Increasing sensitivity
Improving diagnostics

technologies

ApoH

www.apohtech.com

ApoH-Technologies overview

Company Highlights

Unique
- Pre-analytical solution
- Capture, wash and ***concentration*** of micro-organisms
- Innovative technology based on the acute phase ApoH protein

Efficient
- High affinity for non-self
- Allowing ***ultra sensitive*** detection
- Developed and manufactured in France

Universal
- ***Multiple*** pathogens: bacteria, virus, fungi, parasites, prions
- All biological samples
- Compatible with many detection methods

Applications

Human health, diagnostics and vaccine production
Veterinary care
Food industry
Environmental biosafety

ApoH-Technologies overview

ApoH-T solutions for improved diagnostics

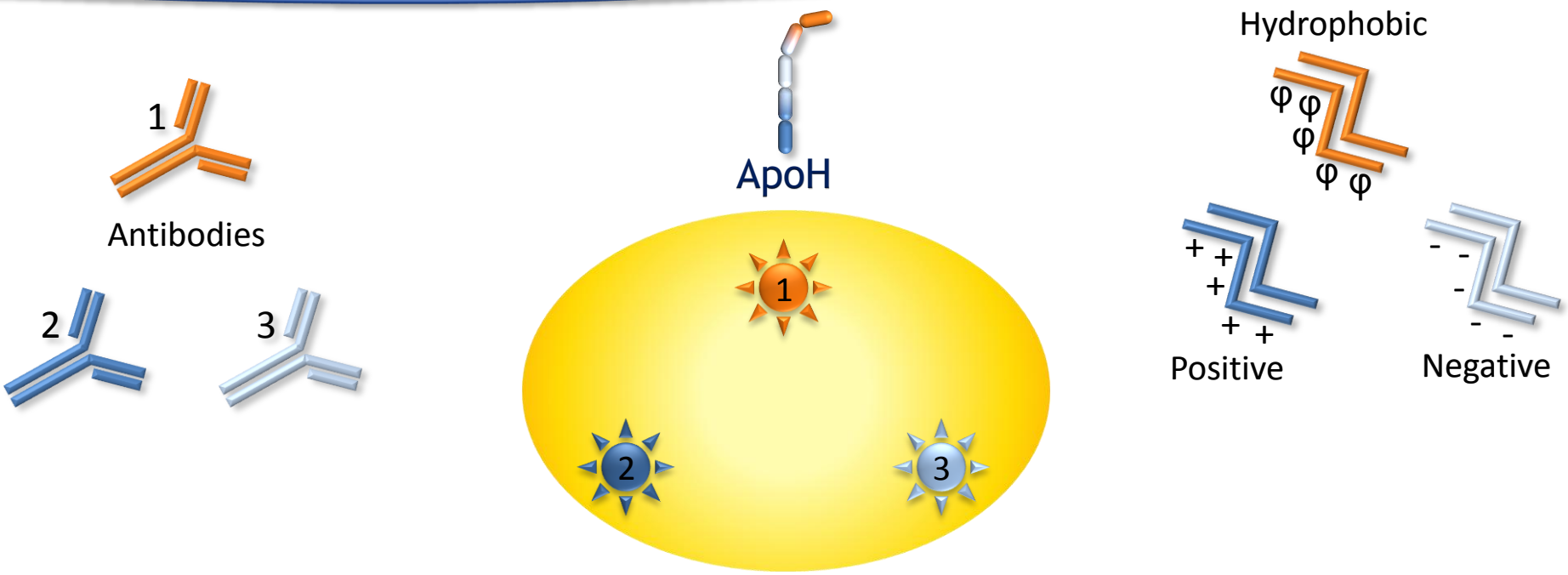
Works on the original sample	***Reduces delays*** in the diagnosis and treatment of infections
Concentrates micro-organisms	Avoids ***false negative results*** when the load is low
Washes away inhibitors	Removes heparin, anticoagulants, leukocytes, antibiotics…
Allows easy ***multiplexing***	Binds all the micro-organisms of a same sample

Improving

- Diagnostic sensitivity
- Patient and epidemic management
- Possible reduction of costs

