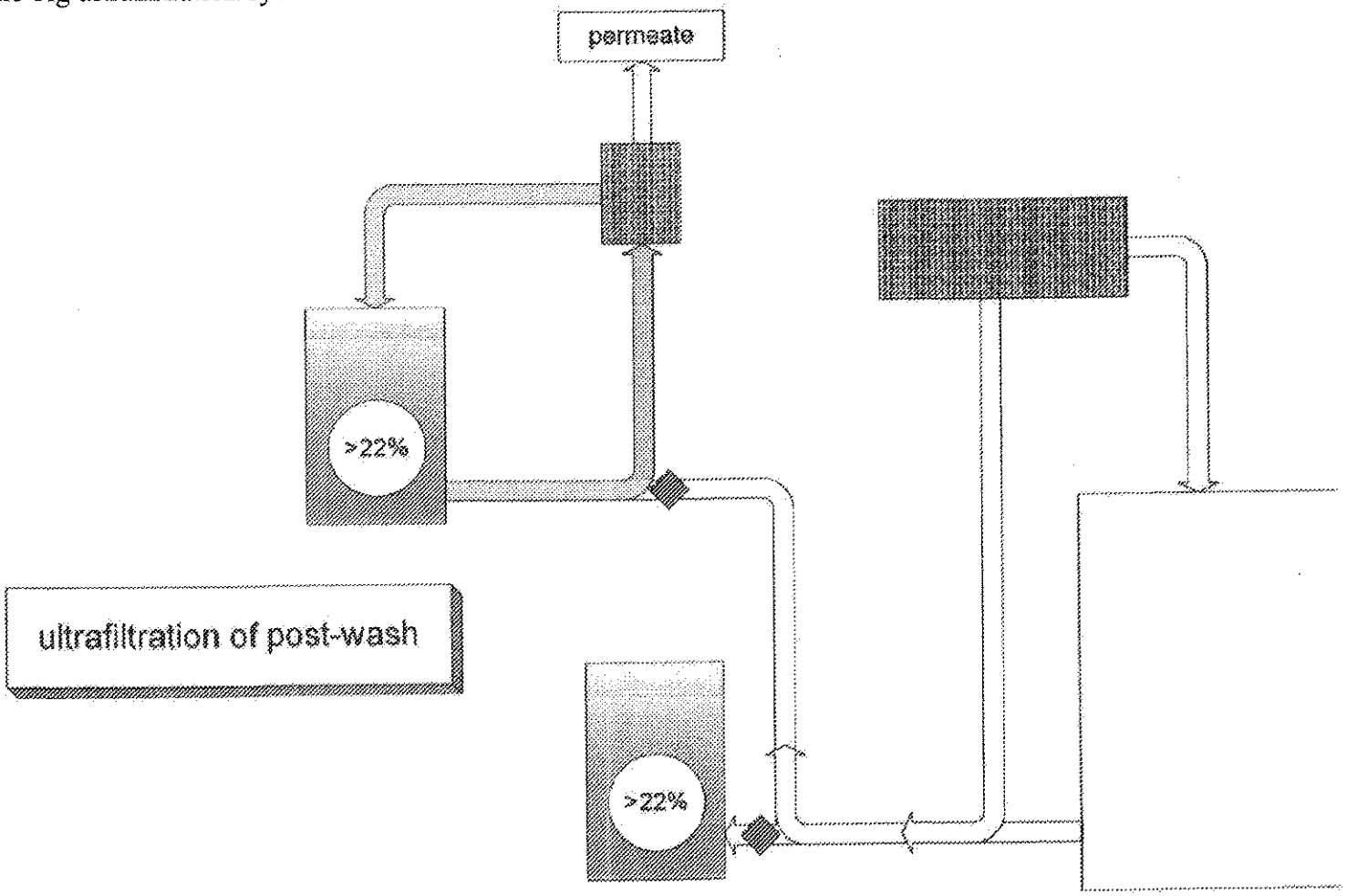
