INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Zpracování lidské plazmy

a bezpečnost koncentrátů plazmových proteinů

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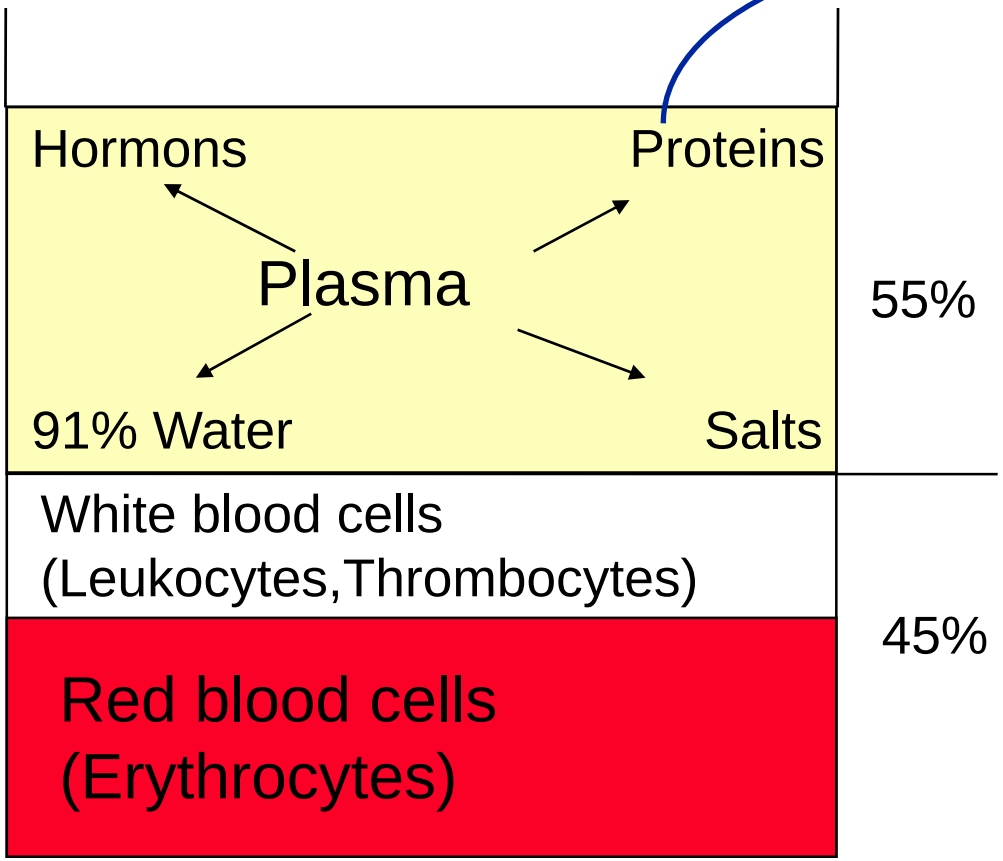
Raw material blood



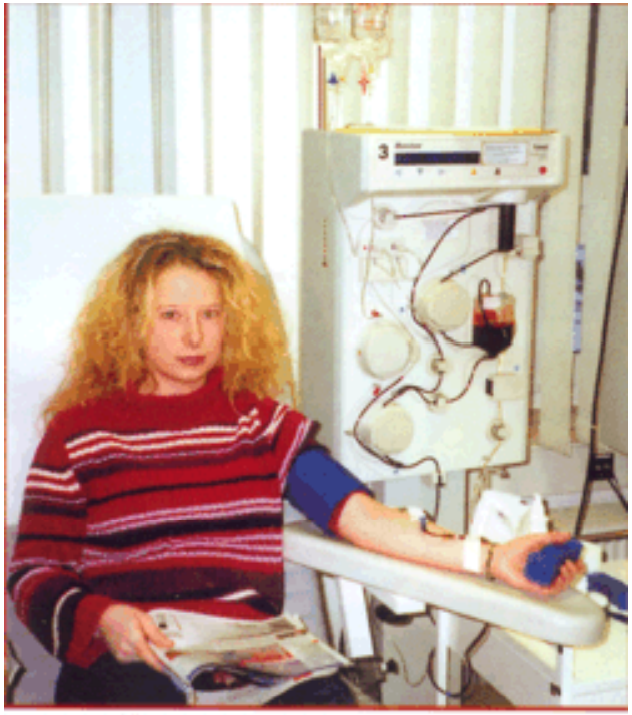
More than 120 plasma proteins
70 - 80 g / Liter

Albumin 60 - 80%
(Transport)

Globulins 20 - 40%
(Immune system)

Fibrinogen 4 %
(Coagulation)

Blood or plasma donation?



Plasma can be donated up to 30 times a year (plasmapheresis)
Blood can be donated 3 times a year

Recovered versus source plasma

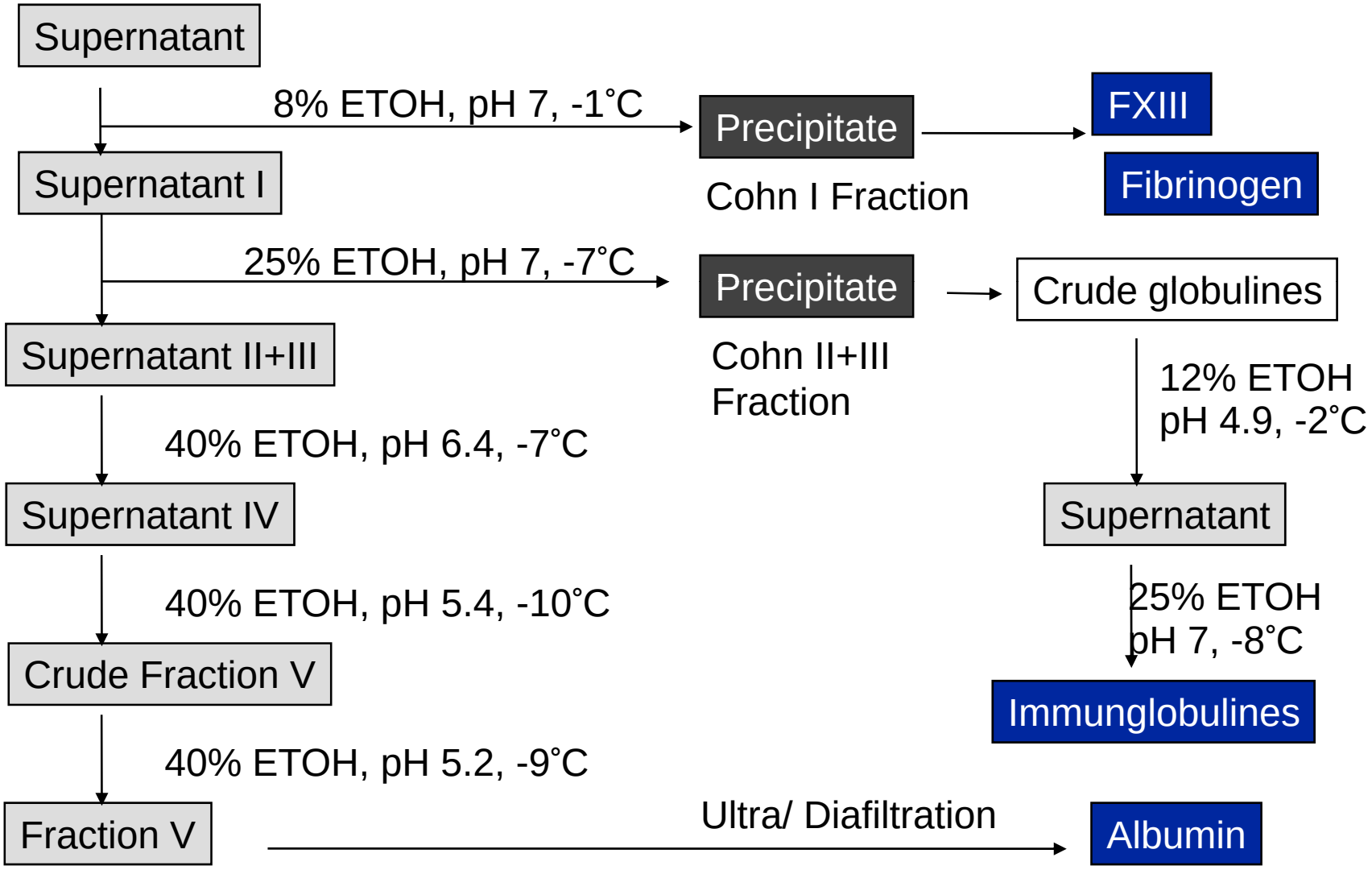
Recovered plasma:
produced from full blood donations
(Red Cross):
300-400 ml/donation