ApoH-Technologies overview

ApoH vs Competitors

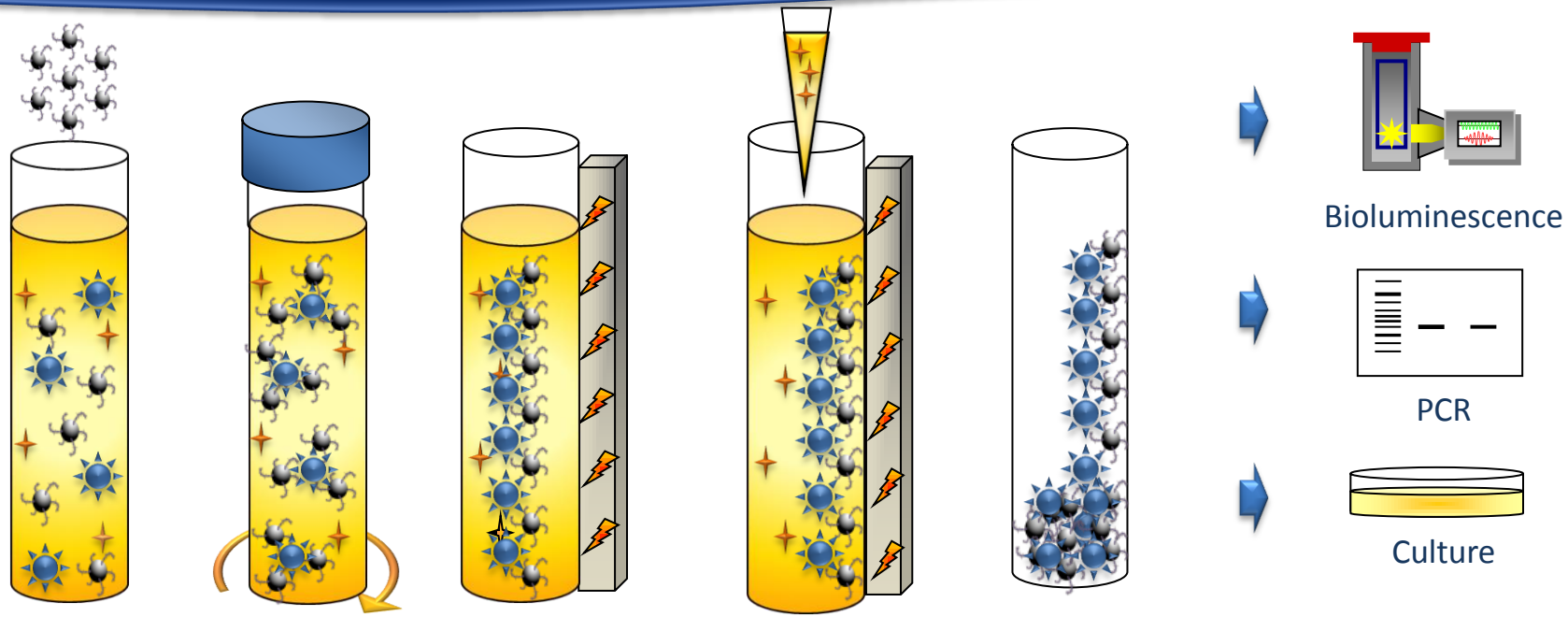


Sample with 3 different micro-organisms

Multiple Capture	3 different antibodies	3 charged surfaces	1 ApoH ligand
Cost	Hight	Medium	Low
Easy	++	+++	++++
Sensitive	++	++	++++

ApoH-Technologies overview

Simple, fast and highly efficient workflow



Inhibitors removal
Concentration of micro-organisms

ApoH-Technologies overview

ApoH-T products

➢ **Various solid supports coated with native or synthetic ApoH*α* :**

- ApoHα-coated magnetic beads:

For molecular and culture assays

Human ApoHα:
APOH-CAPTOBAC (for bacterial capture)
APOH-CAPTOVIR (for viral capture)
APOH-CAPTOFUN*** (for fungal capture)

Synthetic ApoHα named Peps6:
PEPS6-CAPTOBAC (for bacterial capture)
PEPS6-CAPTOVIR (for viral capture)
PEPS6-CAPTOFUN *** (for fungal capture)

*** coming soon

- Tailored supports :

For custom applications
Membranes, chips, chromatography gels...

