A Universal Virus Capture Method for a Rapid & Ultrasensitive Detection of Pathogens for Early & Late Post-Exposure Countermeasures

Francisco Veas

EC H2020 "IF-EBOLA action" – Ebola preparedness
EC H2020 EDCTP "PANDORA"- preparedness

Faculty of Pharmacy-University of Montpellier
Montpellier – France

PANDORA
Pan-African Network For Rapid Research, Response and Preparedness for Infectious Diseases Epidemics

IF·EBOLA
INNOVATIVE TECHNOLOGIES TO FIGHT EBOLA

Global context & Health:
Epistemological bases to contextualize the problematic:

Uncontrolled Global Demography, productivity & waist chaos (eg.: Palm oil)

Human demography growth OUT OF CONTROL *with desastrous consequences*

1800 : 1B (Jenner & Pasteur) ; 1900 1.5B; 1960: 3B; 2017: 7.5B to go ***very soon 10 B!. (***

The present population of humans consume 100 to 1000-times more energy than a human in 1800. Africa will double its demography.

The pop density of Bangladesh in France (67 M ➔approx. 900 M)

In Europe in 30 years, -80 % insectes & in 15 years -30% birds

Orangutans' poaching is done deliberately as a policy made by palm oil corporations.

Over **50 Orangutan are killed per week** due to deforestation (heavy machinery or fire).

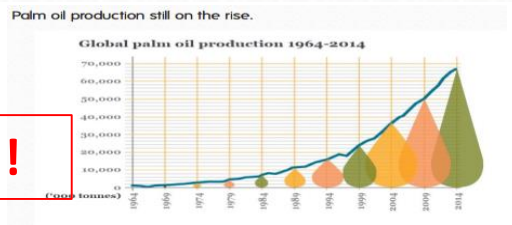
Orangutans (=pest for oil palm companies) **are left starved in plantations searching for** young palm plants as food.

Elephants are often killed by poisoning. Due to habitat loss and lack of food, elephants wander onto plantations and into villages and destroy crops. Their **habitat is cleared for oil palm**. Plantations and local villages often poison the elephants.

Tiger and Rhino numbers are so low due to habitat loss and is rapidly nearing.

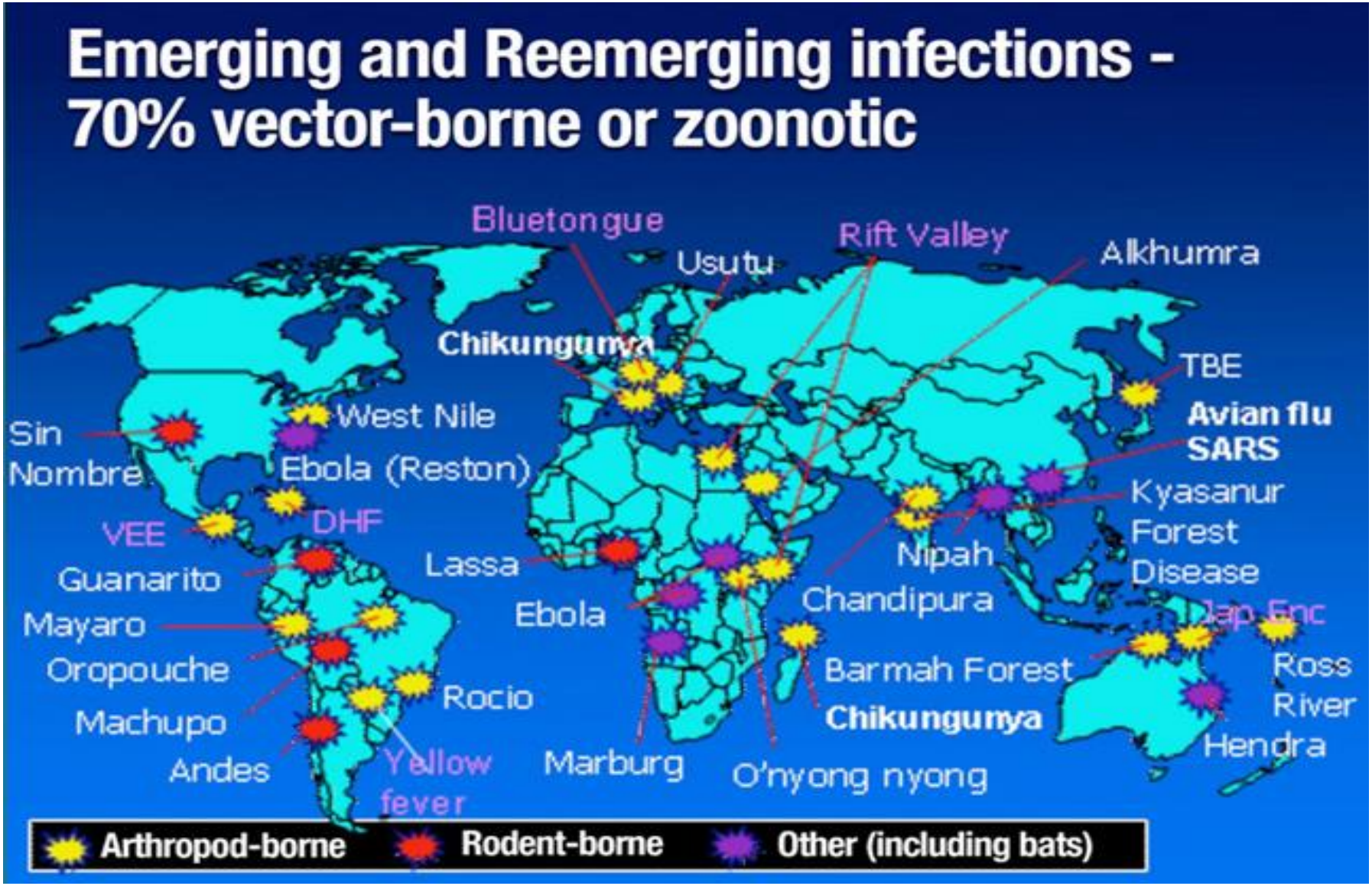
Water pollution *from an oil palm plantation in Indonesia*

Oxygen reduction due to fertilizers, pesticides, etc



The world has lost -50% of its flora & fauna these last 40 years !

Urgent need to set up a prepared rapid One-Health approach to adopt efficient epidemic surveillance & countermeasures



The absence of learning lessons from latest important outbreaks

Virus outbreak/ Transmission	Year	Location	Main symptoms
Hantavirus/reservoir	1993	Americas	fever &severe cardio-pulmonary dysfunctions
SarsCoV/reservoir	2003	China	fever with severe respiratory distress
H1N1/reservoir	2009	Mexico /the World	fever with broncho-pulmonary dysfunctions
MERSCoV/reservoir	2012	Middle East /Korea	fever with severe respiratory distress
Chikungunya/vector	2014	Brazil/Caribbean	fever and joint pain and dysfunctions
Ebola/reservoir?	2014	West Africa	fever and severe vascular dysfunctions
Zika/vector	2015	Americas/Brazil	fever, rash, but 80% asymptomatic / fetus with MC

From WHO reports

Most of them have evolved on very large areas much quicker
than **the slow and inefficient responses** on both
diagnostic (with a lot of false-negative diagnoses) and therapeutic or vaccine (ChikV example)

The European IF-EBOLA action (H2020)
2014-2017

“An early Ebolavirus detection for an early post-exposure passive immune therapy”

Institut de Recherche pour le Développement, France; **Orion Integrated Biosciences**, USA; **Absiskey**, France; **Ministry of Health and Sanitation**, Sierra Leone; **Metabiota** Inc., USA; **Fab’entech**, France; **Pasteur Institute**, France; **Spallanzani Institute**, Italy; **Ben Gurion University**, Israel; **Manitoba University**, Canada.

