





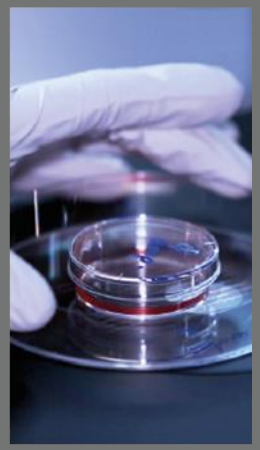


HEV RISK ASSESSMENT FOR PLASMA PRODUCTS

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INTRODUCTION

- Proven HEV transfusion risk (France, Japan, UK, Germany…)
  - +20 notifications in France (ANSM)
  - **12 Post – transfusion notifications (HEV RNA +ve Plasma) received at LFB**

    ➔ 11 donations fractionated; 3 on stock (discarded)

- Confirmed high seroprevalence and incidence in France (geno. 3)
  - endemic (hyper- in some areas): 22,4 % (8-86,4 %) *(Mansuy, Hepatology 2016)*
  - 1 HEV RNA +ve / 2,218 donations *(Gallian, EID 2014)*
  - **5 Look-back information from SD plasma HEV RNA testing**

    ➔ 4 donations fractionated; 1 on stock (discarded)

- No transmission documented from plasma derivatives
  - risk assessment for PDMP

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ASSESSING THE RISK FOR VIRUS TRANSMISSION

- Guideline on plasma-derived medicinal products EMA/CHMP/706271/2010 - Chapter 9
  - *…where possible, include a quantitative estimation of the probability of a virus contaminant being present in a defined dose of final product*
  - *"overall virus inactivation/removal capacity" of the mfg process*
    - CPMP guideline on virus validation (CPMP/BWP/268/95)
    - for emerging viruses:
      - relevance of model viruses (ie HAV 27 – 30 nm for 15/20 N Nanofiltration)
      - investigational studies recommended
  - *"potential virus input": potential amount of virus that may be present in the amount of starting material needed to manufacture a single dose of product*
    - number of viraemic donations in the mfg pool, volume of individual donations, titre of a viraemic donation
    - amount of plasma for production of one vial of product

OUTLINE

- Introduction
- Parameters of Risk assessment for PDMP
  - Exposure: Viremia in HEV RNA +ve donations ➔ IU/ml
  - Reduction: virus Inactivation / Elimination steps ➔ Infectivity
    - HEV infectivity assay
    - Nanofiltration 35 nm
    - Efficacy of VI/VE steps (summary)
  - Correspondance between HEV RNA and Infectivity
    - Towards definition of a *minimum infectious dose* for Risk assessment of PDMP

