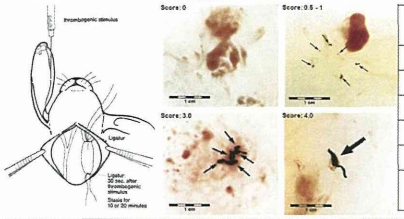
The *In Vivo* Wessler Test

Baxter



Quantitative evaluation of thrombogenicity	Score
no clot	0
few macroscopic strands of fibrin	0.5 - 1
several small thrombi	2
two or more large thrombi	3
several large thrombi	3.5
single thrombus forming a complete clot in vein segment	4

Product	Wessler Score				Average
	male	male	female	female	
Gammagard Liquid	2	1	2	0.5	1.38
Competitor 1 (tested in 2009)	4	3.5	4	4	3.88

Conclusions:

In the Wessler *in vivo* test KIOVIG showed a very low thromboembolic potential

The *in vivo* test results are consistent with the *in vitro* test results

Pro-coagulant Activity Removal: Conclusions

Baxter

- **Baxter´s KIOVIG process has an excellent removal capacity for procoagulant activities**
  - NAPTT, chromogenic substrates and the *in vivo* Wessler test were already used for the development of KIOVIG
  - Only 1.9% of FXI zymogen / FXIa present in Cohn pool are found in Precipitate G
  - In the PptG intermediate procoagulant activities are already low
  - The downstream chromatographic process removes procoagulant activities to an acceptable low level even if 100 U/L FXIa were spiked into PptG suspension
- **Low procoagulant activity in KIOVIG final container were confirmed *in vivo* with the Wessler test and *in vitro* with a broad range of tests established at Baxter**
- **KIOVIG/Gammagard Liquid was shown to have the lowest thrombotic adverse events in the study published in Transfusion by Daniel G.W. et al.: "Immune globulins and thrombotic adverse events as recorded in a large administrative database in 2008 through 2010"**

G.W. Daniel, M. Menis, G. Sridhar, D. Scott, A. E. Wallace, M. V. Ovanesov, B. Golding, S.A. Anderson, J. Epstein, D. Martin, R. Ball, and H. S. Izurieta, (2012) Transfusion http://onlinelibrary.wiley.com/doi/10.1111/j.1537-2995.2012.03589.x/pdf

Conclusions

Baxter

Baxter has almost 60 years of fractionation experience

Donor, donation and manufacturing related safety measures guarantee the high safety standard of Baxter plasma products

Baxter delivers albumin in glass vials and in unique flexible containers with improved efficiency, safety and ease of administration

Pharmacovigilance data demonstrate the excellent safety of Baxter´s albumin

KIOVIG/Gammagard Liquid was used as a reference preparation having the lowest thrombotic adverse events in the study published in Transfusion by Daniel G.W. et al

Acknowledgements

Baxter

Experiments:	Ursula Mais-Paul
	Brigitte Talir
In vitro tests:	Johan Deprez
	Martina Simon
	Alfred Weber
Wessler test:	Eva-Maria Muchitsch
Coagulation expertise:	Hans-Peter Schwarz
Global Pathogen Safety:	Gerhard Pölsler
Stability:	Dagmar Racz

資料３：「Significant Differences of Properties between Model viruses and target viruses in HAV,HEV and B19 during manufacturing processes of plasma derivatives」
Mikihiro Yunoki

Significant differences of properties between model viruses and target viruses (wild type) in HAV, HEV and B19 during manufacturing processes of plasma derivatives

Speaker: Mikihiro Yunoki [1,2,3]
Co-authors: Katsuro Hagiwara [2] and Kazuyoshi Ikuta [3]

[1] Research and Development Division, Japan Blood Products Organization, Japan.
[2] School of Veterinary Medicine, Rakuno Gakuen University, Japan.
[3] Department of Virology, Research Institute for Microbial Diseases, Osaka University, Japan

Guidance for virus inactivation and/or removal

Base guidance (also see others)

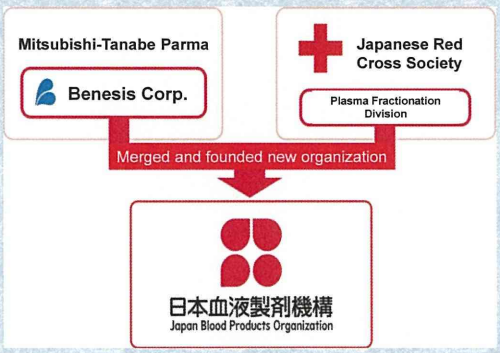
- ICH Topic Q5A (R1), 1999 (ICH)
- WHO Technical Report, Series No. 924, 2004 (WHO)
- CPMP/BWP/268/95r1, 1996 (EMA)
- Iyakuhatsu1047, 1999 (MHLW Japan)

Guidelines required to use model viruses in accordance to GLP/GMP with notify the differences of virus properties between clinical isolated virus and the model.

http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q5A_R1/Step4/Q5A_R1__Guideline.pdf
http://www.who.int/bloodproducts/publications/WHO_TRS_924_A4.pdf
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003684.pdf
http://www.mhlw.go.jp/new-info/kobetu/iyaku/kenketsugo/5l.html

Japan Blood Products Organization

2012.10.1



Purpose of virus clearance study

Evaluate
the virus inactivation/removal ability of the step

Clarify
the characteristic features
the mechanism such as inactivation and/or removal of the step

Confirm
whether constructed more than two inactivation and/or removal steps during the manufacturing (for enveloped viruses)

Virus clearance study is an evaluation, do not guarantee/validate the virus inactivation and/or removal ability of the clinical virus threat.

