

A flow cytometry-based immunoassay for several Polycyclic Aromatic Hydrocarbons (PAHs) in foods

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Goal

- PAHs are known carcinogenic & mutagenic chemical contaminants.

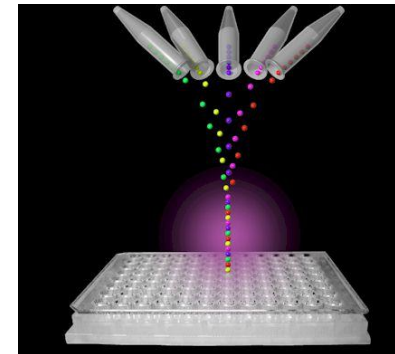


- Existing measurements are time-consuming, laborious and expensive.



- A good alternative can be Flow Cytometry (FC) in combination with the xMAP technology (Luminex).

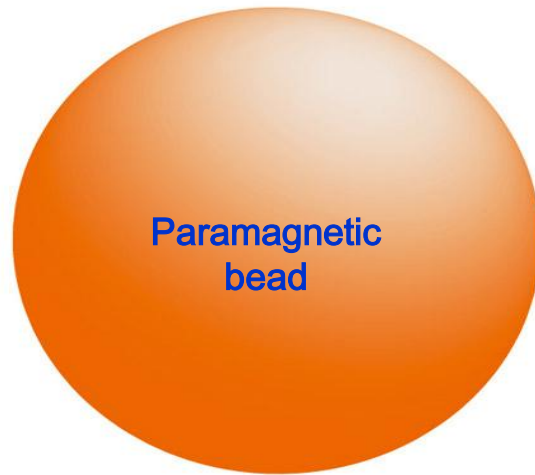
Main advantage is the detection of **multiple** analytes in parallel.



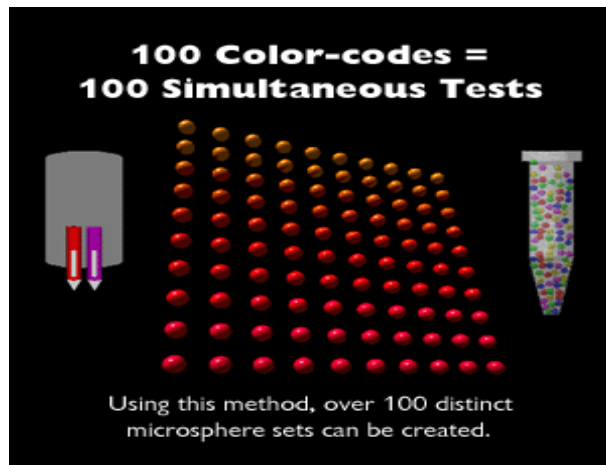
<http://www.labodia.com/>



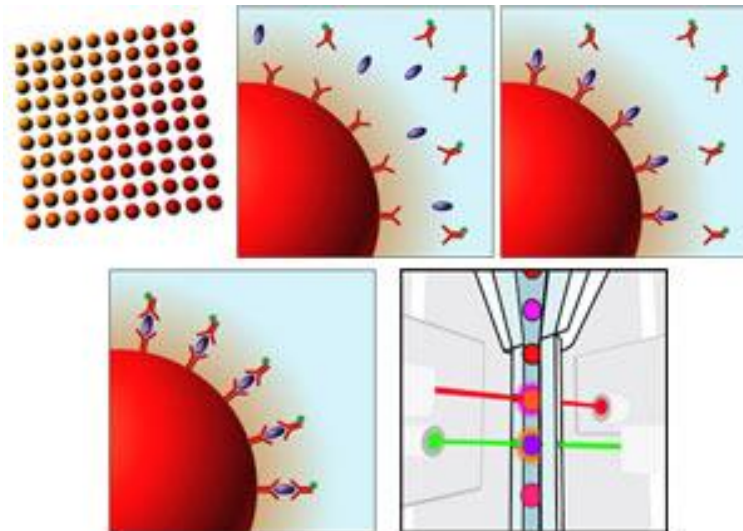
Luminex-based PAH inhibition immunoassay



The microsphere is a 6,5 μm polystyrene paramagnetic bead with two fluorescent dyes incorporated into different ratios and with carboxyl groups on the surface.

