THE APOH TECHNOLOGY, A SIMPLE & POWERFUL PATHOGENS PRE-ENRICHMENT SYSTEM ALLOWING ULTRASENSITIVE DETECTION OF PATHOGENS

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MOTIVATION

Accurate diagnostic is a key and fundamental element to adopt appropriate countermeasures against pathologies. In the field of infectious old and new (re)-emerging diseases, a lot of progress has been done, mainly and successively with technologies such as the PCR, the microfluidic and the NGS. However, some critical situations escape to this ideal situation, giving rise to false-negative diagnostics, which, in turn, generate dramatic individual or public health situations.

SYSTEM OVERVIEW

We have discovered, ApoHa or apolipoprotein H or β2-glycoprotein I, a conserved protein with pleiotropic functions. ApoH is an innate immunity component exhibiting a role of scavenger protein. ApoHa is activated with a proprietary procedure to capture pathogen elements, including proteins, phospholipids, myristoiled or palmytoiled groups. ApoH interact specifically with micro-organisms including infectious viruses, bacteria, fungi, parasites & prions. The ApoH characteristics: plasmatic concentration 200mg/L; molecular mass 50kDa; 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 (near nano-molar) and efficiency of through novel physico-chemical conditions

RESULTS

We have generated results on ultrasensitive detection of pathogens from different experimental and clinical samples and different kind complex biological samples, including mucosal swabs, urine, feces whole blood, serum, plasma and tissues.
We have shown that any kind of pernicious microorganism, such as Gram+ or Gram– bacteria (Table 1, Figure 2) as well as enveloped or non-enveloped viruses (Figure 3- 5) can be ultrasensitively sensitively detected, once the ApoH pre-enrichment technology is carried out. Thus, using ApoH-coated solid supports, such as nano-magnetic beads or plates we are able to avoid false-negative diagnostics.
This sensitivity and rapidity are not only important to rapidly apply appropriate treatment to patients in their early phase of infection, but also in public health issues, like the recent Ebola outbreak where countermeasure as their early isolation and their potential treatment must be done as soon as possible.

Figure 1: Workflow of the enrichment of pathogens from complex samples

ApoH capture of microorganisms
Enabling their ultrasensitive PCR detection &/or isolation in culture conditions

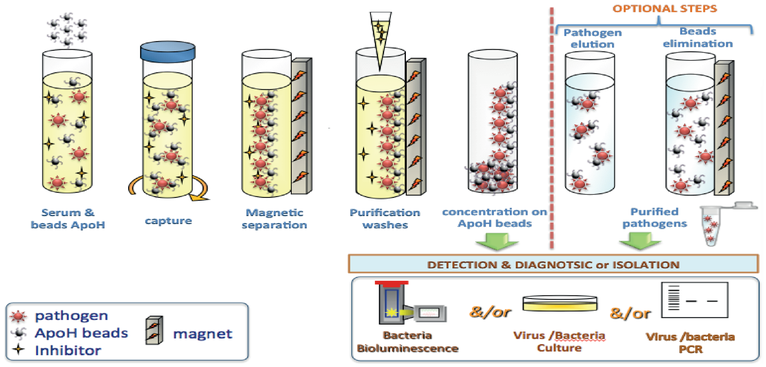


Figure 2: Structural composition the ApoH scavenger acute phase protein

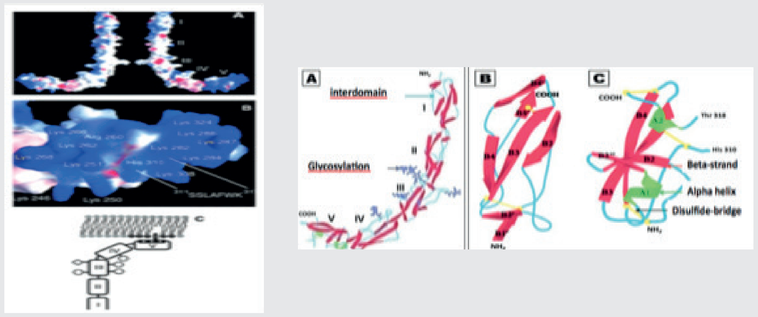
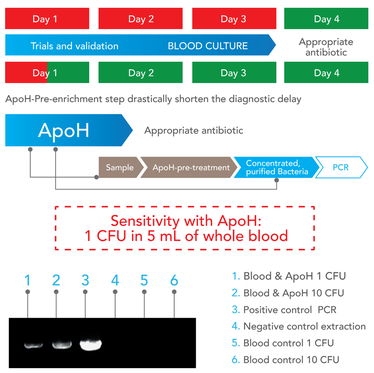


Figure 3: Increased and fastened bacterial detection sensitivity with ApoH: 1 CFU in 5 mL of whole blood

