



Contribution to the Global Virus Safety Against Emerging Viruses

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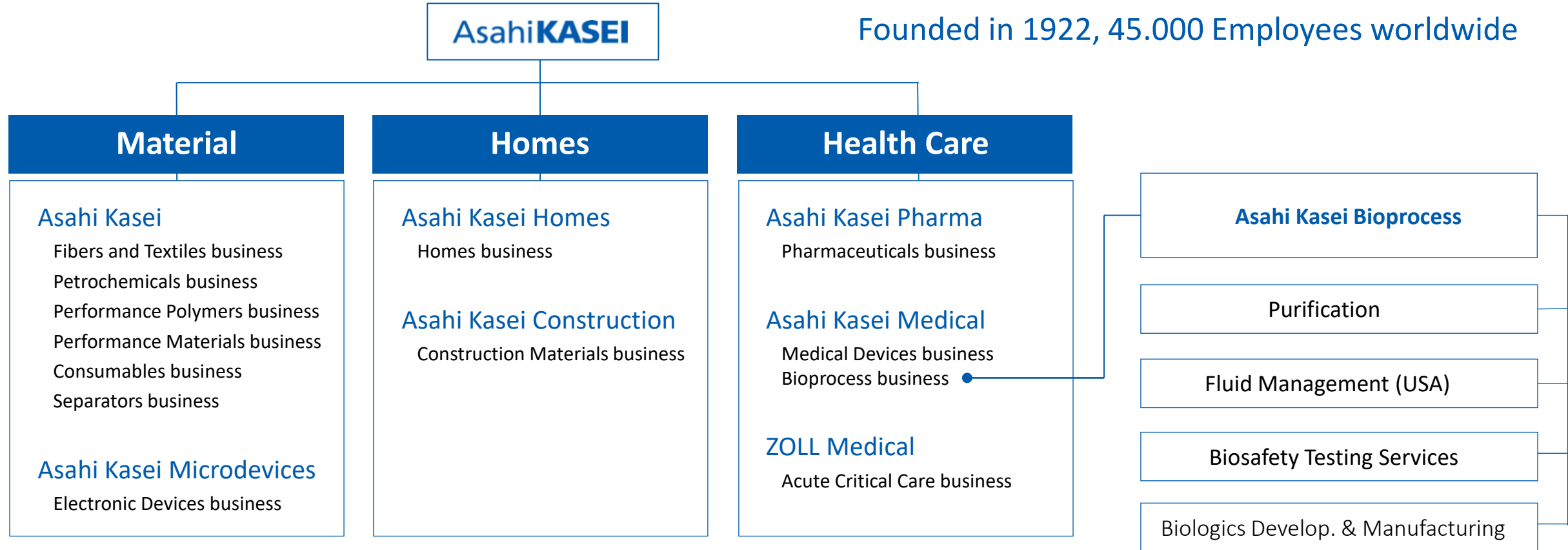
- HCV, Zika, Chikungunya, Mayaro and HEV -