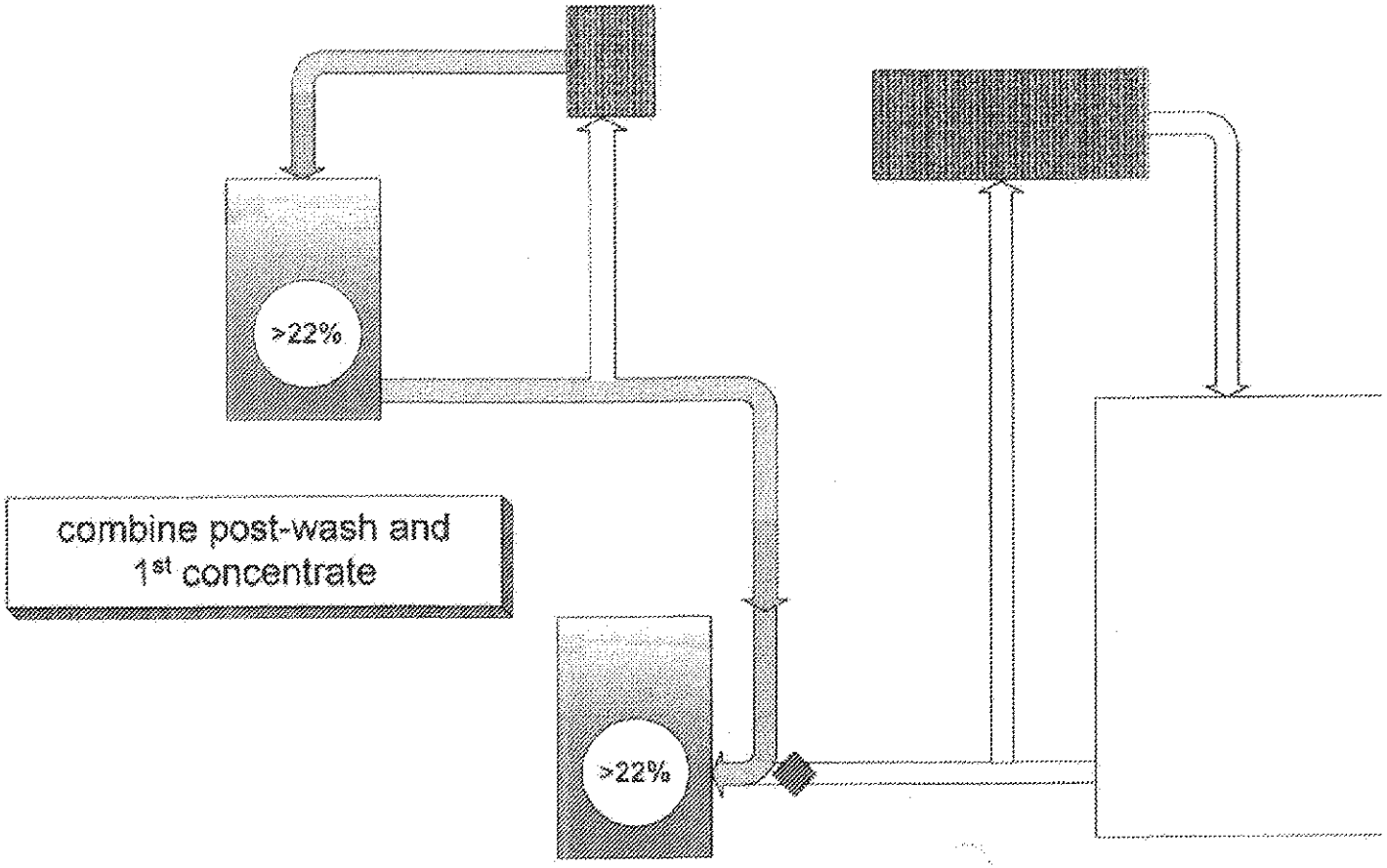