Source Plasma:
generated by plasmapheresis
700-800 ml/donation

Predominantly used for plasma products

Frozen plasma donations for fractionation

Cohn Fractionation



Protein yields from 1,000 l plasma

Albumin	22 - 28 Kg
Immune globulins	3 - 5 Kg
PCC	300,000 – 500,000 Units
FIX	250,000- 350,000 Units
FVIII	140,000- 270,000 Units
AT III	130,000- 280,000 Units
Protein C	120,000- 150,000 Units

F VIII usage for treatment of severe hemophilia A

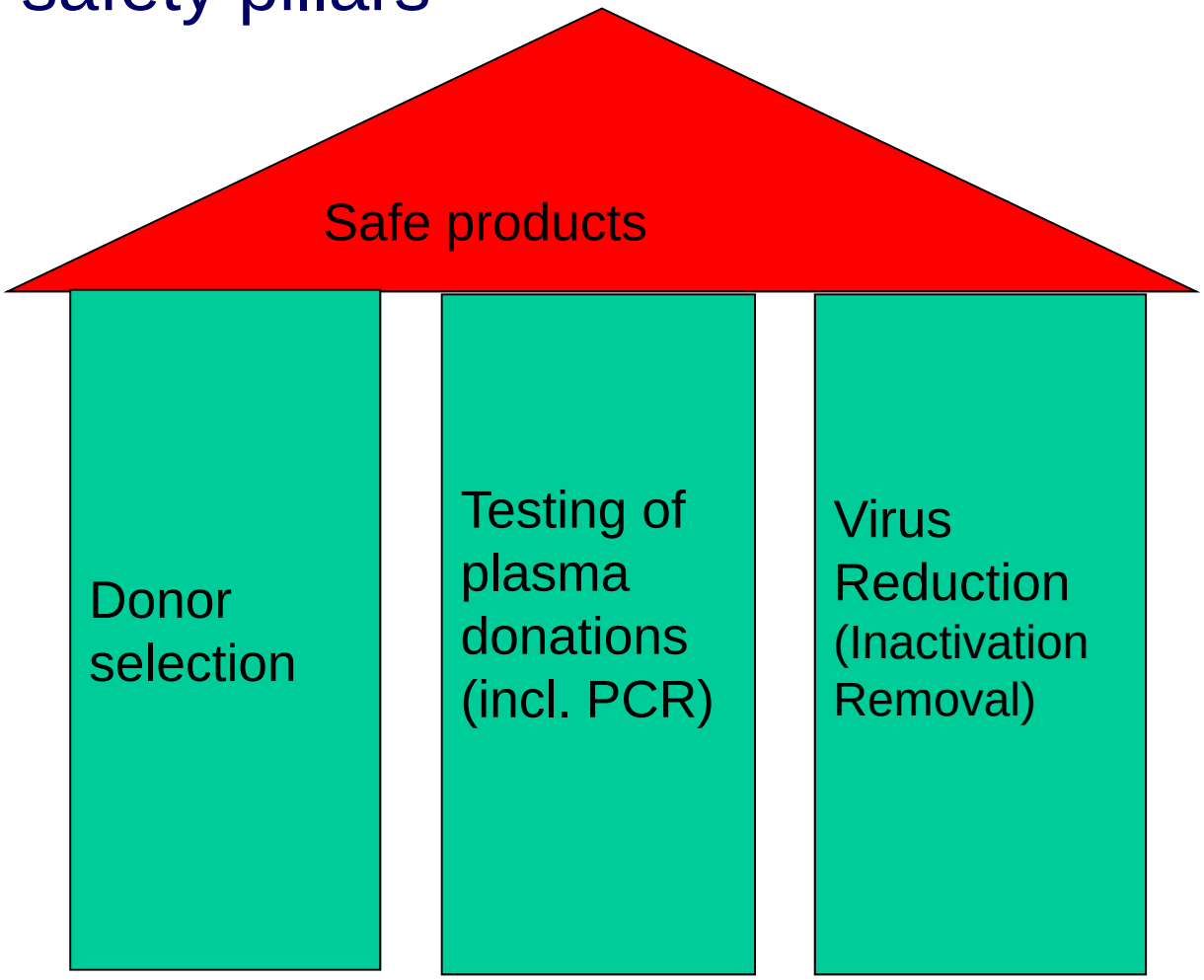
A patient with 60 kg body weigth and severe hemophilia A needs on average:

a) Prophylaxis: 230 000 - 370 000 IU FVIII / year
(10- 50 Einheiten U/kg every 2-3 days)

b) On demand therapy: 20 000 - 640 000 IU FVIII / year
Which corresponds to 100 to >3,000 liter plasma/patient/year

> 25 Mil. liter´plasma per year are fractionated worldwide

Today‘s plasma products are safer than ever based on 3 safety pillars



Plasma origin

Approx. 60 plasmapheresis centers in USA and in Europe (Germany, Austria, Sweden, Czech Republic)

All centers
- are licensed by local authorities
- are iQPP* certified
- adhere to standard operating procedures (SOPs)
- are subjected to regular inspections

* iQPP = International Quality Plasma Program.
Standard developed to guarantee safe and high quality plasma

The main requirements of iQPP

- Only plasma sourced from Qualified Donors can be used for manufacture into plasma derived medical products
- Collection centres should recruit donors from the local community and exclude donors at increased risk of HIV
- Collection centres should participate in the PPTA National Donor Deferral Registry (NDDR) to ensure that rejected donors do not donate elsewhere
- Collection centres should recruit qualified staff and implement in-depth initial and on-going training in plasmapheresis and regulatory compliance
- Facilities present a quality, professional medical appearance and are properly maintained and designed to adequately and safely facilitate donor processing
- Centres must be in compliance with PPTA Source's Viral Marker Alert limits for HIV, HCV, and HBV (alert limits defined by PPTA for qualified donors as seroconversion rates per 10^5 donations)
- Centres must satisfy PPTA inspection and compliance requirements
- Manufacturing systems have been designed, documented, and implemented that consistently guarantee a quality product.

Plasma sourcing policies with regard to CJD

Donor selection criteria according to **US regulations**:

permanent deferral of any...
... person with CJD and CJD in their family
... person with an indication of vCJD and person with this disease in their family
... recipient of dura mater or cornea transplants
... person who has been treated with human pituitary growth hormone
... person who has undergone brain surgery
...person who has received blood transfusion in the UK from 1980 until today

Indefinite deferral of any..
... person who has resided in the **UK** for **3 months** or more cumulatively from 1980-1996
...person who spent time in **France** that adds up to **4 years** or more since 1980
...person who spent a total of **6 months** or more from 1980-1990 associated with a military base in **Belgium, NL** or **Germany** or from 1980-1996 in **Spain, Portugal, Turkey, Italy** or **Greece**

meets these requirements for it‘s plasma sourced in the US
BioLife plasma services SOP # DIS-1119.20

Plasma sourcing policies with regard to CJD

Donor selection criteria according to **European regulations**:
http://www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf

permanent deferral of any...
... person with CJD and CJD in their family
... person with an indication of vCJD and person with this disease in their family
... recipient of dura mater or cornea transplants
... person who has been treated with human pituitary growth hormone
... person who has resided in the **UK** for **6 months** or more cumulatively from 1980-1996 (Please note: **Guidelines request only 1 year)**
... person who has undergone brain surgery
...person who has received blood transfusion in the UK from 1980 until today

exceeds these requirements for it‘s plasma sourced in Europe[1].