3. Conclusion

1

Introduction

Introduction to Asahi Kasei



Asahi Kasei Bioprocess Portfolio

Asahi Kasei Bioprocess

PURIFICATION

PLANOVA™



Virus Removal Filters

Assurance Beyond Expectation

BioOptimal™ MF-SL



Microfilters

FLUID MANAGEMENT



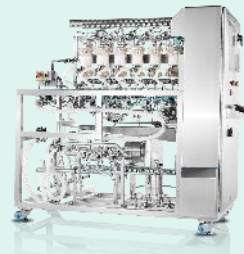
Oligonucleotide
Synthesis



MOTIV™
Inline Buffer
Formulation



Chromatography



Virus
Filtration

Built For You™

BIOSAFETY TESTING SERVICES

VIRUSURE



Virus/Prion Clearance Studies

bi/nique



Mycoplasma Testing Services

Quality is no coincidence

BIOLOGICS DEVELOPMENT & MANUFACTURING

Bionova
Scientific®



End-to-end Process Development

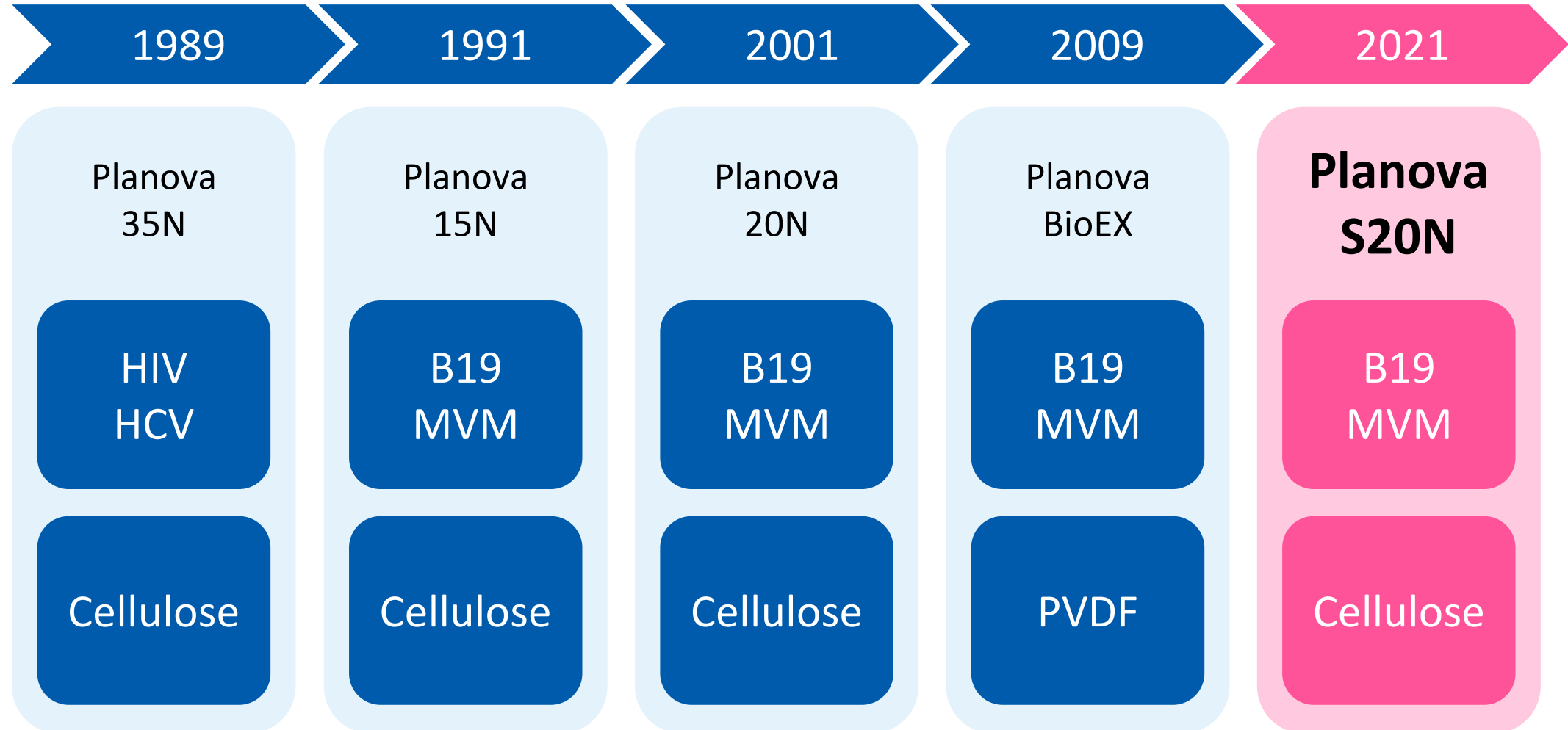


GMP Manufacturing

Where concept becomes cure

History of Planova Products

Pioneering virus removal filtration technology since 1989.