The IF-EBOLA Preparedness toolbox

(i) **an EBOLA ultrasensitive diagnostic** to detect low viral loads by using a sample prep prior a PCR (the FDA-approved EBOLA USAMRIID rt-PCR). The purpose of this approach is to detect ebola virus as early as possible to isolate contaminated patients and reduce viral spreading . Automized **POINT OF CARE**

(i) **an anti-EBOLA passive immunotherapy** based on horse anti-EBOLA polyclonal F(ab')2, that has been highly efficient in well-established EBOLA infection of small animal models, respectively a 7-days life-span of infected mouse model in the Gary Kobinger's lab (BSL4@NML, Winnipeg Canada) and a 7-days life-span of infected guinea pig in the Heinz Feldman's Lab (BSL4@NIH, Montana, USA). 100% of survival in both models after F(ab')2. In particular, when these animals are treated **3 days after infection** (they have lost 30% of their weight) they completely recover up to day 42 (where they must be sacrificed due to ethical rules @ NIH). The European Medical Agency, EMA, have considered that this product perfectly fulfils Pharmaceutical GMP production rules. 900 litres represent individual treatments for about 20-to-30 000 patients. **WHO–FABENTCH Collaboration & EMA have declared this product as one of the 5 most promising therapies**

(i) **Accurate metagenomic analyses** thanks to sample prep coupled with new algorithm (**RIGEL**) and a new data bank **(22-times higher than gene bank!)** to assess Ebola strains and co infections.

(i) **a disruptive technology of « Plasma Water Sanitation System » to provide 4800 literes of drinkable water** to hospital and schools in regions or countries with poor resources; (avoid plastic waste

EBOLA virus diagnostic during the outbreak

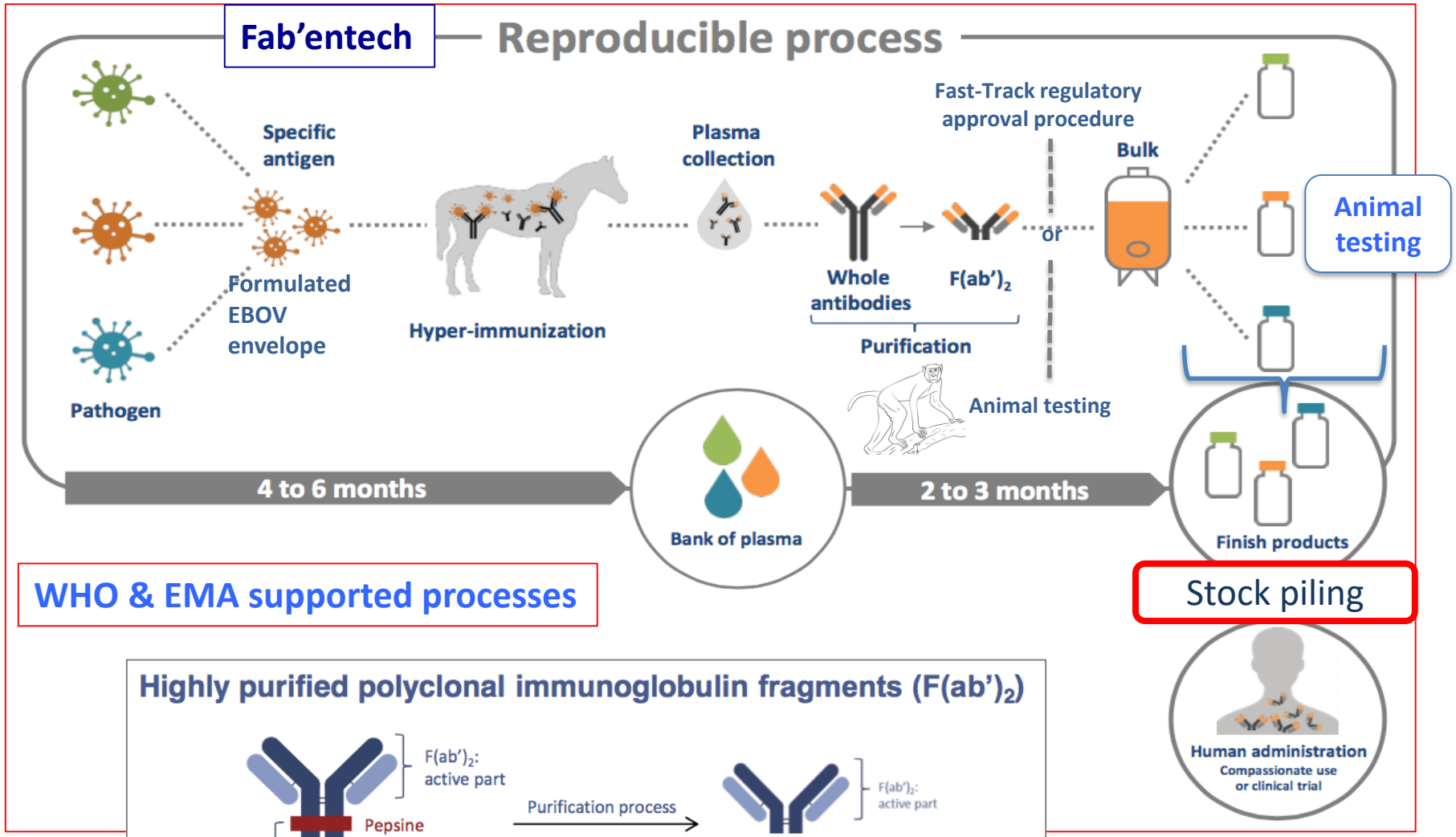
KGH Laboratory

rt-RT-PCR,
Kenema,
Sierra Leone

Clinical Data
Recording

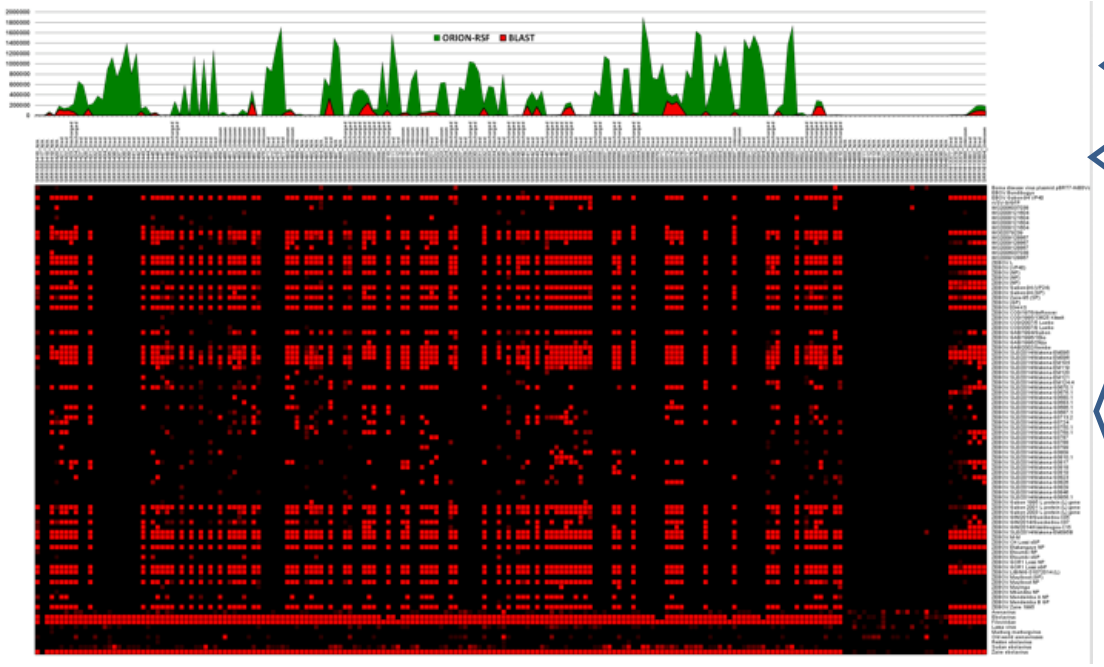
Fab'ntech produce GMP horse anti-EBOV antibodies

