ApoH as a tool for ultrasensitive detection of pernicious microorganisms and to extract metagenomic data in low concentration pathogens

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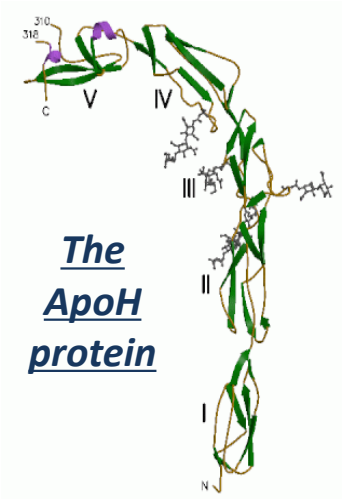
Willy Valdivia, CEO
Orion Integrated Biosciences, NY, US

ApoH capture: a proprietary technology

A poly-specific capture of microorganisms



The ApoH protein

ApoH*a* or apolipoprotein H or β2-glycoprotein I

A conserved protein with pleiotropic functions

- ✓ An innate immunity component exhibiting **a role of scavenger protein**
- ✓ Regulate blood coagulation pathway
- ✓ Regulate the migration of endothelial cells during angiogenesis
- ✓ Auto-antibodies against ApoH are associated with the anti-phospholipid-syndrome such as lupus erythematous

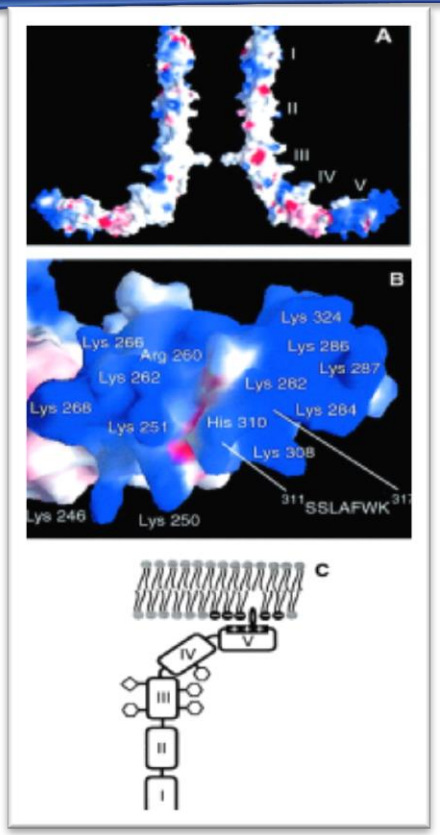
ApoH*a* is ***a***ctivated with a proprietary procedure to capture pathogens elements, including proteins, phospholipids, myristoiled or palmitoiled groups ➔

interacting specifically with micro-organisms including infectious viruses, bacteria, fungi, parasites & prions

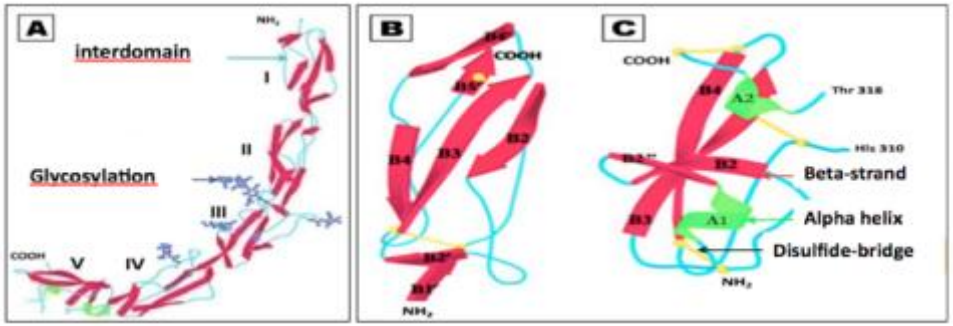
ApoH capture: a proprietary technology

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, HCV..)
- ✓ **Protein-Protein interactions** (Sbi of *S. aureus*; Microbiol 1999, 145: 177-); protein H of *S. pyogenes;* Mol Microbiol. 2008, 67(3): 482-92)
- ✓ **High microorganism capture affinity and efficiency of through novel physico-chemical conditions**



(EMBO Journal. 1999, 18 (19) : 5166–)



ApoH capture a proprietary technology

… enabling ultra-sensitive microbiology

ApoH*a* key features & advantage on other "concentration" methods (Ultracentrifugation, membrane concentration, cationic surfaces, etc)

- Efficiently captures **pernicious** bacteria, viruses, parasites and prions independently of their antigenic variation
- Concentrates micro-organisms from **any complex sample**
- Cleanses the sample from inhibitors or antibiotics for **optimal detection**
- Simple, fast and highly profitable
- Used fixed on various solid supports (magnetic-coated beads, ELISA plates...)
- A same support can be used to bind several and different pathogens, **enabling multiplexing**

ApoHa increases the sensitivity of **any** currently existing detection method (PCR, ELISA, Culture, etc…)

➔ **Asserted results through multiple studies**

Virus concentration & purification using the ApoH-based technology

Workflow

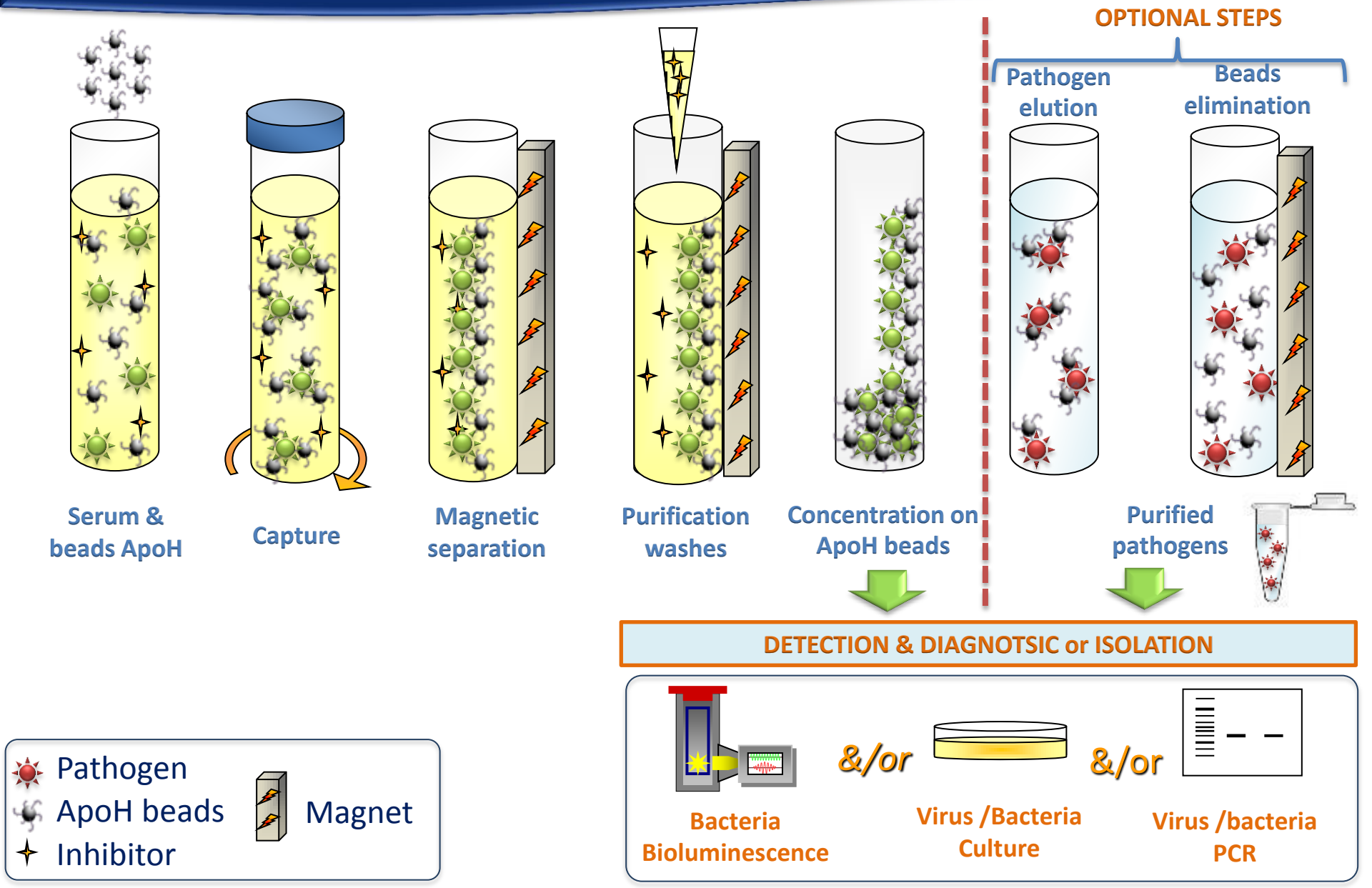
1- Viral or bacteria **capture** (ApoH CaptoVIR kit or CaptoBac)

2- Viral or Bacterial **cleaning & concentration** on ApoHa-magnetic beads

3- Viral or Bacterial **detection** using any appropriated method

ApoH capture

Enabling ultrasensitive micro-organism culture or PCR

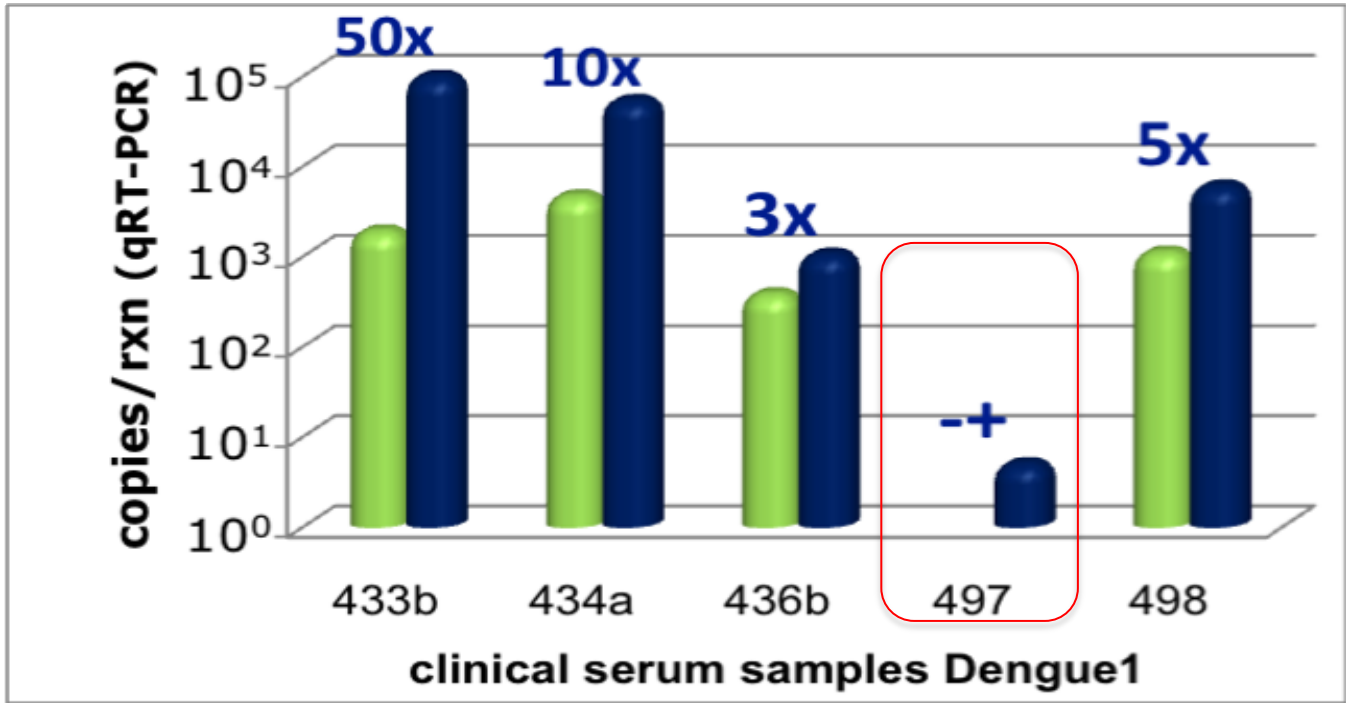


Sylvia Tigrett - Gonzalez

ApoH for ultrasensitive detection of clinical viral infections

ApoH & clinical detection of viral infections

The USDEP European Project — Dengue



QPCR on DENV suspected sera from five German patients having been submitted (blue bars) or not (green bars) to a previous pre-analytical ApoH step. Higher values were obtained for four of them with the ApoH pre-analytical method. One false- negative was solved by ApoH.