- ApoHα-coated microplate:

For immunoassays and viral culture
ELISA plates : APOH-PL

ApoH for clinical diagnostics of bacterial infections

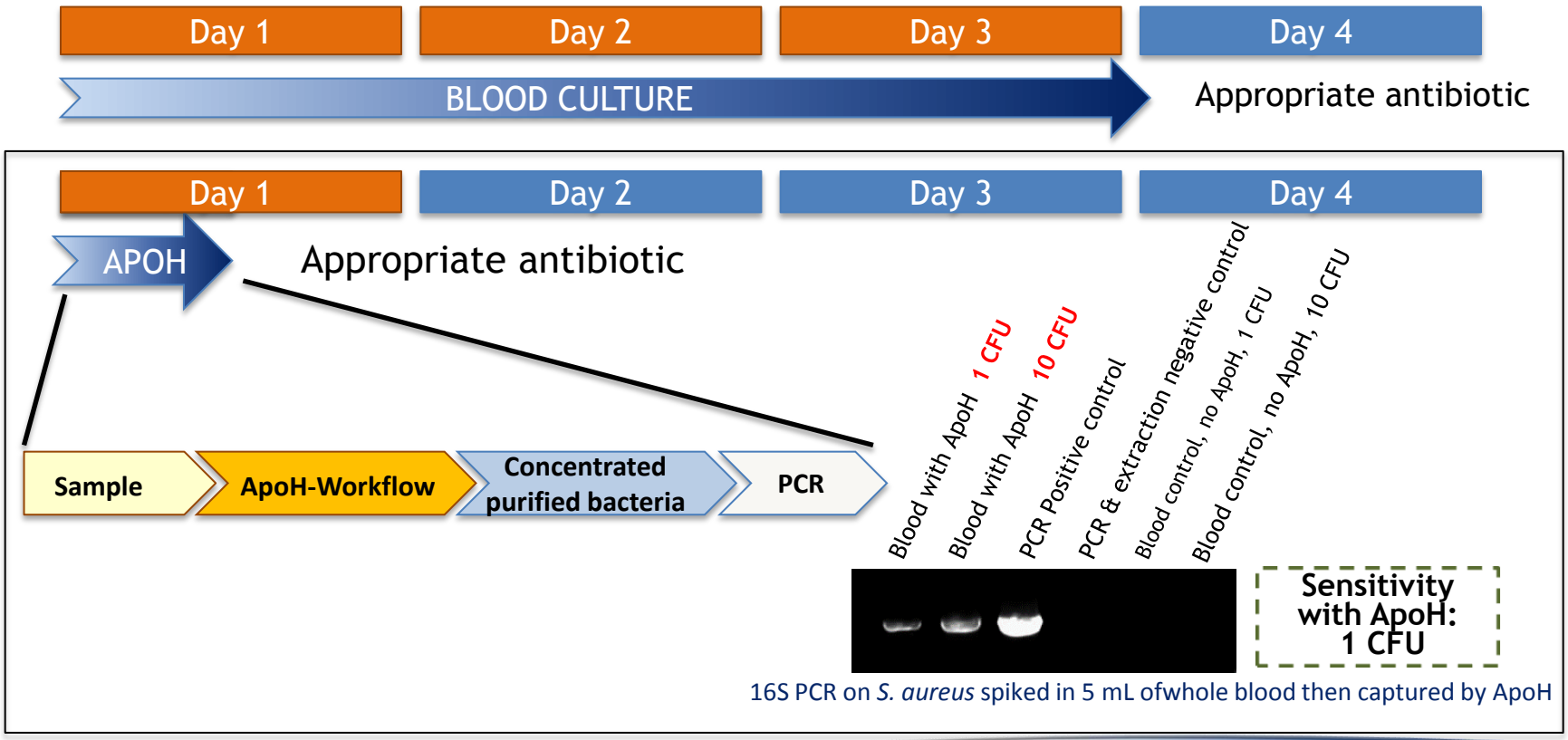
Features for bacteriology: BROAD SCOPE

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.*	*Vibrio cholerae*

ApoH for clinical diagnostics of bacterial infections

Example of sepsis detection

Usually: bacterial load of 0 to 10 CFU/mL → Huge need to increase sensitivity through concentration