Fig. 1D

5. Step: Combine concentrated post-wash and first concentrate



Step 6: Post-wash the smaller ultrafiltration system and use this post-wash to adjust the final protein concentration of the bulk solution to 20.4 ± 0.4% w/v (not shown graphically).

Sterile filtration and filling is done similar to the GAMMAGARD® LIQUID process.

Fig. 1E

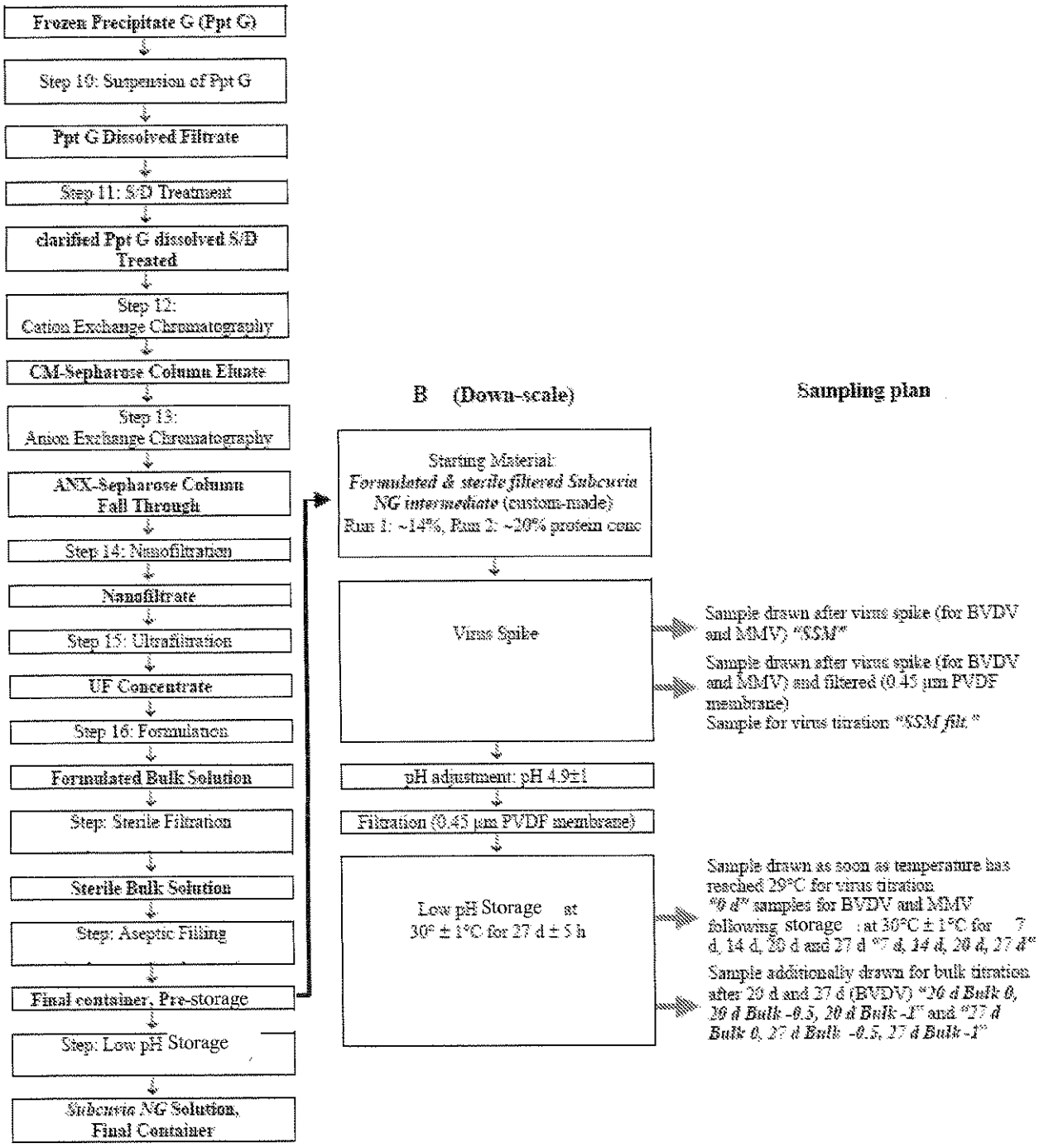

A (Large-scale manufacturing)
Frozen Precipitate G (Ppt G)
Step 10: Suspension of Ppt G
Ppt G Dissolved Filtrate
Step 11: S/D Treatment
clarified Ppt G dissolved S/D Treated
Step 12: Cation Exchange Chromatography
CM-Sepharose Column Eluate
Step 13: Anion Exchange Chromatography
ANX-Sepharose Column Fall Through
Step 14: Nanofiltration
Nanofiltrate
Step 15: Ultrafiltration
UF Concentrate
Step 16: Formulation
Formulated Bulk Solution
Step: Sterile Filtration
Sterile Bulk Solution
Step: Aseptic Filling
Final container, Pre-storage
Step: Low pH Storage
Subcuvia NG Solution, Final Container
B (Down-scale)
Starting Material: Formulated & sterile filtered Subcuvia NG intermediate (custom-made) Pam 1: ~14%, Pam 2: ~20% protein conc
Virus Spike
pH adjustment: pH 4.9±1
Filtration (0.45 µm PVDF membrane)
Low pH Storage at 30° ± 1°C for 27 d ± 5 h
Sampling plan
Sample drawn after virus spike (for BVDV and MMV) "SSM"
Sample drawn after virus spike (for BVDV and MMV) and filtered (0.45 µm PVDF membrane) Sample for virus titration "SSM filt."
Sample drawn as soon as temperature has reached 29°C for virus titration "0 d" samples for BVDV and MMV following storage at 30°C ± 1°C for 7 d, 14 d, 20 d and 27 d "7 d, 14 d, 20 d, 27 d"
Sample additionally drawn for bulk titration after 20 d and 27 d (BVDV) "20 d Bulk 0, 20 d Bulk -0.5, 20 d Bulk -1" and "27 d Bulk 0, 27 d Bulk -0.5, 27 d Bulk -1"