Virus marker rates of qualified donors are lower than that requested by PPTA standards

Virus	Seroconversion rate per 10^5 donations		
	donor applicants[1]	qualified donors[2]	requested[3]
HIV	67	0.4	5
HBV	74	2.1	12
HCV	1285	1.5	14

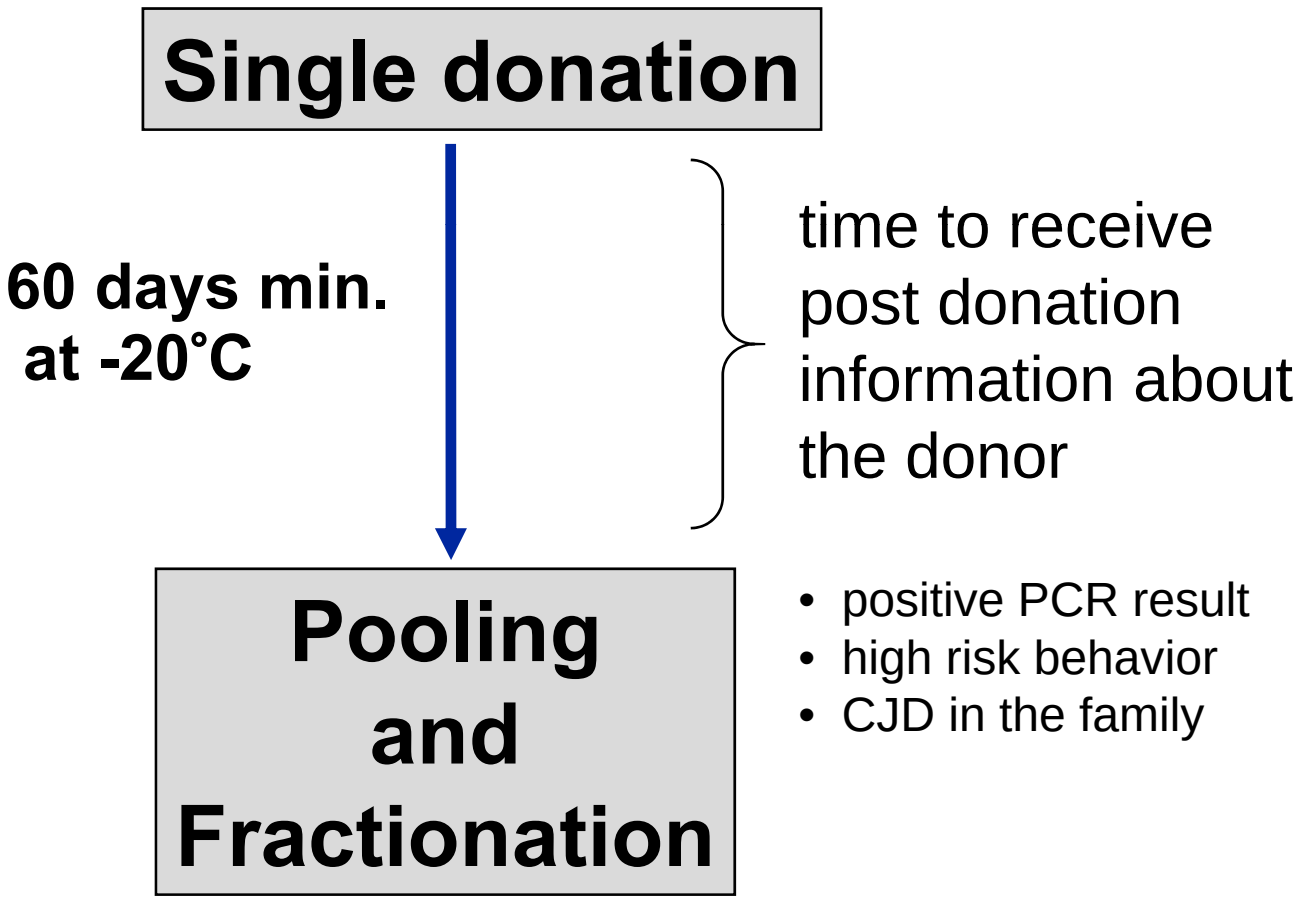
[1]calculation based on 131,077 donations
[2]calculation based on 1,405,375 donations
[3]Viral Marker Standard for Qualified Donors by **P**lasma **P**rotein **T**herapeutics **A**ssociation (PPTA) (Revised 2/2002)

Single donation test program for markers of disease

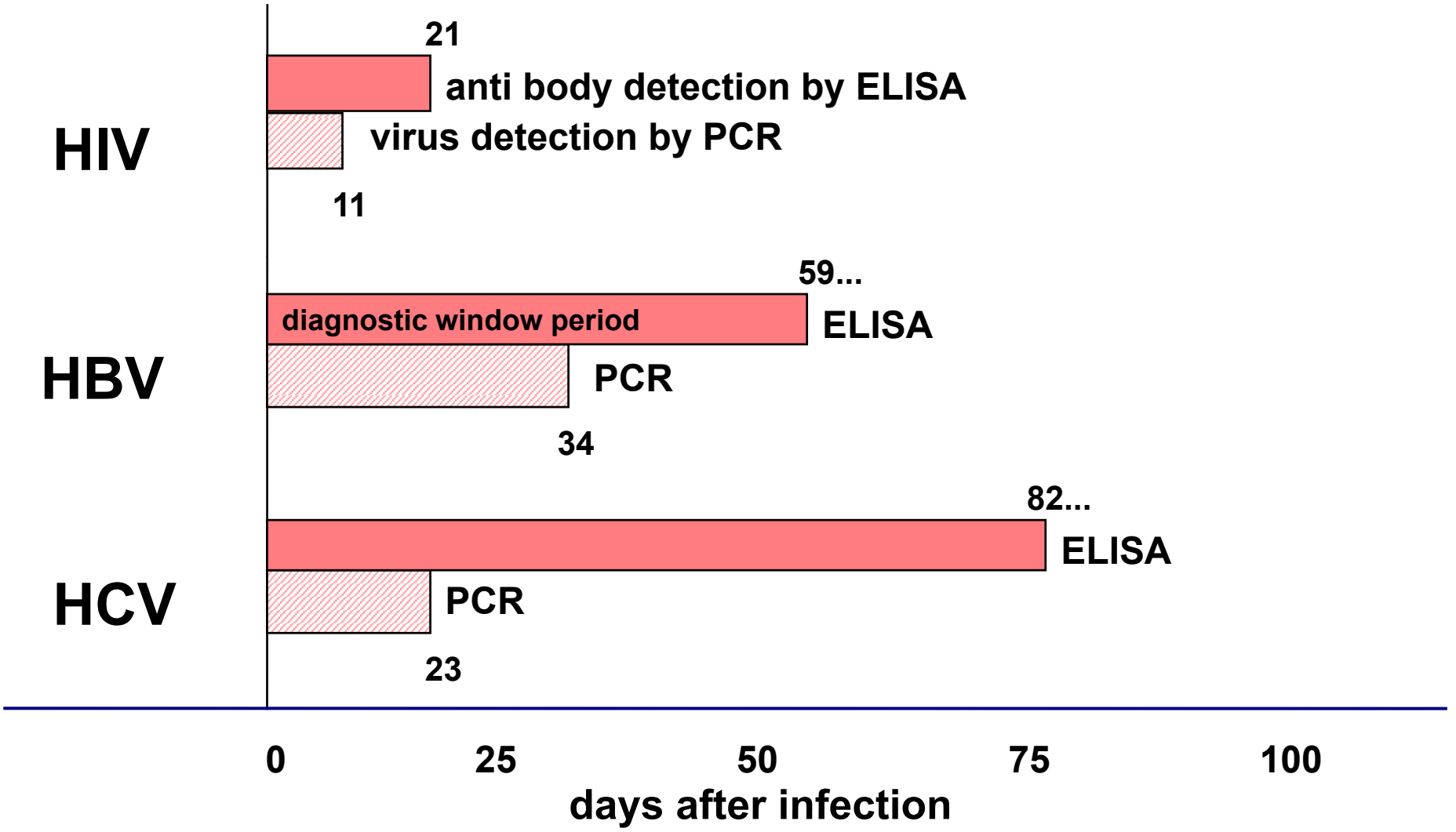
Determination by conventional ELISA

Assay	Requested results
HIV-1 antibodies	non reactive
HIV-2 antibodies	non reactive
HCV antibodies	non reactive
HBs antigen	non reactive

