Processing of Viral Vaccines: Scale up of centrifugation processes vaccines
Alfa Wassermann Bio-purification systems Continuous flow Ultracentrifugation

Separation Technologies

KII & PKII

Continuous Flow Ultracentrifuges

RAND alliance, ltd.

Alfa Wassermann Pharma products, Separations & Diagnostics

- **Privately owned business**
- **Founded in 1948**
- **HQ in Bologna Italy**
- **Pharmaceuticals primary business**
- **Represented in over 60 countries**
- **TOTAL # EMPLOYEES > 1500**

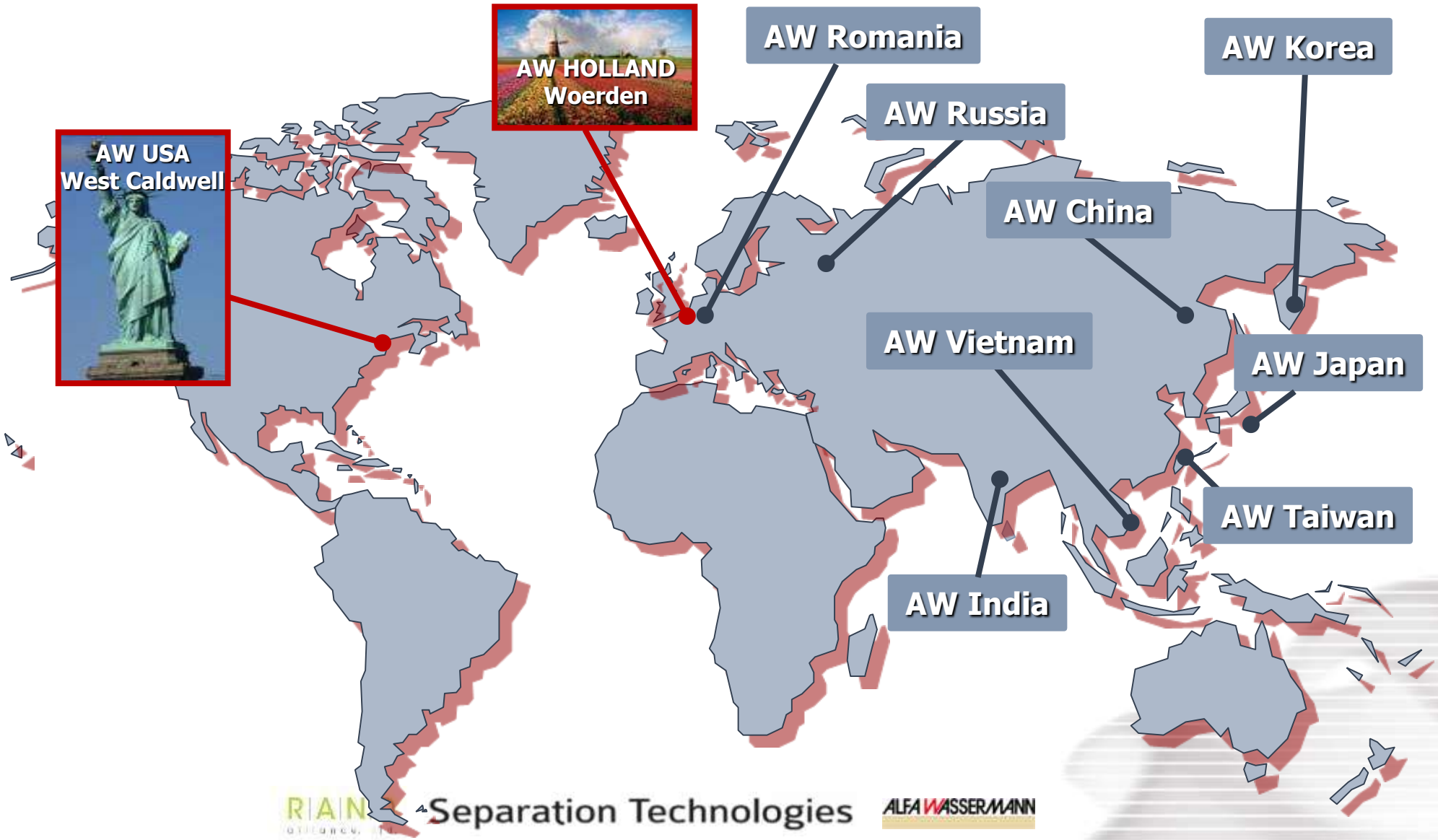
SEPARATION TECHNOLOGIES

Continuous Flow Ultracentrifuges for virus purification in vaccine related virus research, new vaccine development and GMP/FDA processing

DIAGNOSTIC TECHNOLOGIES

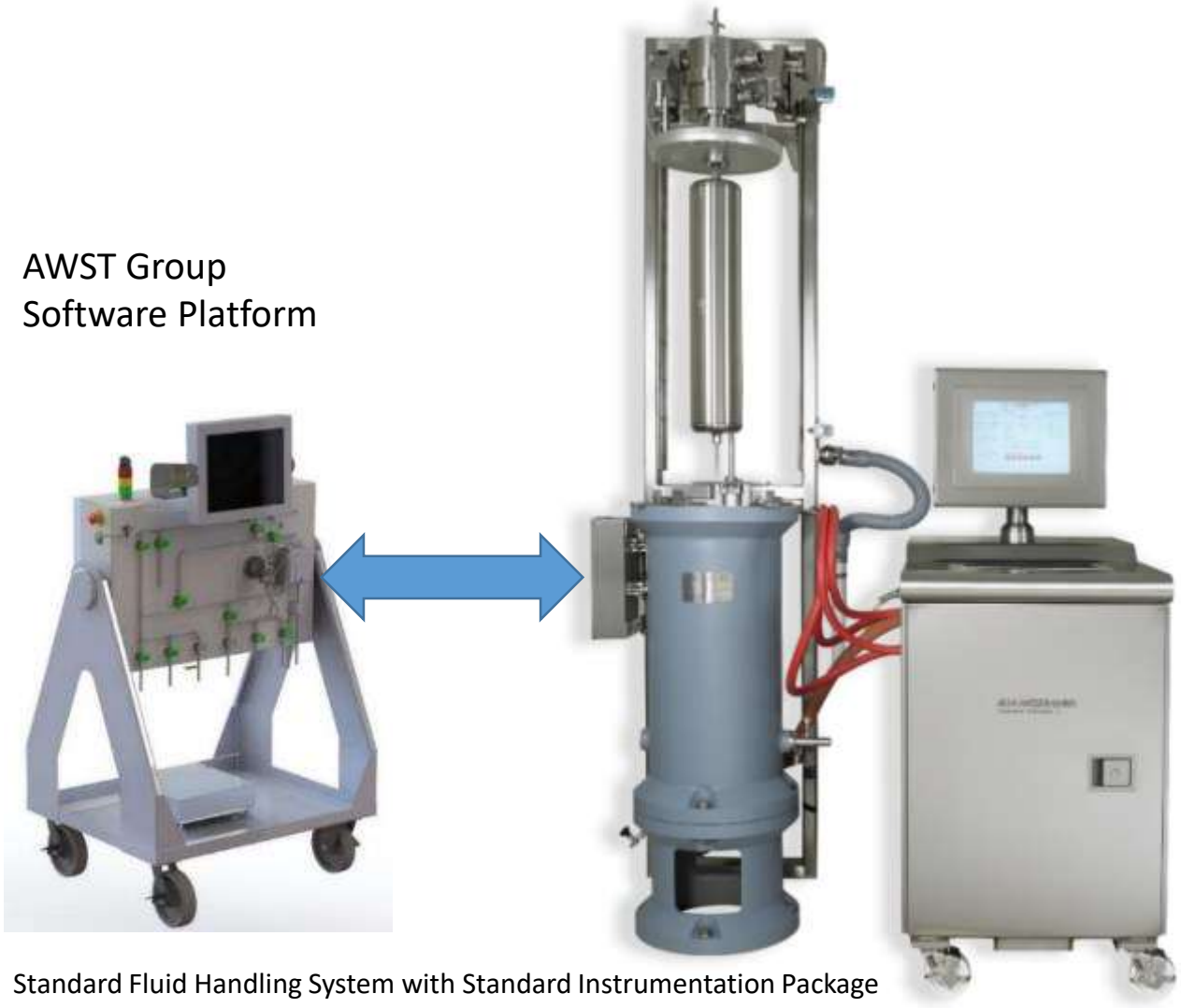
Bio Chemistry Analyzers for patient blood testing

Alfa Wassermann Separation Technologies Groups



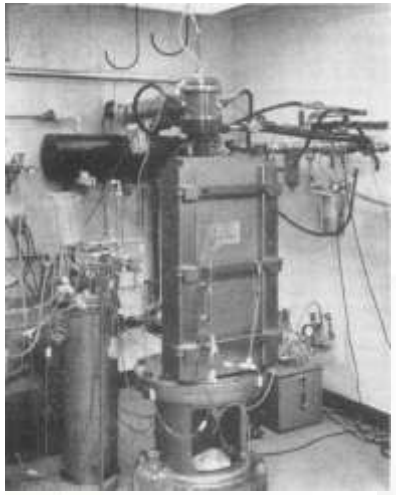
Continuous Flow Preparative Ultracentrifuges

AWST Group
Software Platform



Standard Fluid Handling System with Standard Instrumentation Package

KII Ultracentrifuge History



1969 : K Ultracentrifuge commercially produced (by ENI, New Jersey)

