Robust Inactivation of Yellow Fever Virus 17D Vaccine Strain can be achieved by Photochemical Treatment of Platelet Concentrates

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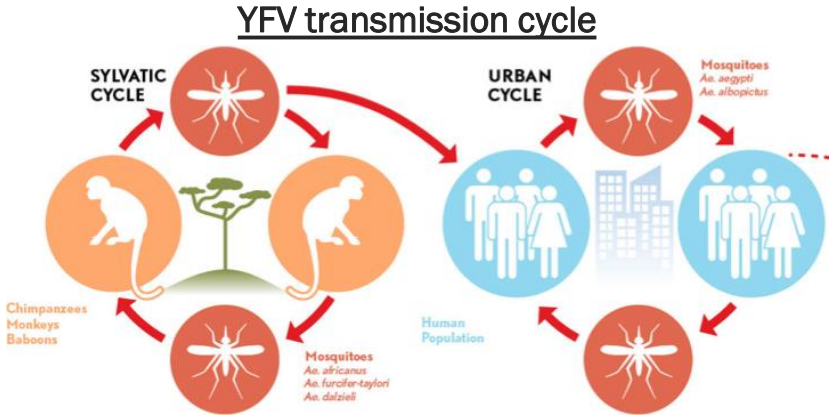
Contents

- Yellow Fever Virus (YFV) characteristics, distribution and outbreaks
- Yellow Fever: a threat to the blood supply
- The INTERCEPT™ Blood System
- Pilot YFV inactivation studies
- Inactivation of arboviruses

Disclaimer:
I am an employee of Cerus. Cerus is commercializing the INTERCEPT™ Blood System.

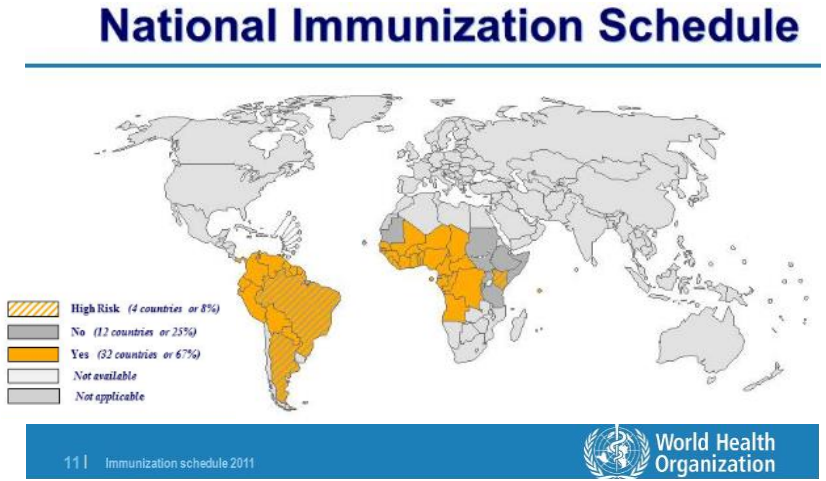
Yellow Fever Virus (YFV)

- Virology:
  - *Flavivirus* / *Flaviviridae*
    - Like Dengue, West Nile, Zika virus
- Clinical outcomes:
  - Mild
  - Hemorrhagic fever
  - Neurotropic / Viscerotropic
- Transmission:
  - Sylvatic (mosquitoes / monkeys)
    - *Haemagogus sp*, *Sabethes sp*,
  - Urban (mosquitoes / humans)
    - *Aedes sp* (*aegypti* and *albopictus*)
  - Transfusion-transmission