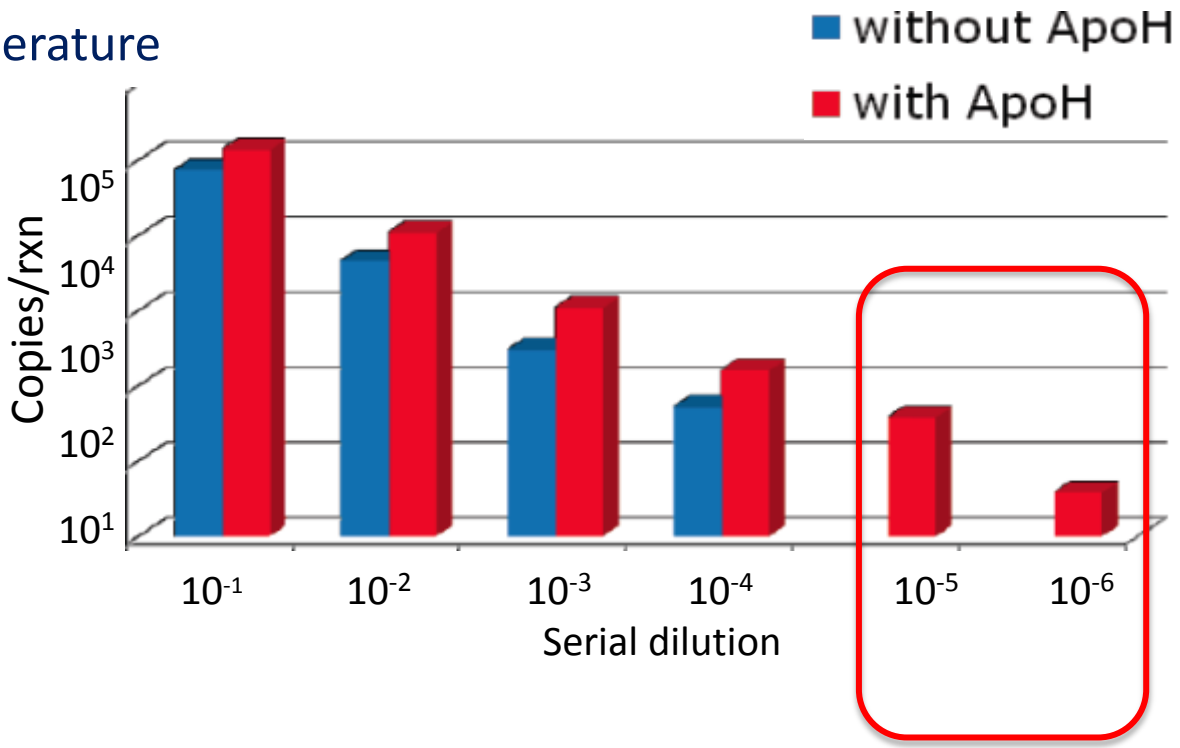
ApoH detection of respiratory viruses

The USDEP European Project — 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



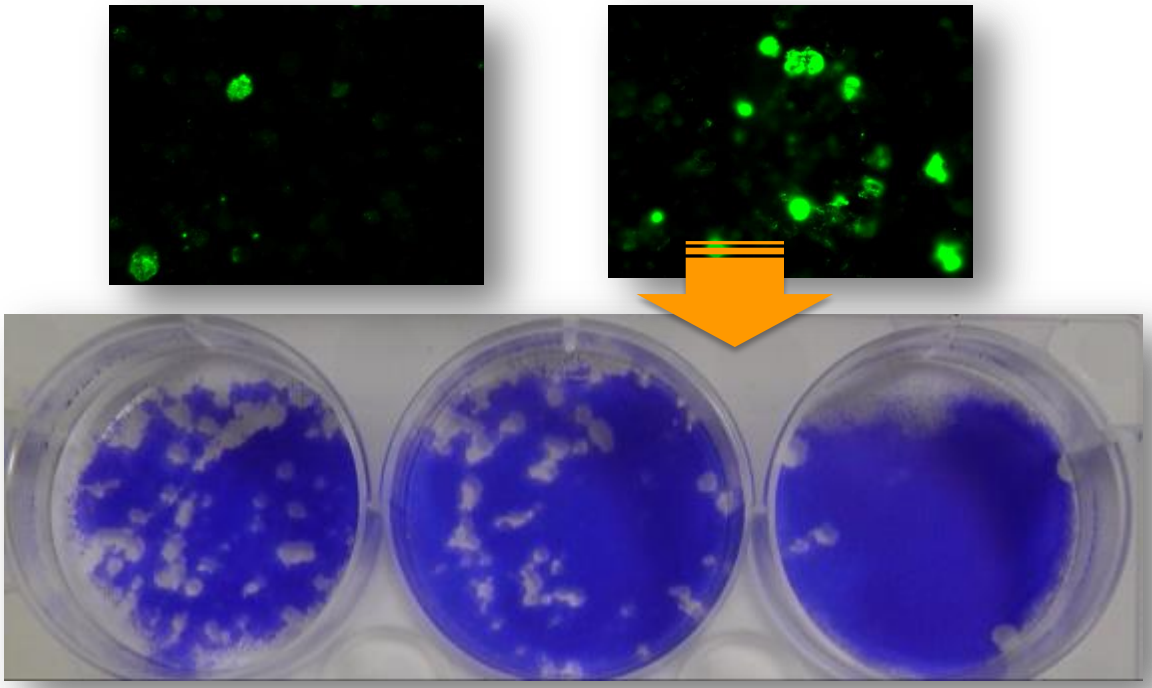
→ **Functional protocol established to enrich respiratory viruses from nasal swabs**

ApoH 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



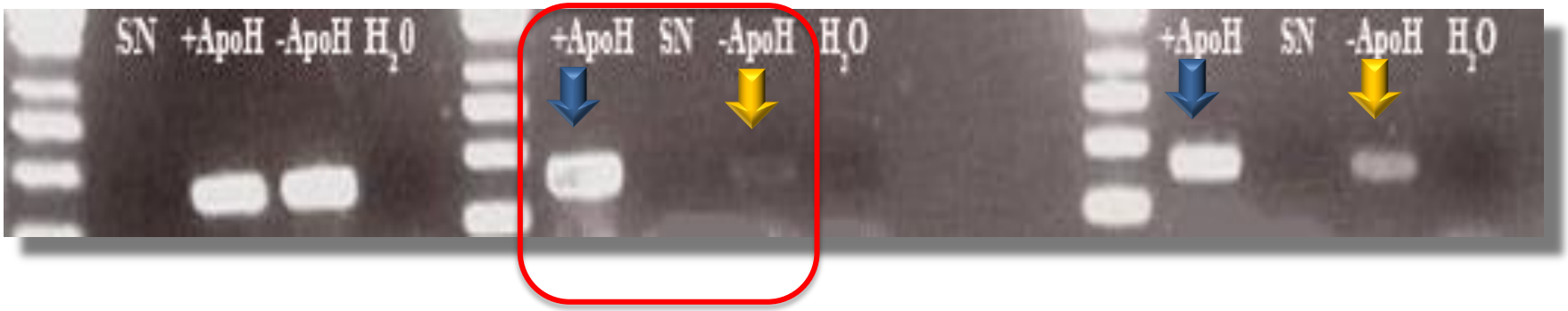
ApoH-captured of a cultivated H3N2 strain & subsequent infection of its target cells ➔ cytopathogenic effects

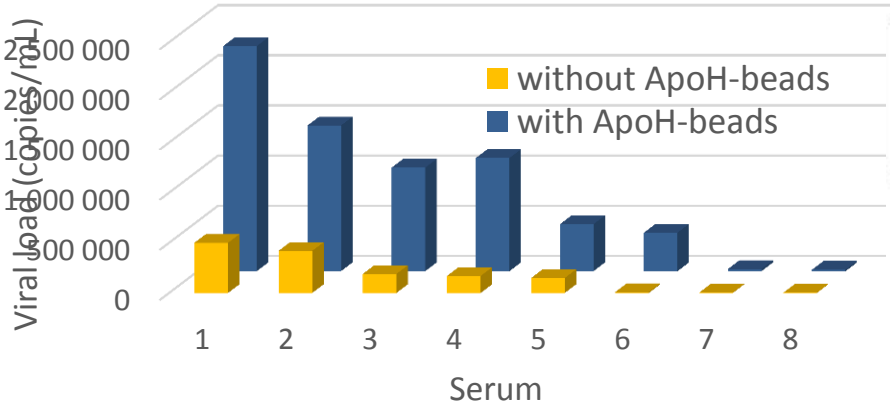
ApoH for clinical detection of low viral infections

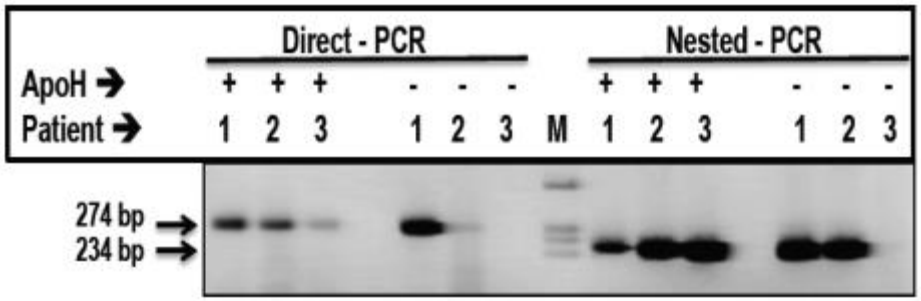
The USDEP European Project

HCV

Highly positive Plasma | Suspected Plasma | Low positive Plasma







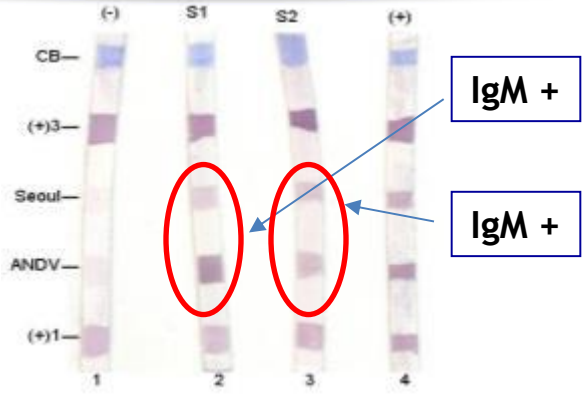
ApoHa-viral capture strongly enhances the HCV-detection sensitivity