E*In vivo* virus neutralizing F(ab')2 antibodies and survival rescue of infected animals: POC



IF-EBOLA action

NGS-Metagenomic analyses ➔ EBOLA-LASSA virus co-infections



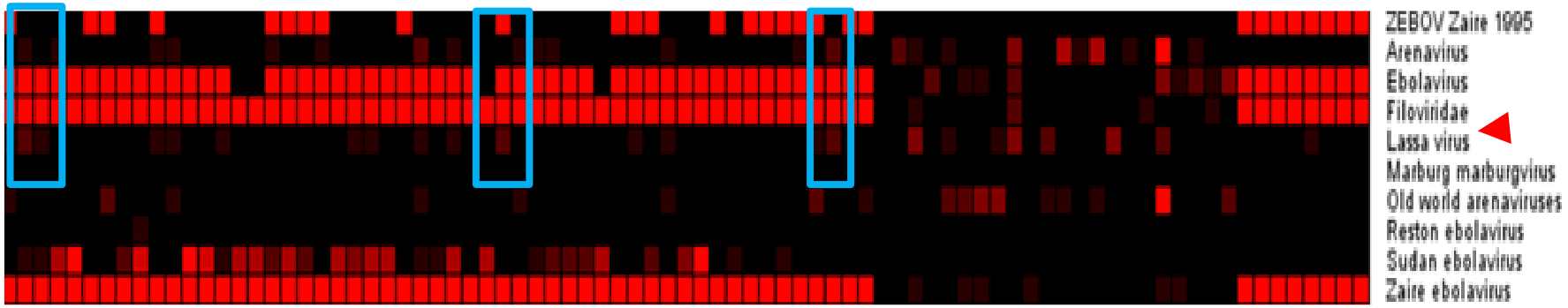
Rigel/Blast

Sample #

Pathogen reads

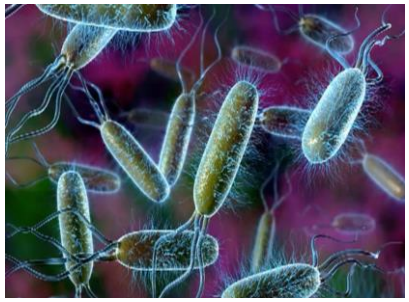
Red dot: pathogen specific reading

Blue square: **Lassa/Ebola signal (18%!!)**

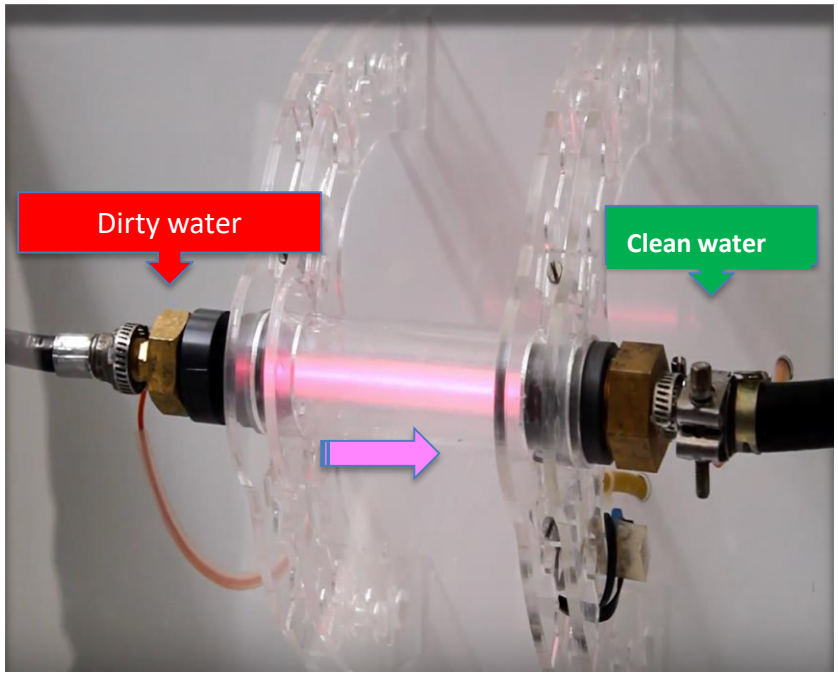


Valdivia // Veas @Gordon Research Conferences, 2015 and 7th Filovirus Symposium 2015

Plasma Water Sanitation system (PWSS)



PWSS : (1) high pressure (100 bars) transform H20 into liquid-gas biphasic state; (2) ionization provide plasma state generating high temperature spots, shocking waves, ozone, ROS, UV rays that change the position of all electrons in all components (of dirty water), thus destroying microorganisms



PWSS developed by AIC

- No chemicals
- No taste no odor vs chlorine & pollution
- Destroy 100% micro-organisms (VS much less with chlorine or filtration methods)
- Only 100 Watts to produce 35 liters in 5 min (5 000 lt/day) (VS expensive filtration)
- Less plastic going to water (rivers & oceans)

Impact water quality water on Immune status & microbiota

PANDORA
Pan-African Network For Rapid Research, Response and Preparedness for Infectious Diseases Epidemics

A universal platform to improve of diagnostics of pathogens

A universal poly-specific capture of pernicious microorganisms

A sample preparation tool to concentrate samples with low pathogen loads for both:

(i) ultrasensitive detection of pernicious microorganisms to **avoid false-diagnoses**

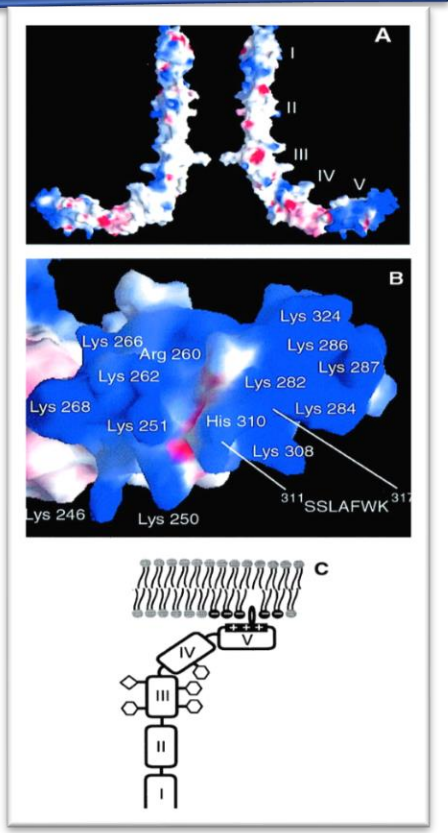
(ii) metagenomic extraction data to accurate detect & identify **the presence of known or unknown pathogens**