Today's presentation

- Purpose of virus clearance study.
- Relationship of the model viruses and clinical isolated viruses.
- Characteristic comparison of B19 and the model virus.
- Characteristic comparison of HAV and the model virus.
- Characteristic comparison of HEV and the model virus.
- Points to consider in the evaluation of specific viruses inactivation and/or removal using the model viruses.

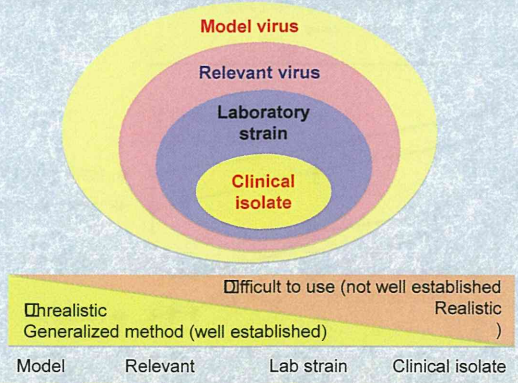
Model / Relevant virus cases for clearance study

Model / Relevant virus	Model for	Size (nm)	Nucleic acid	Envelope
HIV-1 Human immunodeficiency virus-1	HIV-1/2	100–120	ss-RNA	Yes
BHV Bovine herpes virus type-1	Herpes (HBV)	150	ds-DNA	Yes
BVD Bovine viral diarrhoea virus	HCV (HBV)	40–60	ss-RNA	Yes
EMC Murine encephalomyocarditis virus	HAV	30	ss-RNA	No
CPV Canine parvovirus	Parvovirus B19	20–26	ss-DNA	No

Case of former Benesis Corp

Bioplasma World Asia, Bali

Relationship of the model, relevant, lab strain and clinical isolated viruses



We should pay attention to the difference properties of these viruses

Bioplasma World Asia, Bali

Considering model viruses for B19

	Human parvovirus B19 (B19)	Porcine / Canine Parvo virus (PPV / CPV)
Family	Parvoviridae	Parvoviridae
Genome	ssDNA	ssDNA
Particle size	22-24 nm	21-26 nm
Envelope type	none	none

Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses
Clin Microbiol Rev. 2002 July; 15(3): 485–505.

Bioplasma World Asia, Bali

Recently topical viruses of threat

Plasma derivatives

- Human parvovirus B19 (B19)
- Hepatitis A virus (HAV)
- Hepatitis E virus (HEV)

Biologicals derived from cultured cells

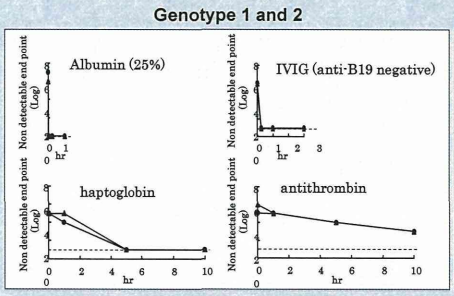
- Mouse minute virus (MVM)
- Porcine circovirus (PCV)

Small particles and resistant to the virus inactivation and/or removal treatment

Bioplasma World Asia, Bali

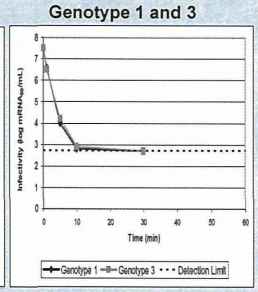
Comparison of heat sensitivity of B19 genotypes 1, 2, 3 by pasteurization

Genotype 1 and 2



Viruses in 25% albumin, IVIG, haptoglobin and antithrombin solution and heat treated at 60 ° C for 10 hr

Genotype 1 and 3



Viruses in 5% albumin solution and heat treated at 56.5 ° C for 60 min. Performed by PEI.