ApoH*a* & occult Hantavirus cardiopulmonary infections

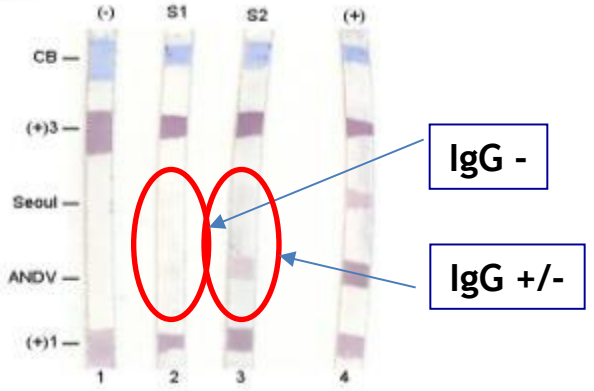
The USDEP European Project

Hantavirus

A

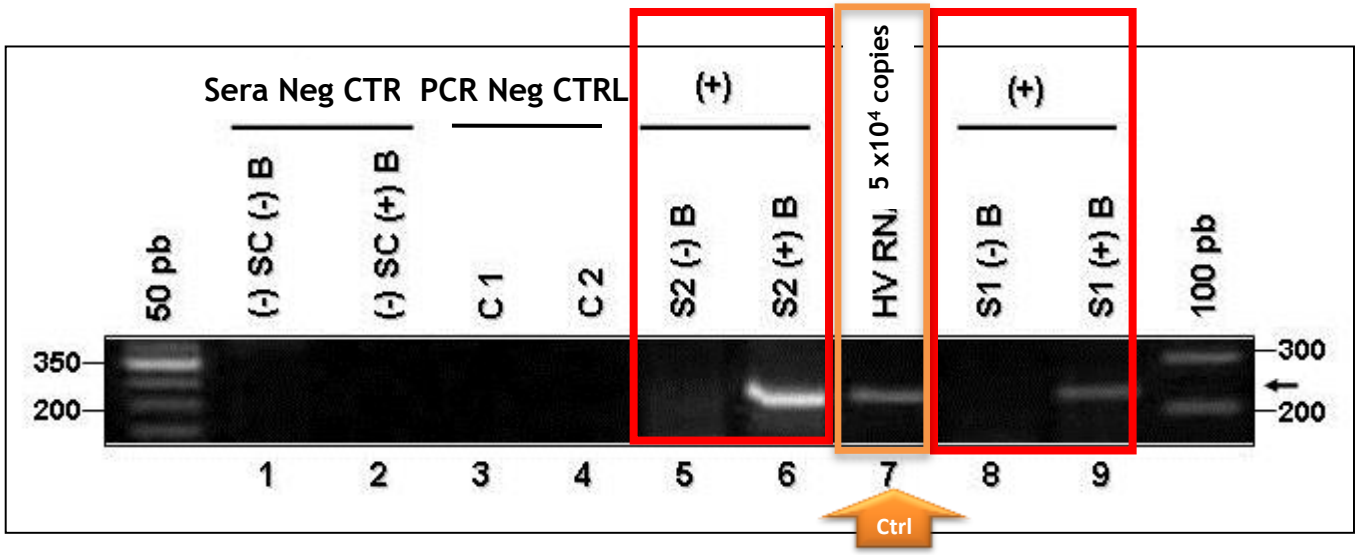


B



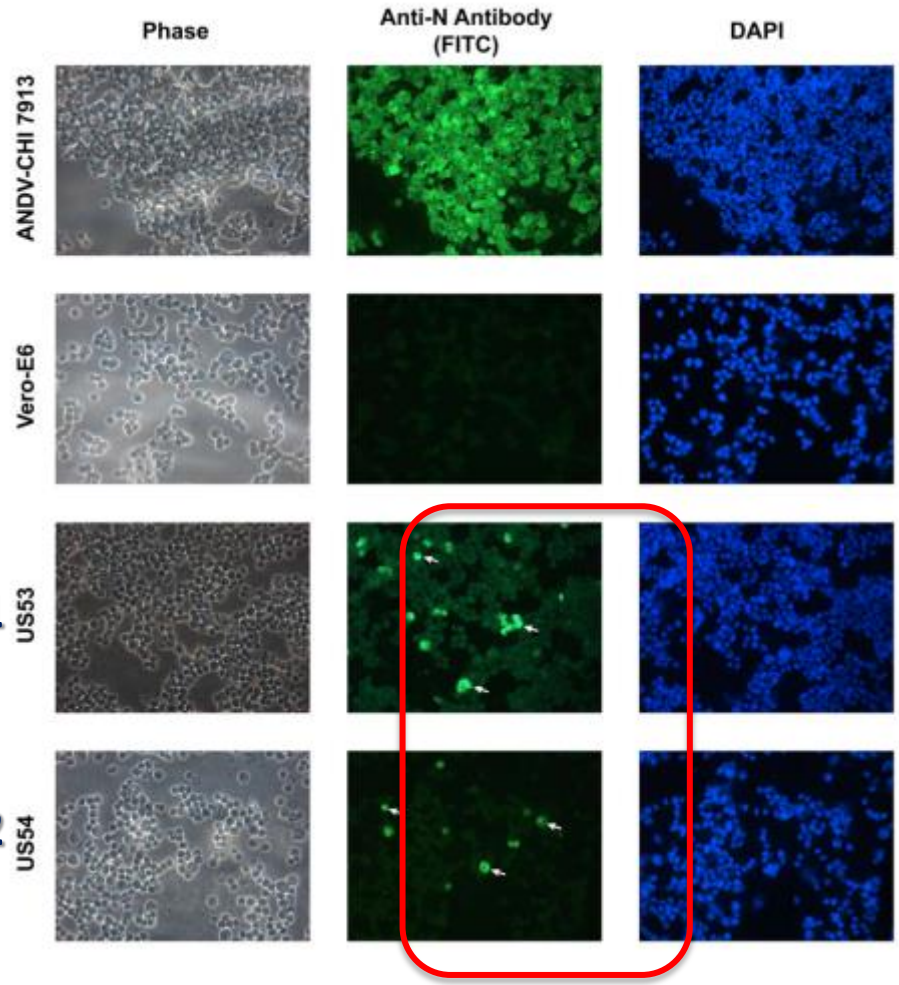
Andes virus PCR after ApoH capture from the suspected samples S1 &S2 : (+)B]= ApoH+ VS (-)B=ApoH-

C



ApoH-pretreatment solved 10% of Hantavirus false negative **diagnostics in Chile**

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 for clinical diagnostic of viral infections

Other examples

Biological psychiatry 2008, 64:1019-23

PRIORITY COMMUNICATION

Endogenous Retrovirus Type W GAG and Envelope Protein Antigenemia in Serum of Schizophrenic Patients

Hervé Perron, Lila Mekaoui, Corinne Bernard, Francisco Veas, Ilias Stefas, and Marion Leboyer

Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease

Hervé Perron, Raphaëlle Germi, Corinne Bernard, Marta Garcia-Montojo, Cécile Deluen, Laurent Farinelli, Raphaël Faucard, Francisco Veas, Ilias Stefas, Babs O Fabriek, Jack Van-Horssen, Paul Van-der-Valk, Claire Gerdil, Roberta Mancuso, Marina Saresella, Mario Clerici, Sébastien Marcel, Alain Creange, Rosella Cavaretta, Domenico Caputo, Giannina Arru, Patrice Morand, Alois B Lang, Stefano Sotgiu, Klemens Ruprecht, Peter Rieckmann, Pablo Villoslada, Michel Chofflon, Jose Boucraut, Jean Pelletier and Hans-Peter Hartung

Mult Scler published online 28 March 2012

Adlhoch *et al. Virology Journal* 2011, **8**:63
http://www.virologyj.com/content/8/1/63

VIROLOGY JOURNAL

RESEARCH Open Access

Highly sensitive detection of the group A Rotavirus using Apolipoprotein H-coated ELISA plates compared to quantitative real-time PCR

Cornelia Adlhoch[1*†], Marco Kaiser[1,2†], Marina Hoehne[3], Andreas Mas Marques[3], Ilias Stefas[4], Francisco Veas[5], Heinz Ellerbrok[1]

ApoHa as a pre-analytical solution for ultrasensitive diagnostic of bacterial infections from clinical and food samples

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 (septicaemia…) or for HAI screening.

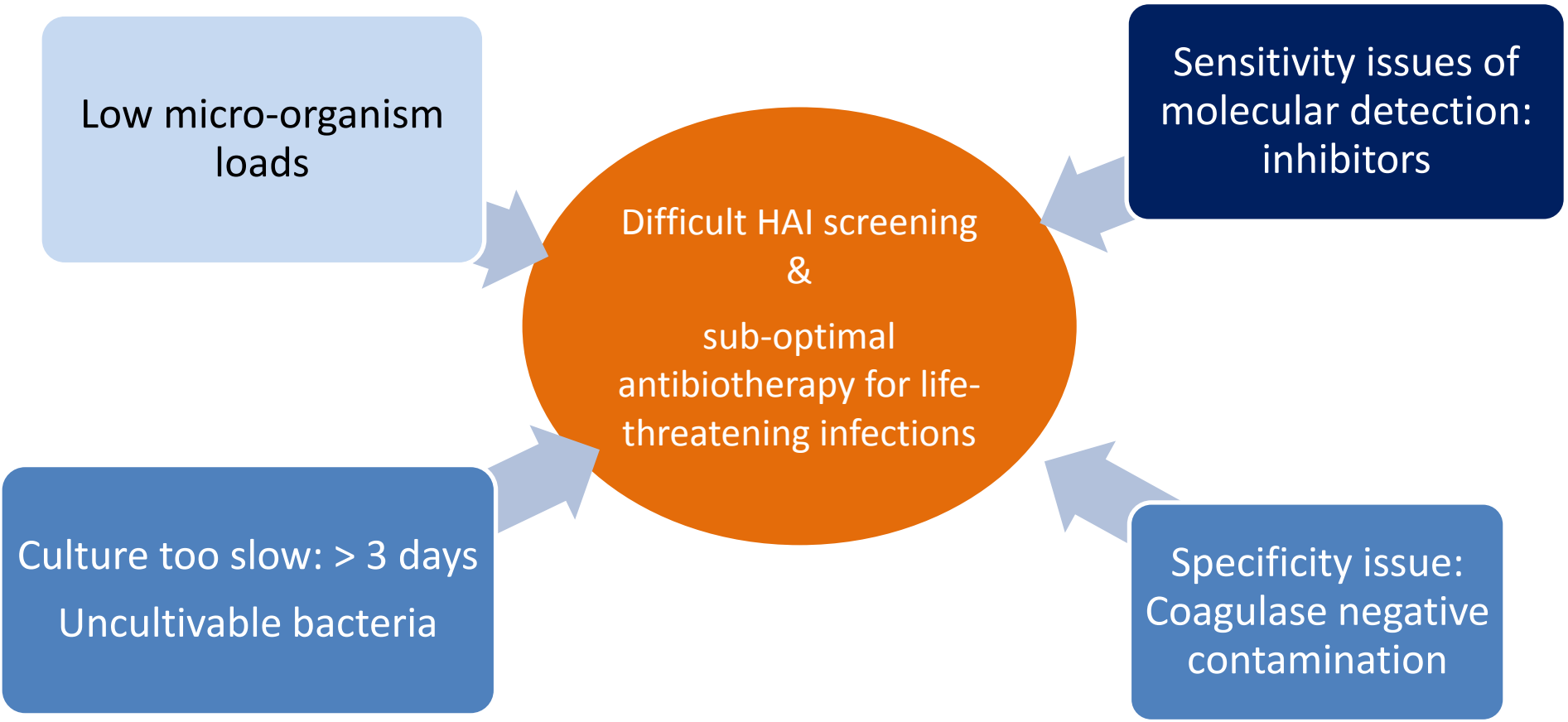
Main concerns:

- **Blood culture** based diagnosis
  - could **take 2 days or more**,
  - **lack of sensitivity** (ex.: false negatives due to presence of antibiotics…),
  - **an issue for non-cultivable bacteria**
  - **specificity** (ex.: Coagulase-Negative *Staphylococcus* contamination)

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

ApoH use in clinical bacterial infections

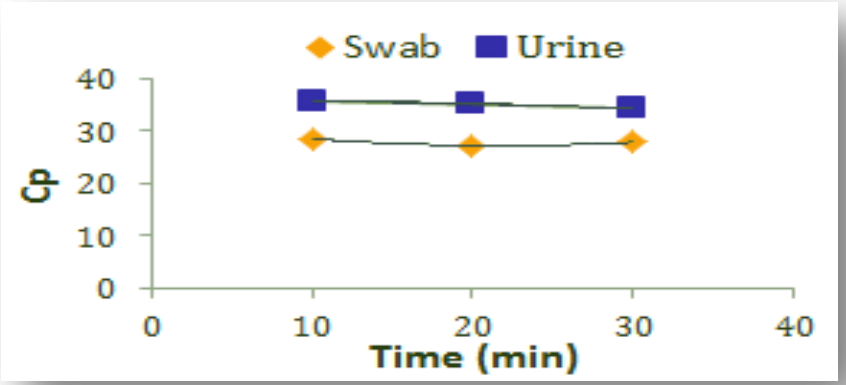
Critical unmet needs



ApoH use for clinical or food bacterial infection

ApoH work with different complex target matrices

- ✓ **Whole blood** : sensitivity as hig as 1-2 bacteria/mL (spiked)
- ✓ **Feces** : *Mycobacterium avium spp paratuberculosis*
- ✓ **Food**: Reducing the detection timing of Salmonella from raw milk meat from 20-24 hr up to 8-10 hr (we presently run work to get diagnostic within 2 hr).
- ✓ **Urine:** *Chlamydia detection with* qPCR after ApoH beads step



→ **ApoH exhibit a high affinity for infectious bacteria, but a low affinity for commensal bacteria from the gut or from collections (ATCC).**

ApoH use in clinical bacterial infection

Very large capabilities of capture of pathogenic bacteria

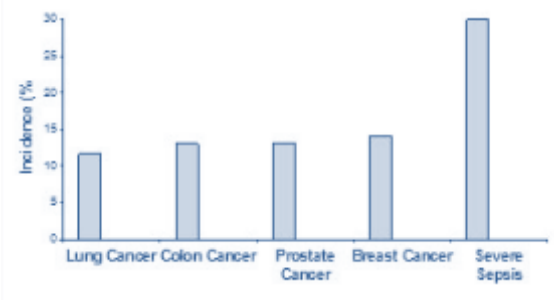
Acinetobacter baumannii	*Corynebacterium sp.*	*Mycobacterium abscessus*	*Salmonella typhimurium*
Acinetobacter lwoffii	*Corynebacterium xerosis*	*Mycobacterium chelonae*	*Serratia marcescens*
Acinetobacter sp.	*Enterobacter aerogenes*	*Neisseria cinerea*	*Sphingomonas paucimobilis*
Bacillus cereus	*Enterobacter 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 …*

ApoH use for clinical or food bacterial infection

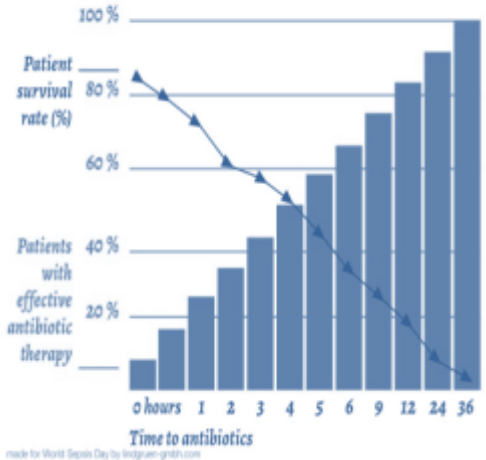
Sepsis

***Sepsis**: Severe widespread infection syndrome of the human body caused by pathogenic germs.*

Sepsis affects more than 20 millions people throughout the world per year



Sepsis is one of the major cause of death for hospitalized patients : with a mortality rate of 28.3% to 41.1% in North America and Europe.



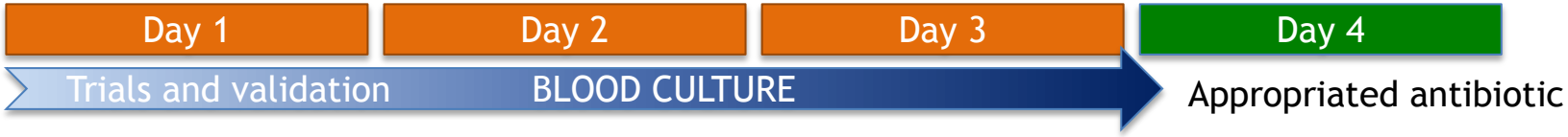
Current procedures:

1. **Culture**: visualization through bacterial growth (colonies forming).
   Limitation : duration, only 30% of bacteria are positive in culture…
2. **Sensitive methods** (i.e. : PCR, RPA)
   Limitations: complex sample sensitivity issues due to presence of inhibitors and low pathogen load

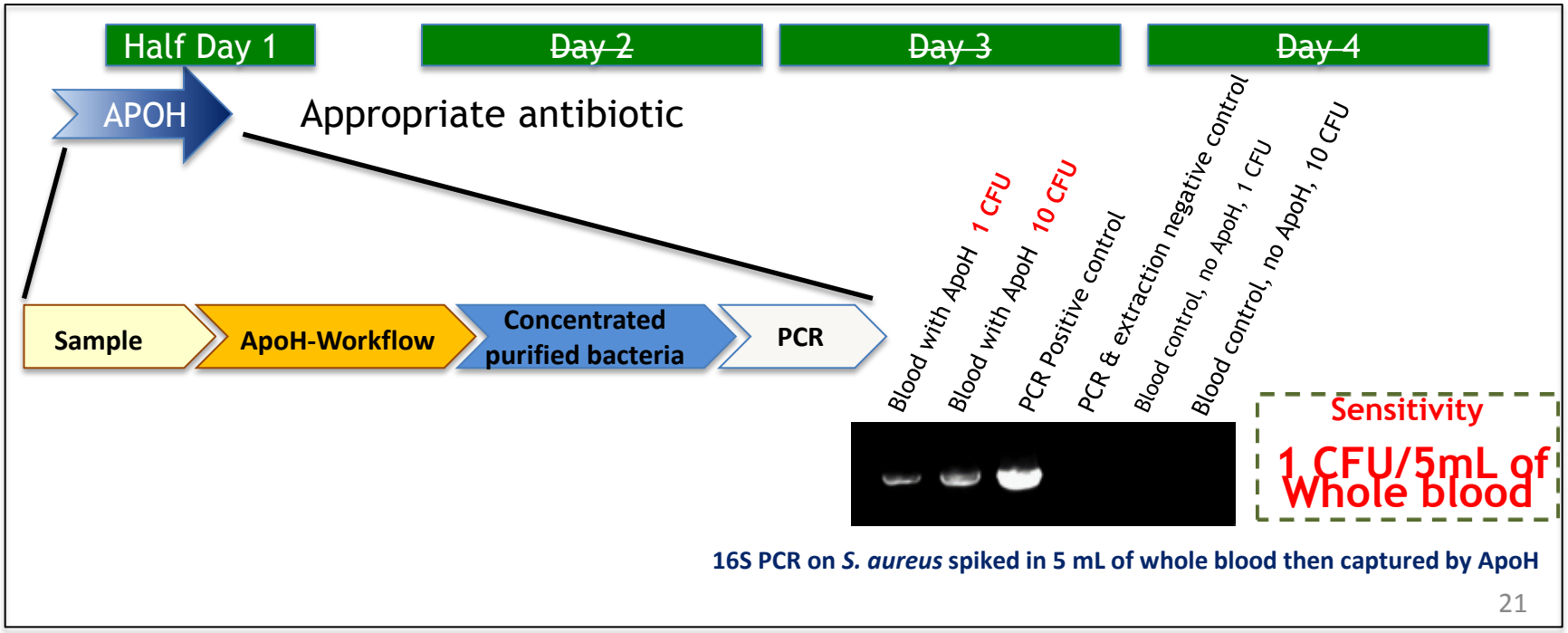
ApoH use for clinical or food bacterial infection

ApoH & sepsis high sensitive detection in whole blood

For a current bacterial load of 0 to 10 bacteria/mL of whole blood, there is a huge need to increase sensitivity through concentration.