➔ **Adopt adapted outbreak & therapy countermeasures**

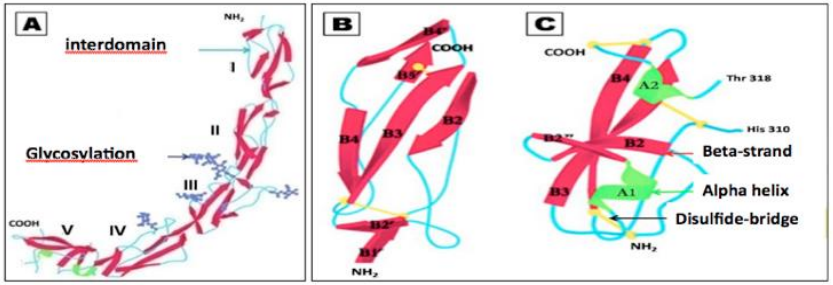
Scavenger ApoH acute phase protein to capture pathogens

Main characteristics

- ✓ molecular mass varying 43 -54 **kDa (Glycosylation)➔345aa**
- ✓ plasmatic concentration ➔ **200 mg/L**
- ✓ ApoH comprises **5 sushi domains**: 4 SCR (short consensus repeats) from CCP (complement control protein) module type & a fifth **lysine rich** domain (with a large patch of 14 positively charged residues)➔**electrostatic interactions**
- ✓ unusual composition with **6.2 % cysteine and 8.3 % proline**
- ✓ **Hydrophobic interactions** with anionic phospholipids (PS, Cardiolipin, some of which are present in HIV, HBV, HCV, (Stefas //Veas 2001, 2011, 2012, 2015)
- ✓ **Protein-Protein interactions** (Sbi of *S. aureus*; Microbiol 1999); protein H of *S. pyogenes;* Mol Microbiol. 2008, 67(3): 482-92)
- ✓ myristoiled or palmitoiled groups (Stefas //Veas 2001)
- ✓ **High affinity & efficiency to capture pernicious microorganisms:**
  - **viruses**
  - **bacteria**
  - **Fungi**
  - **parasites**
  - **prions**



(EMBO Journal. 1999, 18 (19) : 5166



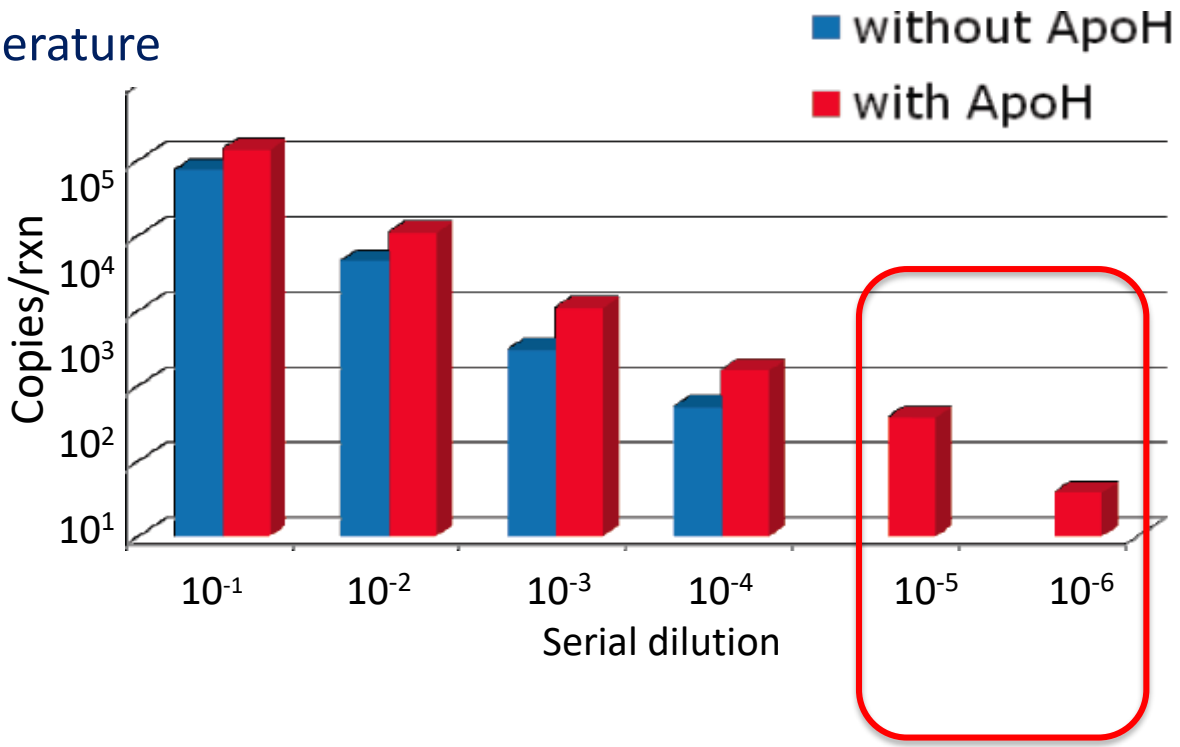
ApoH detection of respiratory viruses

Influenza

Swabs spiked with H3N2 Influenza virus:

- Spiked with cell cultured viruses
- Stored for 24 h at room temperature
- Diluted in 4 mL MEM
- Without ApoH
- With ApoH-beads

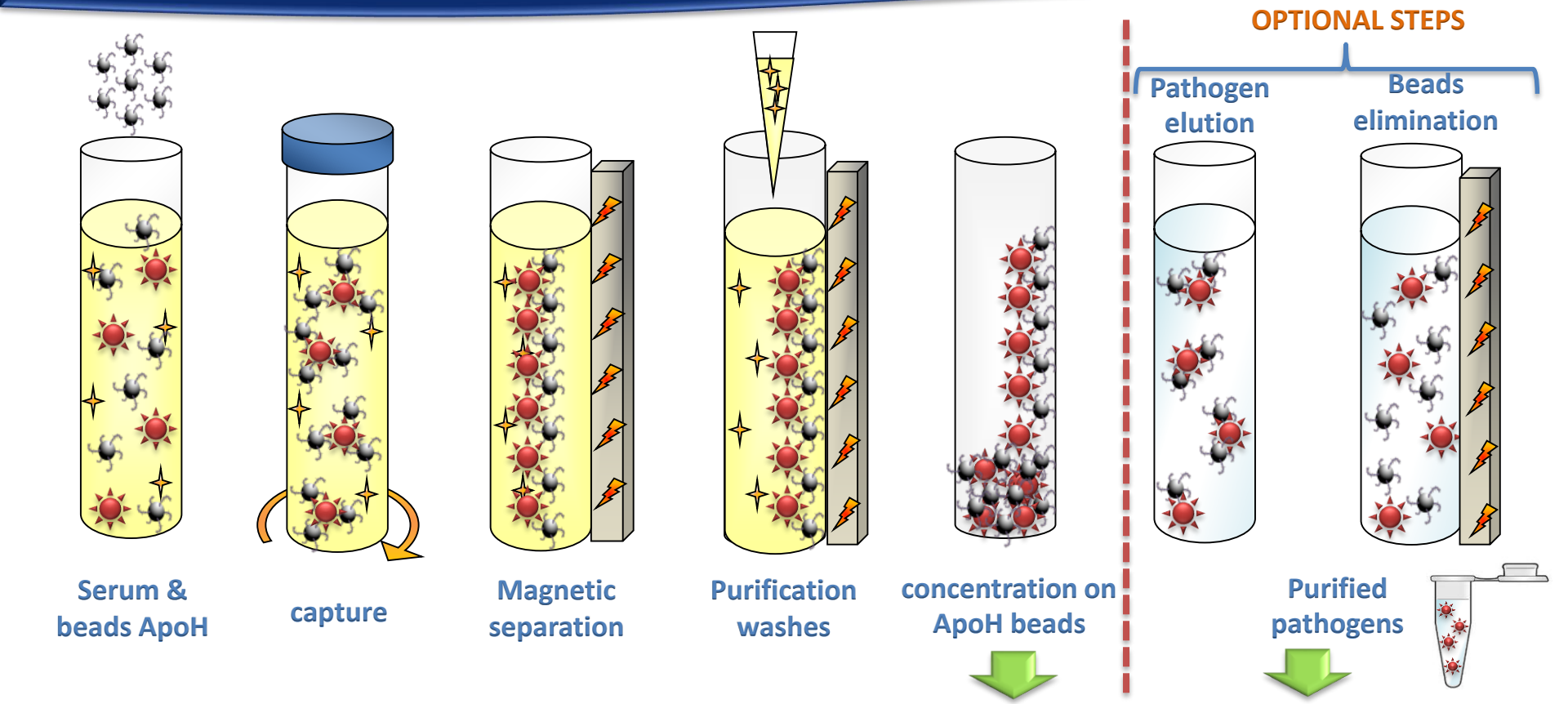
Patient sample copies/rxn	
Without ApoH	With ApoH
1.7^{E}+05	1.8^{E}+06