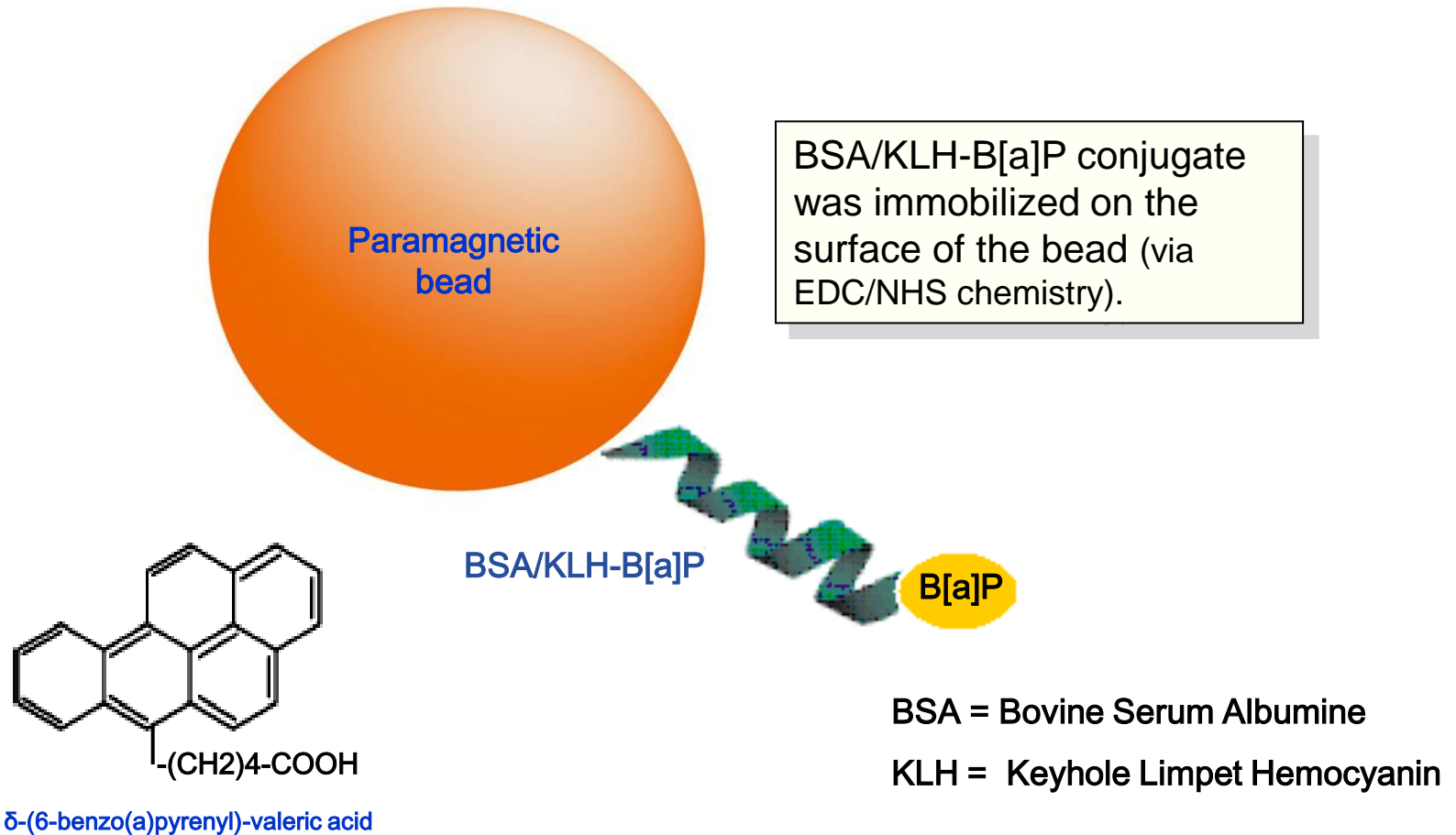


<http://www.luminexcorp.com/>



www.upci.upmc.edu

Luminex-based PAH inhibition immunoassay



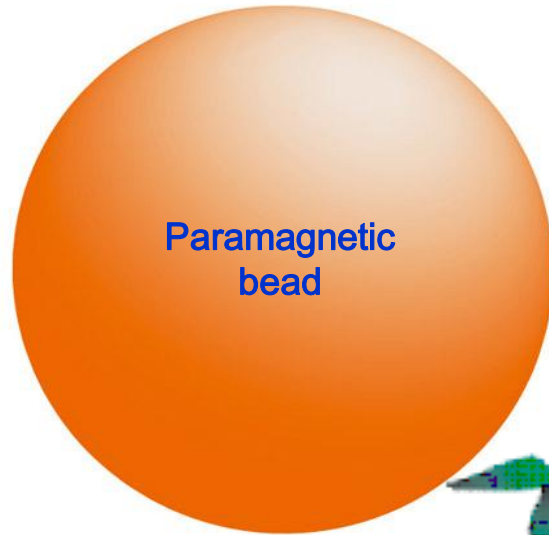
BSA = Bovine Serum Albumine
KLH = Keyhole Limpet Hemocyanin

Matschulat *et al.*, 2005

Institute of Hydrochemistry in Munich



Luminex-based PAH inhibition immunoassay




Paramagnetic bead

A mouse monoclonal anti-B[a]P is binding to the immobilized B[a]P conjugate.