B19 genotype 1, 2 and 3 showed same heat sensitivity

Vox Sang. 2012 Feb;102(2):93-9.
Transfusion. 2012 Jul;52(7):1490-7

Bioplasma World Asia, Bali

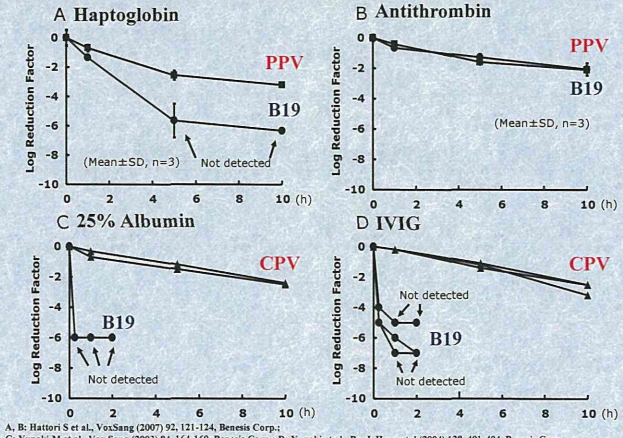
Characteristic comparison of specific virus and the model viruses

B19 and CPV/PPV case (Parvoviruses)

B19: Clinical isolates
PPV/CPV: Established strains (ATCC)

Bioplasma World Asia, Bali

Heat inactivation kinetics of B19 and the model viruses in different solution at 60° C for 10hr

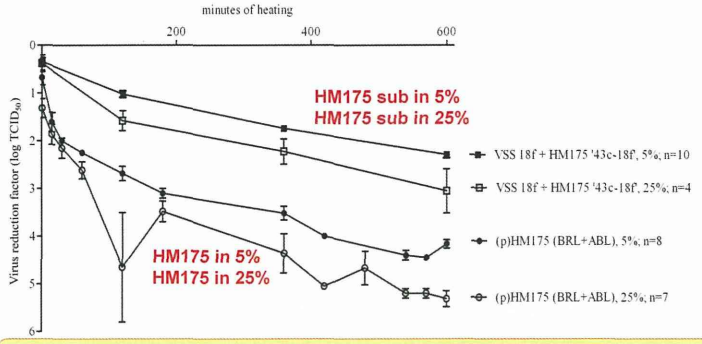


A, B: Hattori S et al., VoxSang (2007) 92, 121-124, Benesis Corp.;
C: Yunoki M et al., Vox Sang (2003) 84, 164-169, Benesis Corp.; D: Yunoki et al., Br. J. Haematol (2004) 128, 401-404, Benesis Corp.

Summary of this part

- No difference of heat sensitivity with B19 genotype 1, 2 and 3 clinical isolates.
- B19 and the model viruses showed different heat inactivation kinetic patterns in albumin and IVIG solution.
- B19 and the model viruses showed similar heat inactivation kinetic patterns in antithrombin and haptoglobin solution.
- These results indicated that B19 and the model viruses CPV and PPV have different nature of the virus.

Different heat kinetics of HAV HM175 and substrain in 5 and 25% albumin at 60° C for 10hr



HM175 and the substrain showed different nature of heat inactivation

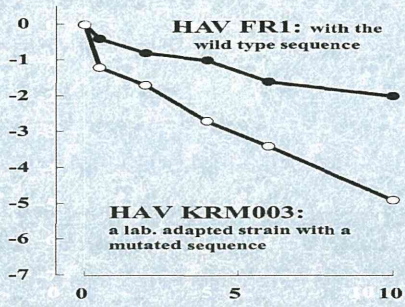
Farcet et al., Transfusion. 2012; 52: 181-187

Characteristic comparison of specific virus and the model viruses

HAV and EMC case

HAV: Passaged lab strains
Wild type lab strain by reverse genetics
Established strain (ATCC)
EMC: Established strain (ATCC)

Different heat kinetics of passaged HAV and wild type HAV by reverse genetics in 25% albumin at 60° C for 10hr



Wild type of HAV could resist to heat inactivation and the lab strain may show heat sensitive nature in the clearance study.

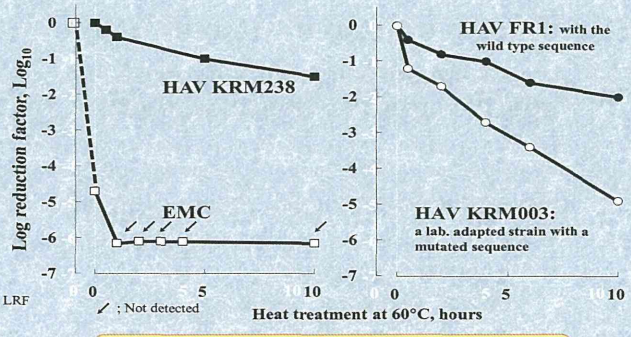
Yunoki et al., Transfusion. 2013; In press

Considering Model Viruses for HAV

	Hepatitis A virus (HAV)	Mouse Encephalomyocarditis virus (EMC)
Family	Picornavirudae	Picornaviridae
Genome	ssRNA (+)	ssRNA (+)
Particle size	27-32 nm	30 nm
Envelope type	none	none

Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses
http://www.who.int/csr/disease/hepatitis/whocdscsredc2007/en/index2.html#morphology

Different heat kinetics of HAV KRM238 and the model virus in 25% albumin at 60° C for 10hr