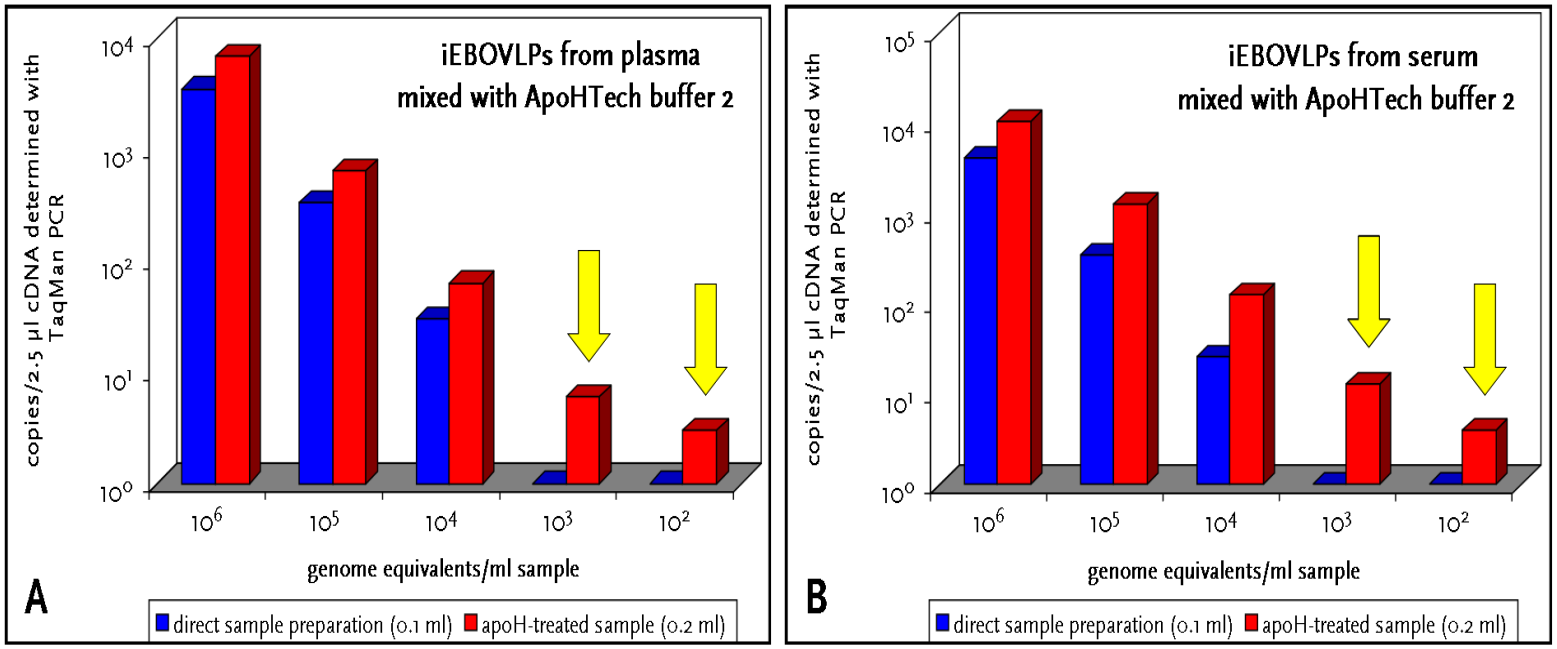
→ **Functional protocol established to enrich respiratory viruses from nasal swabs**

ApoH capture

Enabling ultrasensitive micro-organism culture or PCR



Ultrasensitive detection of iVLP of Ebola Viruses

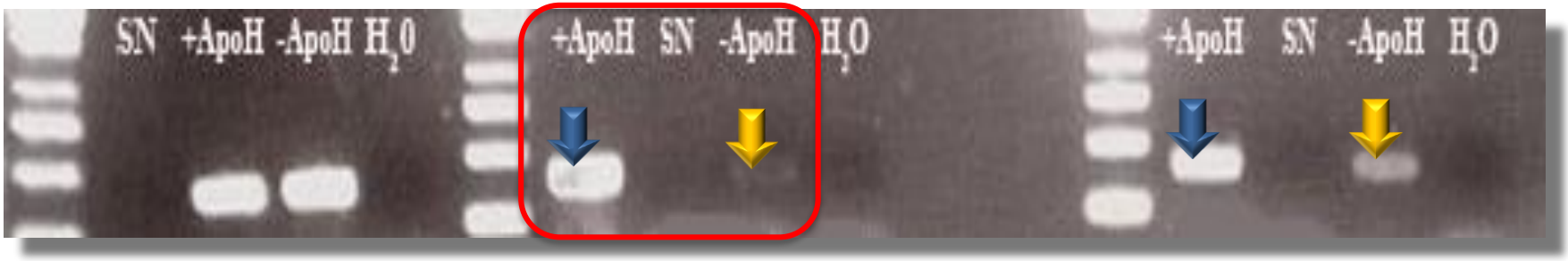


ApoH-enhanced sensitivity of iVLP-detection. Yellow arrows mark the shifted detection limit of serial dilutions of iEBOVLPs spiked in human plasma and serum respectively, due to ApoH-treatment
cDNA of each dilution step was subjected to direct analysis (blue) or post-ApoH-enhanced detection (red) respectively.

ApoH for clinical detection of low viral infections

HCV

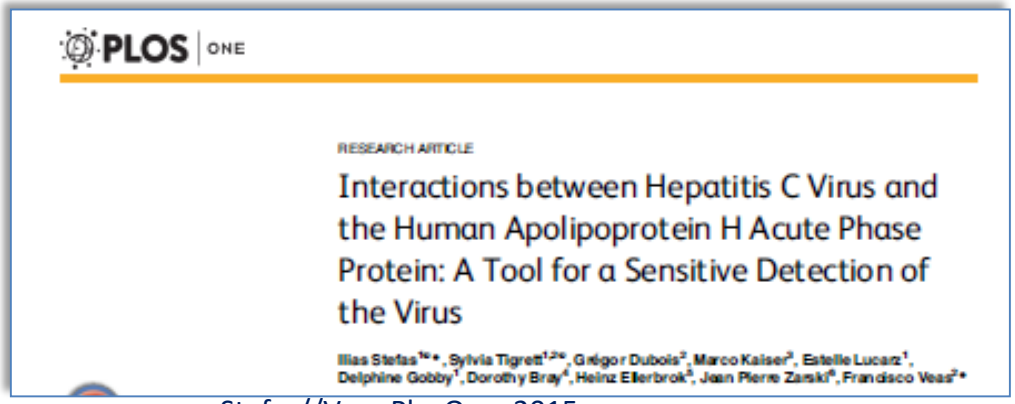
Highly positive Plasma | Suspected Plasma | Low positive Plasma