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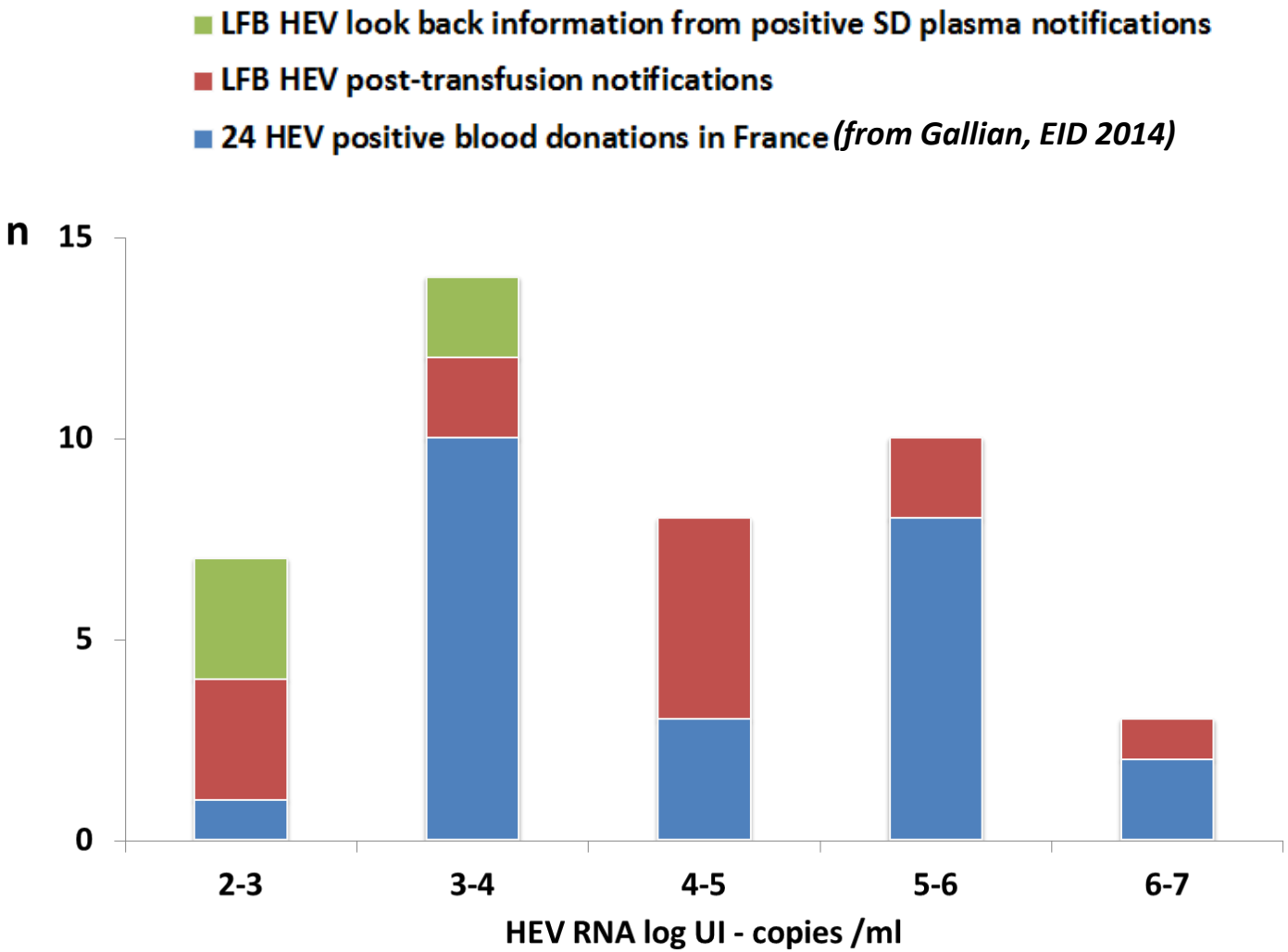
VIREMIA IN HEV RNA +VE DONATIONS



- low to moderate viremia in HEV +ve donations

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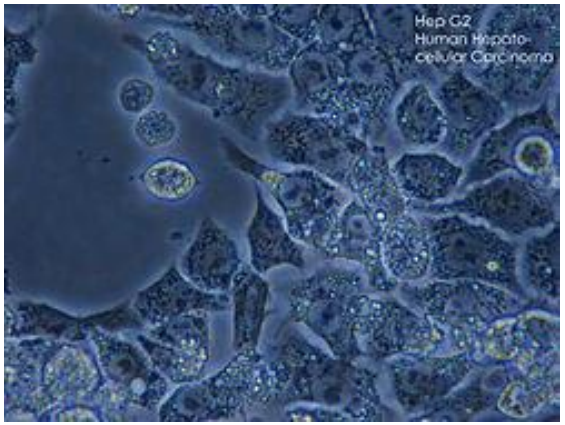
HEV INFECTIVITY ASSAY
(LHOMME et al, J.VIROL 2014)