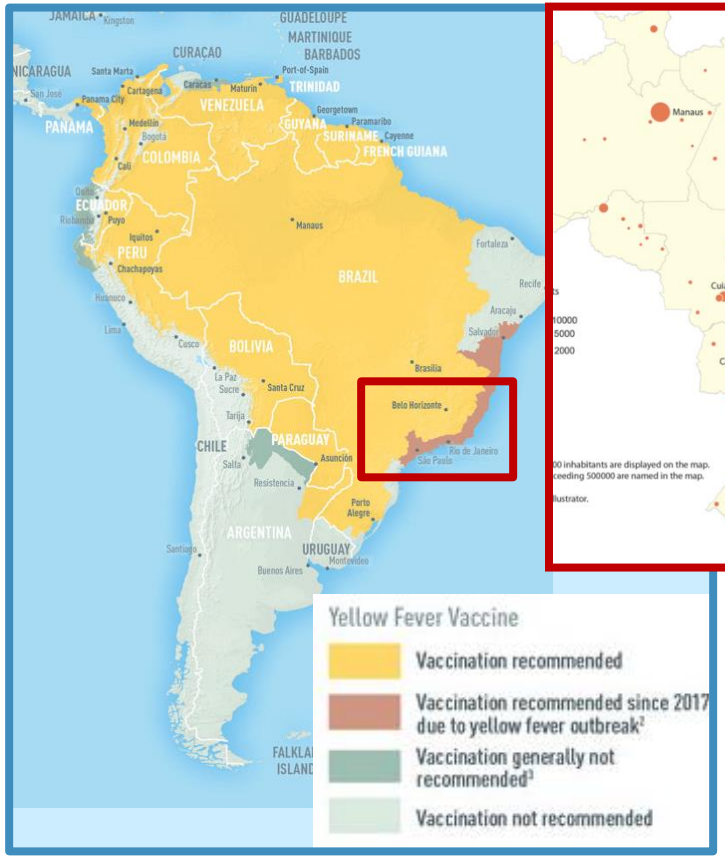


YFV mitigation strategies

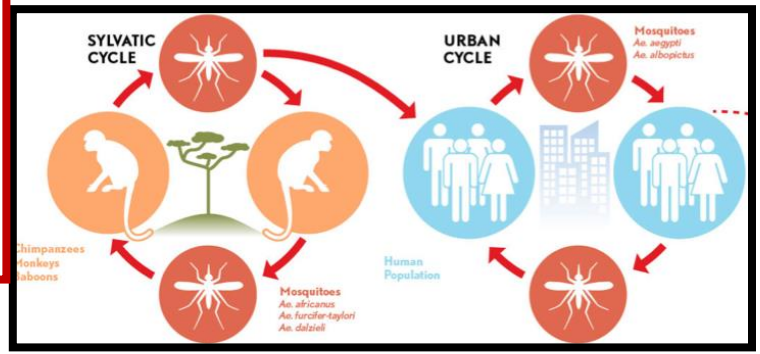
- Epidemiology:
  - Sporadic in South America / Africa
    - Sylvatic transmission (~300 cases/year)
  - Travelers returning from endemic areas
  - Angola 2015-16 / Brazil 2016-18
- No effective antivirals / only supportive
  - Plasma exchange
  - Liver transplantation
- Vaccine (difficult to produce - limited world supply)
  - Live attenuated 17D-YFV vaccine strain
  - Effective
  - Adverse events (Neurologic / visceral invasion)
    - Risk for immunocompromised patients



LATAM at risk areas
2016 - 2018 Brazil YFV outbreak



- June 2016 – May 2018:
- YFV was responsible for
  - 2,034 cases
    - 655 fatalities (32%)
  - Many more unrecognized



- A massive vaccination campaign was undertaken (36.7 million doses delivered)
  - To control the outbreak

First transfusion-transmitted YFV infections in Brazil

Age	Sex	Previous YF vaccine (year)	Blood product received (quantity)	Underlying medical conditions	Symptoms and laboratory abnormalities	YFV IgM	No. of days post-transfusion
Neonate	F	No	Irradiated RBC (4 aliquots)	Prematurity, IVH	None	Neg	37
6 yrs	M	No	Irradiated PC (1 unit)	Wilm´s tumor	None	Pos/ 160	36
66 yrs	M	Yes (1964)	PC (1 unit)	Kidney transplant, diabetes	None	Pos/ 160	33
58 yrs	M	Yes (1975, 1986)	FFP (2 units)	CRF, tuberculosis,psoriasis	None	Pos/ 40,960	26
82 yrs	M	Yes (1959, 1965)	Irradiated PC (1 unit)	B-cell lymphoma	Deceased	NA	-

Data presented by Dr Luiz Amorim, Hemorio at SVTM Swiss congress 2017

Risk of transfusion-transmitted YFV vaccine

- MMWR Jan. 22, 2010 - Evidence of YFV vaccine TTI in the US military
  - 89 military personnel donated blood 4 days after vaccination
    - 6 units were transfused to 5 patients: 1 died, others seroconverted
- Live attenuated virus → viremia after vaccination

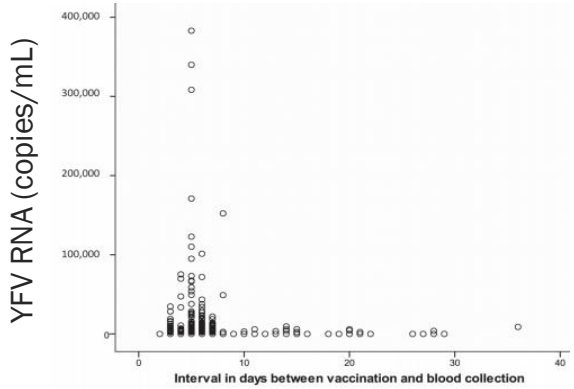


Table 7. Vaccine virus RNA positivity by RT-PCR at specific intervals between vaccination and blood sampling