Among **25 HCV-negative diagnoses** with the **HCV RT-PCR (COBAS™)** done in the Universitary Hospital of Grenoble,

* **11 of them (44%) turn HCV-Positive in a post-ApoH RT-PCR analyses.**

Serum samples hepatitis symptoms	COBAS RT-qPCR*		ApoH + RT-PCR	
	HCV pos	HCV neg	HCV pos	HCV neg
n=25	0	25	11**	14


PLOS ONE

RESEARCH ARTICLE

Interactions between Hepatitis C Virus and the Human Apolipoprotein H Acute Phase Protein: A Tool for a Sensitive Detection of the Virus

Stefas//Veas PlosOne, 2015

ApoH + qPCR = COBAS HCV if **high** loads
ApoH + PCR > COBAS HCV if **low** loads

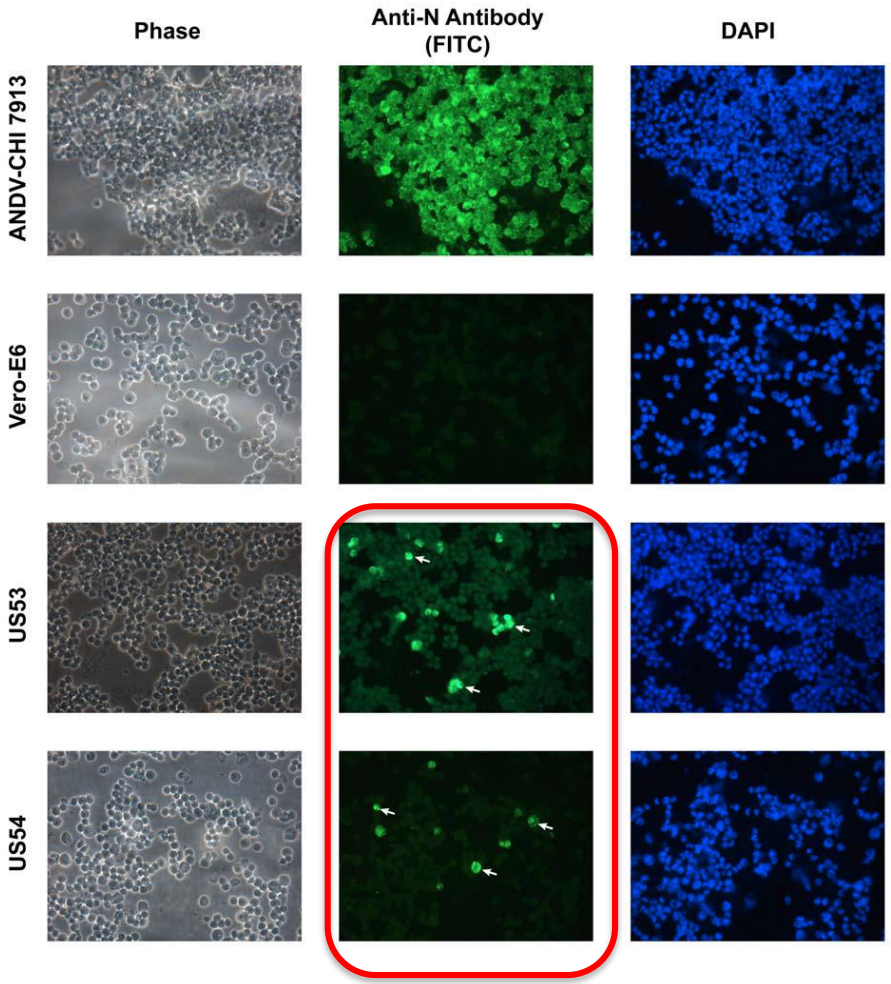
ApoH & isolation of hemorrhagic Hantaviruses

Lab strain

No virus

Isolated strain 1

Isolated strain 2



JOURNAL OF VIROLOGY, May 2009, p. 5046–5055 Vol. 83, No. 10
0022-538X/09/$08.00+0 doi:10.1128/JVI.02409-08

Andes Virus Antigens Are Shed in Urine of Patients with Acute Hantavirus Cardiopulmonary Syndrome▽†

Paula Godoy,[1]† Delphine Marsac,[1]† Elias Stefas,[2] Pablo Ferrer,[1] Nicole D. Tischler,[3] Karla Pino,[1] Pablo Ramdohr,[1] Pablo Vial,[4] Pablo D. T. Valenzuela,[3,5] Marcela Ferrés,[1] Francisco Veas,[6] and Marcelo López-Lastra[1]*

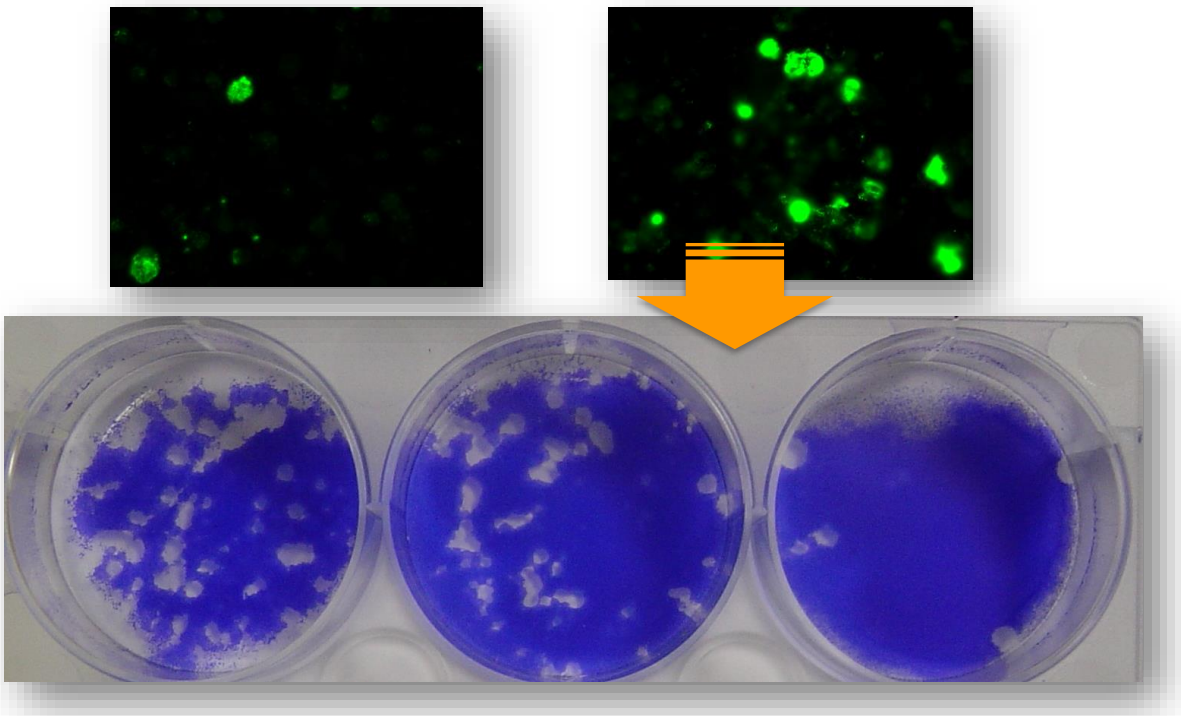
Infection of Vero E6 cells with ApoH-captured Hantaviruses