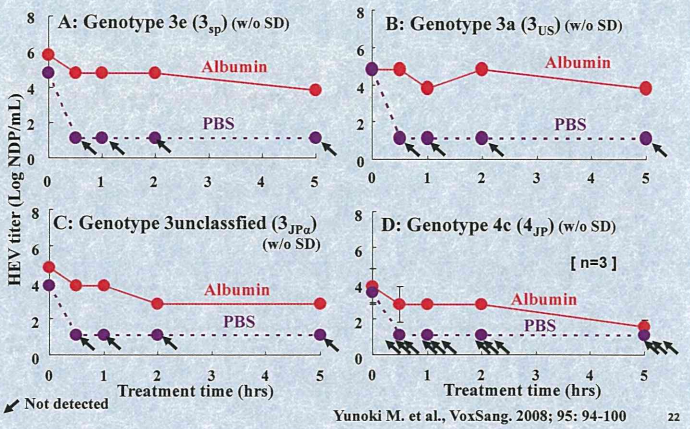
HAV KRM238 and the model virus EMC showed different heat sensitivity

Yunoki et al., Transfusion. 2013; In press

Summary of this part

- Some HAV strains in albumin showed sensitive nature in heat inactivation than wild type strains.
- KRM238 strain showed similar nature with wild type.
- HAV and the model virus EMC showed different heat inactivation kinetic patterns in albumin.
- These results suggested that not only HAV and the model viruses EMC has different nature of the virus but also some HAV lab strains could have different nature.
- We should select the model or relevant virus of HAV for the heat inactivation study carefully.

Inactivation kinetics of four HEV isolates in albumin during 60°C liquid-heating



Yunoki M. et al., VoxSang. 2008; 95: 94-100

Characteristic comparison of specific virus and the model viruses

HEV and EMC/PPV case

HEV: Clinical isolate (human plasma)
Lab strain propagated by animal study (swine feces)
EMC: Established strain (ATCC)
PPV: Established strain (ATCC)

HEV removal by virus removal filters

Load material	Planova Filter	HEV in PBS 3unclassfied (3jpα)	3a (3us)	3e (3sp)	4c (4jp)
Detargent treatment, 0.22μm, 0.1μm filtrate	P-75 (73±2 nm)	(6.2/4.7) 1.5	(8. 9/7.5) 1.4	(8.2/6.9) 1.3	(7.6/6.3) 1.3
P-75 filtrate	P-35 (35±2 nm)	(6.2/4.8) 1.4	(6.9/<3.3) ≥3.6	(6.4/3.8) 2.6	(5.6/4.5) 1.1
	P-20 (19±2 nm)	(6.2/<3.3) ≥2.9	(6.9/<3.3) ≥3.6	(6.4/<3.2) ≥3.2	(5.6/<3.0) ≥2.6
	P-15 (15±2 nm)	(6.2/<3.3) ≥2.9	(6.9/<3.3) ≥3.6	(6.4/<3.2) ≥3.2	(5.6/<3.0) ≥2.6

Condition of filtation
P-75N: Planova 75: 0.001m² module, 50kPa, RT
P-35N: Planova 35: 1cm long single module, 50kPa, RT
P-20N: Planova 20: 1cm long single module, 50kPa, RT
P-15N: Planova 15: 1cm long single module, 50kPa, RT

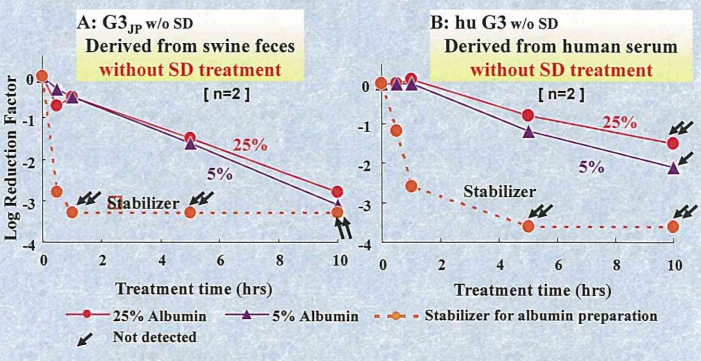
Upper column: Log genome amounts (Before / Filtrate) of HEV were determined by PCR.
Lower column: Log reduction factor

Yunoki M.,et al., , VoxSang (2008) 95, 94-100, Benesis Corp.

Hepatitis E Virus

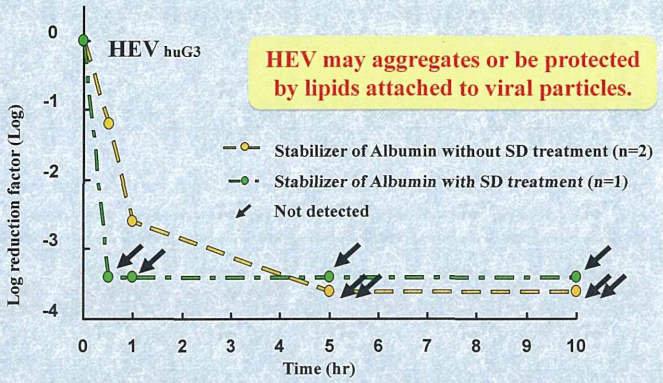
Classification	Family; Hepeviridae Genus; Hepevirus
Pathogenicity	Viral hepatitis
Natural Route of Human HEV Infection	* Food-borne in developed countries * Water-borne in developing countries * Transfusion and Transplantation
Reservoirs	Pig, Wild Boar, Deer etc.
Serotypes/Genotypes	Serotypes: 1 Genotypes: 1 ~ 4 (Genotypes 1, 3 and 4 were found in Japan and major is 3)
Structural Characterization	Non-enveloped ssRNA virus Spherical viral particle, 27 ~ 34 nm in size
Resistance to Inactivations	Low pH: Yes Detergents: Yes Heat: ? (conditions for heat-stability are remained obscure)