VIROLOGY Laboratory, CH TOULOUSE, FRANCE, Pr J. IZOPET

- HEV (genotype 3f) sources:
  - faecal sample from acute HE patient
  - cell supernatant
- HEV replicating Cells: Hep G2/C3A (ATCC)
- Virus stock: 10^8 – 10^{10} HEV RNA copies/ml - ~ 5 log $TCID_{50}$ / ml
  - HEV RNA/$TCID_{50}$ Ratio = 4,28 log copies +/- 0,91 (n=7)
- Detection and quantification of HEV RNA
  - in house RT-PCR *(Abravanel, J Clin Microbiol 2012)*
- Titration
  - Infectivity Assay ($TCID_{50}$/ml)
    - *de novo* HEV RNA (RT-PCR) in cell culture
    - infectivity titration (+ve wells): Reed et Muench
  - Q-PCR (nanofiltration experiments)



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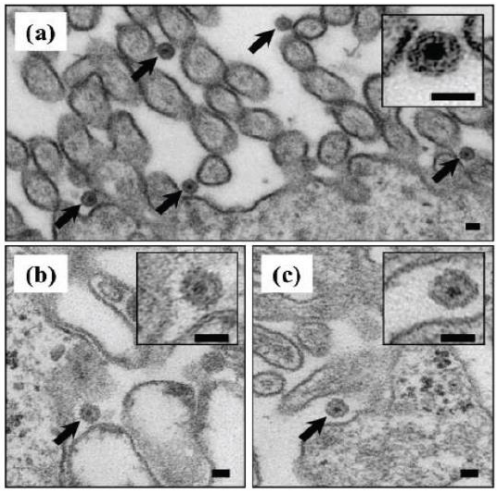
HEV ~ ENVELOPED VIRUS: IMPACT ON SIZE AND SERONEUTRALIZATION

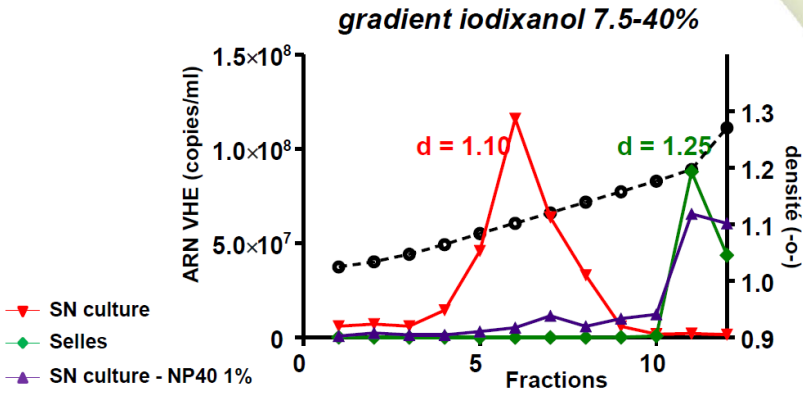
Nagashima et al, *J Gen Virol* 2014 95 2166-2175,
Takahashi et al, *J Clin Microbiol*, 2010, 48 1112-25

- HEV in feces and bile: non enveloped
- In circulating blood and culture supernatant, HEV covered with a cellular membrane similar to EV
- Estimated Size (TEM)
  - membrane associated HEV particles ~50 nm (secretion associated with exosomes)
  - without outer membrane: 30 – 35 nm diameter
- Buoyant density (gradient)
  - HEV in serum (and culture): 1,15 - 1,16 g/ml
  - HEV in faeces: 1,27 – 1,28 g/ml
- HEV in serum non neutralizable by immune sera the difference to HEV from feces



PLC/PRF/5 (a), HepG2 (b) and A549 (c) cells infected with cell culture-produced HEV (JE03-1760F strain). Arrows = extracellular membrane-associated virus-like particles.

Scale bars: 50nm.