1970 : 1st Influenza vaccine marketed that was purified using K Ultracentrifuge

1975 : Introduction RK and KII Ultracentrifuge, operated by analogue console

1995 : RK and KII compliant with CE and CSA

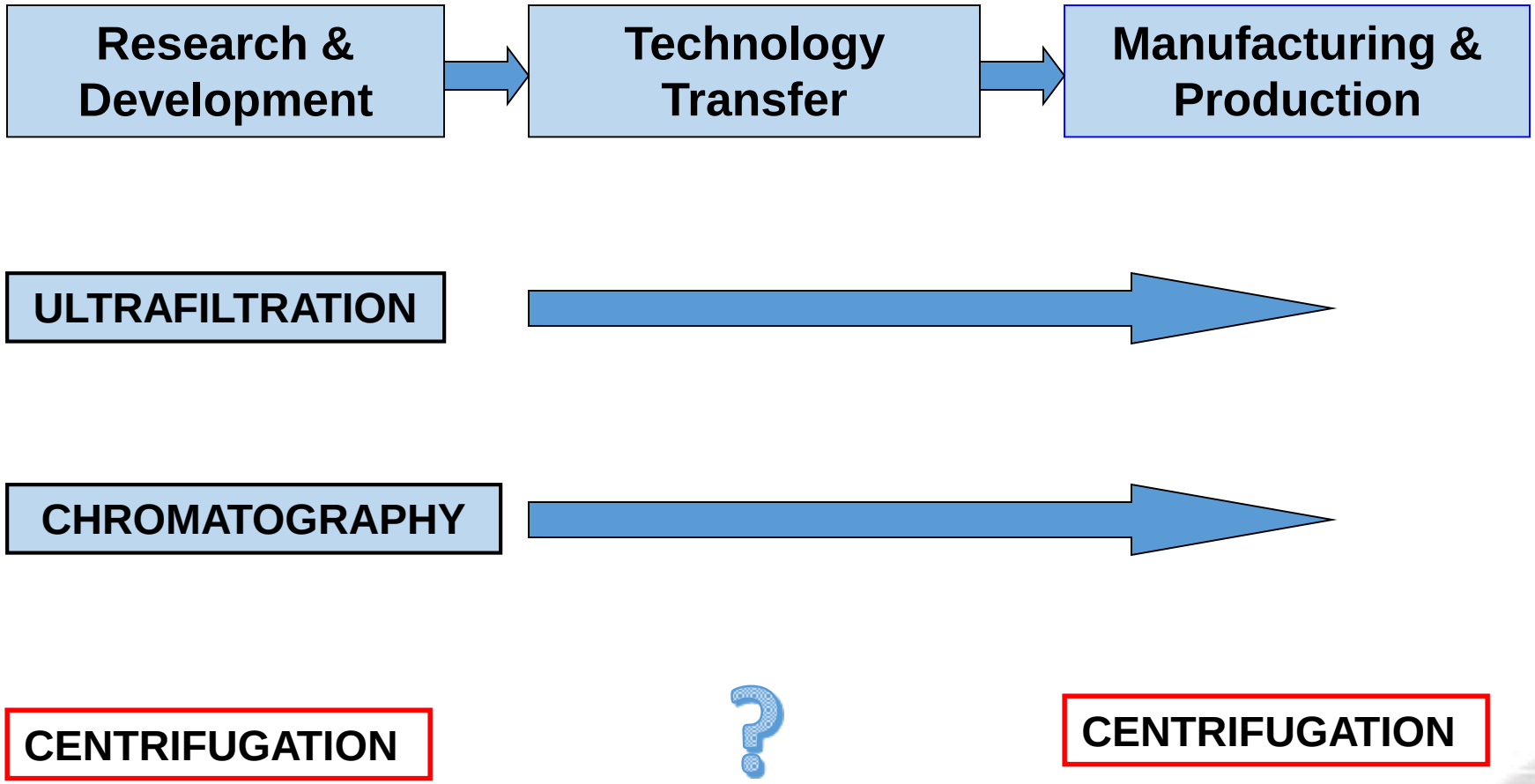
1998 : Introduction of Computer Control and GAMP compliant software

2002 : PK and KII Ultracentrifuges enhanced with clean room and BL2+ features for cGMP vaccine manufacturing

2006 : Electric Drive Promatix, ePK and eKII.

2017 : Automated Fluid Handling System

Bioprocess method pipelines



Bioprocess Downstream Processing

Upstream Process

Vector Construction
Strain Selection
Media optimisation
Fermentation

Harvest & Clarification

Harvest
Cell Removal
Clarification
Primary Extraction

Cells / Supernatant
Cell lysis
Cell debris removal
DNA clearance
Ammonium Sulphate extraction
Solvent extraction

Downstream Process

Remove insolubles
Isolate product
Purify product
Polish product
Sterile Filtration

Centrifugation
Ultrafiltration/Diafiltration
Chromatography

Fill and Finish

Formulation
Filling
Final Release

Buffer Exchange
Blending
Adjuvant Addition
Filling to Containers

Key process scale considerations

Production Volume	Process Technology		
	Filtration	Chromatography	Ultracentrifugation
			Scaleable
1 L	50 cm^2 TFF	50 mL	PX-230 mL
5 L	0.1 m^2 TFF	250 mL	PKII-400 mL
10 L	0.5 m^2 TFF	500 mL	PKII-800 mL
100 L	3.0 m^2 TFF	1000 mL	KII-3200 mL

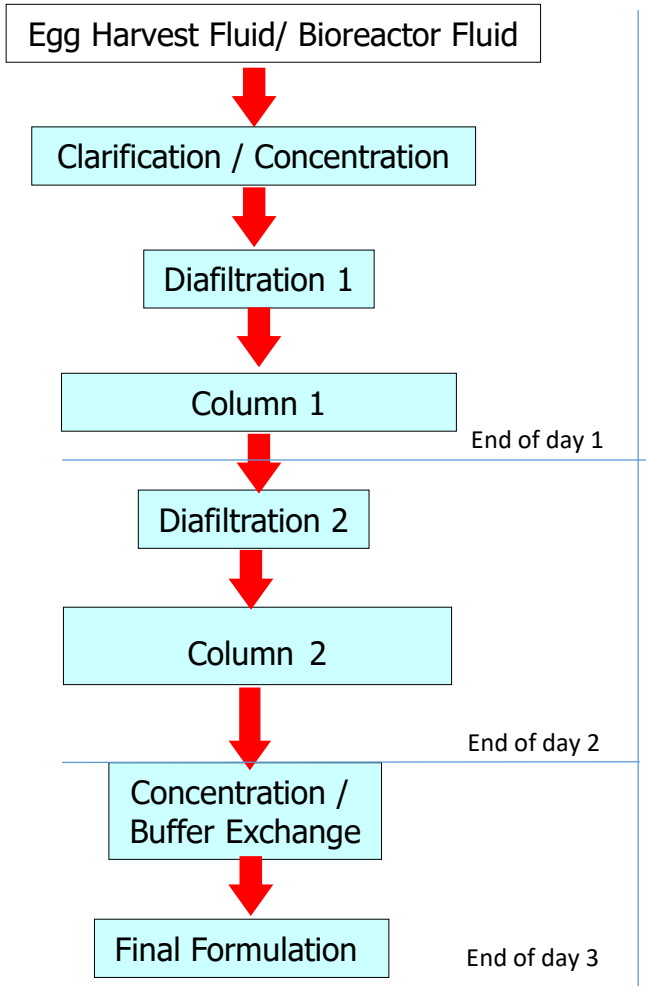
The implication of multiple step processing

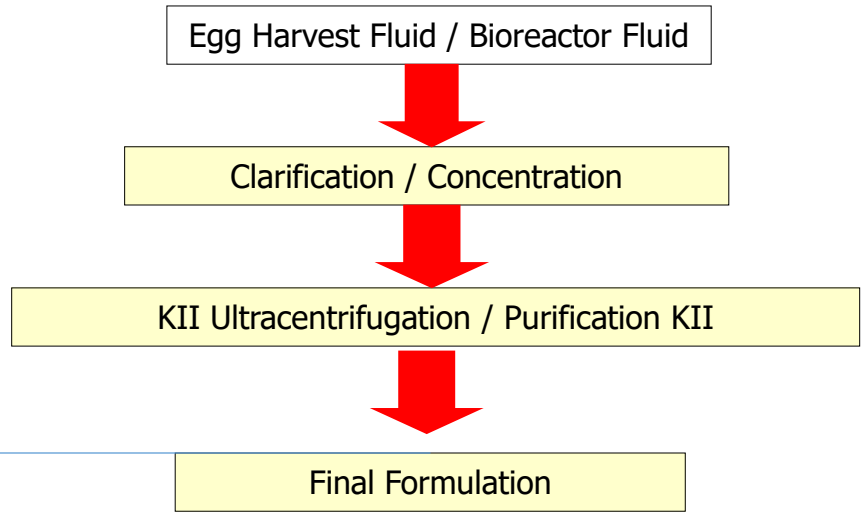
Number of Steps

	1	2	3	4	5
Step Yield					
90	**90**	**81**	**73**	**66**	**60**
80	**80**	**64**	**51**	**41**	**33**
70	**70**	**49**	**34**	**24**	**17**
60	**60**	**36**	**22**	**13**	**8**

The best scenario only one step with 100% recovery.
With each step and with normal efficiencies a 10% loss will incur as a minimum.
It can be seen that a minimum of purification steps is preferable for purification.