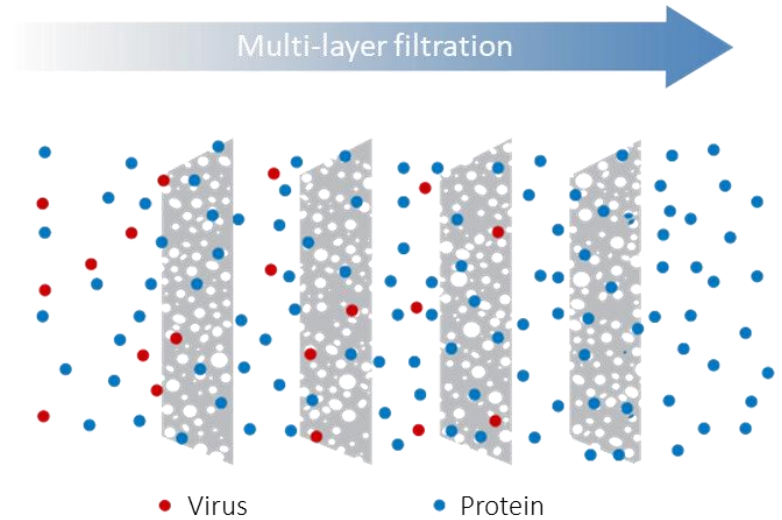
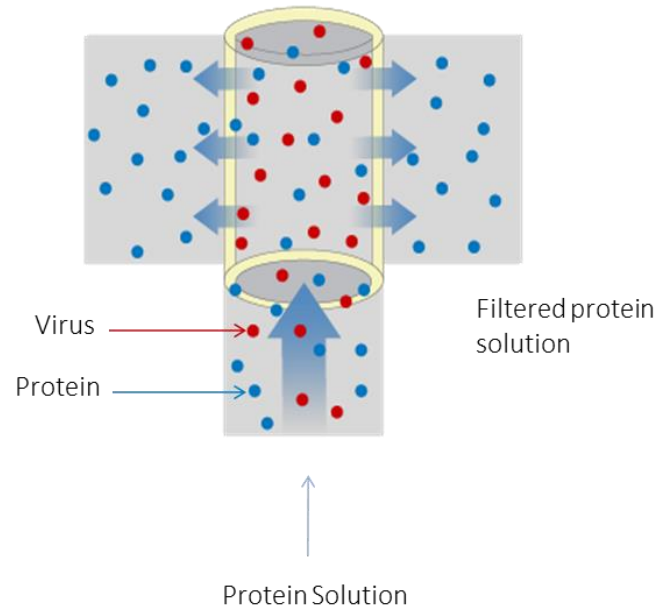
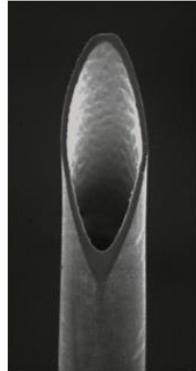
Filtration Mechanism: Size Exclusion

Size exclusion enabled by pore structures across the membrane that trap viruses and allow protein molecules to pass through.