35 N NANOFILTRATION OF VWF

Study Design

HEV CELL SUPERNATANT + NP40 1%

HEV_{NP40} + 0.1µm pre-filtration 5,4 log $TCID_{50}$

Spiking (1%) of VWF solution (0.2 µm pre-filtered**)**

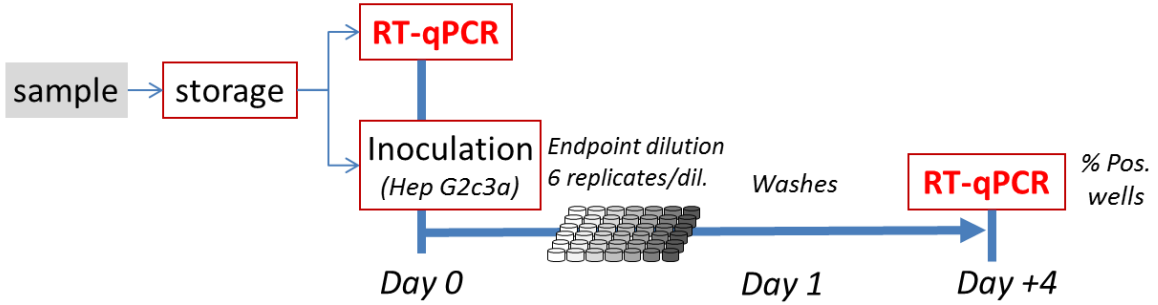
0.1 µm pre-filtration

Load

35nm Nanofiltration

Filtrate

Analysis of samples



HEV REDUCTION – NANOFILTRATION PLANOVA 35N

PRODUCT	Von Willebrand Factor (VWF)	
Virus (treatment)	HEV Cell Supernatant (+ Detergent - NP40)	
	Viral load (log $TCID_{50}$)	**RNA copies (log)**
Spike	5,4	9,5
Load (0,1 µm)	5,3	9,3
(Load 2 – after low pH)	-	-
35 N Filtrate	2,8	7,2
RF	**2.5**	**2,1**

HEV REDUCTION – NANOFILTRATION PLANOVA 35N

PRODUCT	Von Willebrand Factor (VWF)		VWF
Virus (treatment)	HEV Cell Supernatant (+ Detergent - NP40)		**HAV** (+ CHCl3)
	Viral load (log $TCID_{50}$)	**RNA copies (log)**	**Viral load (log $TCID_{50}$)**
Spike	5,4	9,5	7,4 (load 1)
Load (0,1 µm)	5,3	9,3	7,4
(Load 2 – after low pH)	-	-	-
35 N Filtrate	2,8	7,2	7,0
RF	**2.5**	**2,1**	**0,4**

HEV REDUCTION – NANOFILTRATION PLANOVA 35N

PRODUCT	Von Willebrand Factor (VWF)		VWF	IgG
Virus (treatment)	HEV Cell Supernatant (+ Detergent - NP40)		**HAV** (+ CHCl3)	HEV Cell Supernatant (+ EtOH 22%)
	Viral load (log $TCID_{50}$)	**RNA copies (log)**	**Viral load (log $TCID_{50}$)**	**RNA copies (log)**
Spike	5,4	9,5	7,4 (load 1)	9.4
Load (0,1 µm)	5,3	9,3	7,4	9.5
(Load 2 – after low pH)	-	-	-	9.2
35 N Filtrate	2,8	7,2	7,0	6.1*
RF	**2.5**	**2,1**	**0,4**	**3.1**

** free RNA accdg to RNase digestion studies*

- Significant reduction of infectivity of HEV through 35 nm nanofiltration of VWF
- HEV reduction on 35 nm filter > HAV (~ no reduction)
- (at least) 3.1 log HEV RNA copies reduction through 35 nm nanofiltration of IgG

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HEV TRANSFUSION CASES (SUMMARY 1/2)

Reference	Country	Component	Volume (ml plasma)	HEV RNA (log IU/ml)	HEV RNA (total – log IU)
Matsui, Hepatol Res 2014	Japan	Platelets	-	-	**6,8**
Hauser, Blood 2014	France	FFP (Intercept)	*Min. 200*	***? (estimate 100 IU/mL)***	***?** (> 4,3)*
Haïm-Boukobza, J Hepatol 2012	France	Platelets	*(estimate 3 – 30)*	**4,2**	**~ 5**
Matsubayashi, Transfusion 2008	Japan	Platelets	-	-	**5,4**
Colson, EID 2007	France	RBCU	**310**	***? (estimate 100 IU/mL)***	***?** (> 4,5 *)*

***data from literature**

HEV TRANSFUSION CASES (SUMMARY 2/2)