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

ApoHα for clinical diagnostics of bacterial infections

Bacterial infection from different hospital services

Hospital test:
Blood culture

147 CO_2-producing hemocultures

64 positive hemocultures	**56 negative** hemocultures	**27 false positive** hemocultures (CO_2 positive but negative subcultivation)
No ApoH: 64 positives With ApoH: 64 positives	No ApoH: 0 positive With ApoH: **11 positives**	No ApoH: 0 positive With ApoH: **9 positives**
Confirmation of all 64 positive cases	**Detection of 11 occult infections** n= 1 *Micrococcus sp.* n= 1 *Pseudomonas sp.* n= 1 *S. aureus + S. epidermidis* n= 1 *S. captitis + Dermabacter* n= 3 *Staphylococcus sp.* n= 1 *Tropheryma whipplei* n= 1 Unidentified yeast n= 2 Positive PCR 16S undetermined	**Detection of 9 occult infections** n= 1 *Bacteroïdes 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 Positive PCR 16S undetermined n= 1 Positive PCR 18S undetermined

ApoH test :
Subcultivation & PCR

Increases sensitivity
Avoids 20/147 (13.6%) false negative diagnostics

ApoH for clinical diagnostic of viral infections

Broad scope of tested viruses

Human viruses		Animal Viruses (*also found in plants)
DNA	RNA	
Adenovirus	HIV	Porcine Parvovirus PPV
Epstein-Barr Herpes HHV-4 Pseudorabies Virus	Rotavirus, Mammalian rthoreovirus 3	Salmon infectious Pancreatic Necrosis IPNV Infectious Salmon Anemia ISAV
Hepatitis B	Hantavirus	Herpes Oyster virus : OsHV-1
Orthopox Vaccinia, Callpox	Flu : H3N2, H1N1, H3N3, Yamagata-like (Florida) Victoria-like (Malaysia)	Aphtovirus : Foot and mouth Disease FMD A5, O1, C, Asia, Sat1, Sat2, Sat3 Enterovirus : picornavirus, swine vesicular disease
Endogenous retroviruses HERV-K, HERV-W, MSRV	Dengue, Hepatitis C, West Nile, Yellow fever	Porcine reproductive and respiratory syndrome virus (PRRSV) blue-ear pig disease
	Norovirus	Murine-Monkey Norovirus MNV, Tulane
	Ebola Marburg	Vesicular stomatitis Rabdhovirus New Jersey, Indiana1
	Lassa	Bovine Viral Diarrhea virus
	Human respiratory syncytial virus	Grapevine leafroll-associated Baculovirus * GRLaV-1 and 3
		Cydia pomonella granulosis virus *
		Pseudorabies Virus
		Reovirus Bluetongue 2, 8