Planova filter

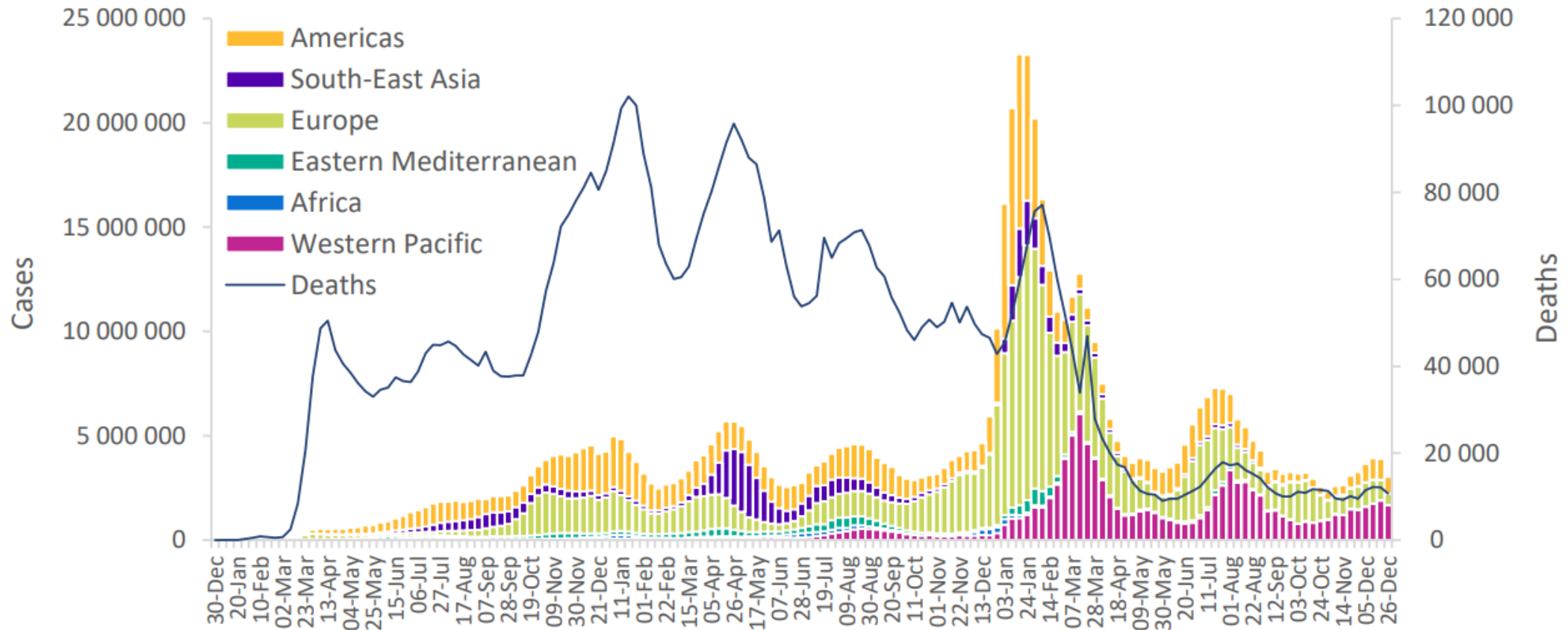


Hollow fiber



Introduction: Global Overview of the SARS-CoV-2

COVID-19 spreads rapidly across the globe within a few years.



Introduction: Outbreak Recurs

Virus outbreak recurs over the years.

Virus	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	Total appearance
Yellow fever																						19
Ebola haemorrhagic fever																						17
Zika virus																						5
Chikungunya																						8
Hepatitis E																						3
Dengue fever																						13
West Nile virus																						5
Poliovirus																						16

Color-filled cells indicate virus outbreak has been reported Disease Outbreak News

Introduction: Potential Risk of the Infection

Emerging viruses are concern of the viral safety of the plasma-derived products.



Emerging Viruses: Proven or Potential Transfusion Transmitted by Blood Products?

Virus	Family	Characteristics
HIV	<i>Retroviridae</i>	Enveloped; RNA
HBV	<i>Hepadnaviridae</i>	Enveloped; DNA
HCV & WNV	<i>Flaviviridae</i>	Enveloped; RNA
CMV & EBV	<i>Herpesviridae</i>	Enveloped; DNA
HTLV-I/II	<i>Retroviridae</i>	Enveloped; RNA
SARS/MERS	<i>Coronaviridae</i>	Enveloped; RNA
WNV	<i>Flaviviridae</i>	Enveloped; RNA
Influenza A	<i>Orthomyxoviridae</i>	Enveloped; RNA
HAV	<i>Picornaviridae</i>	Unenveloped; RNA
Parvovirus B19	<i>Parvoviridae</i>	Unenveloped; DNA
HEV	<i>Hepeviridae</i>	Unenveloped; RNA

Emerging viruses that have actually occurred or are considered to be possible infection cases in the history of virus safety of plasma derived products.