	< 11 d	≥ 11 d to < 14 d	≥ 14 d
N	787	19	65
% RT-PCR positive	42.3%	10.5%	16.9%

Viremia up to 36 days after vaccination

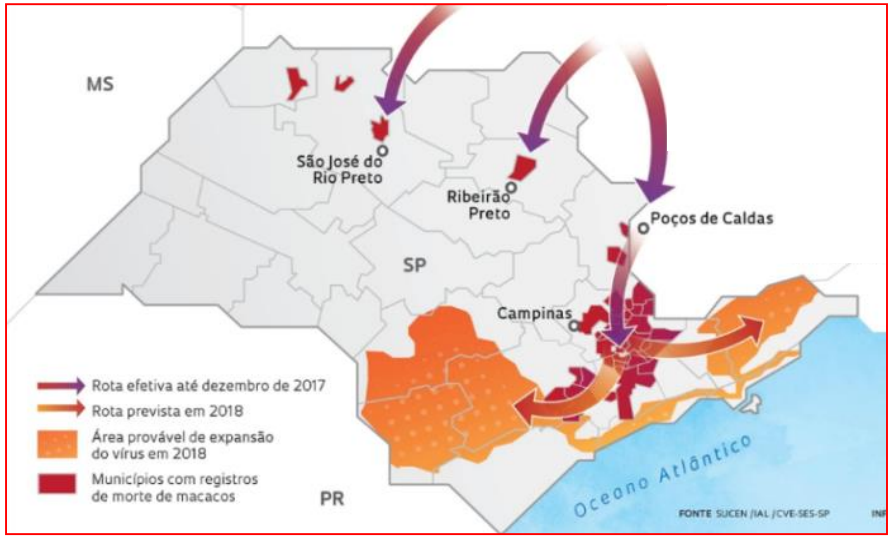
Martins RMM. Human Vaccines & Immunotherapeutics 2013

Data presented by Dr Luiz Amorim, Hemorio at SVTM Swiss congress 2017

- Blood donor deferral period of 2-4 weeks to limit the risk of YFV vaccine TTI

YFV: a threat to the blood supply

- YFV TTI documented for:
  - Wild type strain
  - Live attenuated vaccine strain
- Deferral based on clinical signs:
  - 80% asymptomatic cases
  - Difficult to prevent donation from asymptomatic donors
- Deferral based on risk after vaccine
  - Immunocompromised patients at higher risk for severe outcome



➢ Deferral policies may increase the risk of platelet shortage in areas impacted by massive vaccination campaign

➢ **Pathogen inactivation could be an alternative strategy to help mitigate the risk of YFV TTI and platelet shortage**

INTERCEPT Mechanism of Action Targeting DNA and RNA to Prevent Pathogen Proliferation

1) Amotosalen, a psoralen, penetrates cellular and nuclear membranes and intercalates into helical regions of DNA or RNA.

2) Amotosalen forms covalent crosslinks to nucleic acid base pairs upon exposure to UVA light.

3) DNA and RNA replication is blocked. Pathogens and leukocytes cannot replicate and are 'inactivated.'

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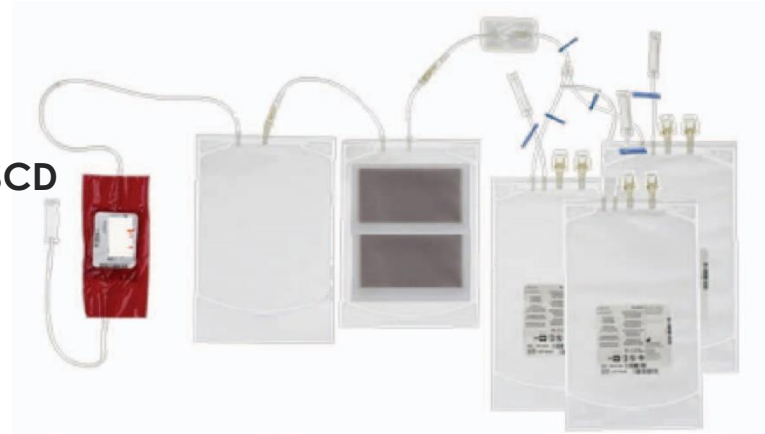
The INTERCEPT Blood System for Platelets

INTEGRATED CONTAINER SET



UVA Illumination Device

- Using a sterile connecting device (SCD), the platelet container is connected to the INTERCEPT kit.
- Amotosalen (1) is added by gravity flow and the platelet mixture is illuminated with UVA light (2).
- Residual amotosalen and its photoproducts in the platelet mixture are reduced to low levels using a compound adsorption device (CAD) (3) before the platelets are transferred to the storage containers.