Difference of inactivation kinetics between two types of HEV isolates during 60°C liquid-heating



HEV in serum tends to more resistant against heat inactivation in first phase period

Change of kinetics pattern of HEV derived from human serum after SD treatment



HEV properties during heat treatments

- No different patterns were shown using 4 isolates.
- Agent in plasma which is dissociated from HEV particle by detergent could protects HEV during liquid heating.
- Heat sensitivity of HEV derived from human serum and swine feces were slightly different.
- Heat stability of HEV was dependent on concentration of albumin.
- HEV showed similar inactivation patterns during heating to HAV and CPV.

Considering Model Viruses for HEV

	Hepatitis E virus (HEV)	Murine Encephalomyocarditis virus (EMC)	Porcine / Canine Parvo virus (PPV / CPV)
Family	Hepeviridae	Picornaviridae	Parvoviridae
Genome	ssRNA (+)	ssRNA (+)	ssDNA
Particle size	27-34 nm	30 nm	21-26 nm
Envelope type	none	none	none

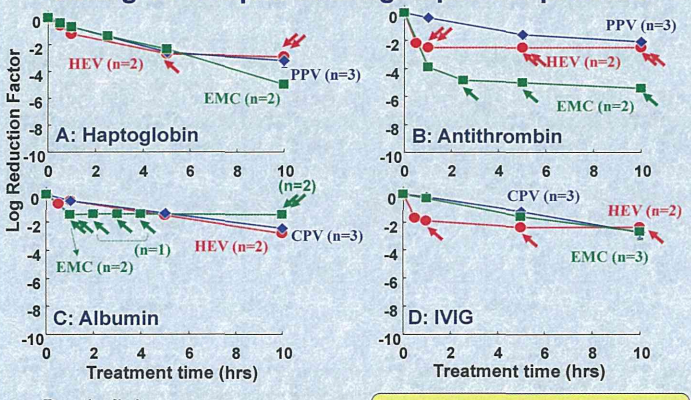
Virus Taxonomy, Ninth Report of the International Committee on Taxonomy of Viruses

Summary of this part

- HEV showed similar inactivation patterns during heating to HAV and PPV.
- The model virus EMC showed more heat sensitive than HEV.

- We should select the model virus of HEV for heat inactivation study carefully.
- Also, should pay attention the derivation and preparation method of HEV

Inactivation kinetics of PPV, CPV, EMC and HEV during 60°C liquid-heating in plasma products



Model viruses of HEV in heat treatment are PPV/CPV?

Summary of virus inactivation and/or removal using small and non-enveloped viruses

Specific viruses:
- Clinical isolates: B19
- Lab strains: HAV, HEV

Model viruses: EMC, CPV and PPV

Virus reduction during ethanol fractionation of albumin preparation

Fractionation Step	Model Virus		Relevant Virus/Origin			
	CPV	EMC	B19 Human Plasma	HAV Cul.Sup	HEV Swine Feces	HEV Human Serum*
I	0.0	0.0	0.0	0.0	0.0	0.0
II+III	≥4.3	2.4	2.9	3.3	2.3	0.0
IV	4.1	5.6	2.5	2.2	0.0	1.3

*: without detergent treatment

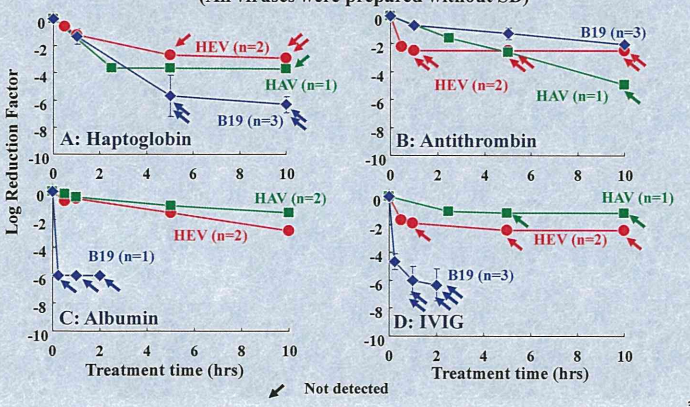
Partitioning property of HEV during ethanol fractionation is not reproducible.