Reference	Country	Component	Volume (ml plasma)	HEV RNA (log IU/ml)	HEV RNA (total – log IU)
Huzly, Eurosurv 2014	Germany	Platelets (apheresis)	**196** **247**	**2,1**	**3,85** **3,95**
		Platelets (apheresis)	*No transmission* **208**	**2,7**	-
Hewitt, Lancet 2014	UK	RBC, Platelets, Plasma	**Transmission** *(min 3mL)*	**4,53**	**> 5**
			No transmission	***2,57***	-
Boxall, Transfus Med 2006	UK	RBCU	**Transmission** **30 mL**	***? (estimate 100 IU/mL)***	***?*** *(> 3,5)*
		Platelets	*No transmission* *3 - 4 ml*		-
Matsubayashi, Transfusion 2004	Japan	FFP	**Transmission** *200-300 mL ?*	***? (estimate 100 IU/mL)***	***?*** *(> 4,5)*
		RBCU	*No transmission* *30-100 mL ?*		-

***data from literature**

HEV TRANSFUSION CASES (SUMMARY 2/2)

Reference	Country	Component	Volume (ml plasma)	HEV RNA (log IU/ml)	HEV RNA (total – log IU)	No transmission
Huzly, Eurosurv 2014	Germany	Platelets (apheresis)	**196**	**2,1**	**3,85**	-
			247		**3,95**	-
		Platelets (apheresis)	No transmission **208**	**2,7**	-	**4,5 log**
Hewitt, Lancet 2014	UK	RBC, Platelets, Plasma	**Transmission** *(min 3mL)*	**4,53**	**> 5**	-
			No transmission	***2,57***	-	***min 3 log***
Boxall, Transfus Med 2006	UK	RBCU	**Transmission 30 mL**	***? (estimate 100 IU/mL)***	***? (> 3,5)***	-
		Platelets	*No transmission 3 - 4 ml*		-	***min 2,7 log***
Matsubayashi, Transfusion 2004	Japan	FFP	**Transmission** *200-300 mL ?*	***? (estimate 100 IU/mL)***	***? (> 4,5)***	-
		RBCU	*No transmission 30-100 mL ?*		-	***min 3,5 log***

***data from literature**

REVIEW OF HEV TRANSFUSION CASES-FRANCE

- Data from 22 transfusion HEV transmission cases in France
  (19 cases from ANSM, 08/2006 – 07/2014, updated from LFB data)
  - imputability: 14/19 certain, 5/19 probable
  - all blood components involved: Plasma (S/D plasma, quarantined FFP, AI FFP), RBC, Platelets (pooled Platelets concentrates, apheresis platelets)
  - HEV RNA Quantification: 20 (?*) – 2,5 10^5 copies/ml
  - Considering minimal plasma volume per component (worst case) total HEV RNA content in donations which transmitted
    - **20/22 cases > 3.9 log IU** (11/22 > 5 log)
    - 2 (probable) cases: 3,16 (?*) - > 3,45 log IU

* *genotype divergence*

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SUMMARY AND CONCLUSION

- No evidence to date that HEV represents a risk to PDMP: no history of residual hepatitis risk through the use of PDMP
- Historical HEV circulation in developed countries with high seroprevalence and incidence in donor populations
- Risk assessment for PDMP (PTC)
  - low to moderate viremia in HEV RNA +ve donations
  - efficient steps towards NEV in PDMP manufacturing processes
  - available data from transfusion cases favour a 4 log HEV RNA correspondence to Infectivity which should be considered in the HEV risk assessment model for PDMP

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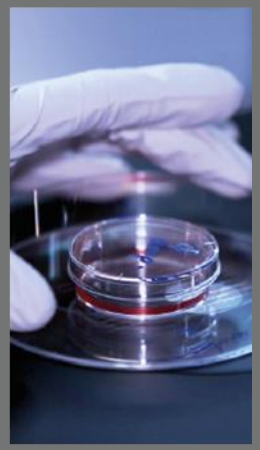
THANK YOU…







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