Andy Baily, Virisure
PDA Workshop on virus filtration, 2018

Our Challenges

To ensure the virus safety of biologicals against emerging viruses...

- Asahi launched Planova, the world's first virus removal filter, in 1989 and produced scientific evidences of virus removal with emerging viruses, HIV and HCV
- It was confirmed that Planova could remove TGEV, the model virus of SARS-CoV. Consequently, the safety of biologicals against SARS-CoV is ensured
- In collaborations with the Paul Ehrlich Institut (PEI), the contribution of Planova virus filters towards safety of PDMPs versus emerging viruses was demonstrated

2

Challenge to Emerging Viruses
- HCV, Zika, Chikungunya, Mayaro and HEV -

Hepatitis C Virus (HCV)

Proof of Removal Capability of Infectious HCV.

- Removal trials with HCV (non-A, non-B hepatitis virus at that time) were done.
- It was a in vivo study with chimpanzees at New York University (1989)
- HCV - infection using a combination of 50 nm and 40 nm pore size membrane, while no infection using 35 nm pore size membrane

Demonstrated removal capability for infectious HCV using Planova filters by size exclusion mechanism.

Hepatitis C Virus (HCV)

The size of HCV (around 35 nm) is smaller than that was considered at that time.

HCV DOSE CID50	FILTER PORE/ STEPS	ANIMAL	POSTINOCULATION SURVEILLANCE					
			ALT ^	HISTOLOGY	ELECTRONMICROSCOPY	EIA	RIBI II	PCR
10 ⁴	None	CH-420	+	Hepatitis	Tubules, Undulating membranes	+		+
	40nm + 50nm	CH-422	+	Hepatitis	Tubules, Undulating membranes	+		+
		CH-424	+	Hepatitis	Tubules, Undulating membranes	+		+
	None	CH-101	+	Hepatitis	Tubules, Undulating membranes	+		+
	35nm	CH-671	+	Hepatitis	Rings, Tubules, Undulating membranes	+	+	+
		CH-565	-	No lesions	No ultrastructural changes	-	-	-
10 ⁵	35nm x 2	CH-520	-	No lesions	No ultrastructural changes	-	-	-
		CH-533	-	No lesions	No ultrastructural changes	-	-	-
	None	CH-418	+	Hepatitis	No ultrastructural changes seen	+	+	+
	35nm	CH-546	-	No lesions	No ultrastructural changes	-	-	-
		CH-569	-	No lesions	No ultrastructural changes	-	-	-
10 ⁵	35nm x 2	CH-587	-	No lesions	No ultrastructural changes	-	-	-
		CH-569	-	No lesions	No ultrastructural changes	-	-	-

Muchmore. E, Manabe. S, Ishikawa. G, Hirasaki. T, Alter. H, Shih. J, Yamaguchi. K and Yamamoto. N.
International Symposium on Viral Hepatitis and Liver Disease (the 8th), May 10-14,1993. (excerpted)

Publication of Collaborative Studies with PEI

The results from the collaborations had been published.

Emerging viruses	Publication	Virus filtration	
Zika	Transfusion 2017, 57, 790-796	Virus size 45-48 nm LRV >6.67	Planova 35N
Chikungunya & Mayaro	Viruses 2019, 11, 234	Virus size 66-67 nm LRV >5.57/6.2	Planova 35N
HEV	Transfusion 2020, 60, 2500-2507	LRV >4.4 (IVIG)	Planova 35N

Hepatitis E Virus (HEV)

Identified in 1981, Orthohepevirus, Hepeviridae
Approximate 7,200 nt (+) ssRNA, CAP + poly A tail
Diameter: 27 - 34 nm

Transmission

Fecal-Oral route from water, infected animals, exposure to body fluid of infected animals, etc.