Chromatography compared to Ultracentrifugation – KII Process Efficiency





Fewer Process Steps => Less Product Loss => Higher Yield

A Quicker KII process has a higher production capacity per day
KII Reduced Process time leads to better product quality by reducing Bio-burden build up

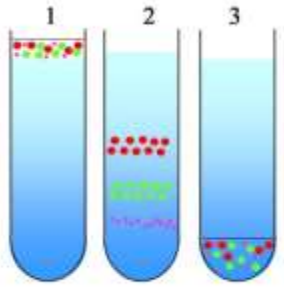
Centrifugation Principle

The primary information of use in centrifugation is sedimentation coefficient (S20,w) of the protein.

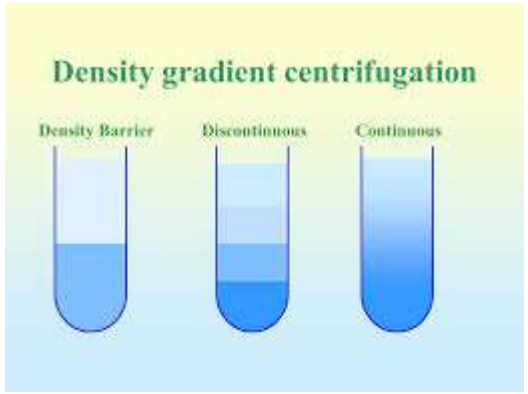
When subjected to the force of gravity the particles will move at a rate which can be calculated from the sedimentation coefficient.

The larger the size and the larger the density of the particles, the faster they separate from the mixture, decreasing the time required for separation.

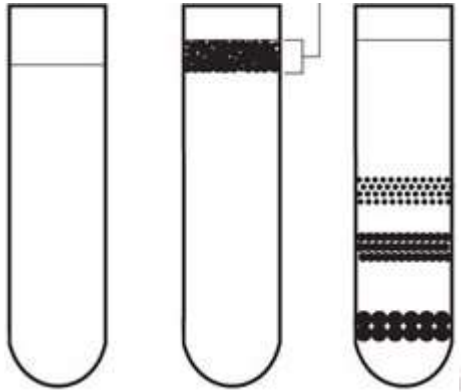
Rate Separation



Types of Density Gradient



Isopycnic Banding



Centrifuges and Ultracentrifuges

Many models of centrifuge exist from a range of equipment manufacturers.

Ultracentrifuge is generally:

- Separation of small particles or large molecules
- High speed / centrifugal force
- Usually runs in a vacuum

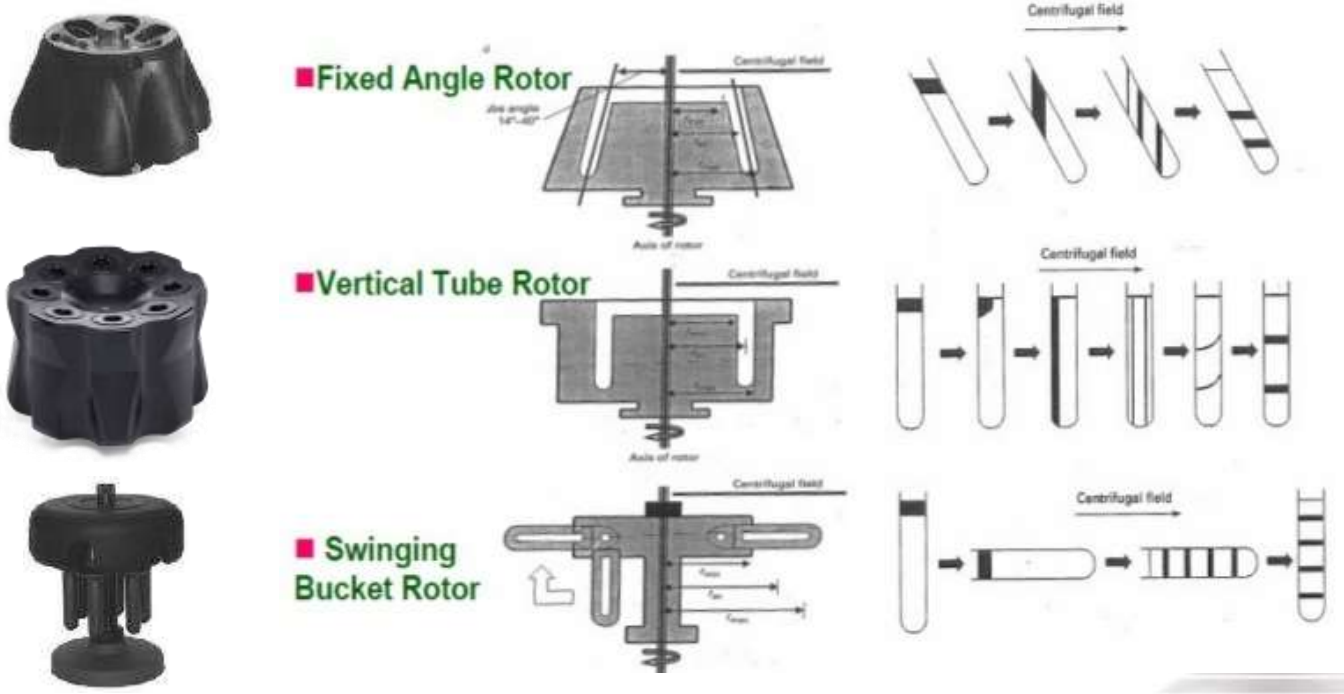
Centrifuge is generally

- For separating sediments, removing moisture
- Lower speed / centrifugal force
- Not typically running in a vacuum chamber

Batch Rotors

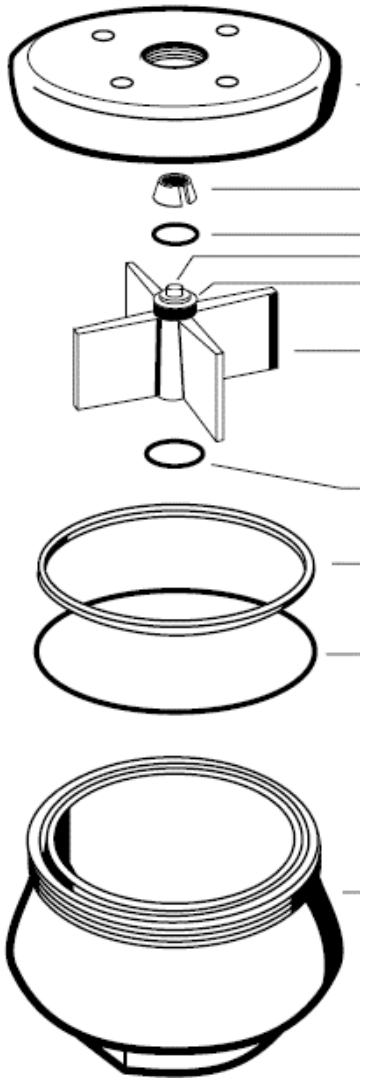
These rotors are limited by their capacity to be useful in vaccine manufacture. Too many runs would be needed to make a batch of vaccine.

Principle: Fill tube – spin tube – collect from tube



RAND Separation Technologies ALFA WASSERMANN

Zonal Rotors



These rotors do not have tubes but sectors/zones;

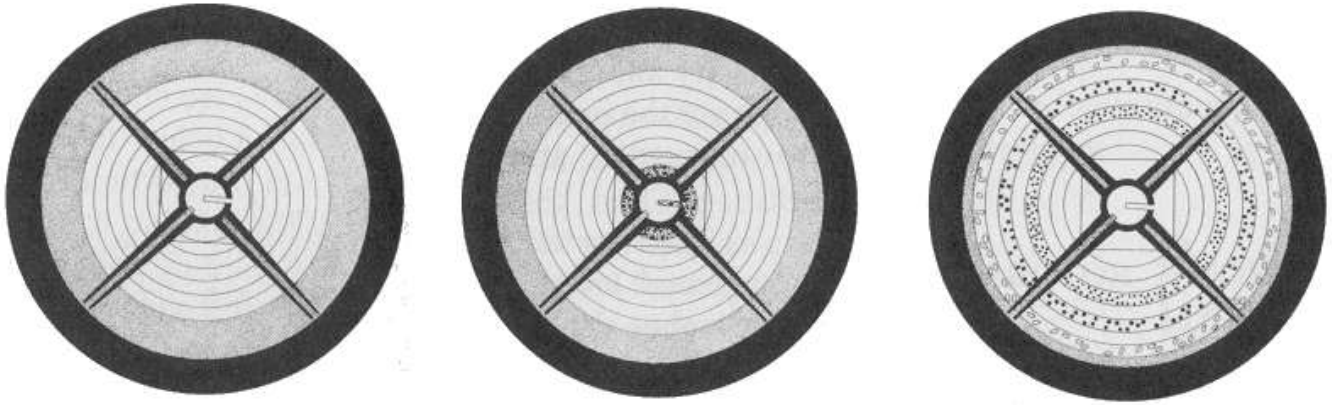
The fluid fills the entire rotor.

A plastic core is used as an insert inside the rotor to create chambers.

The vanes/fins of the core keep the fluid from mixing during rotation.