Enveloped virus
Non-enveloped viruses

ApoH for clinical diagnostic of viral infections

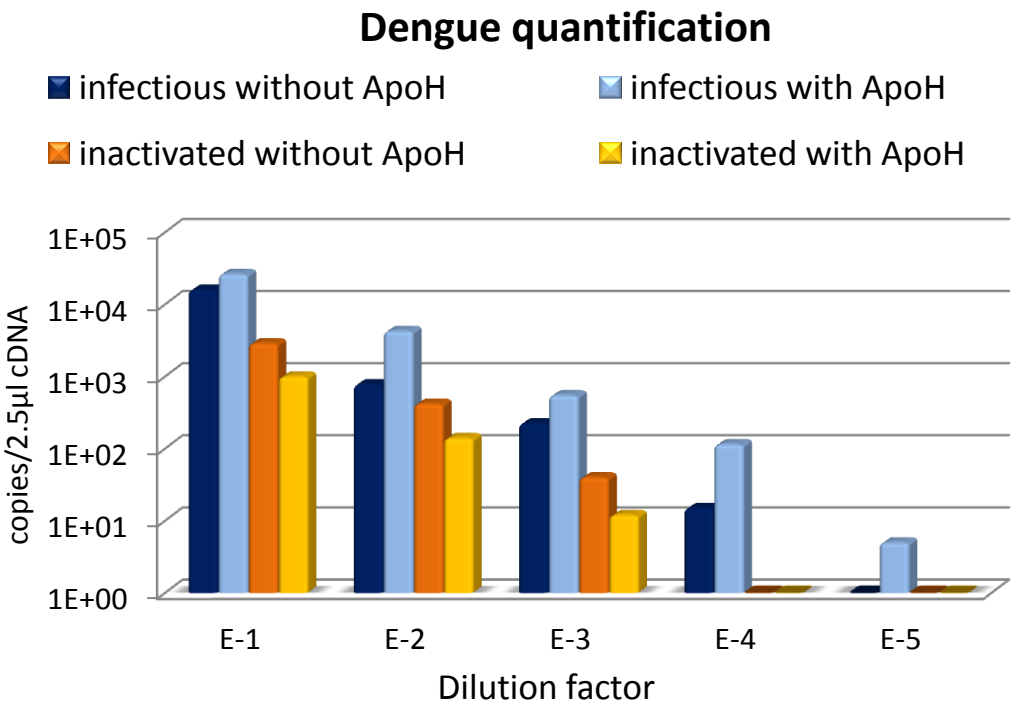
Dengue Viruses

The USDEP European Project

Human serum spiked with the Dengue-4 virus

Heat-inactivation: 1h @ 56°C

ApoH +qPCR | qPCR | ApoH +qPCR | qPCR



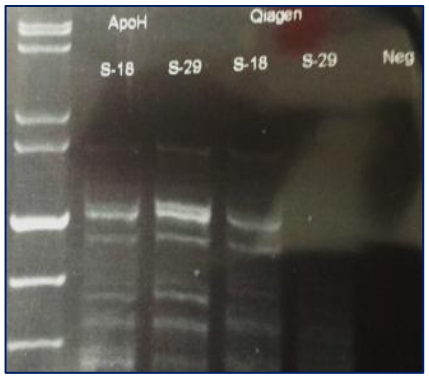
ApoH will capture those viruses having preserved their **functional envelopes**

ApoH will **enhance detection** of infectious viruses

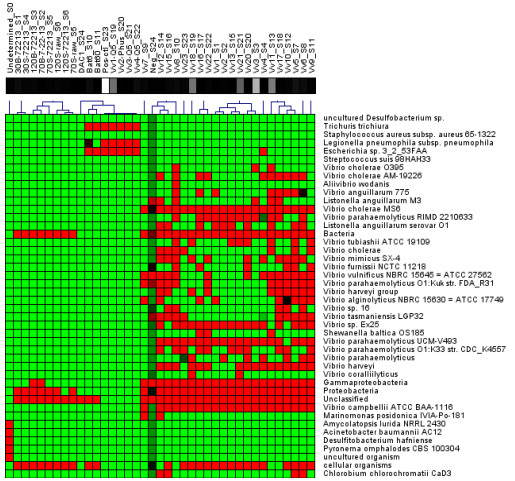
Sewage water

Metagenomics results : ApoH versus filtration

Sample	Number of reads		Gain factor
	Filtration	ApoH	
1	1 163 0	1 139 378 1 047 195	x 1 881
2	486 393	1 852 1 903	x 4,3