BSA/KLH-B[a]P

B[a]P

Mouse monoclonal anti-B[a]P

	IC ₅₀ B[a]P (ppb) ELISA
22F12	0.2
2H3	1.5
commercial	

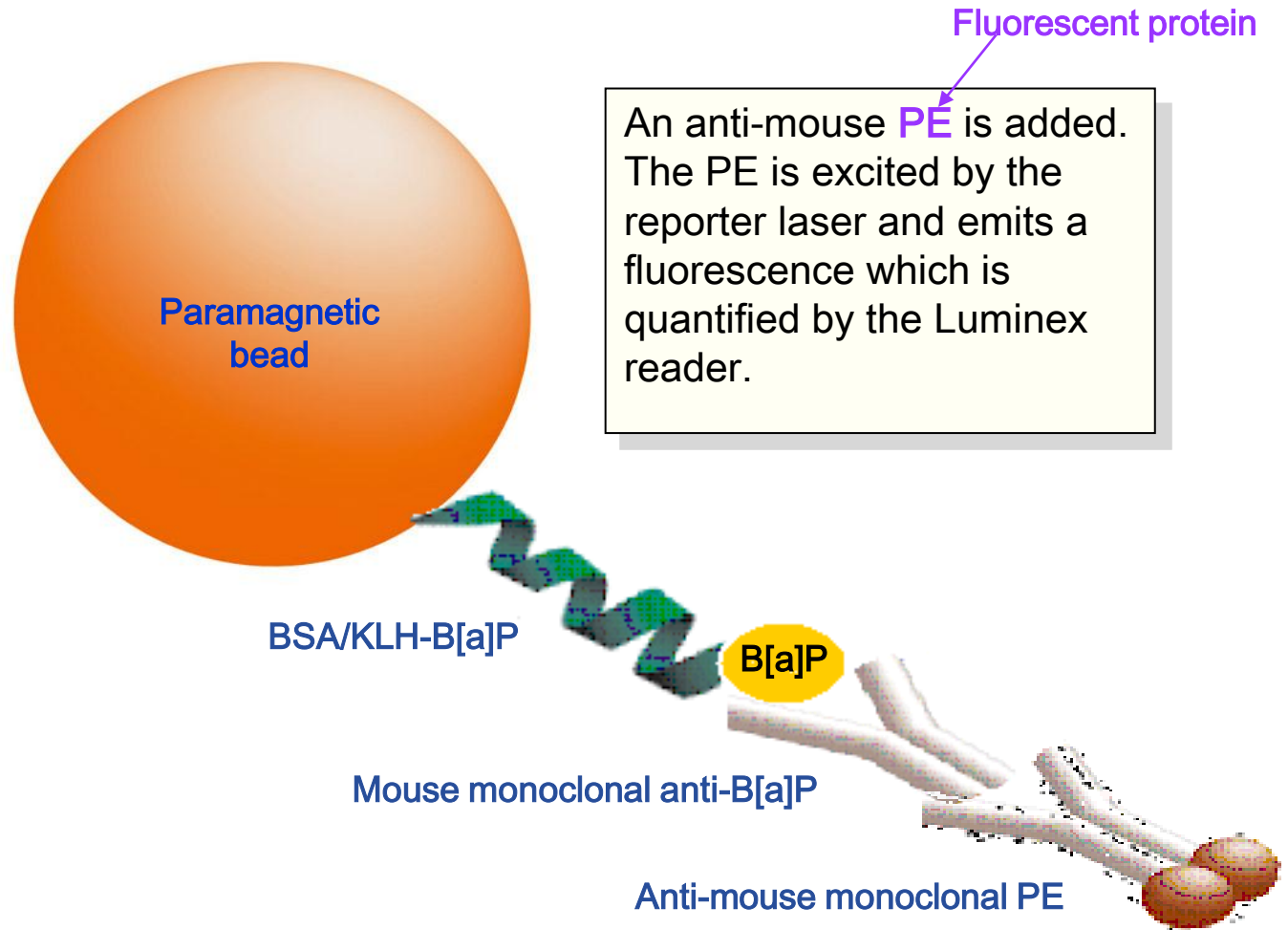
Matschulat *et al.*,2005

IC50 = Half maximal inhibitory concentration

ELISA= Enzyme-linked immunosorbent assay