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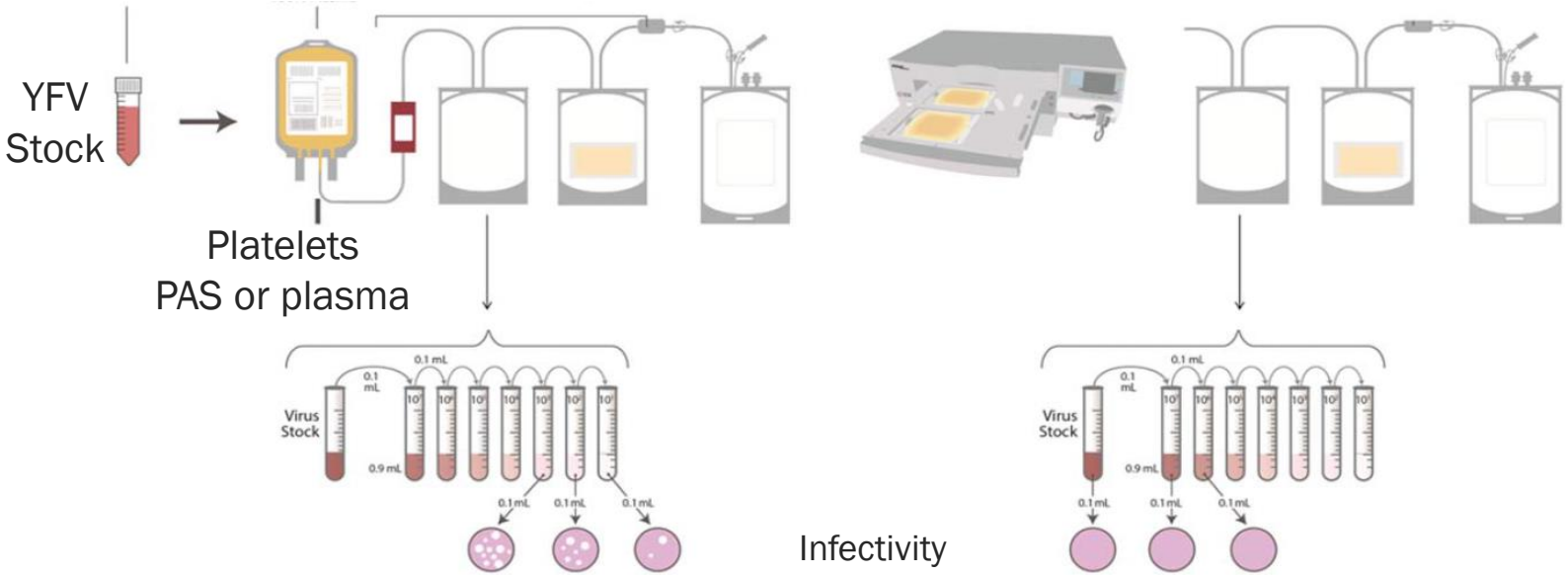
Pilot study objectives

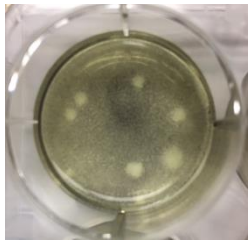
- Evaluate the ability to inactivate 17D-YFV using amotosalen (S-59) and UVA light for pathogen inactivation treatment of platelet components (PC)
- Use infectivity assays with Vero76 cells

YFV vaccine inactivation – Experimental design



Infectivity
by plaque assay
expressed in
plaque forming units
(PFU/mL)

<u>Pre-Inactivation samples</u>
Infectious titer by plaque assay on Vero 76 cells



<u>Post-inactivation samples</u>
Residual infectious titers by plaque assay (Vero cells)

YFV vaccine inactivation - Results

	PC in 35% plasma / 65% PAS (n=4*)	PC in 100% plasma (n=1*)
Pre-PI Treatment (Log_{10} PFU/mL)	4.65 ± 0.6	5.19
Post-PI Treament (Log_{10} PFU/mL)	<-0.70 ± 0.0	<-0.7
Log reduction (Log_{10} PFU/mL)	>4.65 ± 0.6	>5.19
Log reduction (Log_{10})	>5.34 ± 0.6	>5.89