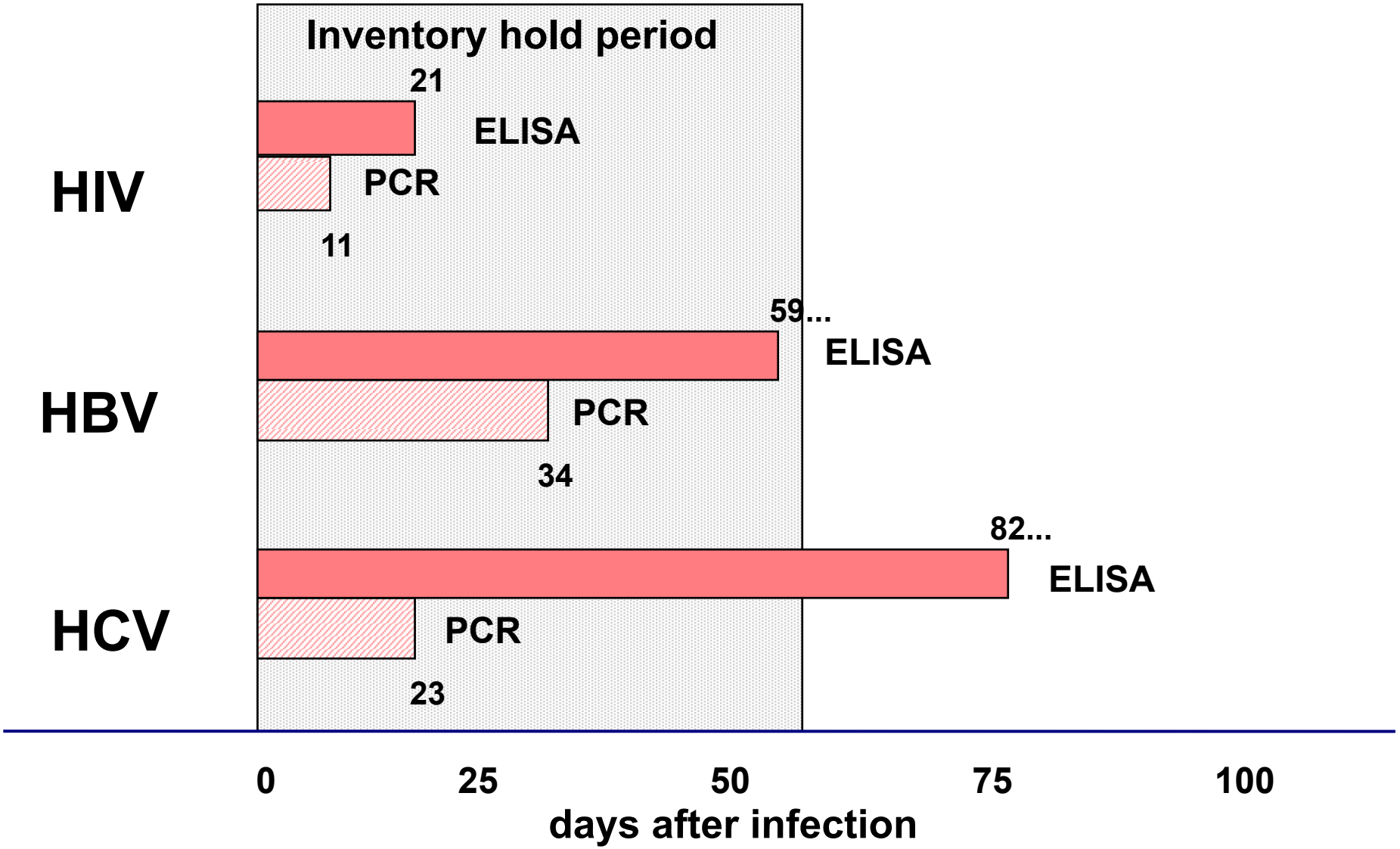
60 days inventory hold



Virus and assays specific diagnostic windows



PCR diagnostic windows covered by 60 days inventory hold



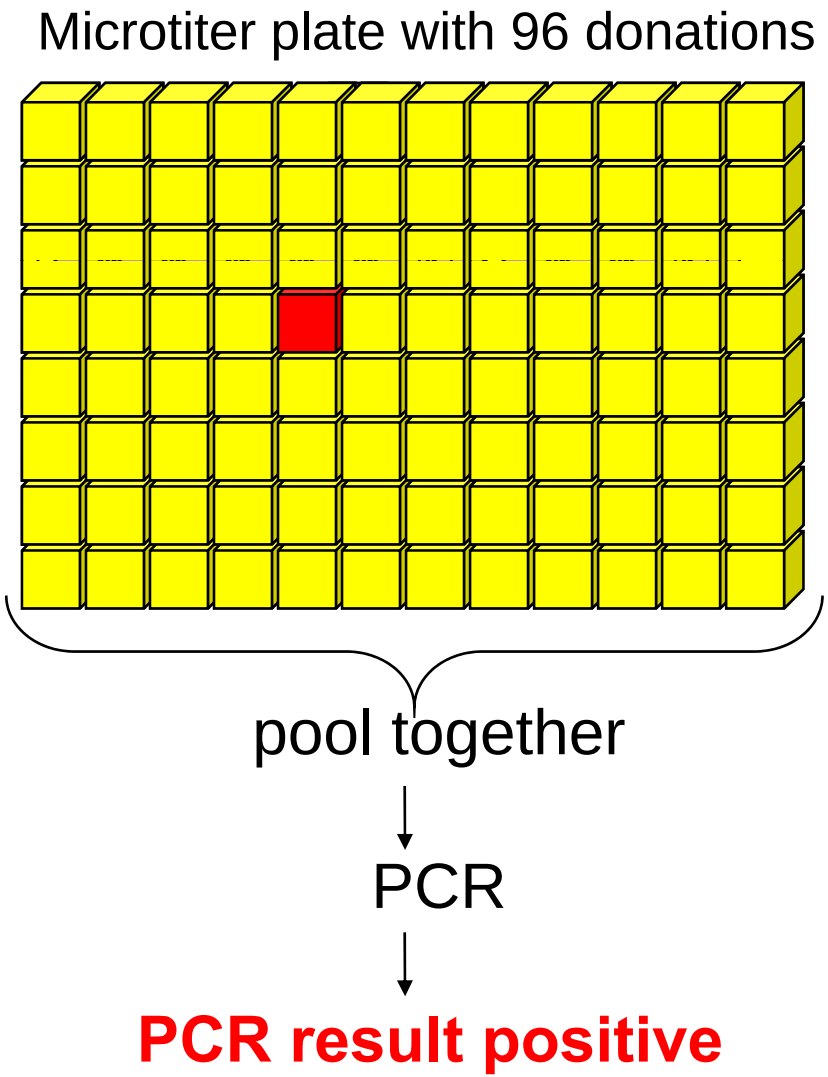
PCR test program on 6 viruses

(started in Vienna in 1995 with HIV, HBV and HCV)

Virus	Disease	Fully implemented
HIV-1+-2	AIDS	Jan 1999
HBV	hepatitis B	Jan 1999
HCV	hepatitis C	Jan 1999
parvo B19	fifth disease	Jan 2000*
HAV	hepatitis A	Aug 2000

* voluntary cut-off: 10^4 parvo units/ml lower than the PPTA standard with cut off 10^5 units parvo / ml

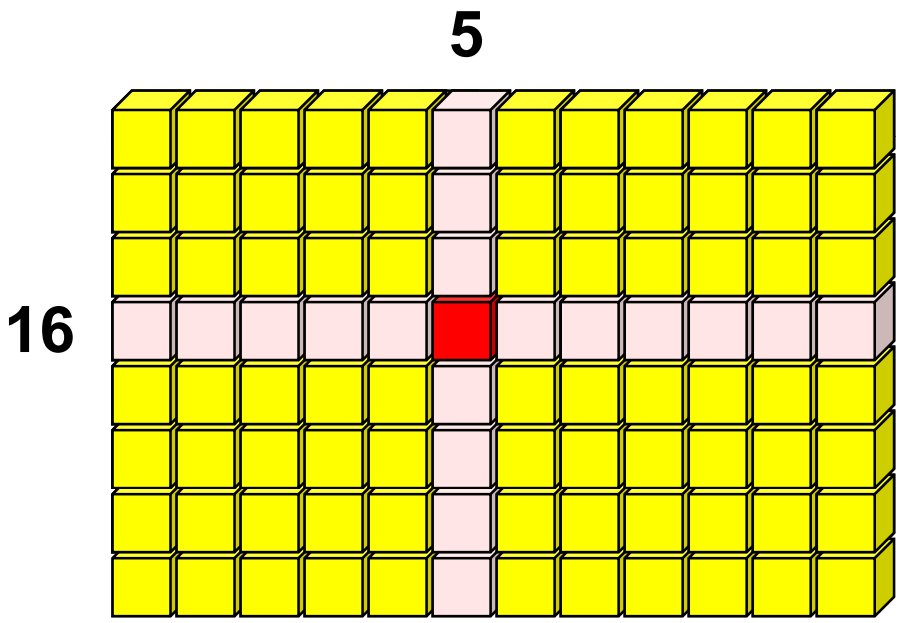
Identification of a contaminated single donation by a 2- dimensional pooling system