PCR



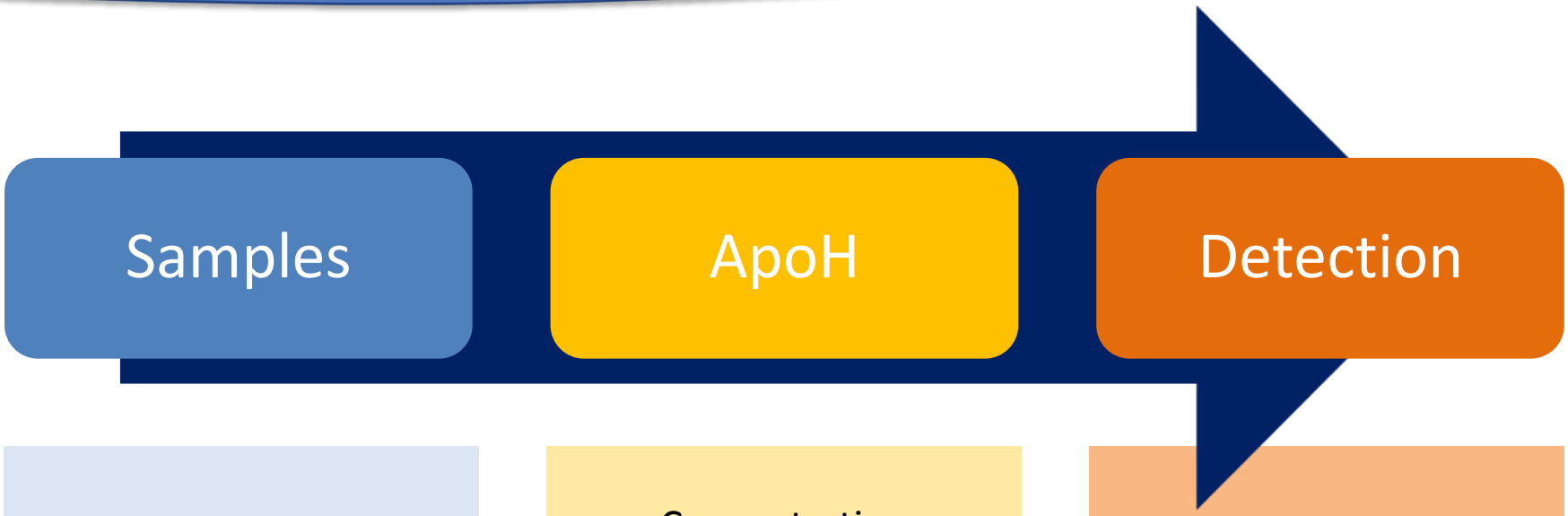
Metagenome heat map

ApoH concentration allows:

- **Huge sensitivity** gain
- More accurate micro-organism detection (coverage and density)
- Better sewage water management