Summary of this part

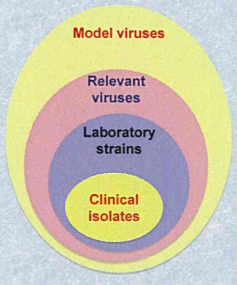
- Model viruses, relevant viruses and clinical isolates could show different nature and result of clearance studies.
- The wild type viruses and lab adapted could show different nature and result of clearance studies.
- Preparation methods of spiking viruses could also affect the viruses nature and the result of clearance studies.
- We should pay attention to the selection of model viruses and preparation methods of spiking viruses and subsequently the interpretation of the result.

Inactivation kinetics of B19, HAV and HEV during 60°C liquid-heating in plasma products

(All viruses were prepared without SD)



Relationship of Model virus, Relevant virus, Lab strain, Clinical isolate



		Relevant virus		Model virus
Target	Clinical isolate	Lab adapted strain	Established strain	Related virus (established strain)
B19	B19 (Heat sensitive in some case)	None	None	PPV/CPV
HAV	None	HAV (Heat sensitive) (Heat resistant)	HAV (Heat sensitive) (Heat resistant)	EMC for virus filter; PPV/CPV for inactivation
HEV	HEV	HEV (Property is different from HEV in plasma)	None	EMC for virus filter; PPV/CPV for inactivation

Virus removal in Albumin and Fibrinogen preparations by virus removal filtrations

Product	Filter	Model virus		Relevant Virus (Origin)		
		CPV	EMC	B19 (Human plasma)	HAV (Culture sup)	HEV (Swine feces)
Albumin	P-15 (15±2 nm)	2.5	≥4.7	5.3	≥3.9	≥3.5
Fibrinogen	P-35 (35±2 nm)	0.0	2.4	0.0	0.0	3.2
	P-20 (19±2 nm)	2.5	≥6.7	1.9	2.2	≥3.9

- Virus amount of CPV and EMC were measured by infectivity assay whereas B19, HAV and HEV were measured by PCR.
- The results were shown as LRF, mean of two experiments.

Research Collaborators

JBPO
Sakai K., Tsujikawa M., Tanaka H., Urayama T., Hattori S., Ideno S., Adan-Kubo J., Ohkubo Y., Yada K., Nishida A., Kashiwara J., Fukunaga U., Miyamoto H., Yamamoto S., Nishigaki H., Takahashi K., Furuki R., Ueda C., Yoshikawa M., Yamamoto I., Tanaka Y., Satake Y., Masuda M., Konoshima Y., Minagi T.

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Other research organizations
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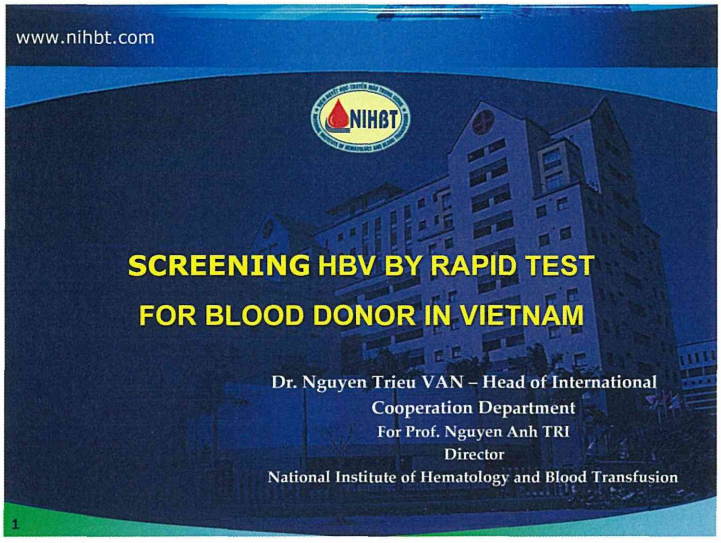
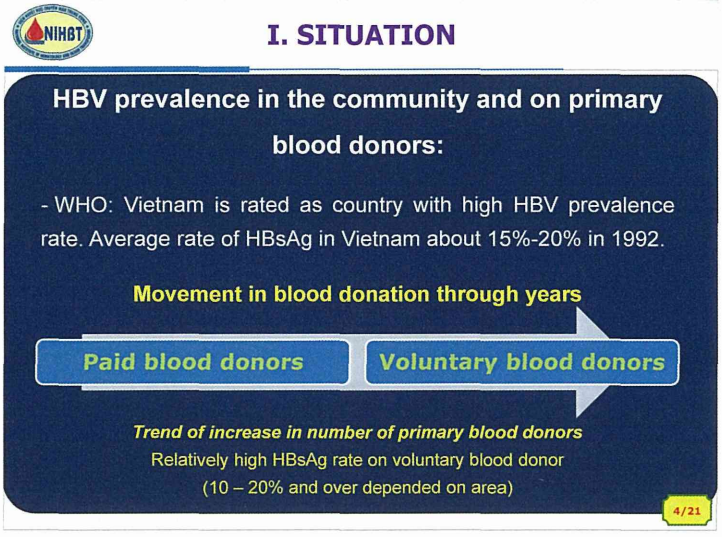
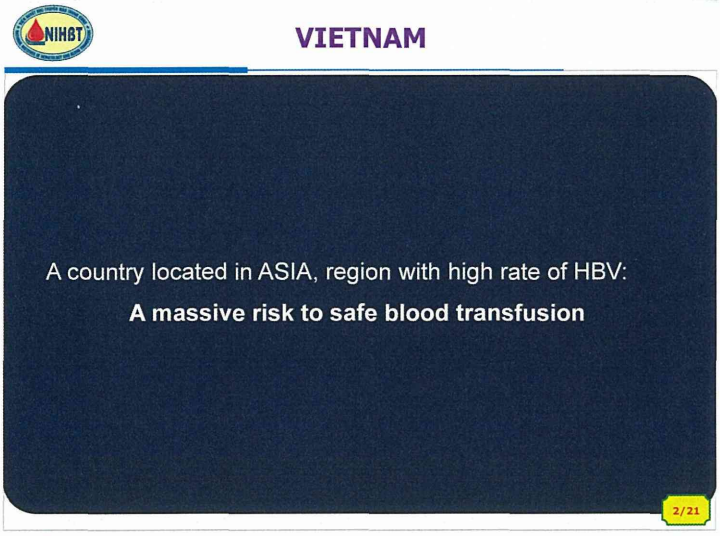
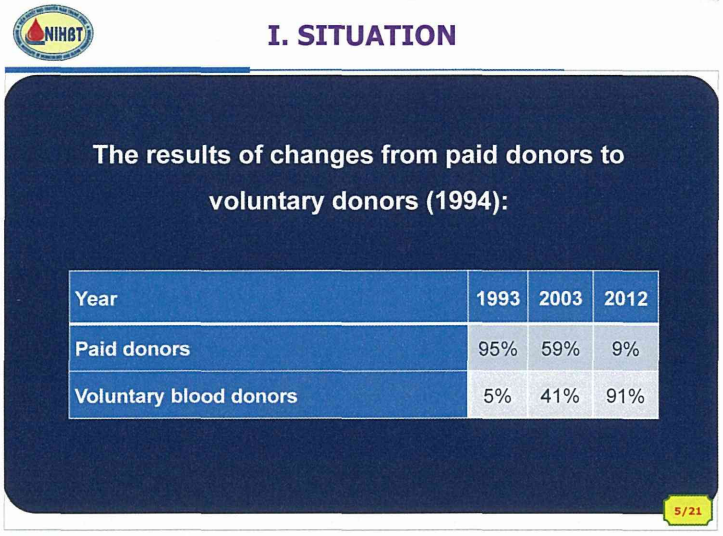
資料４：「Screening HBV by Rapid Test for Blood Donor in Vietnam」