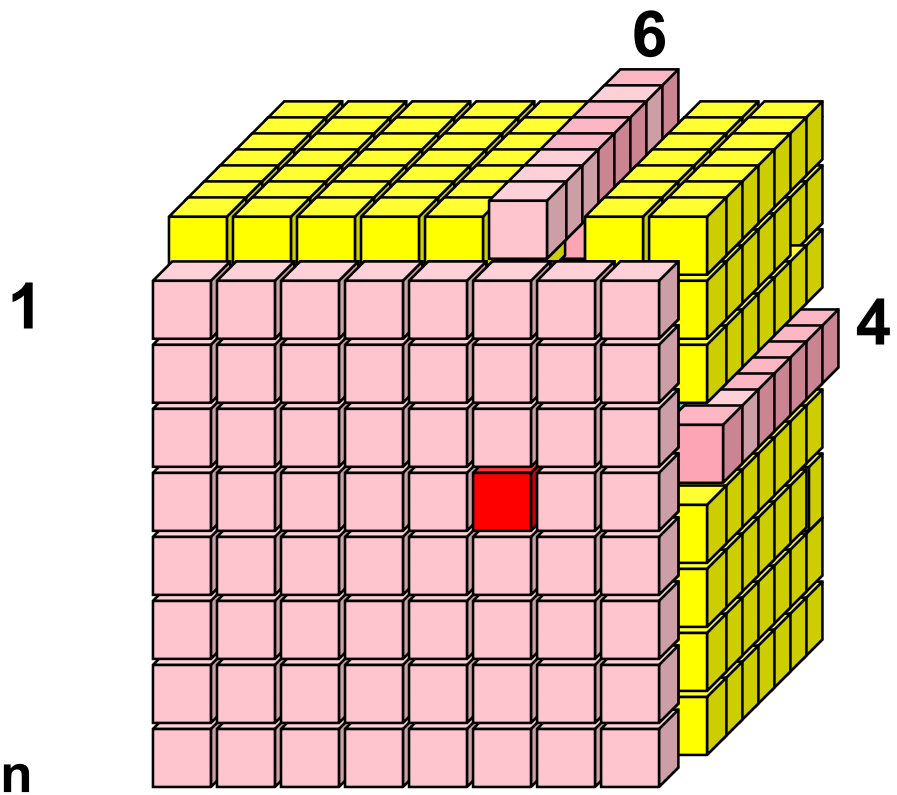
Virus positive donation

Virus negative donation

Identification of a contaminated single donation by 12 + 8 = 20 additional PCR tests

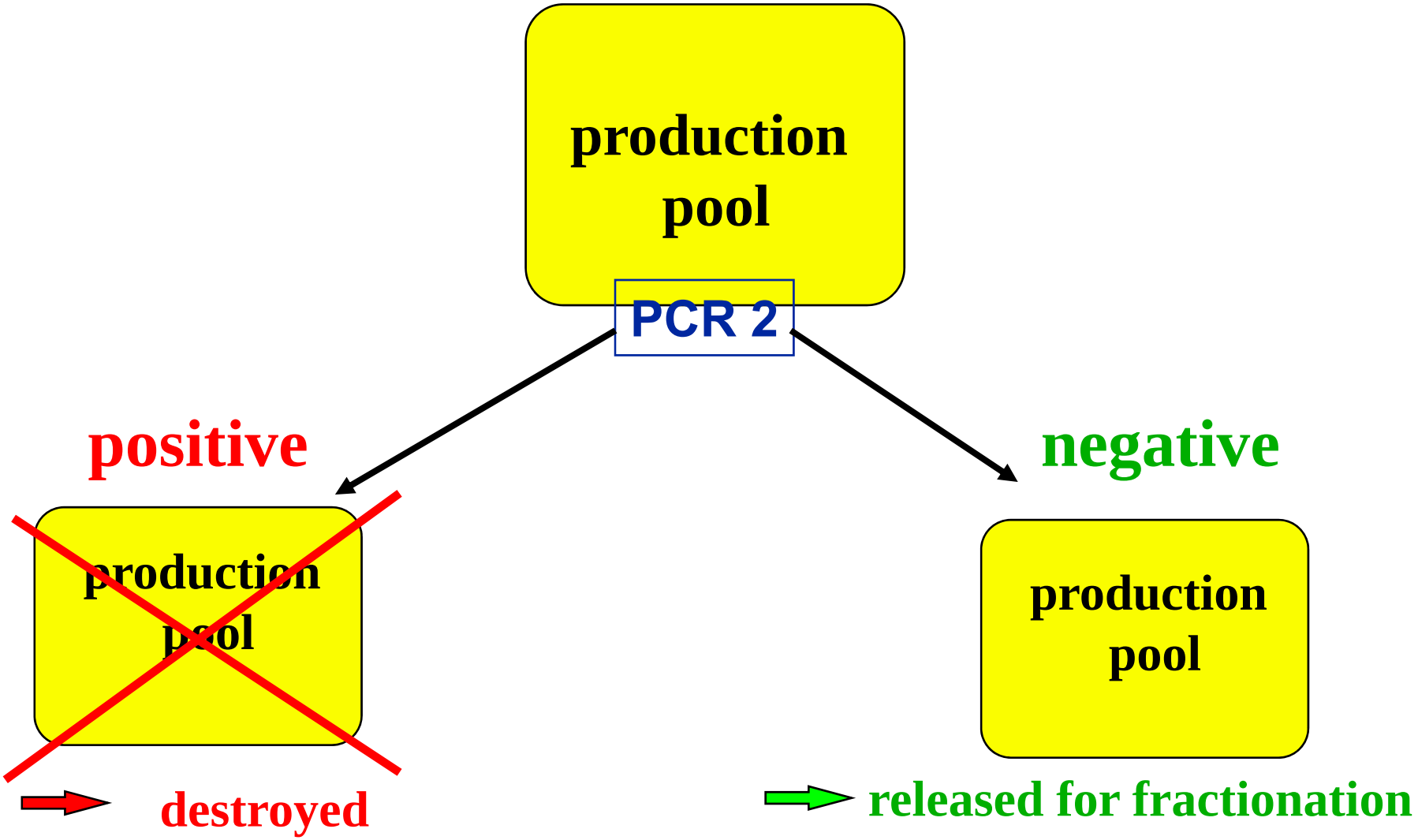


Identification of contaminated single donation



Virus positive
single donation

Screening of plasma production pools by PCR



QSEAL certified

since Nov. 2001 as one of the first companies

QSEAL
Quality Standards of Excellence, Assurance
and Leadership

Certification process for plasma manufacturer
established in 2000
by the Plasma Protein Therapeutics Association
(PPTA)

based on 4 voluntary industry standards agreed on
in 1996 by the PPTA member companies

The 4 voluntary industry standards
(adopted by the PPTA in 1996 to go beyond authority requirements)

- **Qualified donor standard (at since 1994)**
  to ensure a committed, healthy donor population
- **Viral marker standard (plasma center relocation in the US)**
  to demonstrate the quality of the donor population
- **Inventory hold standard (at since 1992)**
  to allow for retrieval of plasma if new post-donation donor-related information becomes available
- **NAT testing standard (since 1995)**
  to allow for detection of certain viruses earlier than current serological screening methods.
  Currently HCV, HIV, and HBV only, PVB19 (cut-off 10^5 /ml)

The QSEAL certificate...

- ...is evaluated by independent third party inspectors
- ...is issued to a company only when **all fractionation facilities** meet the requested standards
- ...is issued for a two-year period
- ...is confirmed by regular inspections

Newly developed standards will be confirmed at the next regular inspection

Why are virus reduction measures needed after all these testing?