The volume is much greater than a tube rotor (approx. 200ml vs 1600ml)

Principle: Fill rotor – spin rotor – empty rotor

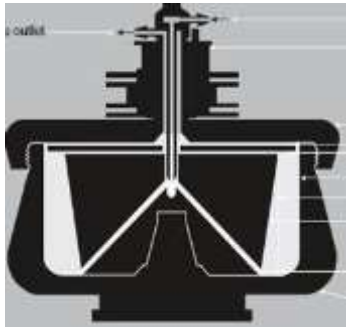


Continuous flow rotors

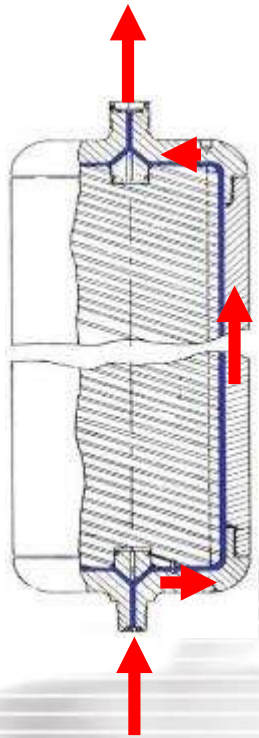
These rotors are not limited by their size as fluid continuously enters and leaves the rotor during high speed operation.

Two types: Disc and Tubular

Principle: Load Gradient – Spin Rotor – Flow Product – Brake Rotor – Collect Gradient



Parameter	CF32	K3
Shape	Disc type	Tubular
Speed max.	32000 rpm	40500rpm
Centrifugal force	102 000xg	121200xg
Capacity	430ml	3200ml
K factor	42	29.7
Flow Path	Loop	Dual inlet
Scaleable	No	Yes
Automated	Manual process	Automated



System Capacities

	Lab Scale Promatix 1000™	Development Scale PKII	Production Scale KII
Typical Feed Flow (vaccine)	0.25 – 2 L/h	up to 15 L/h	up to 30 L/h
Rotor Capacity (separation volume)	PX3 – 120 mL PX3 – 230 mL	PK3 – 400 mL PK3 – 800 mL PK3/PK6 – 1600 mL	K3/K6 – 3200 mL *K10 – up to 8 Liters* *K5 – 8.4 Liters*
Batch Volume (5h feed)	Up to 5 L	Up to 75 L	Up to 150 L
Max. Rotation Speed	35,000 rpm	40,500 rpm	40,500 rpm
Gravitational Forces	Up to 90,500xg	Up to 121,200xg	Up to 121,200xg
Scale Factor	27x scale down 14x scale down	8x scale down 4x scale down 2x scale down	1x scale

How to scale up?

Laboratory

Promatix 1000
R&D

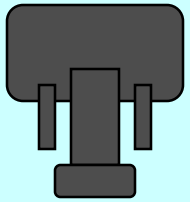
PKII
Pilot Scale

KII
Manufacturing Scale

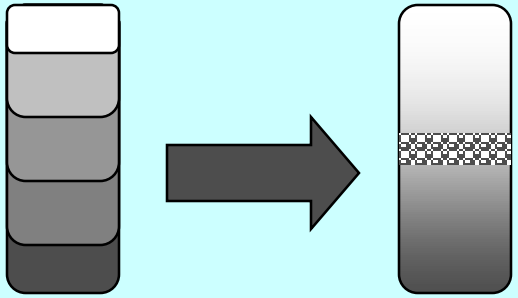
RAND Separation Technologies ALFA WASSERMANN

Small to Large Scale Gradients
从小量到大规模梯度

Tube Rotor

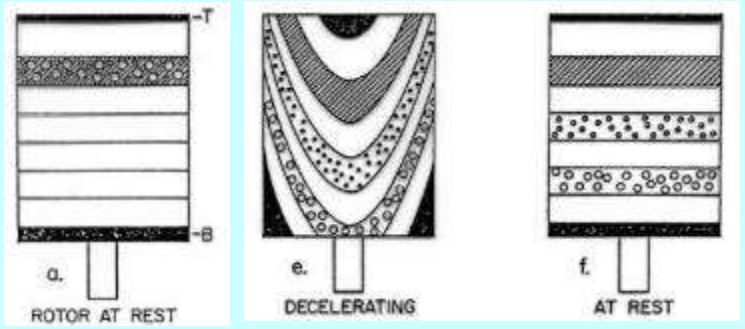


K3 Rotor



228 ml

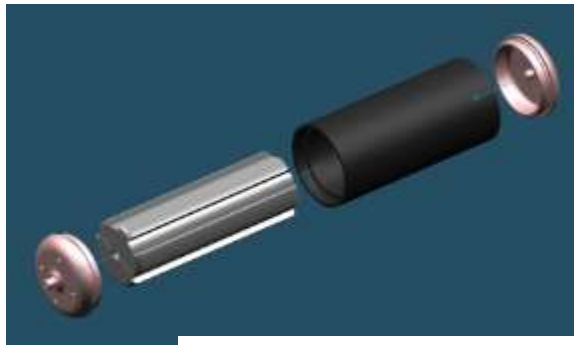
12 ml (x20)



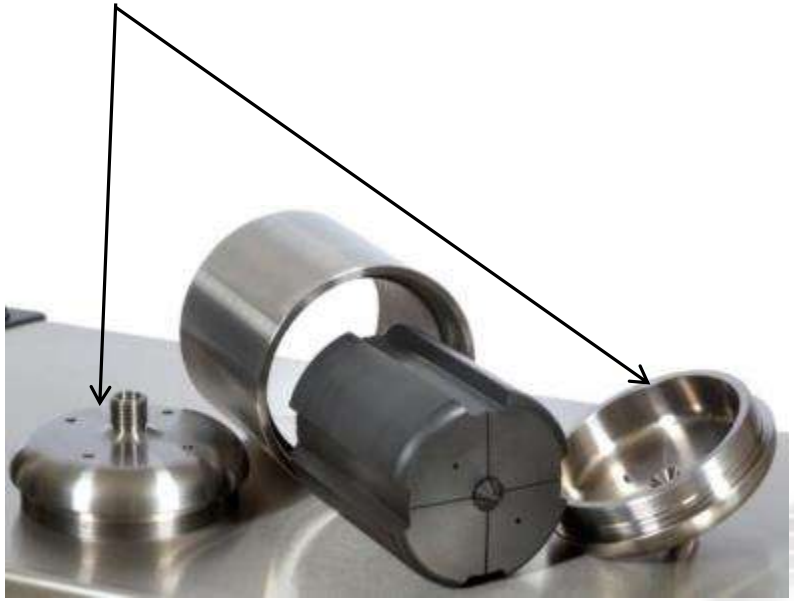
3200 ml

160 ml (x20)

Scaleable centrifugation through rotor technology



Rotor Assembly
Core – Noryl
Rotor Bowl – Titanium
Rotor End caps - Titanium



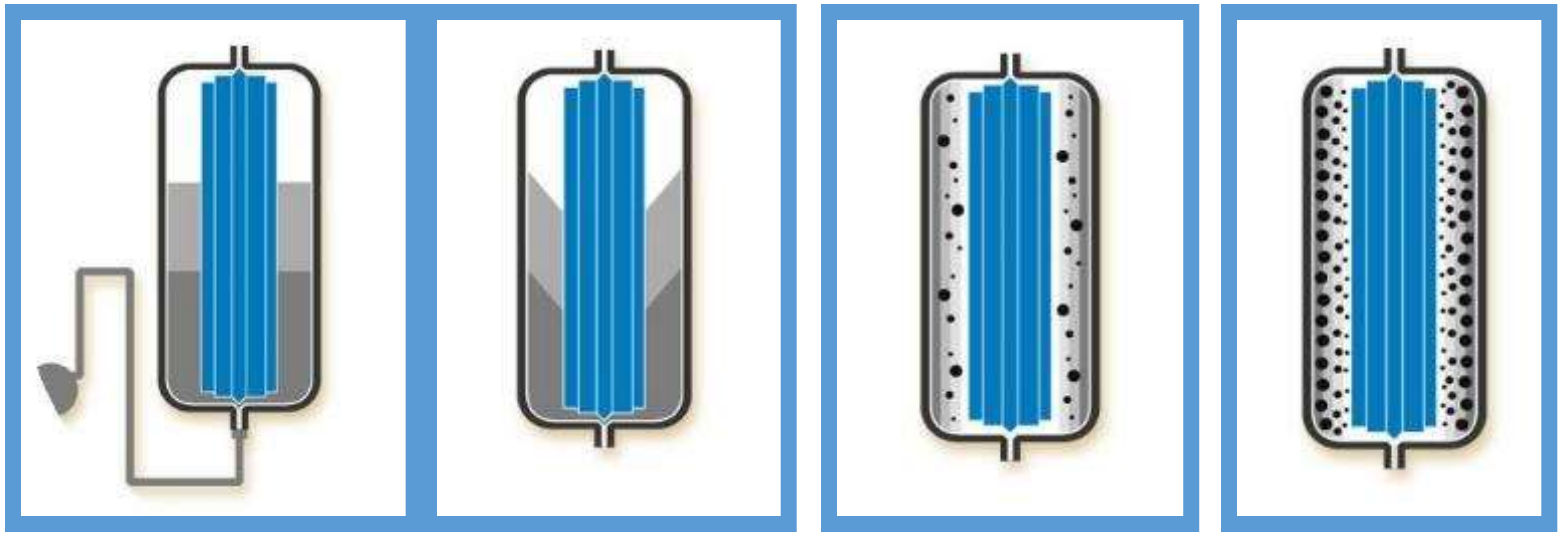
KII Rotor Assembly

Promatix Rotor Assembly

AW KII Separation Technique – Reorienting Gradient
阿尔法韦士曼分离技术---重换方向的梯度

1. **Load the Gradient in the stationary rotor.在静止的转子中加入梯度。**

2. **KII accelerates slowly and the gradient becomes vertical.**
   KII缓慢加速，梯度变为垂直方向。