Hepatitis

The course and clinical presentation are highly variable

- Common symptoms of hepatitis such as fever, nausea, abdominal pain, vomiting, etc
- Jaundice in symptomatic cases (40% for developing countries and 75% for developed countries)
- Possibility to lead to acute liver failure

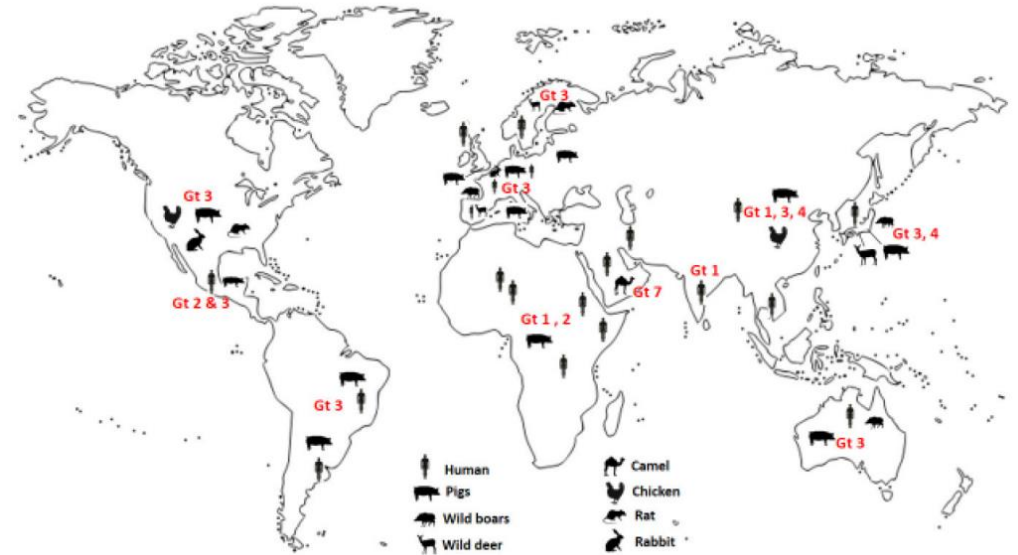


Figure 1. Worldwide prevalence of human hepatitis E cases associated with genotypes 1, 2, 3, 4, and 7 HEV infections.

Genotypes (Gt) 1 and 2 HEV mainly infect humans and are transmitted via fecal-oral route through contaminated water or water supplies. Gt3 and Gt4 HEVs are zoonotic in nature, and are transmitted to human through direct contact with infected animals or through eating undercooked or raw animal meat product. Pigs, wild boar and deer meats are most commonly associated with Gt3 and Gt4 transmission to humans. Gt3 rabbit HEV infection of human is reported from France, and Gt7 camel HEV infection of human is reported from Middle East.

Hepatitis E Virus (HEV)

LE-virus particles were removed with 40-nm filters and 75N.

IM- and NLE-virus particles were removed with 40-nm filters and 35N.

	Cell culture HEV		Human stool HEV
Description	LE 	IM** 	NLE 
Envelope	Lipid enveloped	Non-lipid enveloped	
0.1 µm prefiltration	0.0	0.3	0.1
75N prefiltration	2.8	0.8 (0.5)	0.6 (0.5)
40-nm filtration	>4.4* (>1.6)	3.3 (2.5)	3.6 (3.0)
35N filtration	>5.4* (1.0)	4.8 (1.5)	4.9 (1.3)
30-nm filtration	>5.8 (>0.4)	>5.2 (>0.4)	>5.0 (>0.1)

* Residual PCR signal detected in one of three runs

**IM: S/D treated

*LRVs were determined by RT-PCR

Hepatitis E Virus (HEV)

Removal of HEV IM-phenotype is boosted in the presence of IVIG (boxed area).