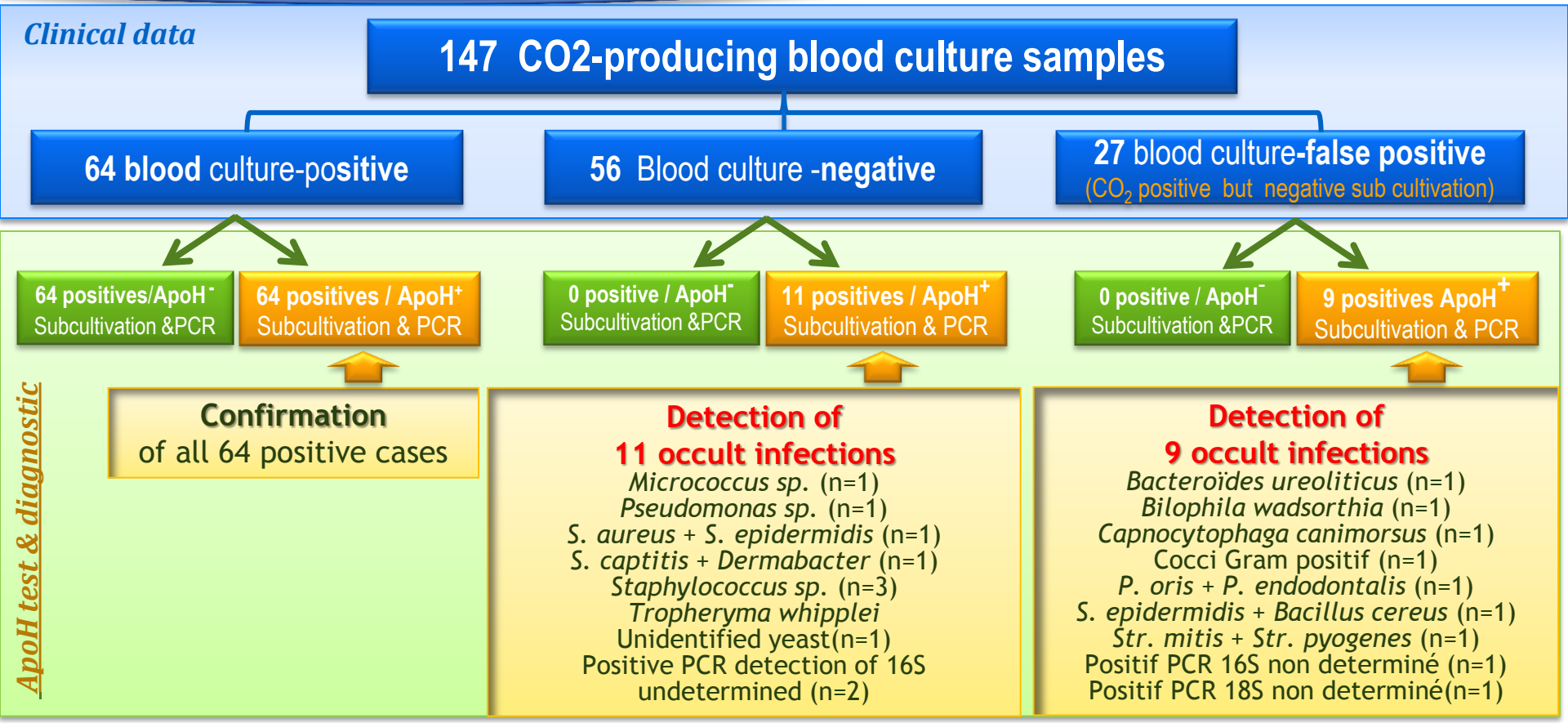
ApoH-T is the only marketed solution able to highly concentrate bacterial load for optimal detection (1 CFU/ 5mL of whole Blood from clinical samples)



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

ApoH for clinical bacterial infection

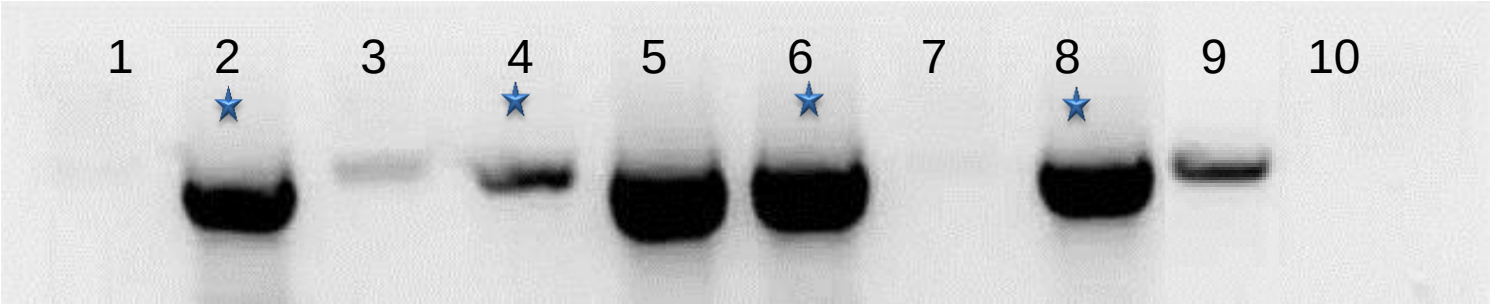
Nosocomial infections from different hospital services



→ApoH beads **strongly increases** the detection sensitivity **of nosocomial infections** consequently **avoiding 20/147 (13.6%) of false negative diagnostics**

ApoH use for clinical detection bacterial infection

ApoH avoid false negatives due to PCR inhibitors



1: *K. pneumoniae* without ApoH beads
2: *K. pneumoniae* **with** ApoH beads
3: *S. aureus* without ApoH beads
4: *S. aureus* **with** ApoH beads
5: *Coagulase-négative* Staphylococcus without ApoH beads
6: *Coagulase-négative* Staphylococcus **with** ApoH beads
7: *P. acnes* without ApoH beads
8: *P. acnes* **with** ApoH beads
9: Positif control (Bacterial DNA)
10: H_2O

→ ApoH beads concentrate bacteria from hemocultures increasing the PCR signal
→ ApoH eliminate inhibitor inducing false negative

P2-138 Concentration of Bacterial Pathogens Using Apolipoprotein H

Tuesday, August 5, 2014
Exhibit Hall D (Indiana Convention Center)

***Erin Almand**, North Carolina State University, Raleigh, NC*
Rebecca Goulter, North Carolina State University, Raleigh, NC
Lee-Ann Jaykus, North Carolina State University, Raleigh, NC

Introduction: Concentration of bacteria from food or environmental samples prior to detection could reduce or even eliminate the need for cultural enrichment. A broadly reactive ligand with the ability to concentrate a variety of microbes from relevant sample matrices could facilitate this type of sample preparation. The human plasma protein Apolipoprotein H (ApoH) has been shown to have a high affinity for a number of Gram- and Gram+ bacteria.

Purpose: To investigate the utility of ApoH conjugated to magnetic beads for the capture and concentration of select foodborne bacterial pathogens.

Methods: Overnight cultures of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* serovar Enteritidis, and *Staphylococcus aureus* were serially diluted in proprietary binding buffer to concentrations of 10^3, 10^5 and 10^7CFU/100 µl. Suspensions were supplemented with 10µl of ApoH conjugated magnetic beads (ApoH Technologies, Villeneuve St Georges, France) and incubated for 60 min at 4°C with rotation. The beads were captured by magnet and washed twice. Both bead and supernatants suspensions were retained for cultural enumeration of bacteria. An aliquot of the beads was also subjected to DNA extraction followed by detection of each pathogen using a SYBR green qPCR method targeting the 16S rDNA gene.

Results: Based on loss to supernatant, the ApoH beads showed high capture efficiency (73.4-100%) for all four pathogens tested and at all three concentrations (10^3, 10^5 and 10^7 CFU/100 µl.) In most cases, there were no statistically significant differences in capture efficiencies when comparing pathogens or initial cell concentration ($P > 0.05$, n = 3). The SYBR green qPCR results were more variable but in general, assay detection limits after ApoH capture and qPCR were approximately one log CFU higher compared to input cell numbers.

Significance: ApoH conjugated magnetic beads show promise for concentration of bacterial pathogens in preparation for detection using cultural methods or qPCR.

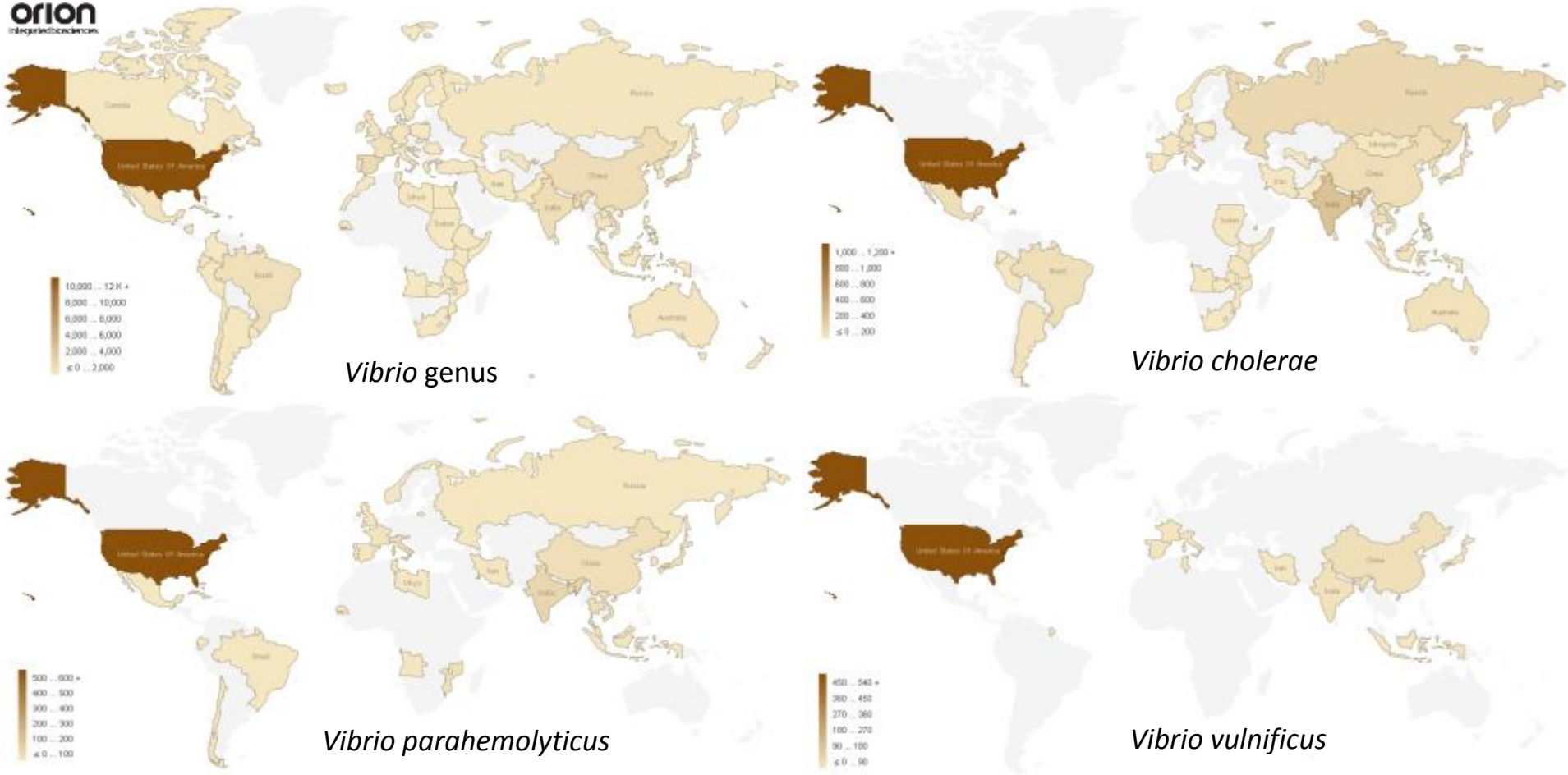
Back to: Poster Session - Pathogens, Epidemiology, Novel Laboratory Methods, Food Defense, Communication Outreach and Education, General Microbiology, Dairy and Other Food Commodities, Food Toxicology