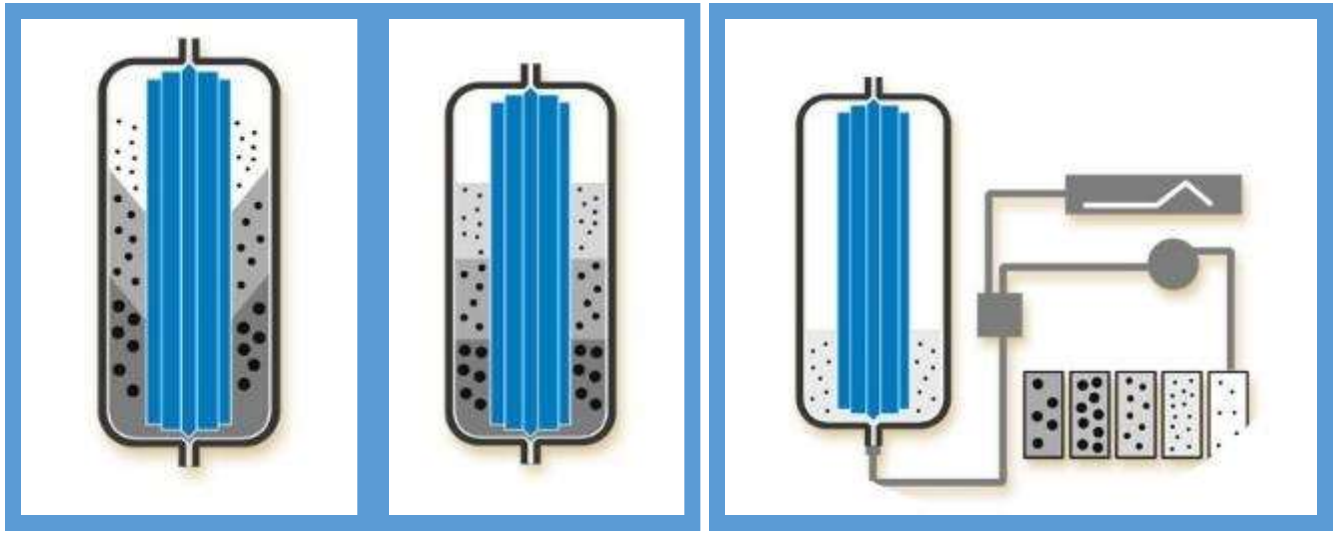
3. **At 35 000 rpm flow allantoic fluid into the rotor to capture the virus in the Gradient. All allantoic fluid waste flows out of the rotor.速度达35000转/分时尿囊液加入转子中，在梯度中捕获病毒。所有的尿囊液废液流出转子。**

4. **At 35 000 rpm flow PBS into the rotor to allow time for the virus to concentrate in a narrow zone.在35000转时向转子中流入缓冲液，为病毒在窄区带浓缩流出时间。**

AW KII Separation Technique – Reorienting Gradient
阿尔法韦士曼分离技术---重换方向的梯度

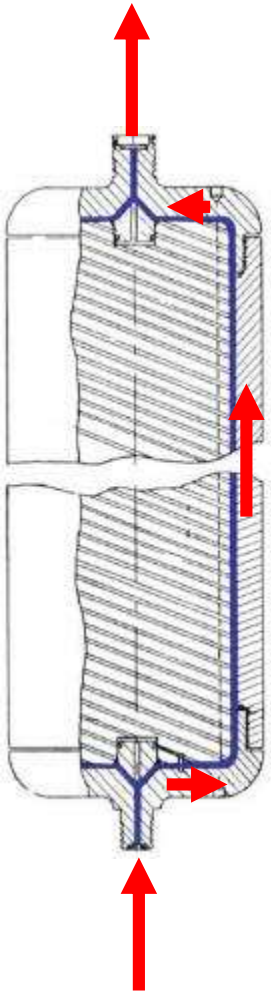
5. Set on the brake and the vertical gradient becomes horizontal again.制动设备，随后垂直方向的梯度又回到水平方向。

6. The layers of virus remain separate in the density gradient.病毒仍然分别呆在不同的密度梯度层中。



7. Collect the gradient using a pump from the bottom of the rotor. Select virus fractions using UV monitor. 用泵从转子底部抽出不同的梯度。用紫外检测仪选择病毒部分。

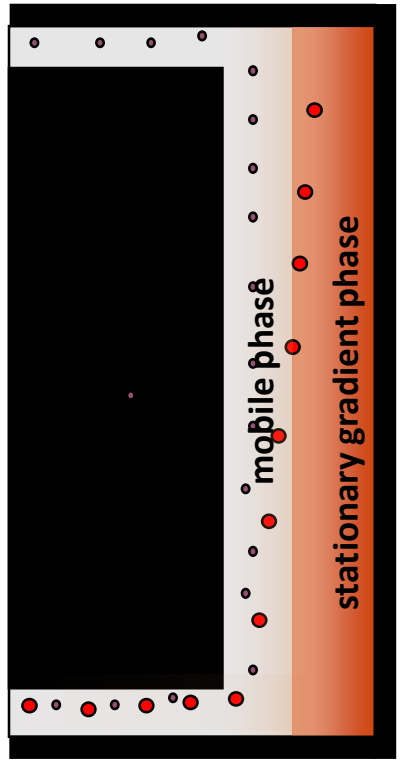
Particle separation during continuous flow operation



Waste:
Allantoic Fluid without Virus Particles

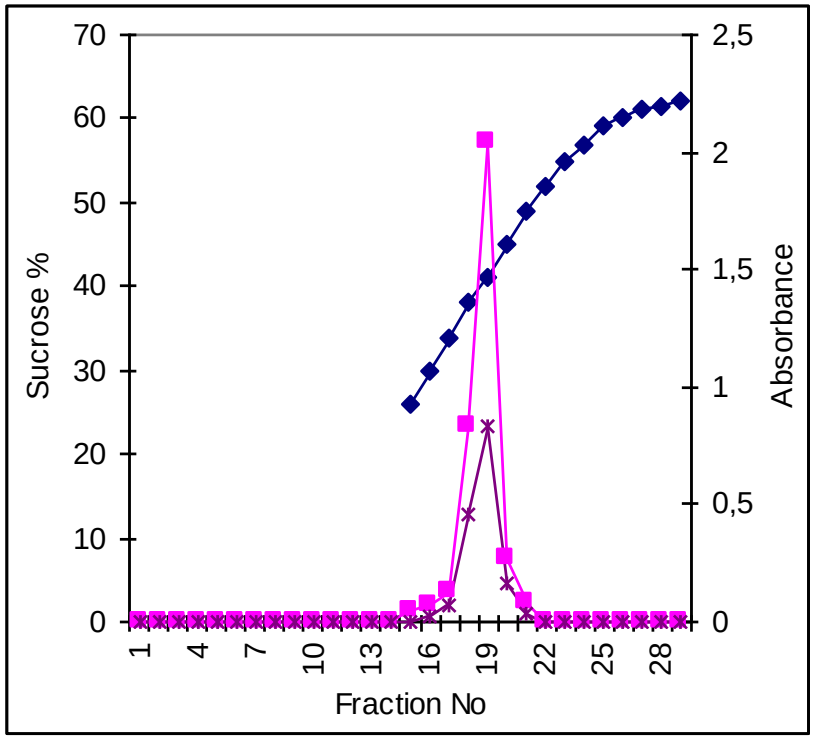
Influenza Viral particles remain in the stationary gradient phase (purification and concentration)

Ovalbumin waste leaves the rotor in the mobile phase



Product Feed:
Clarified Allantoic Fluid

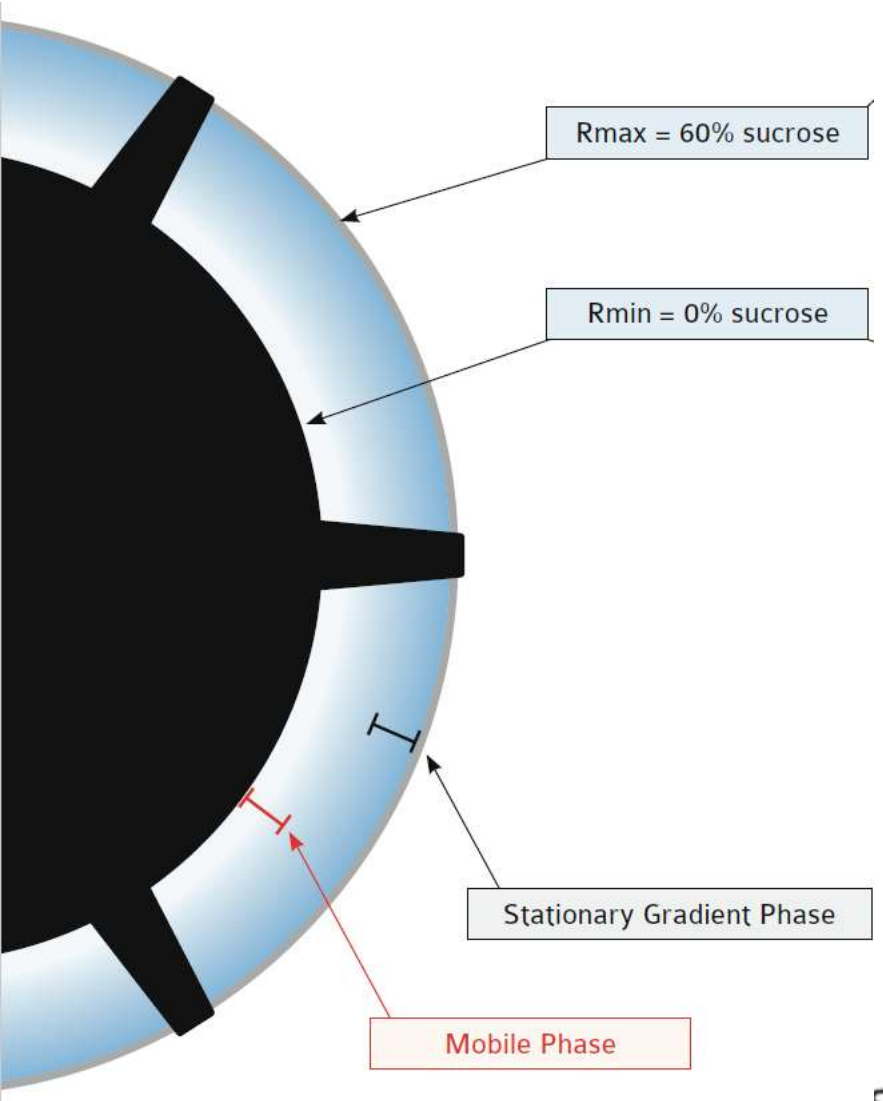
Typical separation profile



Speed: 40 500 rpm
RCF: 121 200 xg
Flow: 29 L/h
Gradient: 0-60% Sucrose
Product Banding: 41% Sucrose
Rotor: K3
Volume: 50 - 150 L
Product: 600-800ml
Product clean-out: 95%
Product Recovery: 70%