*n=4 more replicates in PAS and 100% plasma are planned for regulatory submission

Inactivation of Arboviruses with the INTERCEPT Blood Systems to the Limit of Detection

Arbovirus	Log_{10} Inactivation		
	Plasma	Platelets	RBC*
WNV	≥6.8[4]	≥6.0[5]	NA
DENV	>5.6[1]	≥4.3[6]	>6.6[10]
CHIKV	≥7.6[7]	>6.4[7]	> 7.1[8]
ZIKV**	>6.6[2]	>4.9[9]	>5.8[3]
°YFV (strain 17D)**	NA	>5.9[11]	NA
°MAYV**	NA	>6.9[12]	>6.2[12]
°RRV**	NA	>5.1[13]	>5.5[13]

1. Musso et al.Transfusion 2014;54: 2924-30.
2. Aubry et al. Transfusion 2016;56: 33-40.
3. Laughhunn et al. Transfusion 2017 Feb 5. doi: 10.1111/trf.13993.
4. Lin L, Hanson CV, et al. Transfusion 2005;45(4): 580-590.
5. Singh Y, Sawyer LS, et al. Transfusion 2006;46(7): 1168-1177.
6. Dupuis K, Arnold DJ, Sawyer L. Transfusion 2012;52(3S):225A.
7. Tsetsarkin et al.Am J Trop Med Hyg 2013;88: 1163-9.
8. Laughhunn et al. Transfusion 2017;58;748–757.
9. Santa Maria et al., Transfusion 2017 Aug;57(8):2016-2025.
10. Aubry, et al., TRANSFUSION 2017;57;2888–2896.
11. Laughhunn, et al., Robust Inactivation of the Yellow Fever Virus 17D Strain Can Be Achieved Using Amotosalen and UVA Light for Pathogen Inactivation Treatment (PRT) Of Platelet Components – AABB 2017.
12. Santa Maria, et al., Robust Inactivation of Mayaro Virus in Platelet Concentrates and Red Blood Cells using nucleic acid targeting pathogen reduction technologies (PRT) – AABB 2017.
13. Laughhunn, et al., Effective inactivation of ross river virus in blood components through nucleic acid targeting – AABB 2017.

* INTERCEPT Blood System for RBC is in development and not approved for commercial use.

**Data for pathogen inactivation of Zika, Yellow Fever, Mayaro, or Ross River Viruses by INTERCEPT Blood System have not been submitted to TUV or FDA for review.

Arbovirus Comparison

Characteristic	Dengue[1]	Chikungunya[2]	Zika[3,4]	West Nile[5]	Yellow Fever[6]
Virus family	*Flaviviridae*	*Togaviridae*	*Flaviviridae*	*Flaviviridae*	*Flaviviridae*
Virus genus	Flavivirus	Alphavirus	Flavivirus	Flavivirus	Flavivirus
Serotypes	4	1	1	1	1
Vector	*Aedes aegypti* *Aedes albopictus*	*Aedes aegypti* *Aedes albopictus*	*Aedes aegypti* *Aedes albopictus*	*Culex Pipiens* *C. Tarsalis* *C. quinequefasciaatus*	*Aedes aegypti* *Aedes albopictus*
Symptomatic: Asymptomatic	25:75	75:25	20:80	20:80	~20:80
Illness outcome	Severe dengue – plasma leakage	Rarely severe, Arthralgias	Rarely severe, Guillain-Barré Microcephaly	Neuroinvasive disease Meningitis Encephalitis Polyomyelitis	Rarely severe, but ~15% of patients progress to serious form; hemorrhagic fever, multisystem organ failure

1. http://www.cdc.gov/dengue/
2. http://www.cdc.gov/chikungunya/
3. http://www.cdc.gov/zika/
4. Zika Virus: Information for Clinicians, June 13, 2016, http://www.cdc.gov/zika/pdfs/clinicianppt.pdf
5. http://www.cdc.gov/westnile/
6. https://wwwnc.cdc.gov/travel/yellowbook/2016/infectious-diseases-related-to-travel/yellow-fever

Conclusions

- S-59/UVA-mediated PRT is efficient at inactivating the 17D-YFV vaccine strain in PC independently of resuspension medium
  - Similar to results obtained with other Flaviviruses, including West Nile, Dengue and Zika virus
- Because of high rates of asymptomatic infections, donor deferral is an inefficient mitigation strategy for most arboviruses
- Blood recipients are often immunocompromised
  - At risk for the development of severe clinical outcomes
- Pathogen reduction is a potential mitigation strategy to prevent TTI's and maintain PC availability in areas
  - affected by large YFV outbreaks
  - with widespread vaccination campaigns