- To meet authority requirements
- Donor selection and donation testing methods do have limits of detection
- Virus reduction is the most effective safety measure

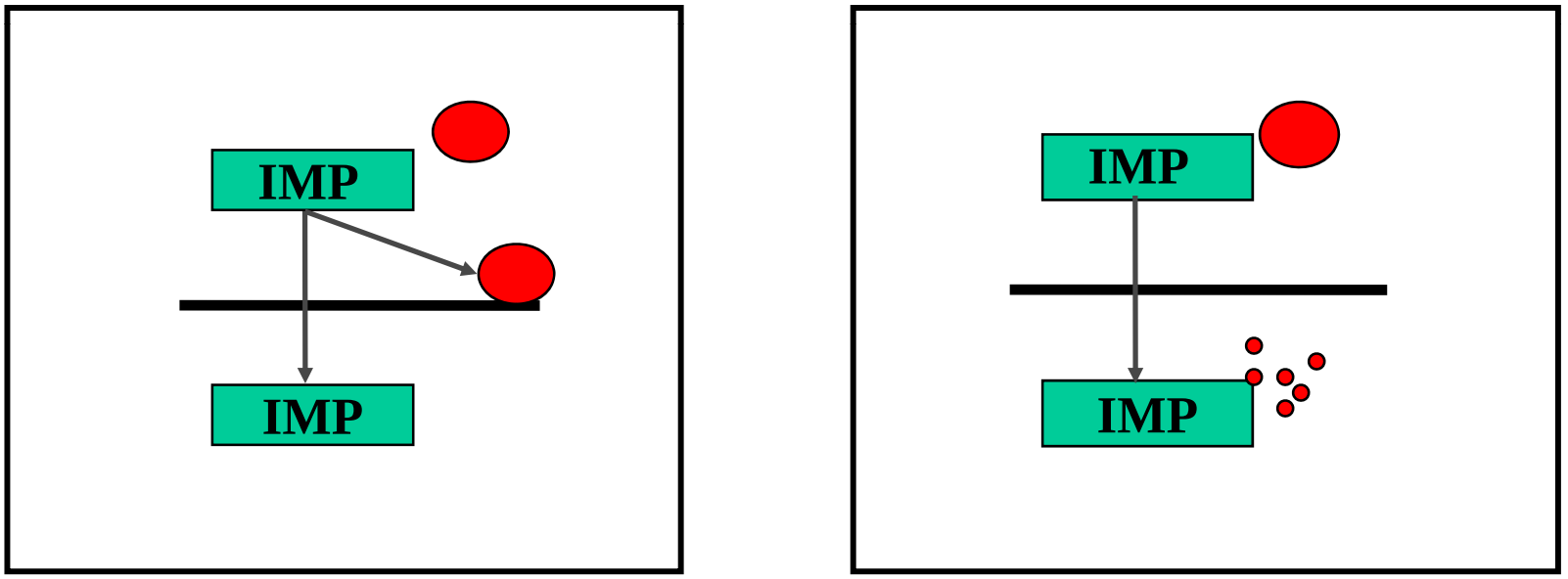
  (on average 100,000-fold virus reduction compared to 100-fold by donor selection or testing)

- To eliminate unknown pathogens

Virus reduction methods

Virus

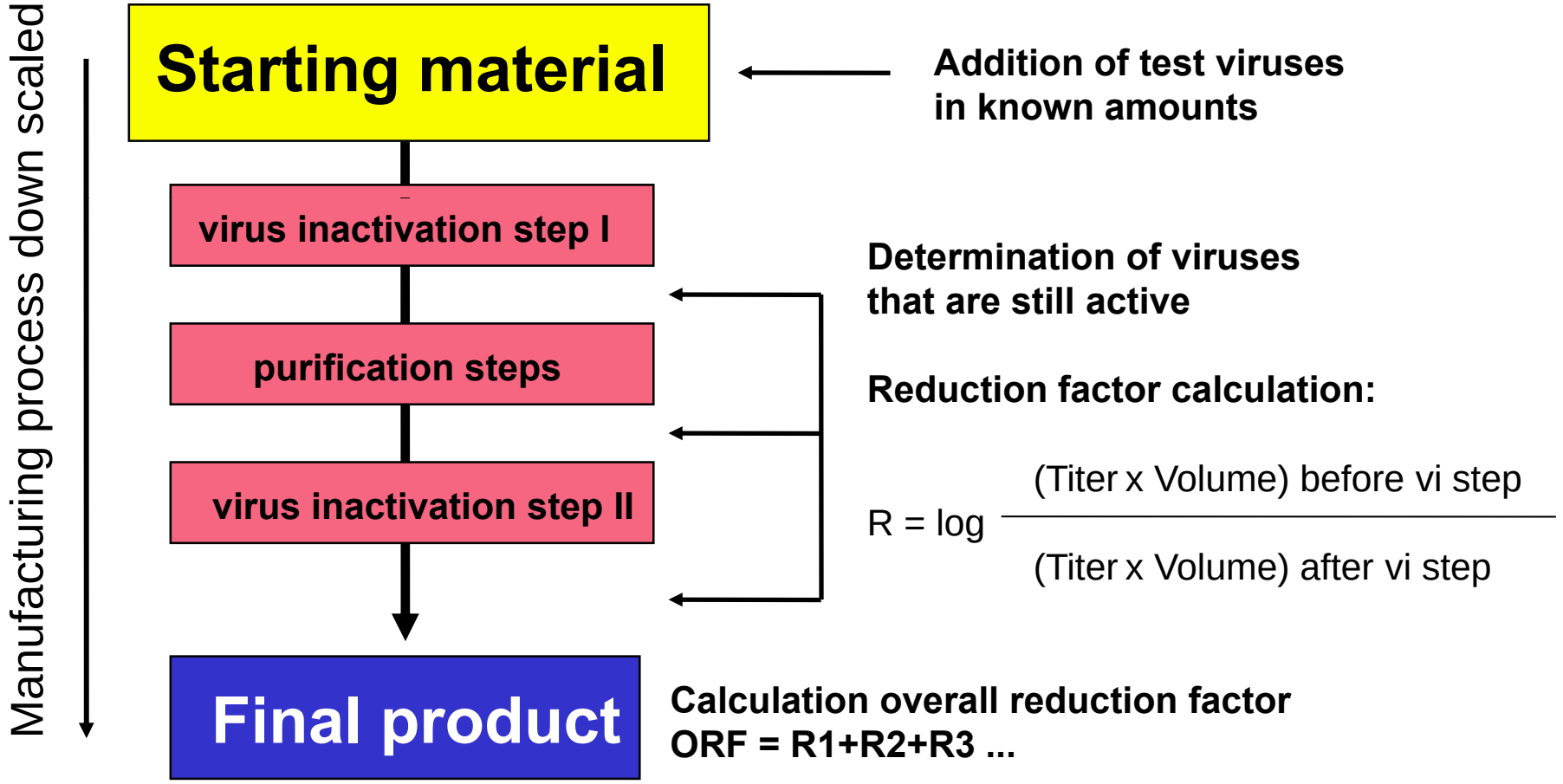
removal inactivation



IMP Intermediate product

virus particle

Demonstration of efficiency of virus inactivation methods by validation studies



Regulatory requirements
(EMEA CPMP/BWP/269/95 rev.3, 2001)

Enveloped viruses

- At least two robust steps (with complementary mode of action) providing ≥4 logs inactivation/removal.

Non-enveloped viruses

- At least one robust step providing ≥4 logs inactivation/removal.

Virus partitioning methods

- **Precipitation steps**

  Cryoprecipitation

  Cohn-fractionation – EtOH/pH,
  PEG

- **Filtration**

  nanofiltration (15-75 nm pore size)