Near Isopycnic banding process - volume reduction and product purification in one step

Critical Scale up parameters of centrifugation



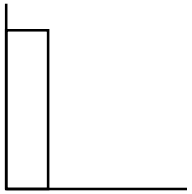
Maximum radius – must not change
Minimum radius – must not change
Gradient load density – must not change
Gradient load volumes – increase volumetrically on scale up
Product flow rate – increase volumetrically on scale up

Particle Range	700S
Flow / Batch	Continuous Flow
Rotor Type	K3 3200 PK3-1600 PK3-800 PK3-400 PX3-230 PX3-120
Flow Rate	28 L/h 14 L/h 7 L/h 3.6 L/h 2.0 L/h 1.0 L/h

Banding of the particles

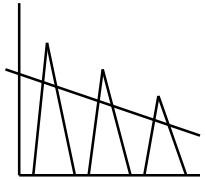
Rate Zonal Centrifugation: Size dependent differentiation of the particles which move in the gradient at different rates but will ultimately band at the same density.

Isopycnic Centrifugation: Density dependent differentiation of the particles which may move at similar rates but will band in the iso-dense layer.



1. *Pelleting:* Removal of the target protein from the process stream to the rotor wall. Pelleting is a method only useful for extremely robust proteins and particles

2. *Sedimenting*: Separates the target protein onto a dense layer 'cushion' where it remains in suspension. This method keeps the protein of interest in a suspension.



3. *Banding:* Resolving the target protein in a gradient allowing separation of impurities at higher and lower densities.

Separation capabilities

Particle Range	< 50S	50-200S	500S	700S
Flow / Batch	Batch	Continuous Flow	Continuous flow	Continuous Flow
Rotor Type	K5 K10	K3 K11	K3	K3
Flow Rate	None	8 L/hr 10 L/hr	11 L/hr	29 L/hr
Time To sediment	3.5 Hr 2hr 50 min	9 min 50s	3.5 min	2.5 min

K10 /K5

K3

K6

K11

Alfa Wassermann Separation Technologies – Process Range
阿尔法韦士曼分离技术---工艺范围

Alfa Wassermann Ultracentrifuge is used globally for manufacture of:

阿尔法韦士曼超高速离心机在全球用于制造：

Influenza vaccine 流感疫苗

Rabies vaccine狂犬疫苗

Hepatitis B Vaccine乙肝疫苗

Meningitis Vaccine 脑膜炎疫苗

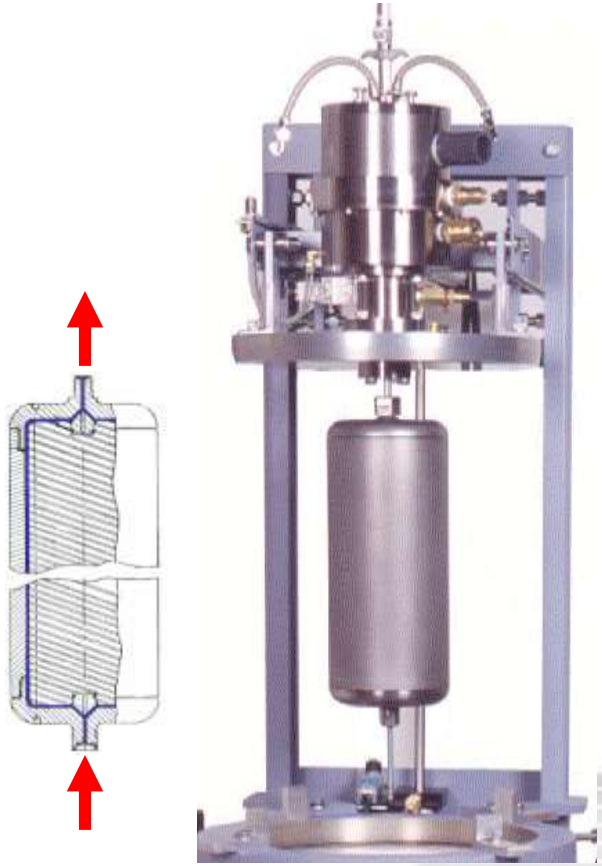
Japanese Encephalitis Vaccine

乙型脑炎疫苗

AWST Continuous Flow Ultracentrifuge – Standard Process Steps
阿尔法韦士曼连续流超高速离心机---标准工艺步骤

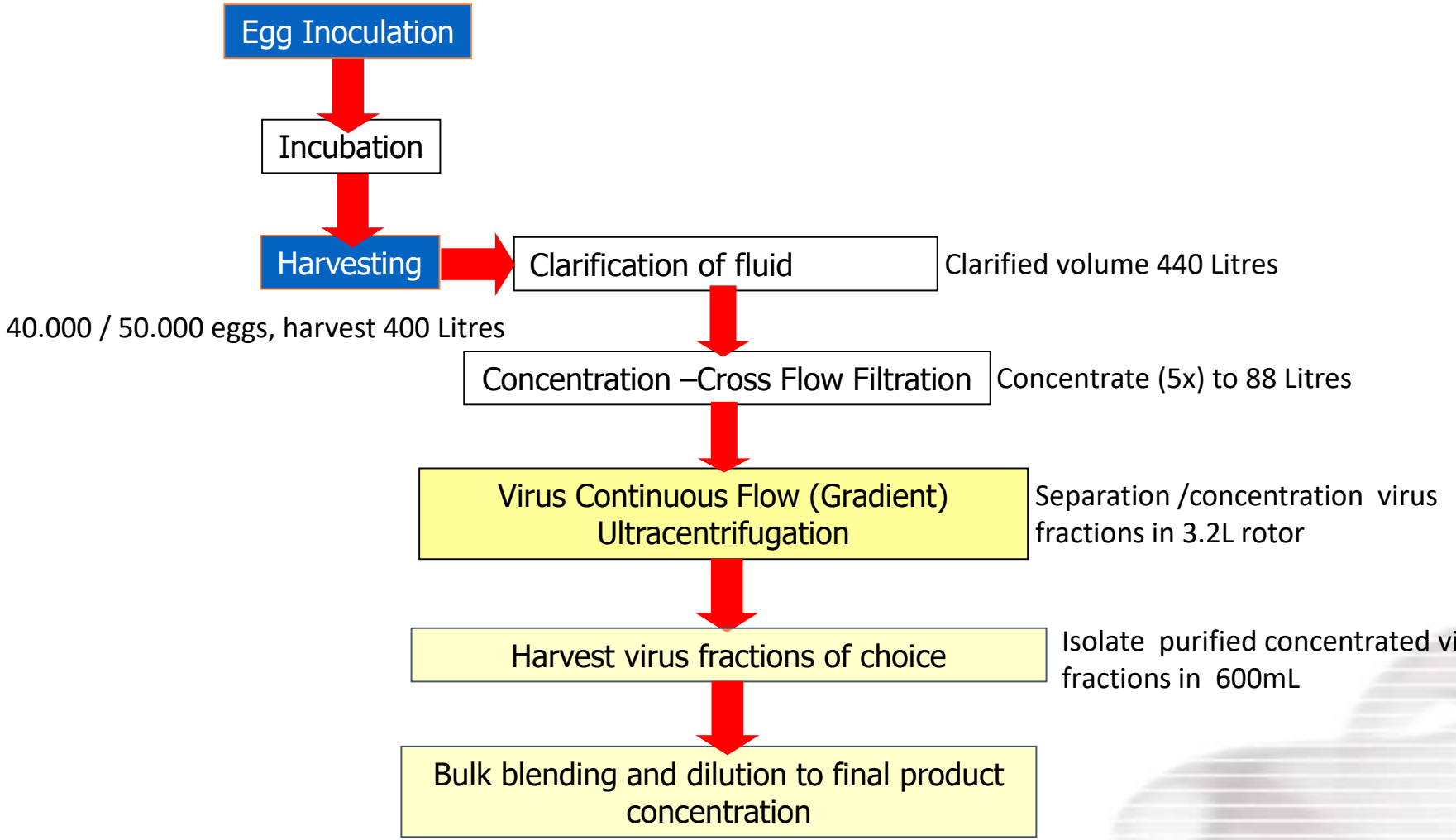
INDUSTRIAL PRODUCTION工业生产

Bioreactor (egg)生物反应器 （鸡蛋）	100%
↓	
Clarification澄清	100%
↓	
Concentration浓缩	95%
↓	
Ultra-Centrifugation 超高速离心	85% >>> 80.8%
↓	
Final Formulation 最终分装	**95% >>> 76.7%**