ApoH isolation of respiratory viruses

Capture & culture of replicating Influenza viruses

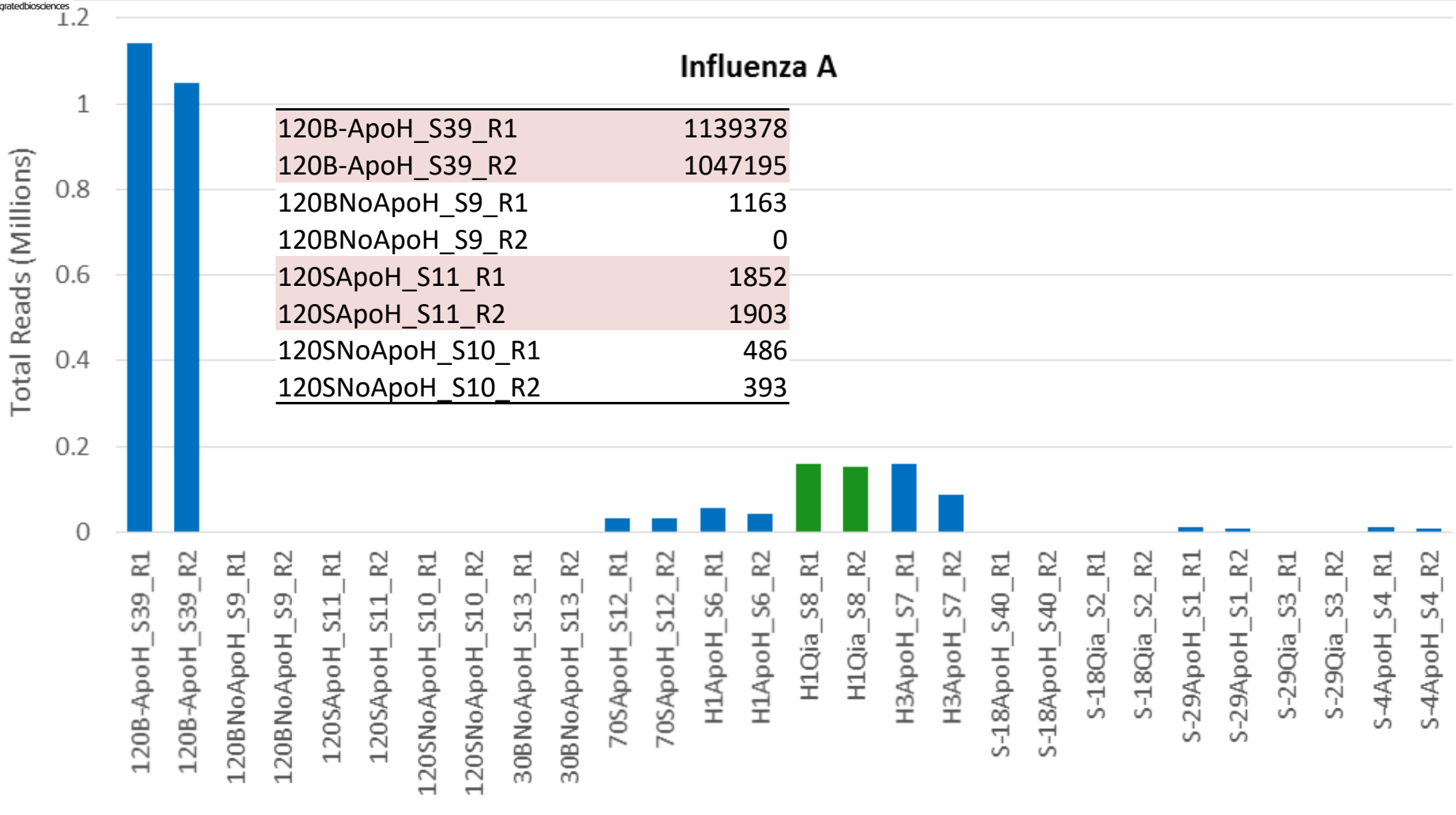
H3N2 infection (without ApoH)
Detection using an anti-H3N2 MAb

Infection with ApoH-captured H3N2
Detection using an anti-H3N2 MAb

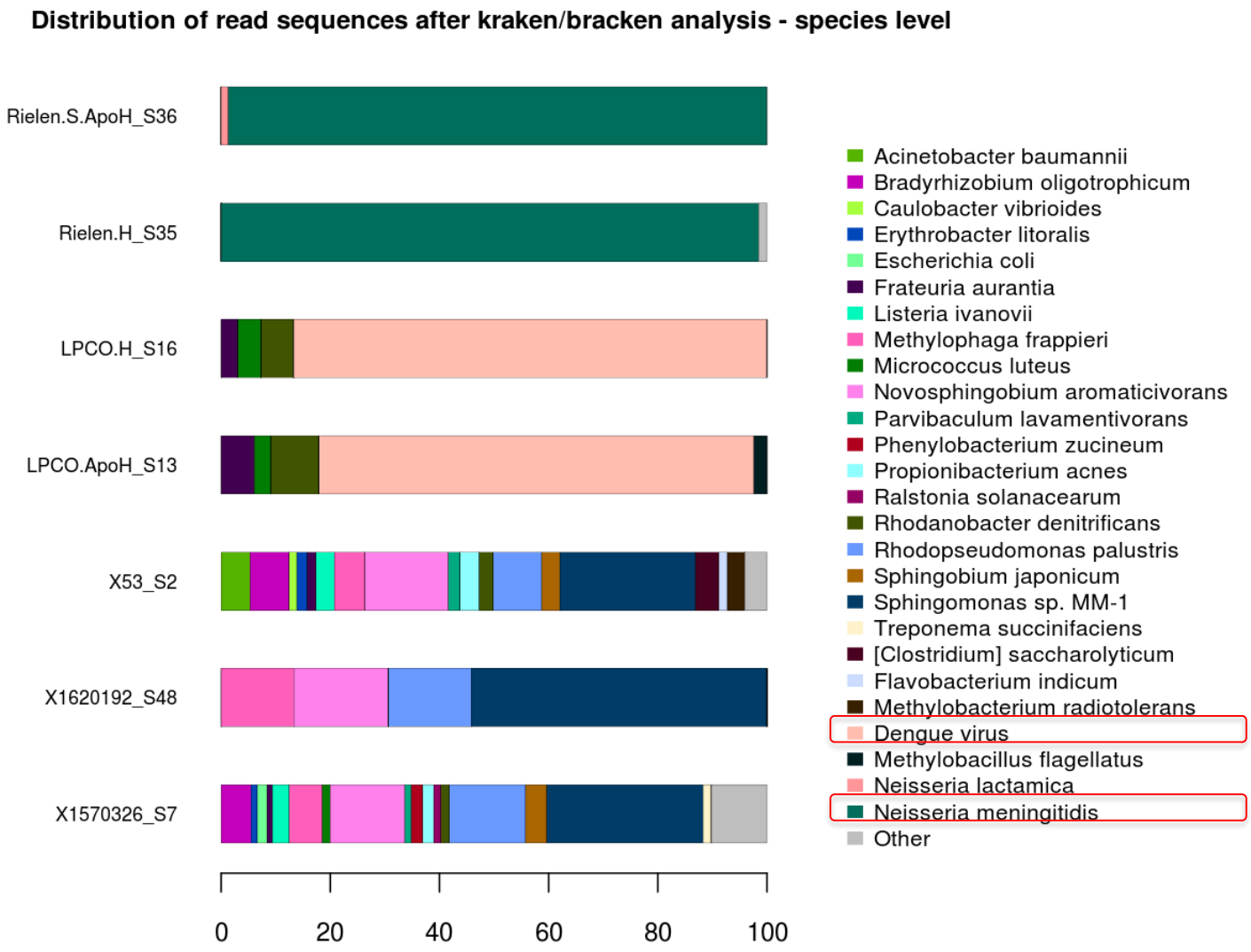


ApoH-captured of a cultivated H3N2 strain & subsequent infection of its target cells ➔ cytopathogenic effects

ApoH sample preparation – environmental FLU
NGS-metagenomic analysis



Unexplained encephalitic fevers (IDDEAA)



ApoH use in clinical bacterial infections

Critical unmet needs

- Current bacterial detection & identification methods are **too slow** and/or **not sensitive enough** to drive anti-biotherapy for life-threatening infections (sepsis…) or for HAI screening.