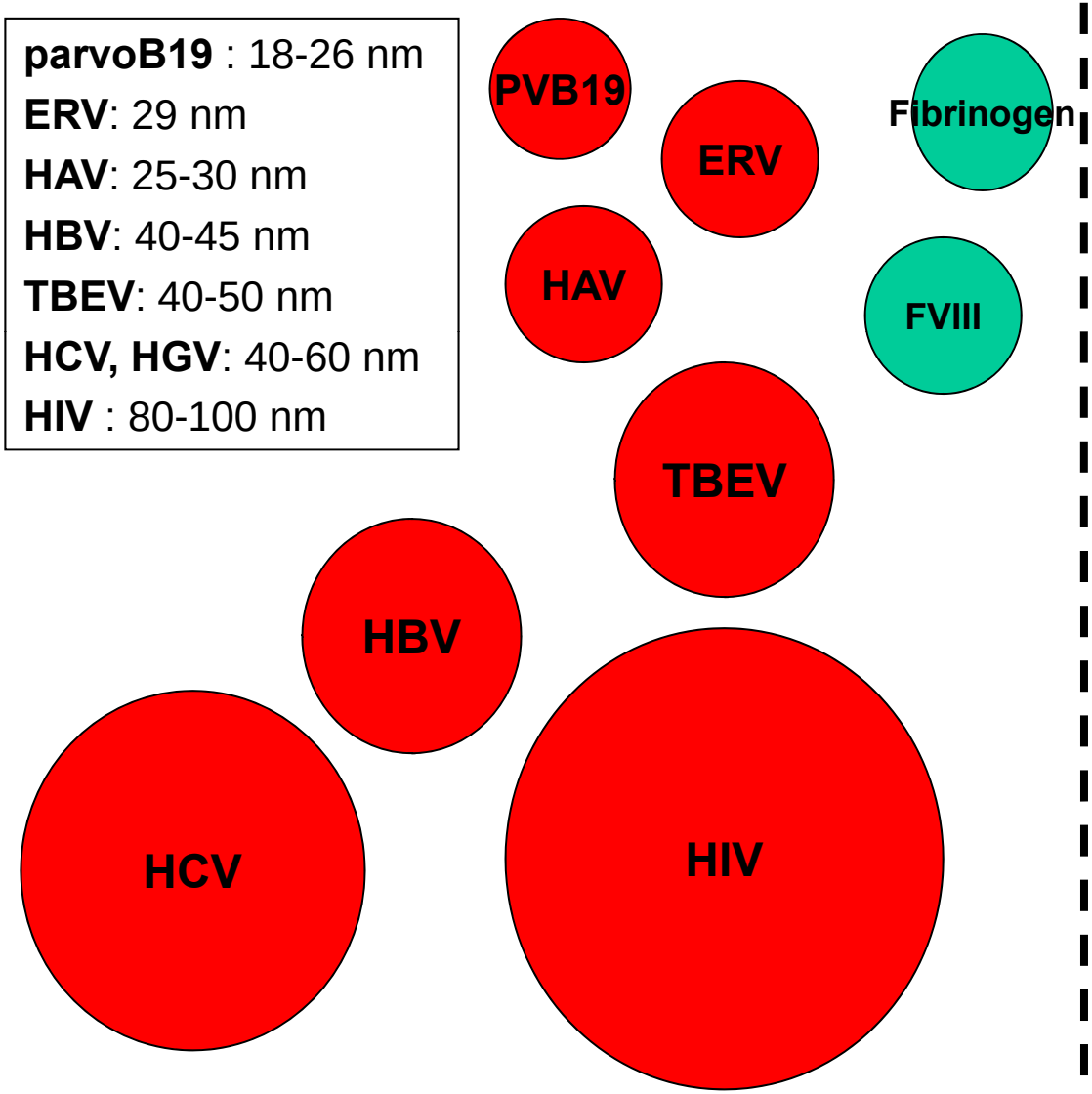
- **Adsorption**

  AlOH adsorption to remove the PTC

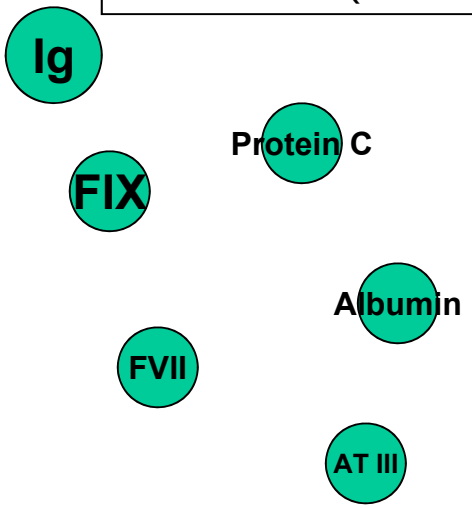
- **Chromatographies (batch or columns)**

Filtration methods (nano filtration 15-75nm pore size)

parvoB19 : 18-26 nm
ERV: 29 nm
HAV: 25-30 nm
HBV: 40-45 nm
TBEV: 40-50 nm
HCV, HGV: 40-60 nm
HIV : 80-100 nm



FVIII: 330 kD (25 nm)
Fibrinogen: 340 kD (25 nm)
IgG : 150 kD (18-20 nm)
FIX: 56 kD (14 nm)
Albumin: 68 kD (14 nm)
Protein C: 56 kD (14 nm)
AT3: 58 kD (14 nm)
FVII: 50 kD (14 nm)



Chromatographies

Ion exchange chromatography

Anion exchange chromatography (DEAE Sephadex, Q-Sepharose)

Kation exchange chromatography (Source F, S-Sepharose)

Immunoaffinity chromatography

monoclonal ab against FVIII, vWF, protein C, heparin

Gel permeability chromatography

(Sepharose); size exclusion

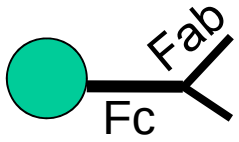
Hydrophobic

(C18-Sepharose, Phenylsepharose); binding to hydrophobic proteins

Protein A, Protein G

protein isolated from Staphylococcus aureus

binding to Fc-part of IgG



Virus inactivation methods

- **Heat treatments**
- **Solvent detergent (SD) treatment**
- **Lyophilization**
- **Immobilized hydrolases**

  (trypsin, chymotrypsin treatment of Ig)
- **Ethanol**

Virus inactivation – heat treatments

- **Dry heat**

  100˚C; 1 h

  100˚C; 30 min

  80˚C; 72 h

- **Vapor heating**

  7-8% humidity, pressure delta190 mbar

  S -TIM3: 10h; 60˚C

  S -TIM4: 10h; 60˚C plus 1h; 80˚C

- **Wet heat = pasteurization**

  10h; 60˚C

Parvovirus B19 inactivation by vapor heating

(FEIBA STIM 4)

	MMV RF	VH1 #1	VH1 #2	VH2 #1	**B19V mean RF**
positive control	**-**	11.2	11.8	n.d.	**-**
spike control	**-**	10.3	10.5	10.5	**-**
after Lyo	**0.6**	9.8	10.0	9.8	**0.6**
3h 60˚C	**n.d.**	7.7	7.6	n.d.	**2.7**
8h 60˚C	**0.6**	6.1	6.5	6.5	**4.1**
55min 80˚C	**0.9**	5.7	5.5	5.8	**4.8**