Fig. 2

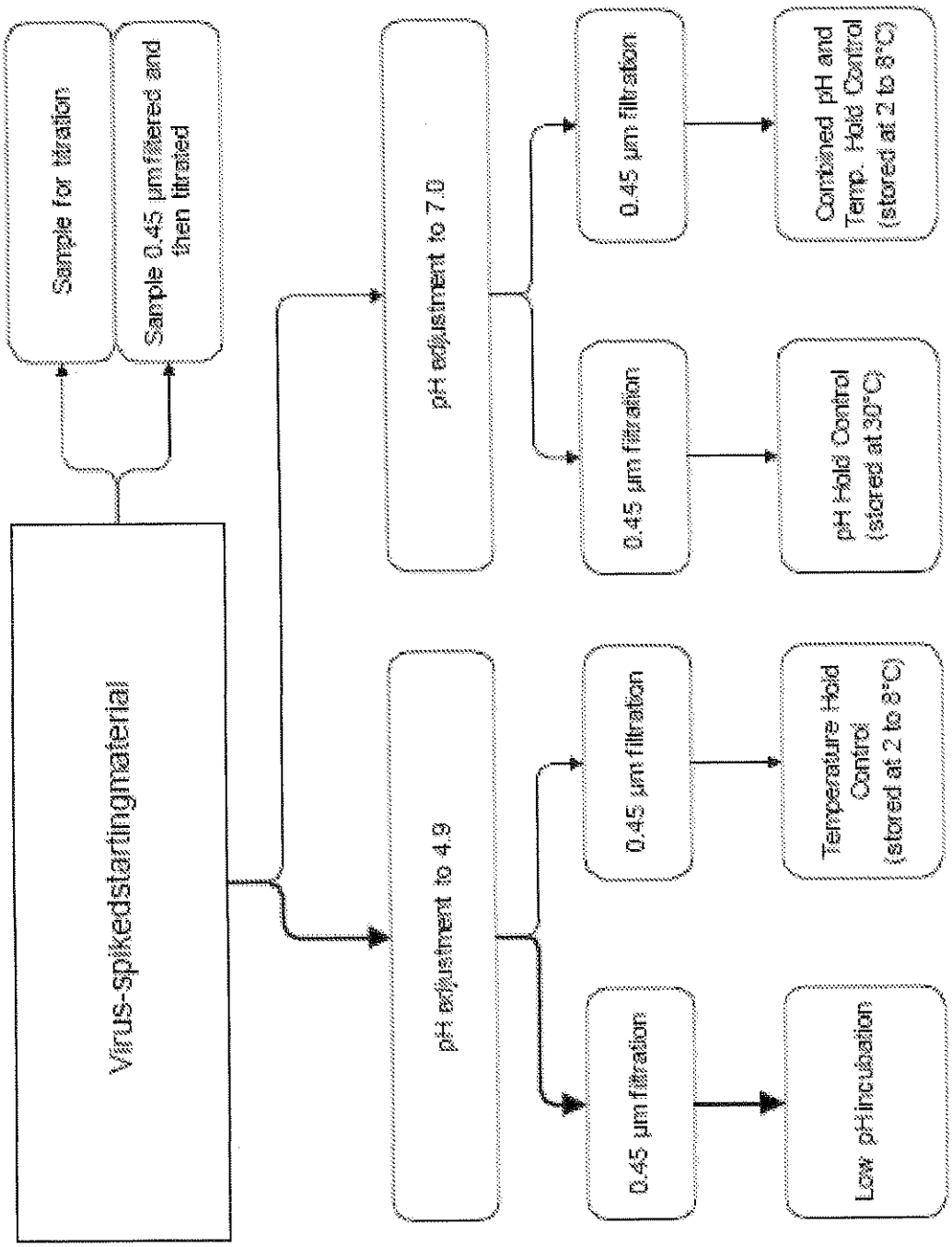

Virus-spikedstartingmaterial
Sample for titration
Sample 0.45 µm filtered and then titrated
pH adjustment to 4.9
pH adjustment to 7.0
0.45 µm filtration
0.45 µm filtration
0.45 µm filtration
0.45 µm filtration
Low pH incubation
Temperature Hold Control (stored at 2 to 8°C)
pH Hold Control (stored at 30°C)
Combined pH and Temp. Hold Control (stored at 2 to 8°C)

Fig. 3