Innovative ApoH solutions

Providing increased sensitivity



Blood, tissue, food, leaf, water, swab, urine…

Virus, bacteria, fungi, spores…

1 µL to 100 mL

Unknown target

Concentration
Inhibitor removal

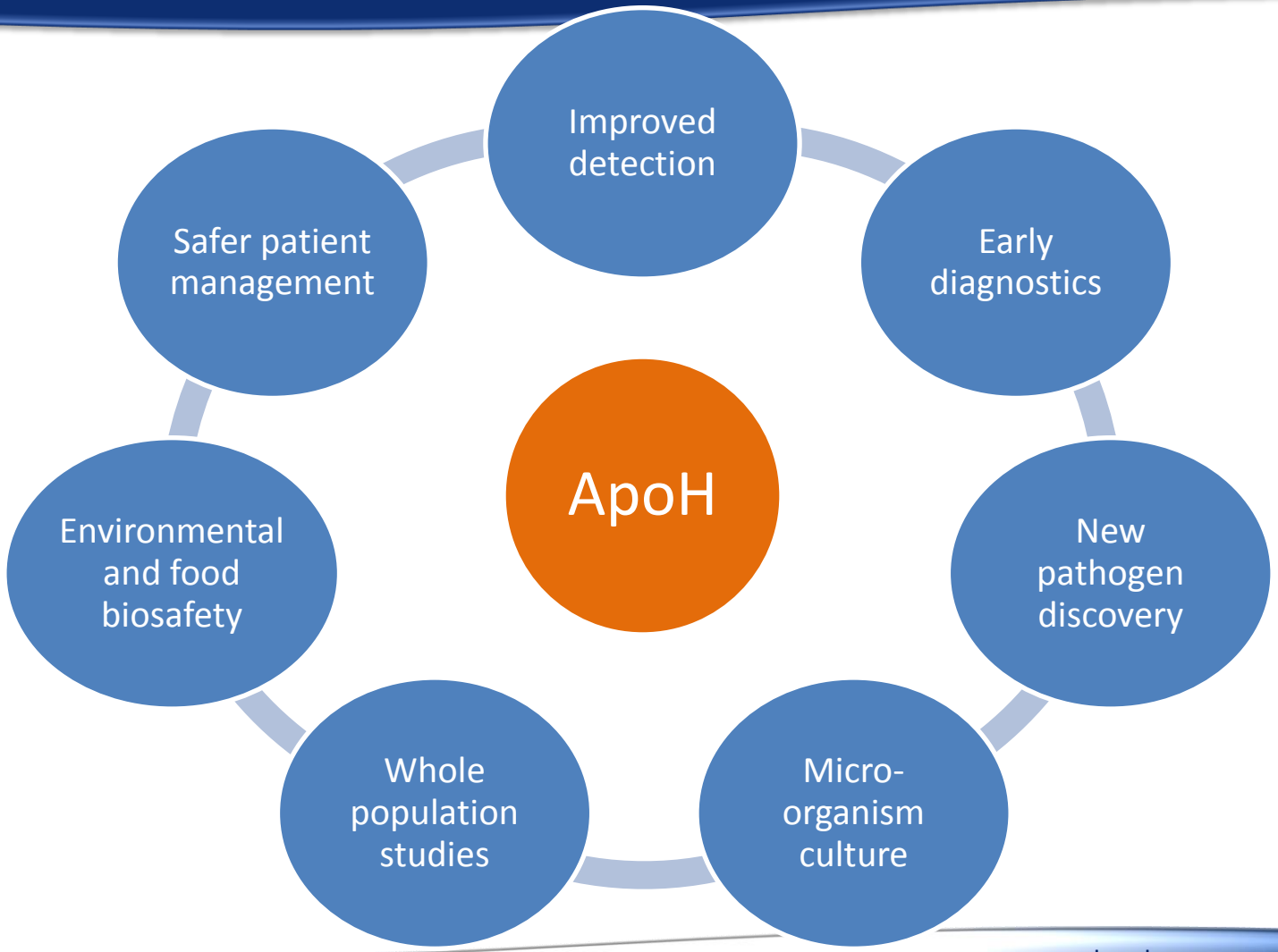
Fast
Easy
Sensitive

High compatibility

Multiplex capacities

Automatable

ApoH multiple applications



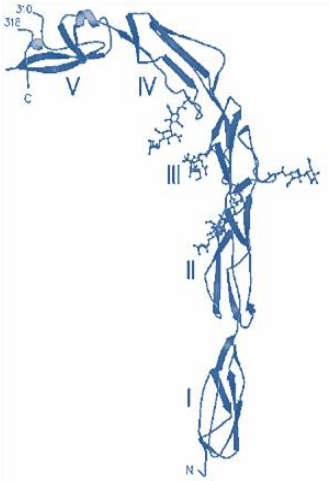
Thank you for your attention !

ApoH-Technologies team :

Dr. Ilias STEFAS, CEO
Emmanuel CHARLES, Sales consultant
Estelle LUCARZ-BRUNEL, Bacterial Project Manager and Production manager
Sylvia TIGRETT-GONZALEZ, Viral Project Manager and Quality manager

ApoH-Technologies overview

Principle



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

- 50 kDa protein close to 200 mg/L in plasma
- an innate immunity component exhibiting a role of **scavenger protein** through
  - electrostatic interactions with negatively charged surfaces (LPS from *E. coli*)
  - hydrophobic interactions with anionic phospholipids (HIV, HCV...)
  - protein-protein interactions (Sbi of *S. aureus*, M1 and H of *S. pyogenes*)

ApoH-Technologies expertise

- Production and ***a***ctivation of the human ApoH protein with a proprietary procedure insuring **high micro-organism affinity**
- Coating on solid supports (96-well plates, magnetic beads, membrane, chips) insuring **high stability**
- Supplier of specific buffers and protocols to insure **efficient micro-organism capture** through precise physical-chemical conditions
- **NEW !** The ApoH protein can be replaced by a synthetic molecule, preserving all capture efficiency: Peps6 products

