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

Francisco Veas, PhD  
Head of the Laboratoire d’Immuno-Physiopathologie Moléculaire Comparée  
Deputy director of UMR-Ministry of defense  
CSO of ApoH-Technologies  
Faculty of Pharmacy-Université de Montpellier  
Montpellier – France

Willy Valdivia, CEO  
Orion Integrated Biosciences, NY, US
ApoH capture: a proprietary technology
A poly-specific capture of microorganisms

ApoH\(a\) or apolipoprotein H or \(\beta 2\)-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 erythematosus

ApoH\(a\) is activated 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–)
ApoHa capture a proprietary technology

... enabling ultra-sensitive microbiology

**ApoHa 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

OPTIONAL STEPS
Pathogen elution
Beads elimination

1. Serum & beads ApoH
2. Capture
3. Magnetic separation
4. Purification washes
5. Concentration on ApoH beads
6. Purified pathogens

DETECTION & DIAGNOSTIC or ISOLATION

Pathogen
ApoH beads
Inhibitor

Bacteria
Bioluminescence

&/or

Magnet

&/or

Virus /Bacteria Culture

&/or

Virus /bacteria PCR
ApoH for ultrasensitive detection of clinical viral infections
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.
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

<table>
<thead>
<tr>
<th></th>
<th>copies/rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without ApoH</td>
<td>$1.7 \times 10^5$</td>
</tr>
<tr>
<td>With ApoH</td>
<td>$1.8 \times 10^6$</td>
</tr>
</tbody>
</table>

→ Functional protocol established to enrich respiratory viruses from nasal swabs
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

Highly positive Plasma

Suspected Plasma

Low positive Plasma

ApoHa-viral capture strongly enhances the HCV-detection sensitivity
ApoHa & occult Hantavirus cardiopulmonary infections
The USDEP European Project

Hantavirus

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

ApoH-pretreatment solved 10% of Hantavirus false negative diagnostics in Chile
ApoH & isolation of hemorrhagic Hantaviruses

Infection of Vero E6 cells with ApoH-captured Hantaviruses
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 García-Montojo, Cécile Deluen, Laurent Farinelli, Raphaël Faucard, Francisco Veas, Ilias Stefas, Babs O Fabric, Jack Van-Horstsen, 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, José Boucraut, Jean Pelletier and Hans-Peter Hartung

*Mult Scler* published online 28 March 2012

**Research**

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

Cornelia Adlhoth1**, Marco Kaiser1,2†, Marina Hoehne3, Andreas Mas Marques3, Ilias Stefas4, Francisco Veas5, Heinz Ellerbrok1
ApoHa as a pre-analytical solution for ultrasensitive diagnostic of bacterial infections from clinical and food samples
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
Critical unmet needs

- Difficult HAI screening & sub-optimal antibiotic therapy for life-threatening infections
- Culture too slow: > 3 days
  Uncultivable bacteria
- Specificity issue: Coagulase negative contamination
- Sensitivity issues of molecular detection: inhibitors
- Low micro-organism loads
ApoH use for clinical or food bacterial infection
ApoH work with different complex target matrices

- **Whole blood**: sensitivity as high 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

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

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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

Clinical data

147 CO2-producing blood culture samples

64 blood culture-positive
56 Blood culture-negative
27 blood culture-false positive (CO₂ positive but negative sub cultivation)

64 positives/ApoH⁺ Subcultivation & PCR
64 positives / ApoH⁺ Subcultivation & PCR
0 positive / ApoH⁺ Subcultivation & PCR
11 positives / ApoH⁺ Subcultivation & PCR
0 positive / ApoH⁺ Subcultivation & PCR
9 positives ApoH⁺

Confirmation of all 64 positive cases

Detection of 11 occult infections
Micrococcus sp. (n=1)
Pseudomonas sp. (n=1)
S. aureus + S. epidermidis (n=1)
S. captitis + Dermabacter (n=1)
Staphylococcus sp. (n=3)
Tropheryma whippelii
Unidentified yeast (n=1)
Positive PCR detection of 16S undetermined (n=2)