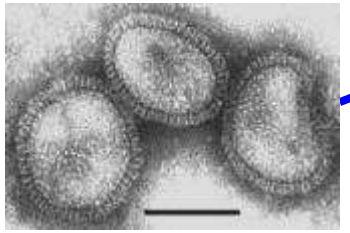


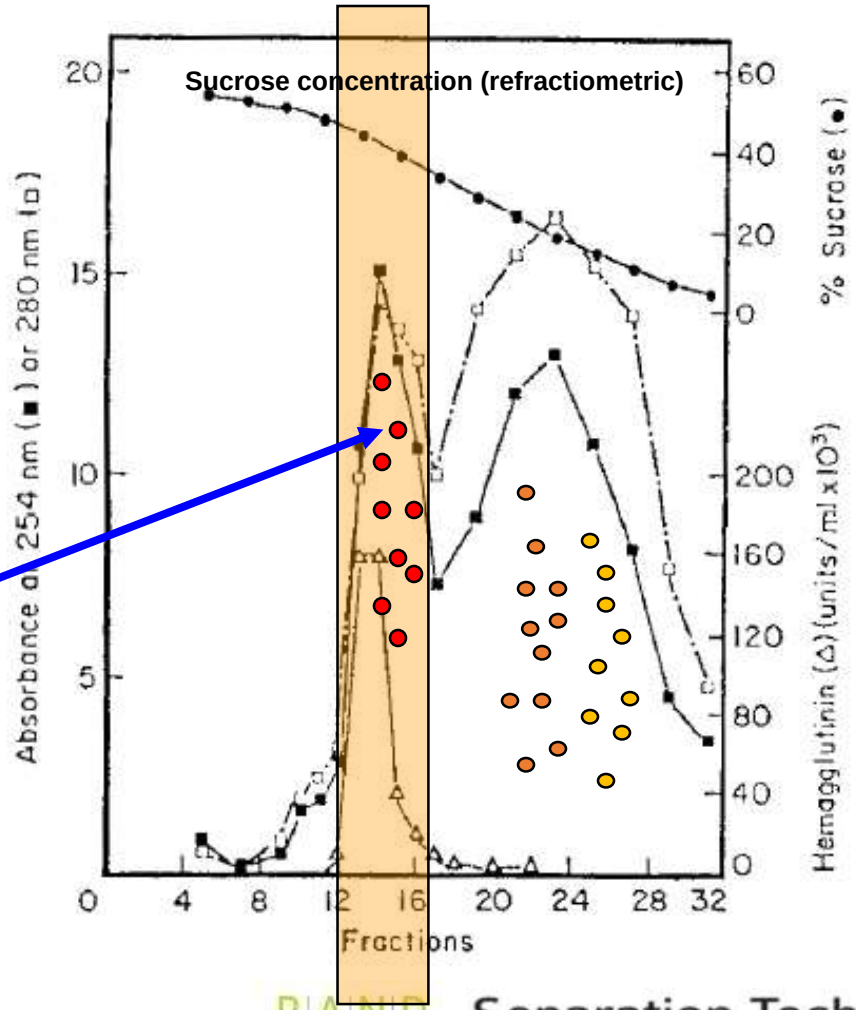
An Example - Influenza Virus Purification

Typical process flow for allantoic influenza manufacturing



Influenza Virus Gradient Purification Results
流感病毒梯度纯化结果





KII Ultracentrifuge KII超高速离心机
K3 Rotor – 3.2L K3转子----3.2升
Process Speed: 35 000 rpm
处理速度： 35000转/分
Process Centrifugal Force: 121 000 xg
处理离心力： 121 000 xg
Gradient: 1.6L PBS
1.6L 60% Sucrose
梯度： 1.6升缓冲液
Product flow rate: 15 L/h
产品流速： 15 L/h
Virus fractions: 12 – 17
病毒区： 12 – 17
Yield 74% in the peak 峰值收率74%
$6x10^4$ HA units/mg protein
$6x10^4$血凝素/毫克

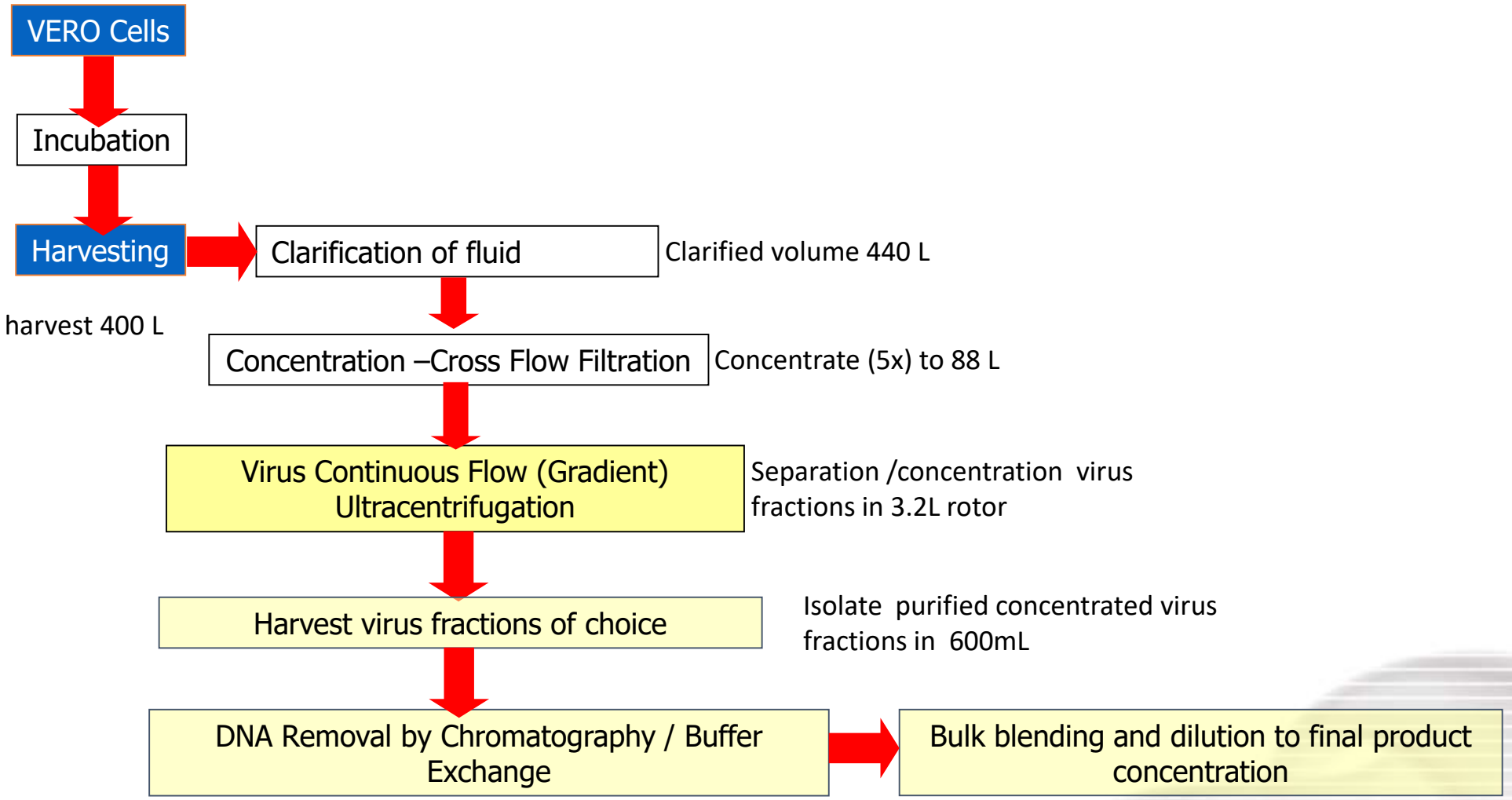
RIAND Separation Technologies ALFA WASSERMANN

Process Summary (Monovalent)