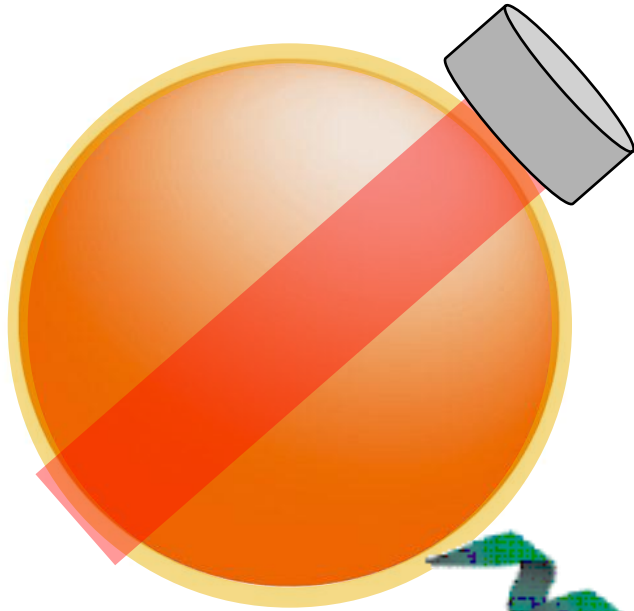


Luminex-based PAH inhibition immunoassay



Luminex-based PAH inhibition immunoassay

Red laser identifies the bead



The complex/ microsphere is then excited by the laser. The bead specific emission is quantified by the Luminex and the bead is identified.