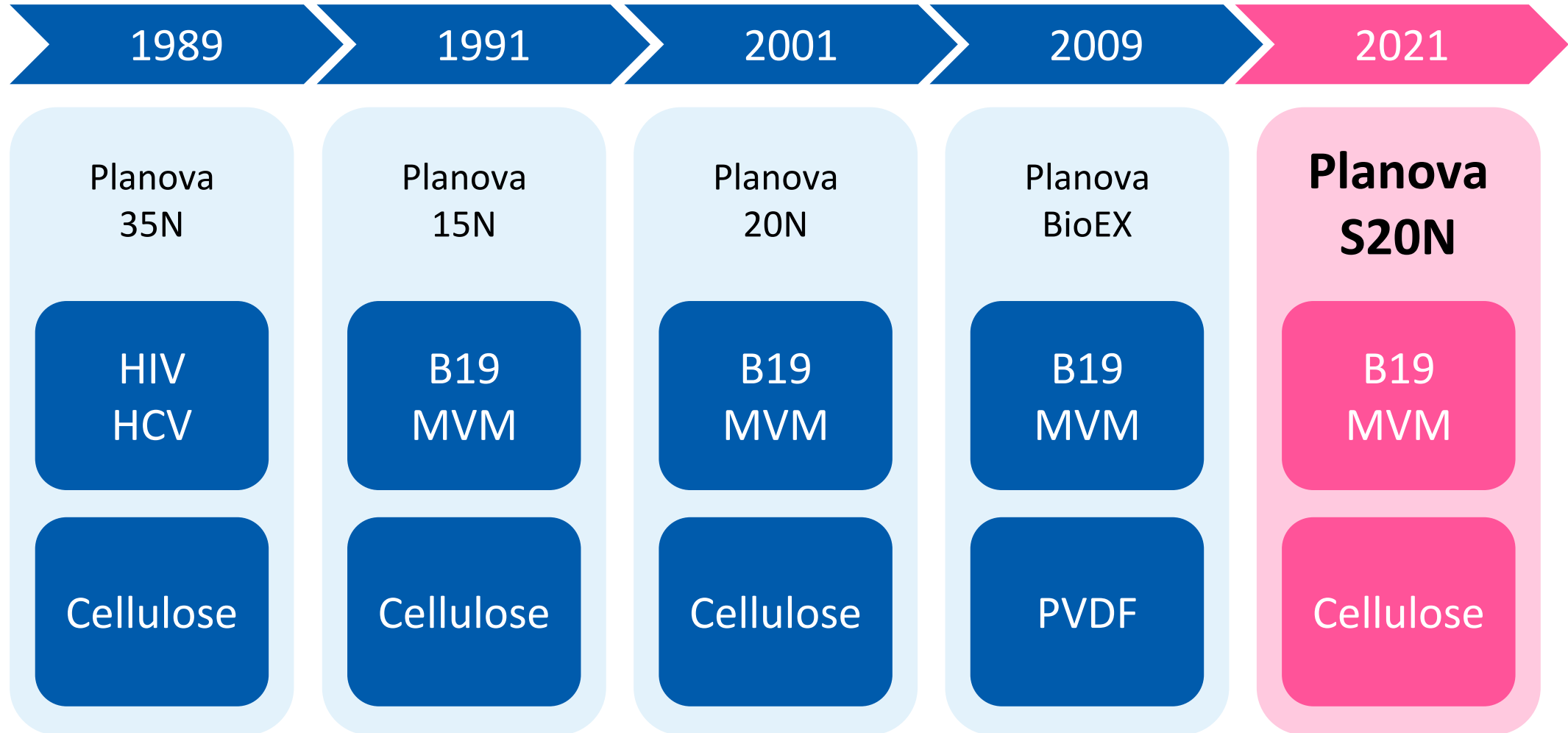
Antibody-enhanced nanofiltration LE-phenotype removal is not impacted by IVIG addition.

Matrix	PBS				IVIG
IVIG added	No	Yes 	No	Yes 	n.a. 
HEV Phenotype	LE 		IM 		IM 
LRV*	4.0	4.1	2.5	5.6	>4.4

*LRVs were determined by RT-PCR

History of Planova Products

Pioneering virus removal filtration technology since 1989.