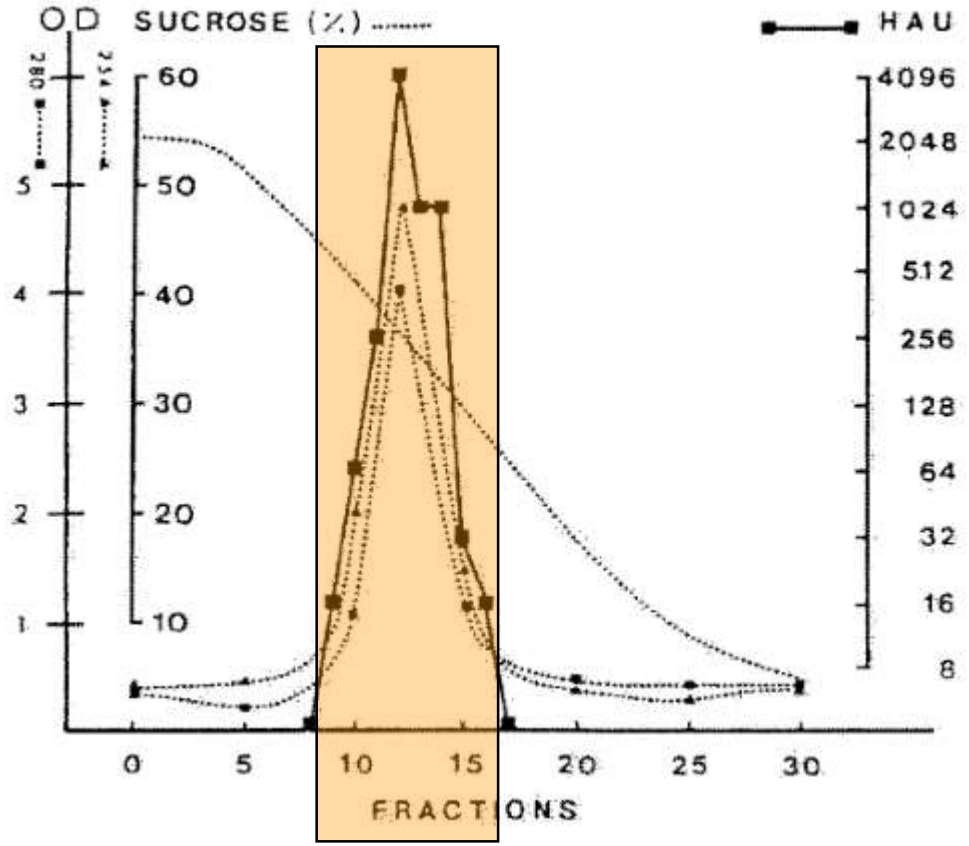
Process	Timing	Remark	Duration	# Units	Volume start	Volume finish
Harvesting	Day 1	12°C	2 – 2.5 h	1	50.000 eggs	500 L
Clarification	Day 1	12°C; log3 reduction bioburden	2 h; 250 L/h	1	500 L	500 L
Concentration	Day 1	12°C; no aggregation; use Thimerosal, VitE, Tween 80, DOC)	2 h	1	500 L	100 L
Inactivation	Day 2	21°C; formaldehyde, BPL, Triton X	overnight	1	100 L	100 L
KII Capture/Conc. whole virus	Day 2	12°C; log 6-7 reduction bioburden	6 h	1	100 L	1.6 L
Diafiltrate/dilute	Day 3	Remove sucrose			1.6 L	30 L
Blending/mixing	Day 3					
Final filling						

Rabies Purification

Typical process flow for rabies vaccine manufacturing



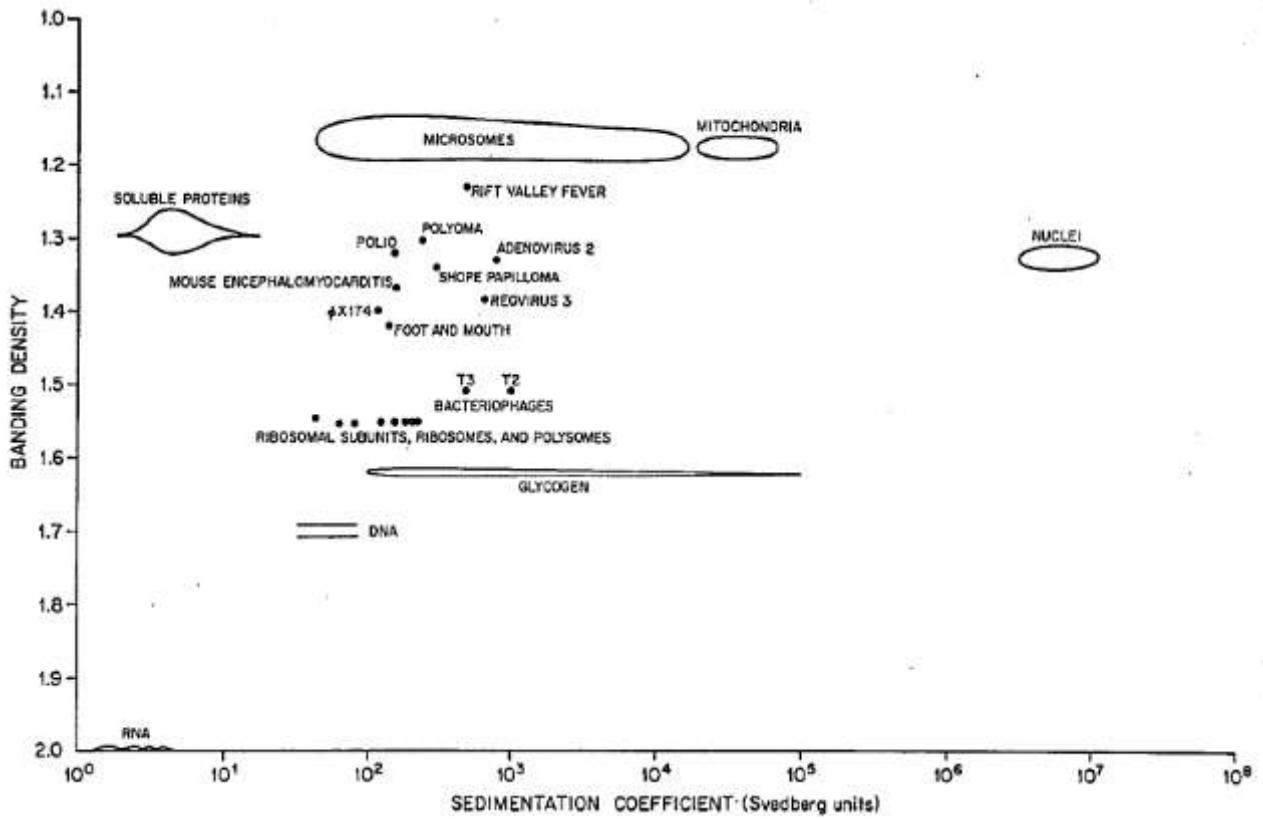
Rabies Vaccine



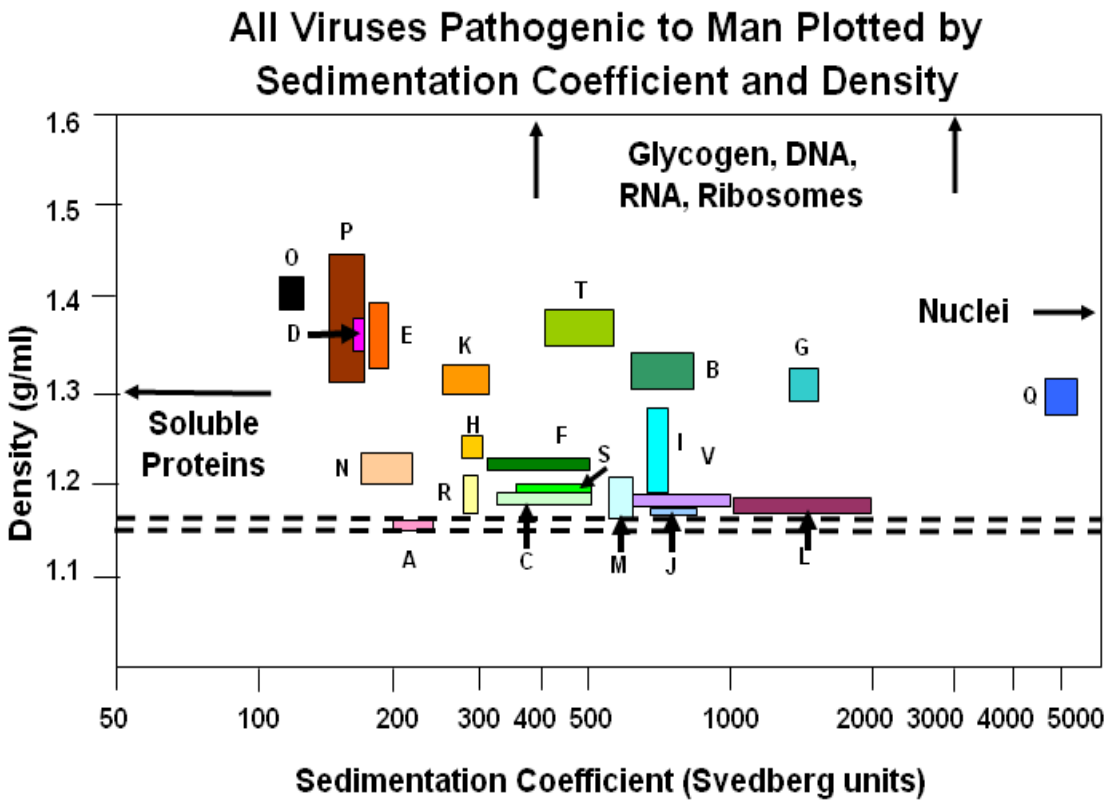
KII Ultracentrifuge KII
RK3 Rotor – 1.6L
Process Speed: 35 000 rpm
Process Centrifugal Force: 90000 xg
Gradient: 0-55% w.w Sucrose
Product flow rate: 7 L/h
Virus fractions: 30-35% sucrose
Yield up to 90% in the gradient

Atanasiu

In summary what can be purified with a centrifuge to make vaccines



Established Protocols for Virus Families



	Family	Virus
B	Adenoviridae	Adenovirus
H	Hepadnaviridae	Hepatitis
I	Herpesviridae	Herpes
J	Orthomyxviridae	Flu
K	Papovaviridae	HPV
L	Paramyxoviridae	Mumps
M	Retroviridae	Various
N	Flaviviridae	Encephalitis
P	Picornaviridae	Polio
Q	Poxviridae	Vaccinia
R	Togaviridae	Semliki
U	Rhabdoviridae	Rabies

AWST continuous flow ultracentrifuges is used to purify virus particles from all virus families in the diagram for manufacutre of viral vaccines.

US Patent 6,051,189 System and Method for detection, identification and monitoring of submicron-sized partlces CM Wick, DM Anderson Apr 18 2000

RAND Separation Technologies ALFA WASSERMANN