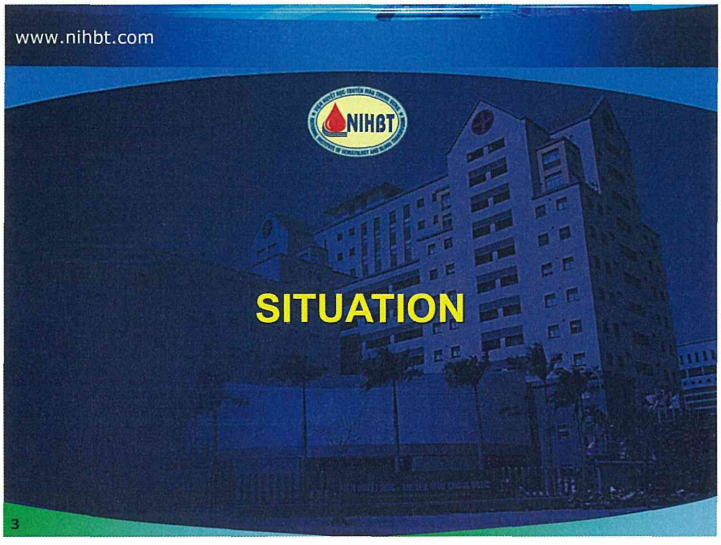
Dr.Nguyen trieu VAN

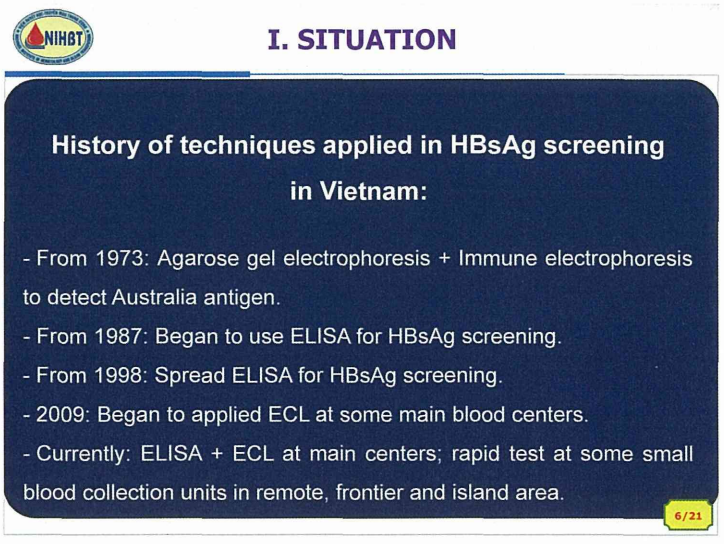












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NUMBER THROUGH YEARS

HBV SCREENING ON VARIOUS TYPES OF BLOOD DONORS

7

NUMBER THROUGH YEARS - HBV SCREENING ON VARIOUS TYPES OF BLOOD DONORS:
Before 1995

Type of donor / Technique	HBsAg rate(+) on paid donors
Agarose gel electrophoresis and Immune electrophoresis	4.14%
ELISA	8-10%