ApoHα for food diagnostics of bacterial infections

Detection of Salmonella

Salmonella spp Q-PCR test by AQMC

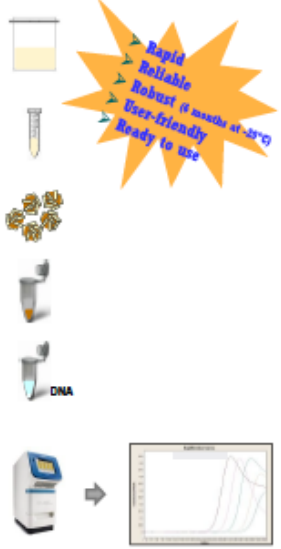
Method for rapid detection in raw milk

Salmonellas are enterobacteria of *Salmonella* genus, pathogenic for man and presenting a strong contagiousness. They are responsible for gastroenteritis, food poisoning and typhoid or paratyphoid fevers (Colin M.P. and Lailler M.R., 2009; Korsak N. and al., 2004). Many methods, validated by AFNOR*, are commercialized on the market for the detection of *Salmonella* spp., however, it turns out that none of these methods are adapted to the specific case of raw milk cheese production, where the response time lag is a crucial problem. The method proposed today by the AQMC laboratory is an innovative *Salmonella* spp. detection method in raw milk, reliable and especially rapid: results in less than 11h. Moreover, the sample volume can be adapted to the user needs. This speedy diagnostic allows the release of raw milk before the rennet process and therefore the milk may be oriented depending on its hygienic quality.

*AFNOR : French Standardization and Certification Association (equivalent to the American ASTM)

Principle

- **Phase 1** ≈ 7h30 — Enrichment in liquid media
- Possible stop point
- *Sample drawing (5 mL)*
- **Phase 2** ≈ 50 min — Bacteria capture on magnetic beads + Apolipoprotein H
- *Magnetization / Concentration*
- Bacteria pellet
- Possible stop point
- **Phase 3** ≈ 40 min — DNA preparation
- *Bacteria lysis*
- *Centrifugation*
- **Phase 4** ≈ 1h40 — Amplification and result analysis



- Rapid
- Reliable
- Robust (6 months at +25°C)
- User-friendly
- Ready to use

Results in less than 11h

Inclusivity

- Inclusivity was assessed on 56 *Salmonella* strains, including:
  - 38 serotypes of the subspecies *Salmonella enterica enterica*
  - 13 strains of other subspecies (*enterica arizonae, enterica diarizonae, enterica houtenae, enterica salamae, enterica indica, bongori*)
  - 5 strains of non-motile variants *Salmonella enterica enterica typhimurium*
- All strains were amplified with the AQMC Q-PCR *Salmonella* spp. method.

Number of tested strains	Results
56	+

Number of tested strains	Results
21	–

Exclusivity

- Exclusivity was assessed on a appropriate range of 21 non-targeted pathogens: the method shows no cross-reactions.

Detectability

- Detectability was evaluated by comparing to the reference method ISO 6579:2002 Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

CFU/25mL sample	1000 cfu	100 cfu	10 cfu	1 cfu	Control
AQMC method	+	+	+	+	–
ISO 6579:2002	Presence	Presence	Presence	Presence	Absence

- Concordance of both methods for infection levels down to 1 CFU per 25 mL sample.

Conclusion

The AQMC Q-PCR *Salmonella* spp. method is presented in form of a ready-to-use diagnostic kit complying with precise criteria: *Salmonella* spp. presence/absence test ideally in less than 11 hours.

Kit composition

- Diluant 1 (DL1): 25 mL vial
- ApoH beads: 1 mL tube
- Lysis buffer 1 (TL1): 20 mL vial
- Lysis buffer 2 (TL2): 2 mL vial
- Mix *Salmonella* 1 (M1 Salmonella): 1.4 mL tube
- Mix *Salmonella* 2 (M2 Salmonella): 200 µL tube
- PCR positive control: 75 µL tube
- PCR negative control: 75 µL tube

Kit for 100 tests

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