Planova S20N

Improved performances

Higher flux and throughput

Water flux: 150 LMH @ 216 kPa

Higher pressure filtration

MAX TMP: 216 kPa

Robust LRV

Parvovirus high LRV possible

No process pause impact*

No low-pressure impact*

Improved Operational Efficiency

Simple IT

Leakage test

No GPT



Traditional Planova Qualities

Filtration consistency and reliability

Stable flux with minimal flux decay

Less pH and conductivity impact

Robust virus removal

Less pH, conductivity and flux decay impact

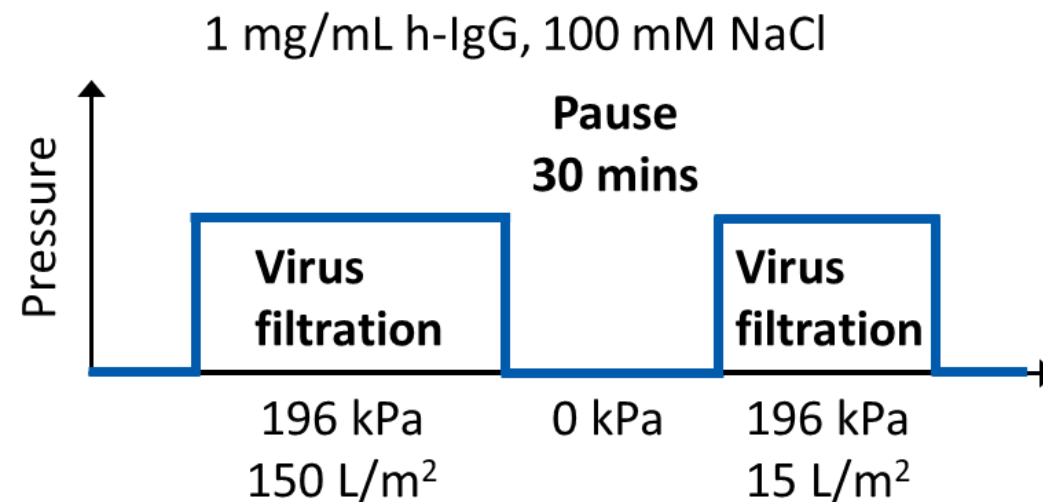
Excellent product recovery

Excellent scalability and high lot to lot consistency

S20N demonstrates high LRV across various viruses

Virus	Genome	Size (nm)	LRV* ¹
MVM	DNA	18 – 24	≥ 5.3
PPV	DNA	18 – 24	≥ 5.8
PRV	DNA	120 – 200	$\geq 5.4^{*2}$
EMCV	RNA	25 – 30	$\geq 5.9^{*2}$
BVDV	RNA	50 – 70	≥ 5.9
HIV	RNA	80 – 120	$\geq 4.1^{*2}$

High LRV for various viruses even with 30 min process pause.



*¹Total Pool LRV after 30 min process pause

*²CRO was contracted to perform the test. Large Volume Assay was introduced to expand the dynamic range of virus testing.

3

Conclusion

Conclusions

To ensure the virus safety of biologicals against emerging viruses...

- Asahi launched Planova including newly released S20N and produced scientific evidence of virus removal with emerging viruses like HCV and HEV.
- Nanofiltration in the downstream process is a crucial step to viral safety, including against emerging viruses, as a robust and efficient method due to the size-exclusion mechanism
- Asahi hopes to continue collaborations to characterize and/or verify virus reduction/inactivation methods to ensure PDMPs safety against emerging viruses

We will contribute to the virus safety of biologicals against emerging viruses.

Acknowledgement

Special thanks to:

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AsahiKASEI

BIOPROCESS

감사합니다

Gracias

Danke

Благодаря

谢谢

Tack

धन्यवाद

Dziękuję

Спасибо

Thank You

Obrigado

Děkuju

Grazie

Ευχαριστώ

Merci

Köszönöm

ありがとうございました

Teşekkür ederim