<< Previous Abstract | Next Abstract >>

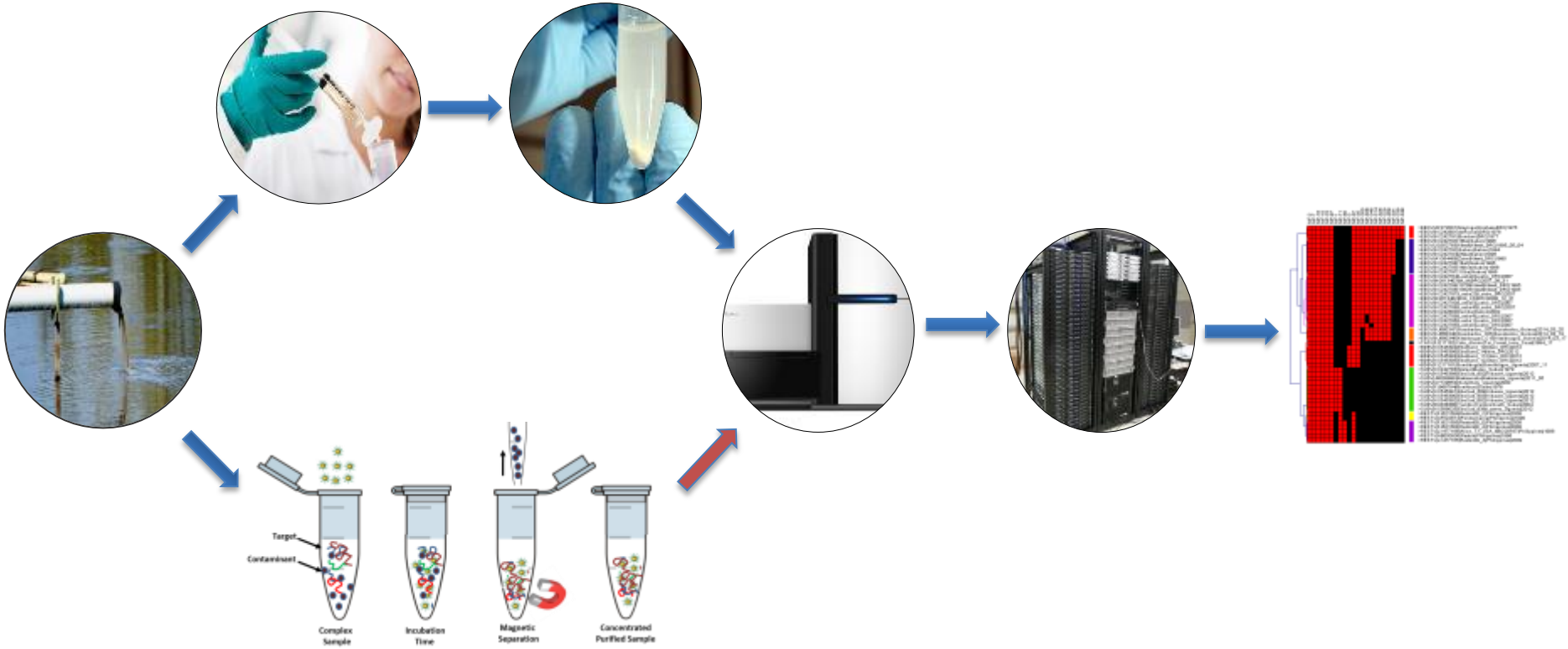
ApoH sample preparation – environmental FLU

NGS-metagenomic analysis

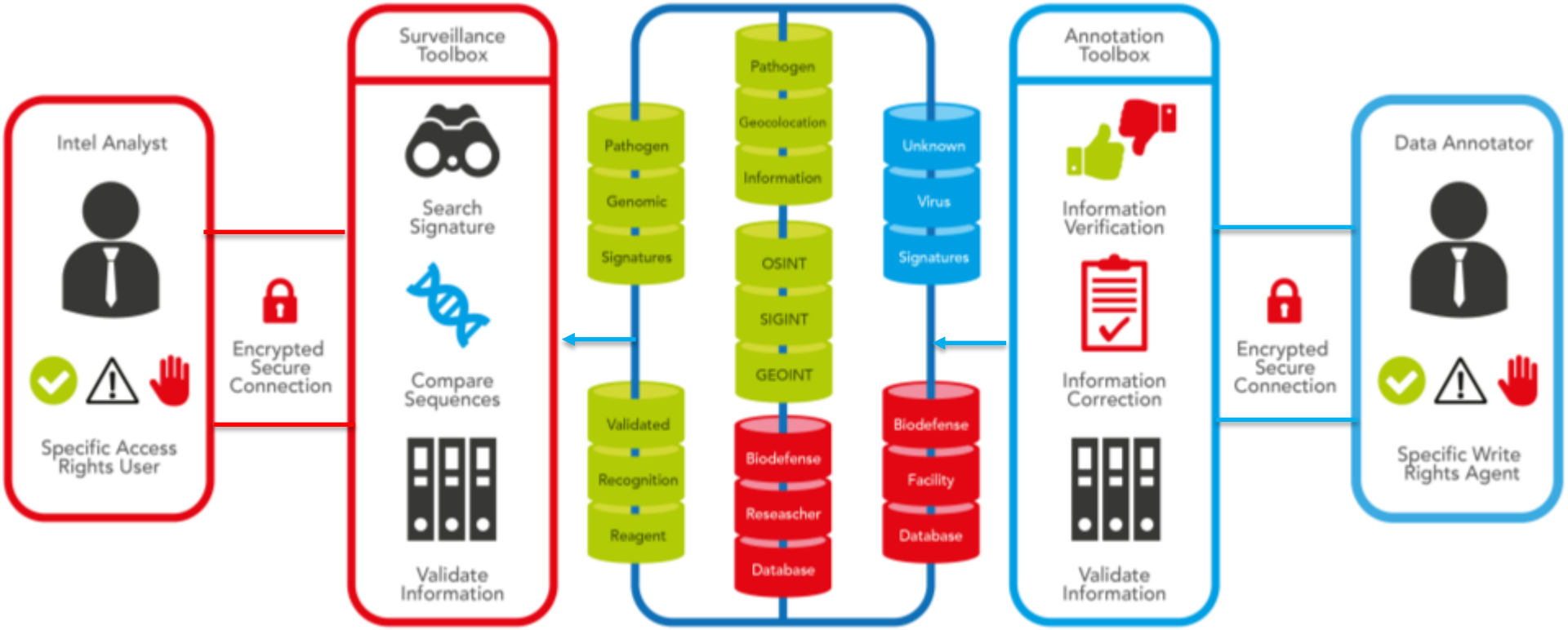
Genomic Origin: *Vibrio vulnificus*



ApoH deployment



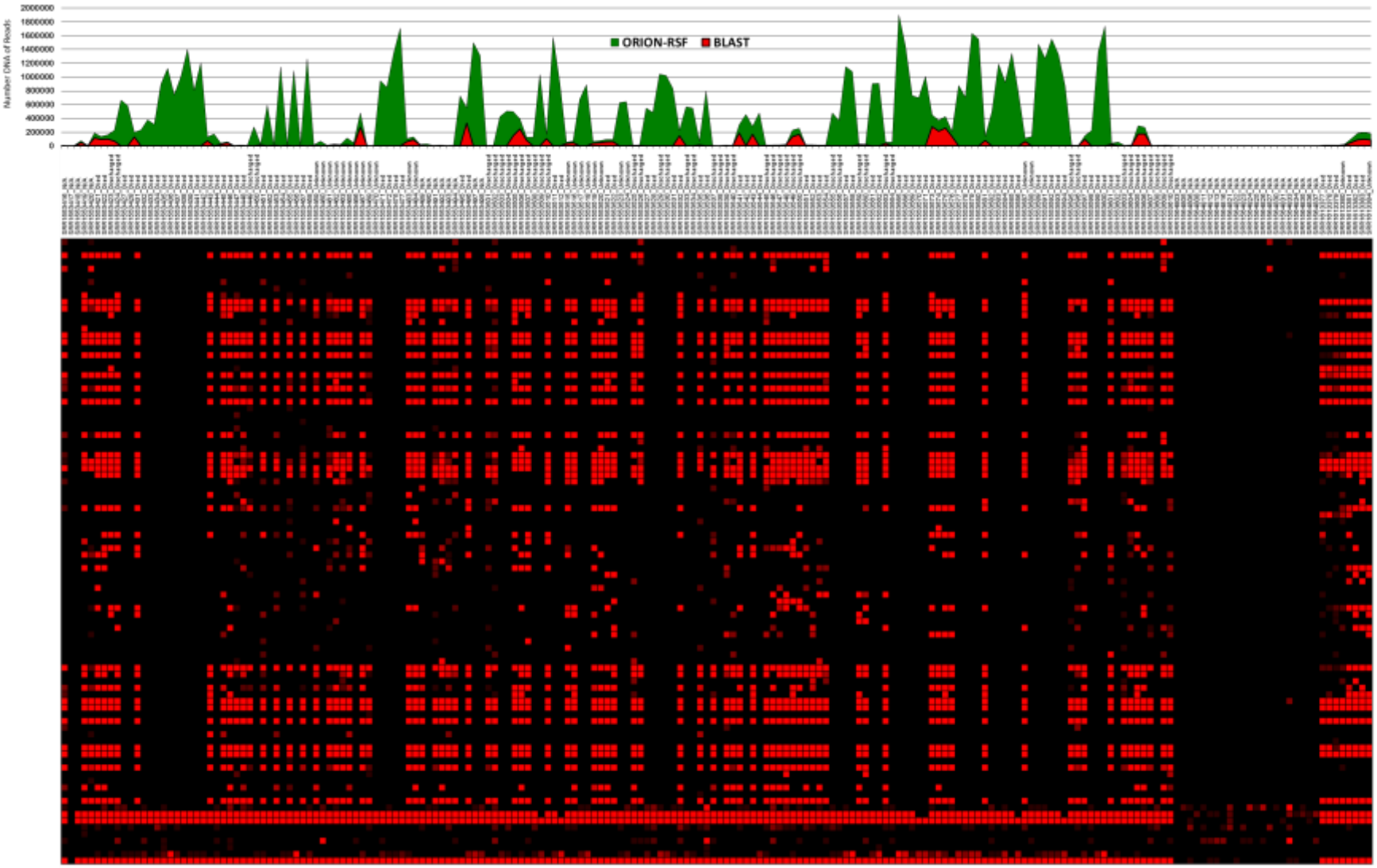
Integrated Biodefense Analysis System



Large Scale Metagenomic Analysis:

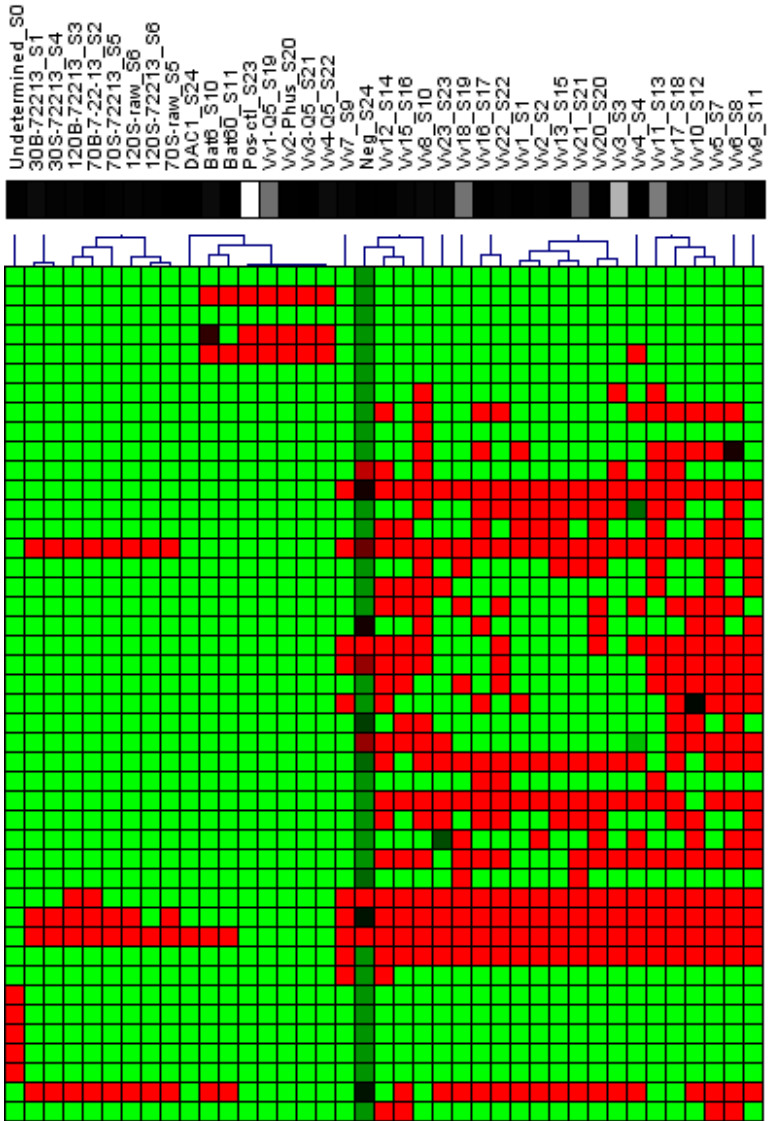
- Quasispecies
- Co-Infection and Biomarker
- Pathogen discovery and threat assessment

MF-discrimination of EBOV outbreaks

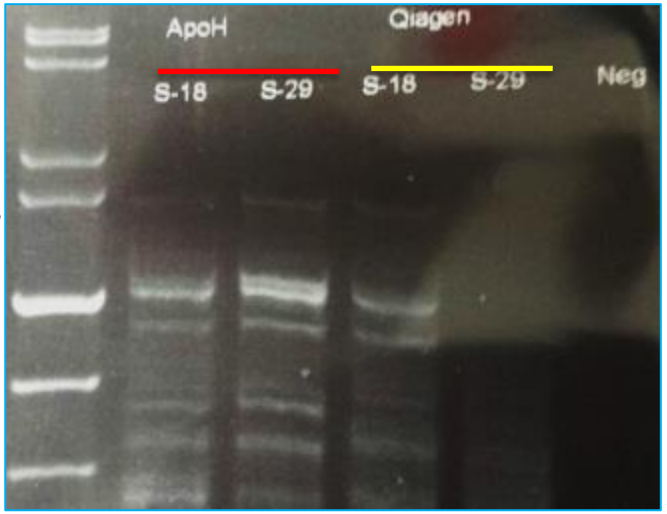


NGS-Metagenomic analyses of Water Samples

Prepared with and without ApoH

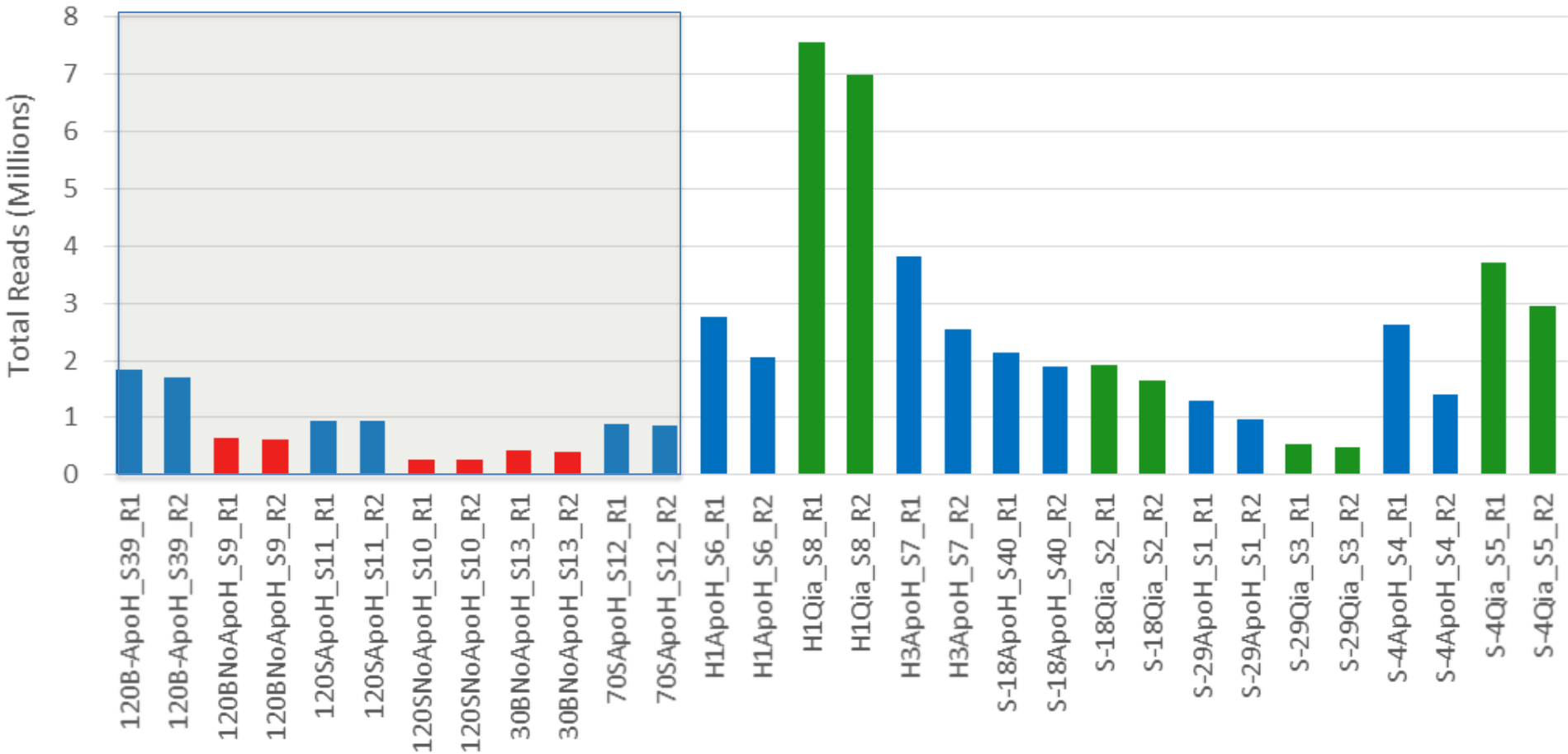


uncultured Desulfobacterium sp.
Trichuris trichiura
Staphylococcus aureus subsp. aureus 65-1322
Legionella pneumophila subsp. pneumophila
Escherichia sp. 3_2_53FAA
Streptococcus suis 98HAH33
Vibrio cholerae O395
Vibrio cholerae AM-19226
Aliivibrio wodanis
Vibrio anguillarum 775
Listonella anguillarum M3
Vibrio cholerae MS6
Vibrio parahaemolyticus RIMD 2210633
Listonella anguillarum serovar O1
Bacteria
Vibrio tubiashii ATCC 19109
Vibrio cholerae
Vibrio mimicus SX-4
Vibrio furnissii NCTC 11218
Vibrio vulnificus NBRC 15645 = ATCC 27562
Vibrio parahaemolyticus O1:Kuk str. FDA_R31
Vibrio harveyi group
Vibrio alginolyticus NBRC 15630 = ATCC 17749
Vibrio sp. 16
Vibrio tasmaniensis LGP32
Vibrio sp. Ex25
Shewanella baltica OS185
Vibrio parahaemolyticus UCM-V493
Vibrio parahaemolyticus O1:K33 str. CDC_K4557
Vibrio parahaemolyticus
Vibrio harveyi
Vibrio coralliilyticus
Gammaproteobacteria
Proteobacteria
Unclassified
Vibrio campbellii ATCC BAA-1116
Marinomonas posidonica IVIA-Po-181
Amycolatopsis lurida NRRL 2430
Acinetobacter baumannii AC12
Desulfitobacterium hafniense
Pyronema omphalodes CBS 100304
uncultured organism
cellular organisms
Chlorobium chlorochromatii CaD3



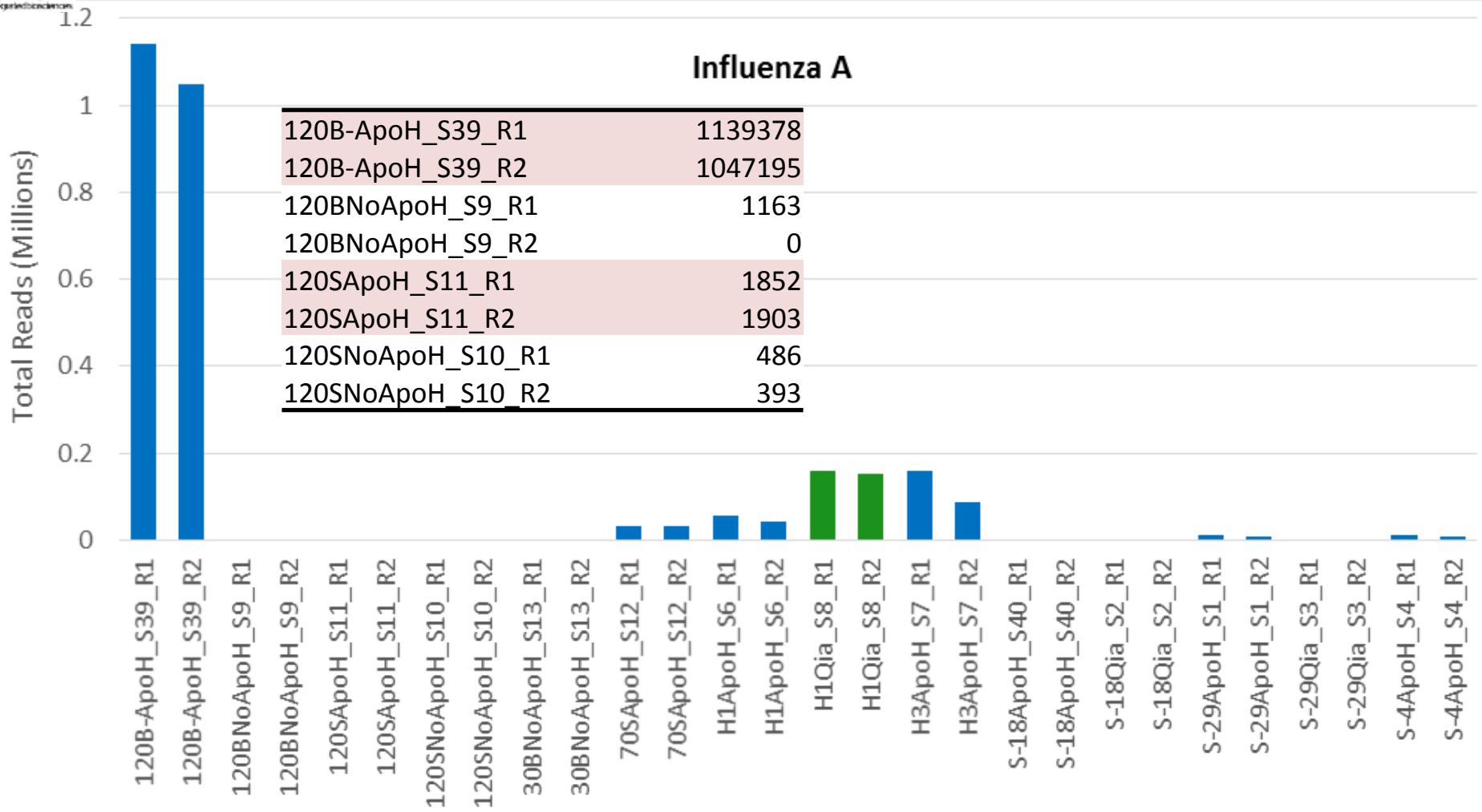
ApoH sample preparation – environmental FLU