Current time to detection vary & could be too long



1. Blood & ApoH 1 CFU
2. Blood & ApoH 10 CFU
3. Positive control PCR
4. Negative control extraction
5. Blood control 1 CFU
6. Blood control 10 CFU

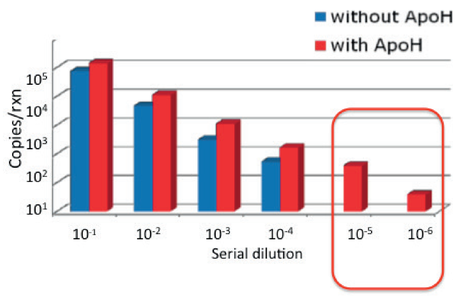
16S PCR on Staphylococcus aureus spiked in whole blood then captured by ApoH

TABLE 1: Some examples of bacteria genus able to be captured by ApoH

Acinetobacter	Enterobacter	Propionibacterium
Bacillus	Escherichia coli	Proteus
Bacteroides	Fusobacterium	Providencia
Bacteroides	Klebsiella	Pseudomonas
Bilophila	Listeria	Salmonella
Capnocytophaga	Micrococcus	Serratia
Chlamydia	Mycobacterium	Staphylococcus
Citrobacter	Ochrobactrum	Stenotrophomonas
Clostridium	Porphyromonas	Streptococcus
Corynebacterium	Prevotella	Tropheryma whipplei

For the most common clinical bacterial load from below 10 bacteria/mL there is a huge need to increase sensitivity through concentration. The ApoH technology is the only solution able to highly concentrate any kind (Table 1) of bacteria for their optimal detection

Figure 4: increased detection of H3N2 virus spiked into nasal swabs preserving its pathogenic effects



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

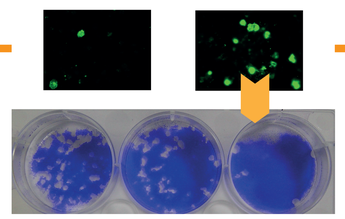


Figure 4: Clinical H3N2 Flu virus isolate spiked and serially diluted into a swab form healthy control (up). ApoH-enriched H3N2 cultivated in primary alveolar cells and detected using FITC-conjugated anti-H2 antibodies. Those ApoH-captured and cultivated viruses preserved their capacity to induce PFU.

Figure 5: Sensitive detection of clinical Dengue and HCV viruses or spiked infectious EBOLA VLPs

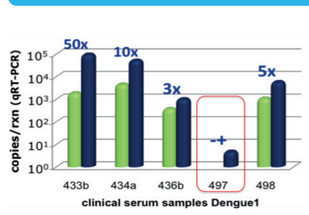


Figure 5A: QPCR on DENV suspected sera from five German patients having been submitted (blue bars) or not (green bars) to a previous pre-analytical ApoH step. Higher values were obtained for four of them with the ApoH pre-analytical method. One false-negative was solved by ApoH.

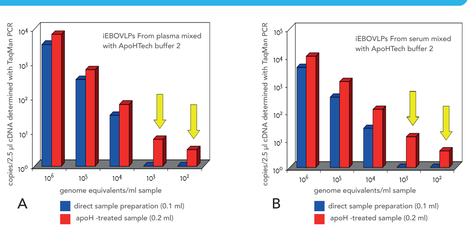


Figure 5B: ApoH-enhanced sensitivity of iVLP-detection. Yellow arrows mark the shifted detection limit of iEBOVLPs spiked in human plasma and serum respectively, due to ApoH-treatment. A serial dilution of the spiked stock sample was generated in plasma and serum. Material of each dilution step was subjected to direct analysis (blue) and ApoH-enhanced detection (red) respectively. Comparable results were achieved for ApoH-Tech buffer 1 and iMARVLPs with ApoH-Tech buffers (not shown).

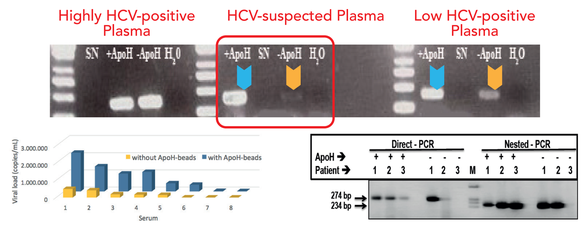


Figure 5C: HCV PCR in 3 patients exhibiting different viral loads, (up); Spiked and diluted (1/10-n) primary HCV isolate into a serum from healthy donor(left); Nested HCV from HCV patients 1, 2 & 3, exhibiting different viral loads and showing that even a Highly sensitive method was only able to detect HCV in the presence of ApoH-sample pretreatment.

CONCLUSIONS

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**
- 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 also **allows** rapid **isolation** and **identification** of unknown pathogens using NGS-metagenomic analysis

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