Detection of 9 occult infections
Bacteroides ureoliticus (n=1)
Bilophila wadsorthia (n=1)
Capnocytophaga canimorsus (n=1)
Cocci Gram positif (n=1)
P. oris + P. endodontalis (n=1)
S. epidermidis + Bacillus cereus (n=1)
Str. mitis + Str. pyogenes (n=1)
Positif PCR 16S non déterminé (n=1)
Positif PCR 18S non déterminé (n=1)

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: H₂O

➔ ApoH beads concentrate bacteria from hemocultures increasing the PCR signal
➔ ApoH eliminate inhibitor inducing false negative
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 $10^7$ CFU/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 supernatant 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.
Genomic Origin: *Vibrio vulnificus*

- **Vibrio genus**
- **Vibrio parahemolyticus**
- **Vibrio cholerae**
- **Vibrio vulnificus**
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 ApoHH
ApoH sample preparation – environmental FLU
NGS-metagenomic analysis
NGS-metagenomic analysis

Influenza A

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Reads (Millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120B-ApoH_S39_R1</td>
<td>1139378</td>
</tr>
<tr>
<td>120B-ApoH_S39_R2</td>
<td>1047195</td>
</tr>
<tr>
<td>120BNoApoH_S9_R1</td>
<td>1163</td>
</tr>
<tr>
<td>120BNoApoH_S9_R2</td>
<td>0</td>
</tr>
<tr>
<td>120SApoH_S11_R1</td>
<td>1852</td>
</tr>
<tr>
<td>120SApoH_S11_R2</td>
<td>1903</td>
</tr>
<tr>
<td>120SNoApoH_S10_R1</td>
<td>486</td>
</tr>
<tr>
<td>120SNoApoH_S10_R2</td>
<td>393</td>
</tr>
</tbody>
</table>
ApoH sample preparation – environmental FLU
NGS-metagenomic analysis

Ilumatobacter coccineus YM16-304
Variovorax paradoxus
Rhodomicrobium vanniellii ATCC 17100
Actinoplanes missouriensis 431
Magnetospirillum magneticum AMB-1
[Cellvibrio] gilvus ATCC 13127
Alphaproteobacteria
Janthinobacterium agaricidamnosum NBRC 102515 = DSM 9628
Streptomyces clavuligerus ATCC 27064
Synechococcus sp. KORD1-100
Arthrobacter arilaitensis Re117
Bukholderia
Rhodopseudomonas palustris HaA2
Guillardia theta CCMP2712
Planktothrix agardhii NIVA-CYA 126/8
Thermodesulfatator indicus DSM 15286
Streptomyces bingchengensis BCW-1
Tsukamurella paurometabola DSM 20162
Betaproteobacteria
Gammaproteobacteria
Sulillus luteus UH-SlLu-Lm8-n1
Synechococcus sp. WH 8102
uncultured bacterium A1Q1_fos_4
Comamonadaceae
Proteobacteria
Fusobacterium nucleatum subsp. polymorphum ATCC 10053
Morus notabilis
Rhodospirillum photometricum DSM 122
Taylorella asinigenitalis 14/45
Helicobacter felis ATCC 49179
Streptomyces
Thiimonas sp. CB2
CONCLUSION

<table>
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<tr>
<th>Sample Description</th>
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</table>
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)
The ApoHa 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, **ApoHa 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
ApoHa 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!

francisco.veas@ird.fr

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

• **ApoHα-coated nano-magnetic beads:**

  - **Human ApoHα:** ApoH-CaptoBAC kit (bacteria)
    - ApoH (ApoH)
    - ApoH-CaptoVIR kit (virus)
    - ApoH-CaptoFUN kit (fungi)
  
  - **Synthetic Peptides:** Peps6-CaptoBAC kit (bacteria)
    - Peps6 (Peps6)
    - 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**

www.apohtech.com