NGS-metagenomic analysis



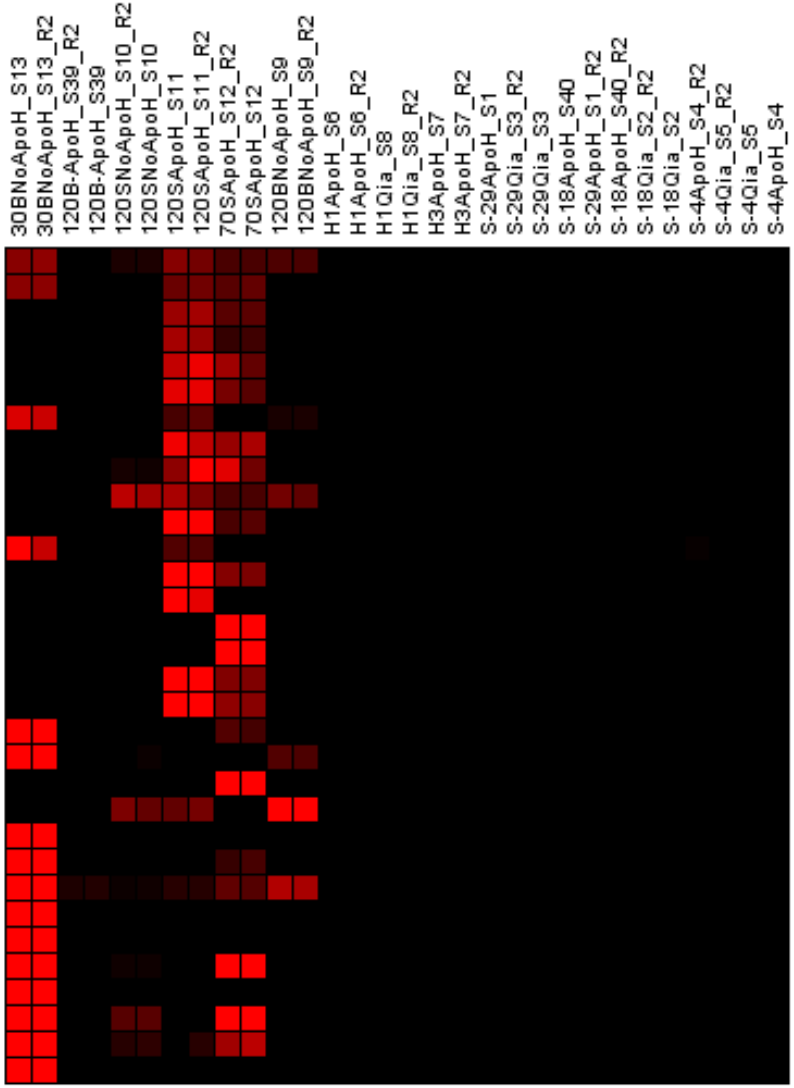
ApoH sample preparation – environmental FLU

NGS-metagenomic analysis



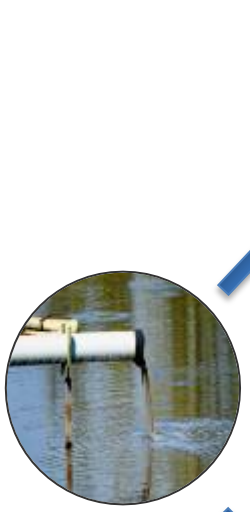
ApoH sample preparation – environmental FLU

NGS-metagenomic analysis

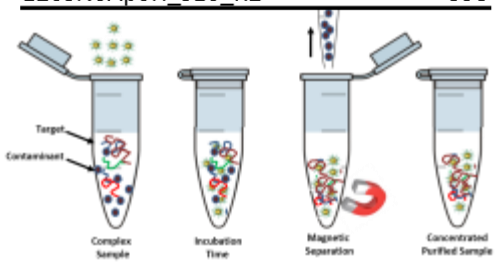


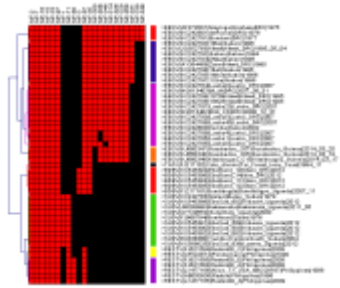
ApoH sample preparation & NGS-metagenomic analysis

CONCLUSION



120B-ApoH_S39_R1	1139378
120B-ApoH_S39_R2	1047195
120BNoApoH_S9_R1	1163
120BNoApoH_S9_R2	0
120SApoH_S11_R1	1852
120SApoH_S11_R2	1903
120SNoApoH_S10_R1	486
120SNoApoH_S10_R2	393





ApoH use for pathogen detection & metagenomics

- Ultra-sensitive ApoH-capture step significantly **reducing the time to result and sensitivity issues of the existing culture and PCR techniques** thanks to:
  (i) bacterial concentration,
  (ii) antibiotic or inhibitors elimination (from blood or tissues).

- In cases of life-threatening infections (septicemia..) or, of HAI screening, ApoH leads to major benefits:

  (i) **early diagnosis of disease** and consequently better individual prompt treatment strategy,
  (ii) **early patient isolation.**

- **ApoH increases the number of metagenomics** reads leading to a more accurate known and unknown pathogen detection (coverage/diversity)

General conclusion 1

ApoH*α* increases performances and value of diagnostic products

The ApoH*α* pre-analytical step is **simple, fast , of broad usage, and compatible with multiplexing detection of viruses & bacteria:**

- by **enhancing the sensitivity** of existing viruses detection **ApoH*α* provides major competitive advantages** for:
  - ✓ **early diagnosis of infection and diseases** leading to better individual prompt treatment strategy,
  - ✓ **Fine tuning of therapeutic monitoring** consequently with an improved adaptation of **therapeutic** protocols
  - ✓ **earlier patient isolation** as needed
- by **improving**:
  - ✓ **epidemiological surveillance,**
  - ✓ **reduction of infectious disease risks** during **transfusions & transplantations.**

These factors are particularly useful in case of life-threatening infections (septicemia..) or, of HAI screening

- For **bacterial contamination**, ApoH allows their capture for ultra-sensitive detection significantly **reducing the time to the results of the existing culture and PCR techniques**

General conclusion 2

ApoH*a* increases performances and value of diagnostic products

- The ApoH-coated beads can be used to **capture, cultivate, detect & identify pathogens (virus & bacteria) from different origins**:
  - ✓ Environment (Water, soil, plants)
  - ✓ Human (any kind of sample)
  - ✓ Animals (including insects, any kind of sample)
  - ✓ Food (security)
  - ✓ Industry (biological productions: vaccines, proteins etc)

- **Different issues** can be considered :
  - ✓ Public health (Epidemiology, Biosecurity , Bioterrorism)
  - ✓ Clinical (Translational research ex Nosocomial infections, diseases evolution & treatment efficacy)
  - ✓ Veterinary issues (bio-security, Food)
  - ✓ Biodiversity
  - ✓ Food security

- In addition, technology of the ApoH-coated nano-magnetic beads with metagenomics **allows** rapid **isolation** and **identification** of **unknown pathogens**!

Special thanks & contacts

info@apohtech.com or francisco.veas@ird.fr

- ✓ **European USDEP project**
- ✓ **University Montpellier 1, France**
- ✓ **The French Research Institute for Development**
- ✓ **The French Bank for Innovation (BPI, formerly OSEO)**
- ✓ **The Region Languedoc–Roussillon – France**
- ✓ **Lab d'Immuno-Physiopathologie Moléculaire Comparée (LIPMC)**
- ✓ **The ApoH-Technologies engineer-team** & Ilias Stefas (CEO)

✓**Biotechnologies-Développement-Conseil** (France, USA, Israel & Japan), **Christian Policard CEO** (former CEO of Pasteur Institute Business Development & former CEO of Sanofi-Pasteur Diagnostics) **cpolicard@biotechdevconseil.com**

THANKS FOR YOUR ATTENTION !

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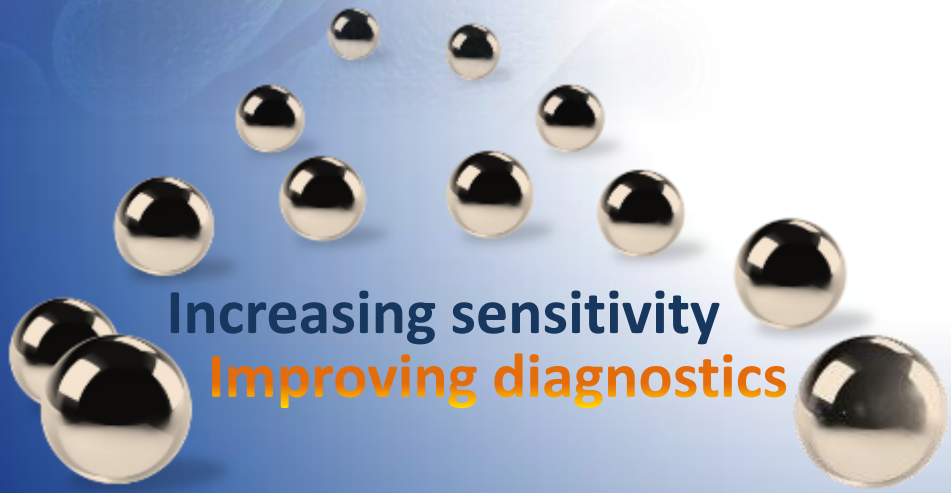


Increasing sensitivity
Improving diagnostics

info@apohtech.com

www.apohtech.com

ApoH capture a proprietary technology

ApoH-Technologies products:

Solutions for samples pre-treatment & microorganisms capture for their ultra-sensitive detection & characterization

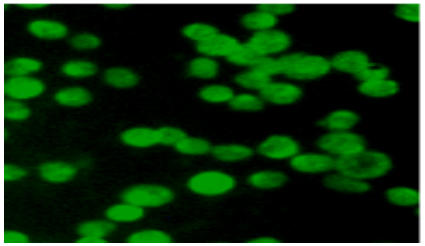
- **ApoH*α*-coated nano-magnetic beads:**

Human ApoH*α*: (ApoH)	ApoH-CaptoBAC kit (bacteria)
	ApoH-CaptoVIR kit (virus)
	ApoH-CaptoFUN kit (fungi)

Synthetic Peptides: (Peps6)	Peps6-CaptoBAC kit (bacteria)
	Peps6-CaptoVIR kit (virus)
	Peps6-CaptoFUN kit (fungi)

→ **Nano-magnetic beads for molecular and culture assays**

- **ApoH-coated micro-plates:**



→ **ApoH-coated micro-plates for ELISA immunoassays**

- **ApoH protein:**

→ **For customized use**