Virus inactivation – SD treatment

Common Solvent Detergent Combinations

a) Tri(n-butyl) phosphate (TNBP) + Tween 80

b) TNBP + Triton X-100 or Sodium cholate

at room temperature; 6 h

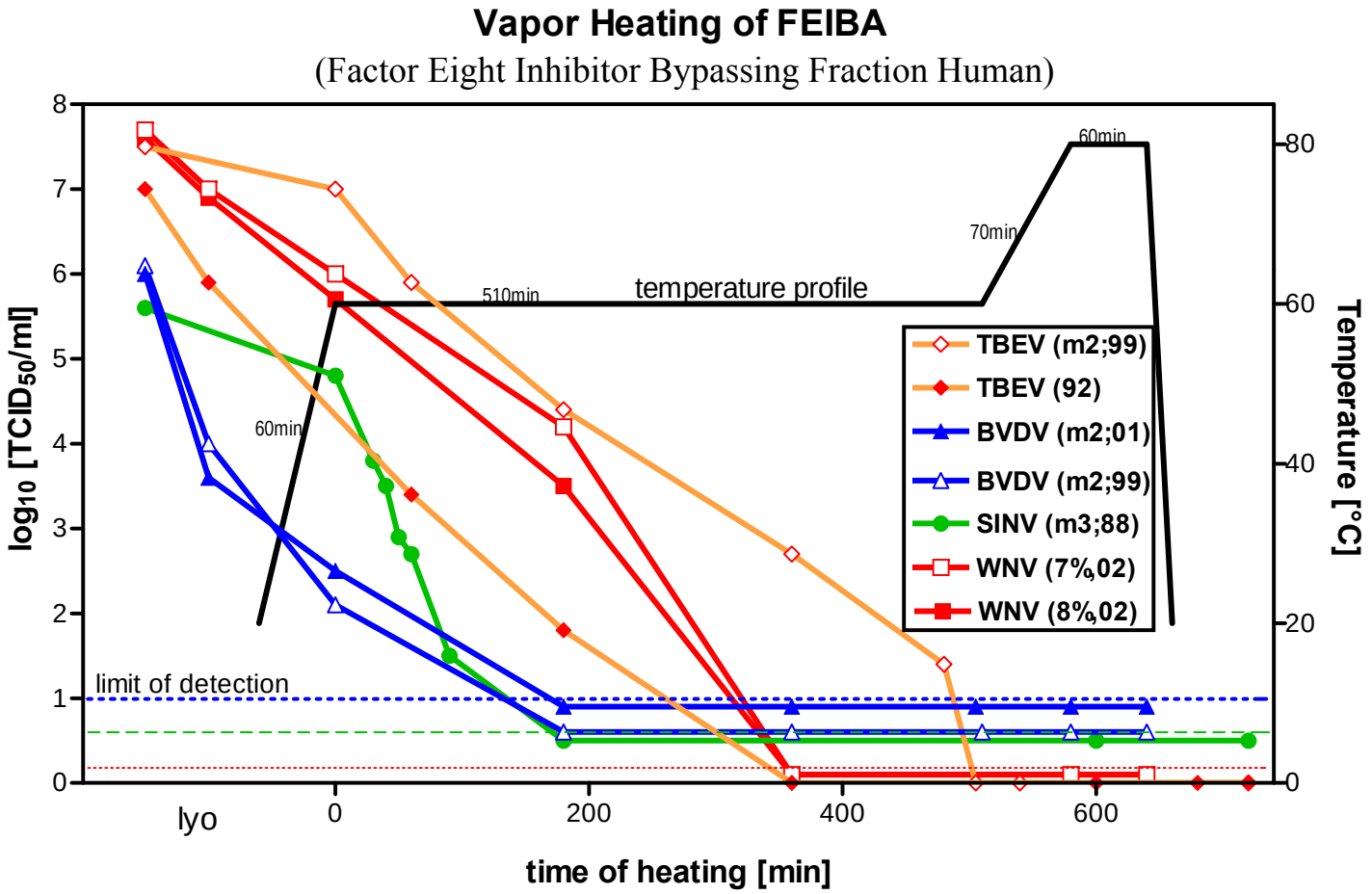
Mode of action

Solubilization of lipid membranes/virus envelopes

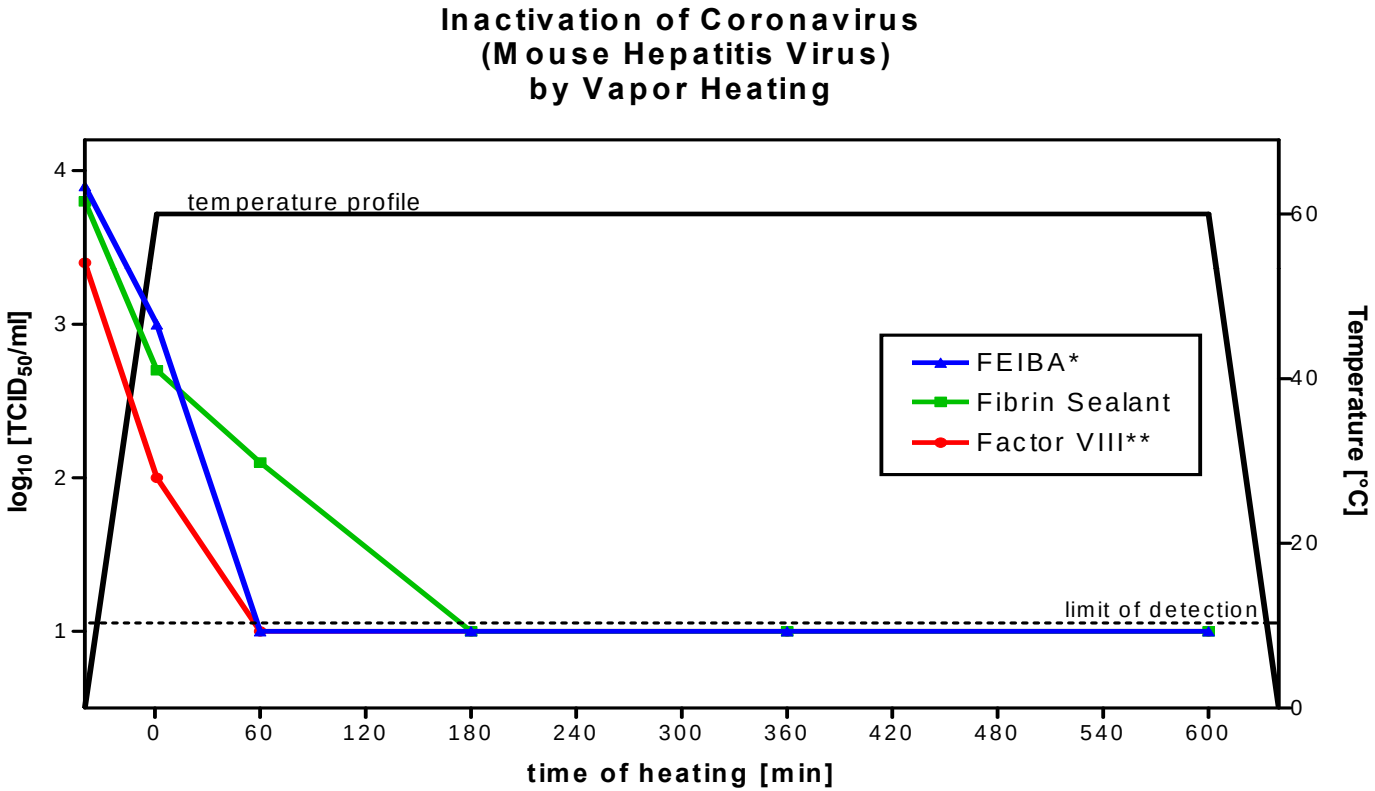
Inactivation technologies are effective against new emerging pathogens?

New emerging pathogens within the last years:
WNV
SARS
H5N1 bird flu

West Nile Virus: complete inactivation by vapor heating



SARS – completely inactivated by vapor heating



*...mean of 2 studies
**..mean of 3 studies

H5N1 influenza virus (orthomyxovirus) completely inactivated by vapor heating (>5.1 log_{10}) after 6 hours like other lipid-enveloped viruses

TABLE 2. Virus inactivation during freeze-drying followed by vapor heating of FEIBA*

	HIV		BVDV		PRV		HsNh	
Residual moisture (%):	7.0	8.0	7.0	8.0	7.0	8.0	7.0	8.0
Virus stock suspension	7.4	7.2	7.0	6.7	8.0	7.7	6.3	6.5
Spiked process intermediate	6.2	6.1	6.0	5.5	7.1	7.0	5.3	5.0
Spiked and lyophilized intermediate	4.6	4.8	3.2	3.5	4.8	4.6	4.4	4.4
Heated to 59.0°C	3.5	3.2	2.7	2.6	1.1	1.4	4.2	4.2
Heated at								
59.5 ± 0.5°C for 180 min	1.9	2.0	1.1	0.9	1.8	<1.1	2.9	2.7
59.5 ± 0.5°C for 360 min	1.7	1.8	<1.1	<0.8	<1.1	<1.1	2.0	<0.8
59.5 ± 0.5°C for 505 min	1.9	1.4	<1.1	<0.8	<1.1	<1.1	<0.8	<0.8
Heated to 79.0°C	0.8	0.8	<1.1	<0.8	<1.1	<1.1	<0.8	<0.8
Heated at								
79.5 ± 0.5°C 30 min	<0.8	<0.8	<1.1	<0.8	<1.1	<1.1	<0.8	<0.8
79.5 ± 0.5°C 55 min	<0.8	<0.8	<1.1	<0.8	<1.1	<1.1	<0.8	<0.8
Reduction factor (log)†	>5.9	>5.8	>5.6	>5.6	>6.7	>6.6	>5.3	>5.1

* Results are reported as $TCID_{50}$ per mL.

† Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

TR Kreil et al., Transfusion 47, 452-459 (2007)

Summary and Conclusion

- Today‘s plasma products have reached a high level of safety
  No transmission of HIV and hepatitis viruses occurred since implementation of testing and inactivation technologies

- There are two groups of safety measures
  - Donor and plasma screening to avoid contamination of plasma pools
  - Pathogen inactivation/removal techniques

- PCR testing of plasma was shown to minimize the risk of virus transmission by shortening the diagnostic window

- Virus inactivation and removal technologies during manufacturing were demonstrated to be effective against lipid and non-lipid enveloped known and new emerging viruses

- The Pathogen Safety Program **exceeds the QSEAL certification criteria** by:
  - a higher quality of plasma (below requested viral marker rate standard)
  - an expanded PCR test program (lower PVB19 cut-off and HAV testing)