8/21

NUMBER THROUGH YEARS - HBV SCREENING ON VARIOUS TYPES OF BLOOD DONORS:
Period 1995 – 2004

Type of donor / Technique	HBsAg rate(+) on paid donors	HBsAg rate (+) on voluntary blood donors
ELISA	6-9%	13-16%

9/21

NUMBER THROUGH YEARS - HBV SCREENING ON VARIOUS TYPES OF BLOOD DONORS:
Period 2004 – 2008

Type of donor / Technique	HBsAg rate(+) on paid donors	HBsAg rate (+) on voluntary blood donors without recruitment screening	HBsAg rate (+) on voluntary blood donors with recruitment screening
ELISA	< 5%	13-16%	< 3%

10/21

NUMBER THROUGH YEARS - HBV SCREENING ON VARIOUS TYPES OF BLOOD DONORS:
After 2008

Type of donor / Technique	HBsAg rate(+) on paid donors	HBsAg rate (+) on voluntary blood donors without recruitment screening	HBsAg rate (+) on voluntary blood donors with recruitment screening
ELISA & CL (Chemil Luminescence)	< 1%	10-15%	< 1%

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LESSON LEARNT

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BLOOD DONATION RECRUITMENT SCREENING HBsAg BY RAPID TEST

Now: *Add Screening by rapid test*

Before: *Implement on the blood bags*

BLOOD DONATION RECRUITMENT → BLOOD DONATION → BLOOD SCREENING → .....

New method: Blood donation recruitment screening

To reduce HBsAg infected blood bags

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BLOOD DONATION RECRUITMENT SCREENING HBsAg BY RAPID TEST

Timeline

2003 – 2004	• Pilot study
2004 – 2007	• Implement at NIHBT
2007	• Issue national guideline including recommendation on blood donation recruitment screening
2013	• New version of National Guidelines: force to apply blood donation recruitment screening for the whole country

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BLOOD DONATION RECRUITMENT SCREENING HBsAg BY RAPID TEST

Achievement

	2004	2005	2006	2007	2008	2009	2010	2011	2012
At NIHBT	1.6	0.9	0.85	0.8	0.8	0.9	0.93	0.8	0.84
Country	5.2	4.7	4.5	3.6	3.2	3.5	3.21	3.29	2.5

Reduce rate of HBV infected blood units and thrown-away blood units

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THE BENEFIT OF BLOOD DONATION RECRUITMENT SCREENING HBsAg BY RAPID TEST

- Protect health, inform on infection risk and consult to people bring HBV virus and their families.
- Save expenses; by using rapid test may increase testing cost but reduce the main cost on throwing away blood which would reduce the total expense. It is evaluated that by using rapid test could reduce 5 – 8% expense for each blood unit.
- Ensure safety for community, environment as reduce throwing away a large quantity of swab with high risk.
- Ensure humanism of blood donation.
- Contribute on reducing HBV infection risk in the community.

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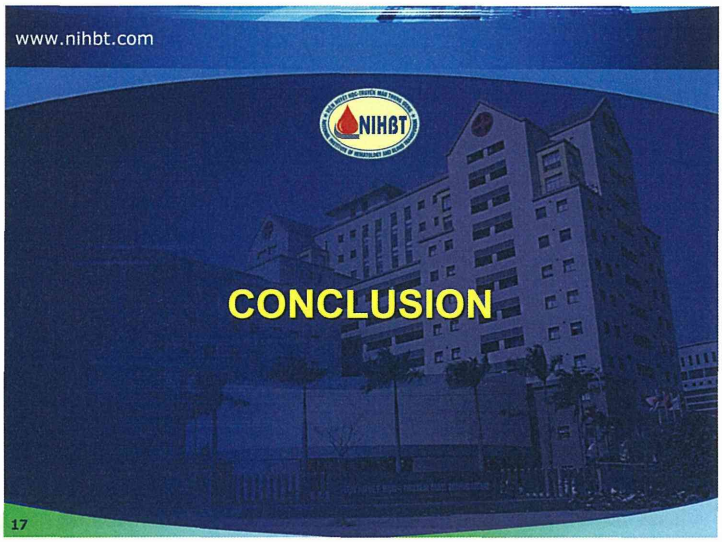

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Application of this method for years prove:

- Reduce on rate of HBV infection on thrown away blood units.
- Save expense on blood collection
- Ensure health for blood donors

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