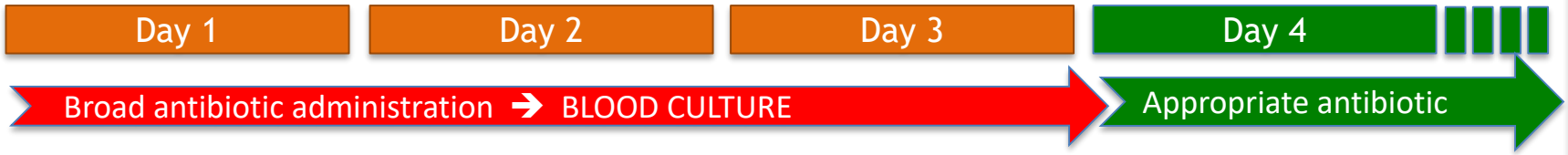
Main concerns:

- **Blood culture** based diagnosis
  - could **take 2 days or more (up 15 days)**,
  - **lack of sensitivity** (ex.: **false-negative diagnoses** due to presence of antibiotics…),
  - **non-cultivable bacteria**

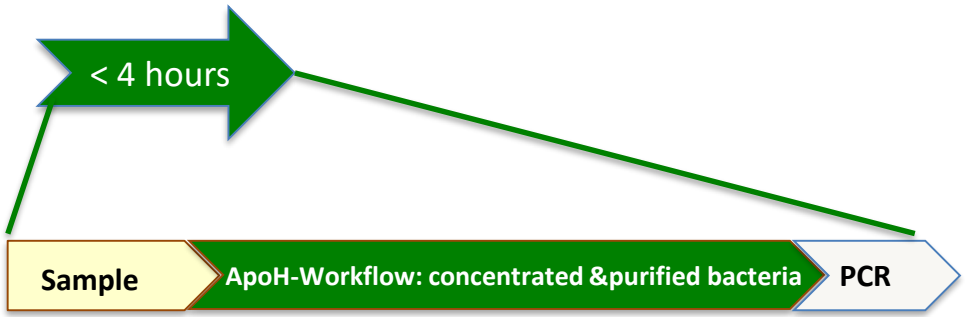
- **Molecular methods still do face a major sensitivity issue** due to:
  - the challenge to concentrate a few pathogens within several ml of blood,
  - the presence of inhibitors

ApoH bring the highest sensitivity bacterial detection for clinical whole blood samples

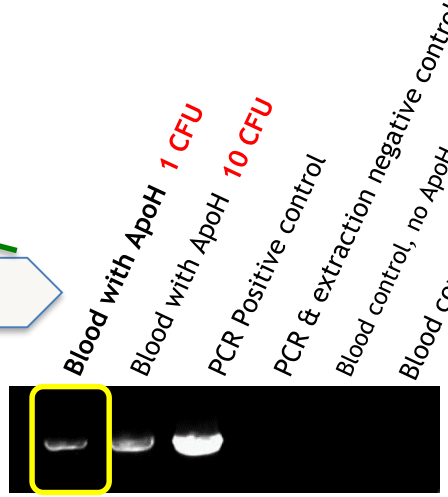
Clinical sensitivity need for sepsis is defined as 10 bacteria/mL of whole blood



ApoH-T is the unique solution able to highly concentrate a bacterial load for a subsequent optimal detection up to 1 CFU / 5 mL of whole blood !



Few hours after bleeding diagnostic+ ➔ administrate appropriate antibiotic therapy



Approved by SIEMENS GmbH

Detection sensitivity 0.2 CFU/mL

16S PCR on *S. aureus* spiked in 5 mL of whole blood then captured by ApoH

ApoH use in clinical bacterial infection

Very large bacterial SCOPE

Acinetobacter baumannii	*Corynebacterium sp.*	*Mycobacterium abscessus*	*Salmonella typhimurium*
Acinetobacter lwoffii	*Corynebacterium xerosis*	*Mycobacterium chelonae*	*Serratia marcescens*
Acinetobacter sp.	*Enterobacter aerogenes*	*Neisseria cinerea*	*Sphingomonas paucimobilis*
Bacillus cereus	*Enterobacter cloacae*	*Nocardia farcinica*	*Staphylococcus aureus*
Bacillus sp.	*Enterococcus faecalis*	*Ocrobactrum anthropi*	*Staphylococcus epidermidis*
Bacillus subtilis	*Enterococcus faecium*	*Parabacteroides distasonis*	*Staphylococcus haemolyticus*
Bacteroïdes fragilis	*Enterococcus gallinarum*	*Porphyromonas endodontalis*	*Staphylococcus hominis*
Bacteroides ureolyticus	*Escherichia coli*	*Propionibacterium acnes*	*Stenotrophomonas maltophilia*
Campylobacter fetus	*Fusobacterium nucleatum*	*Proteus mirabilis*	*Streptococcus agalactiae*
Candida albicans	*Fusobacterium sp.*	*Proteus vulgaris*	*Streptococcus bovis*
Capnocytophaga canimosus	*Klebsiella oxytoca*	*Providencia stuartii*	*Streptococcus D group*
Chlamydia trachomatis	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Streptococcus mitis*
Citrobacter freundi	*Legionella pneumophila*	*Pseudomonas sp.*	*Streptococcus parasanguinis*
Citrobacter koseri	*Listeria sp.*	*Pseudomonas stutzeri*	*Streptococcus pneumoniae*
Clostridium difficile	*Micrococcus luteus*	*Salmonella arizonae*	*Streptococcus pyogenes*
Clostridium perfringens	*Micrococcus sp.*	*Salmonella enteritidis*	*Tropheryma whipplei*
Corynebacterium ammoniagenes	*Mycobacter sp.*	*Salmonella sp.*	*Other ongoing …*

Take home message

The present and future planet context of our Health interventions is made in a **very rapid changing World** with uncontrolled demographic evolution

Highly severe consequences resulting in accelerated uncontrolled destructions of:

- resources & waste production (plastic rivers and oceans
- Fauna (big animals, insects, birds fish etc)
- Flora (deforestation)

Plants and animals suffer to be in a period of coexistence with billions of idiots that think that they control everything

A REAL "One Health meta-approach" implies to urgently consider the planet health (our living milieu)

Consequently what health researchers MUST do?

- We need to generate **actions with impact purposes** on health and education to try to control the demography
- A better health and education in particular to make **young women stronger could impact family planning**

Special thanks

- **European commission**
  - **USDEP project (2006-10)**
  - **EC,H2020 "IF-EBOLA action" (2014-17)**
  - **EDCTP2 "PANDORA" (2018-22)**
- **Montpellier University, France**
- **The French Research Institute for Development**
- **The French Bank for Investments (BPI)**
- **The Region Occitanie – France**
- **Lab d'Immuno-Physiopathologie Moléculaire Comparée (LIPMC)**
- **The ApoH-Technologies** engineers-team & Ilias Stefas (CEO)
- **Biotechnologies-Développement-Conseil** (France, USA, Israel & Japan), **Christian Policard** (former CEO of Pasteur Institute Business Development & former CEO of Sanofi-Pasteur Diagnostics) cpolicard@biotechdevconseil.com