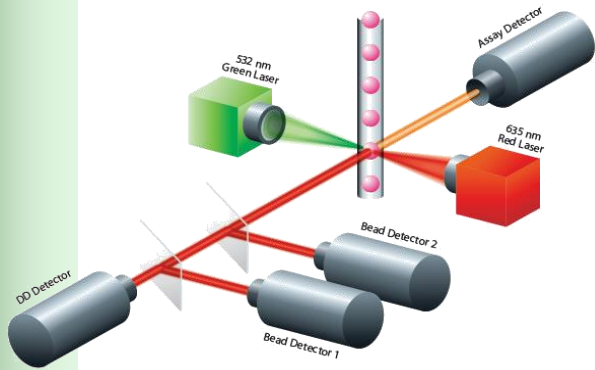
Green laser quantifies the interaction

BSA/KLH-B[a]P

B[a]P

Mouse monoclonal anti-B[a]P

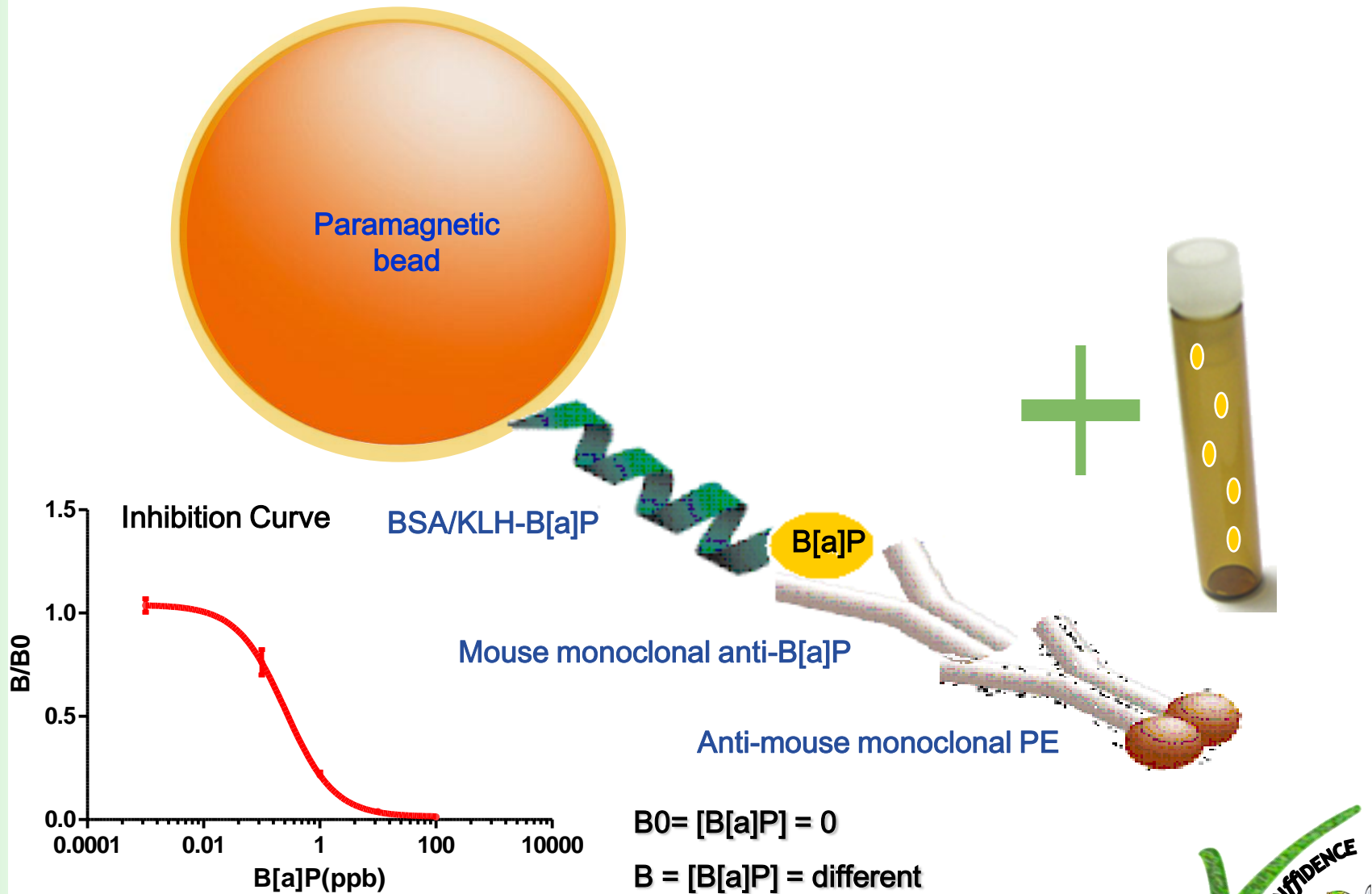
Anti-mouse monoclonal PE



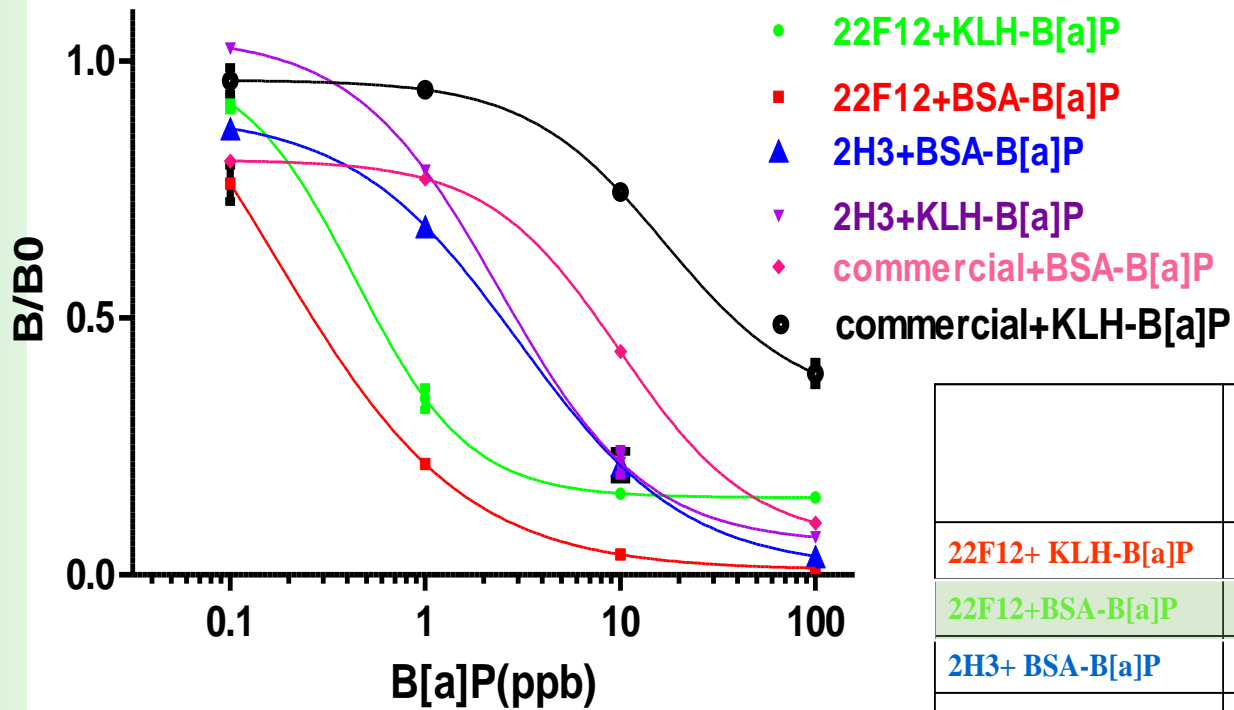
<http://www.panomics.com>



Luminex-based PAH inhibition immunoassay



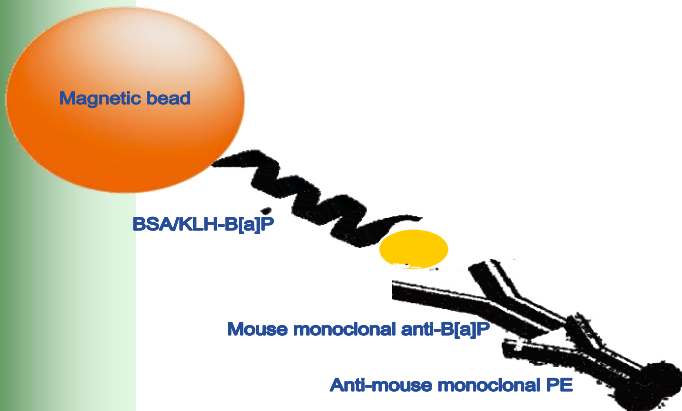
Evaluation of biochemicals



Optimization done for 3 antibodies+2 conjugates

Anti-B[a]P	Anti-mouse PE
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	IC50(ppb)B[a]P	ELISA IC50 (ppb)B[a]P (Matschulat <i>et al.</i> ,2005)
22F12+ KLH-B[a]P	0.4	
22F12+BSA-B[a]P	0.3	0.21
2H3+ BSA-B[a]P	3.2	1.53
2H3+ KLH-B[a]P	2.4	
Commercial+ BSA-B[a]P	10	
Commercial+ KLH- B[a]P	17	



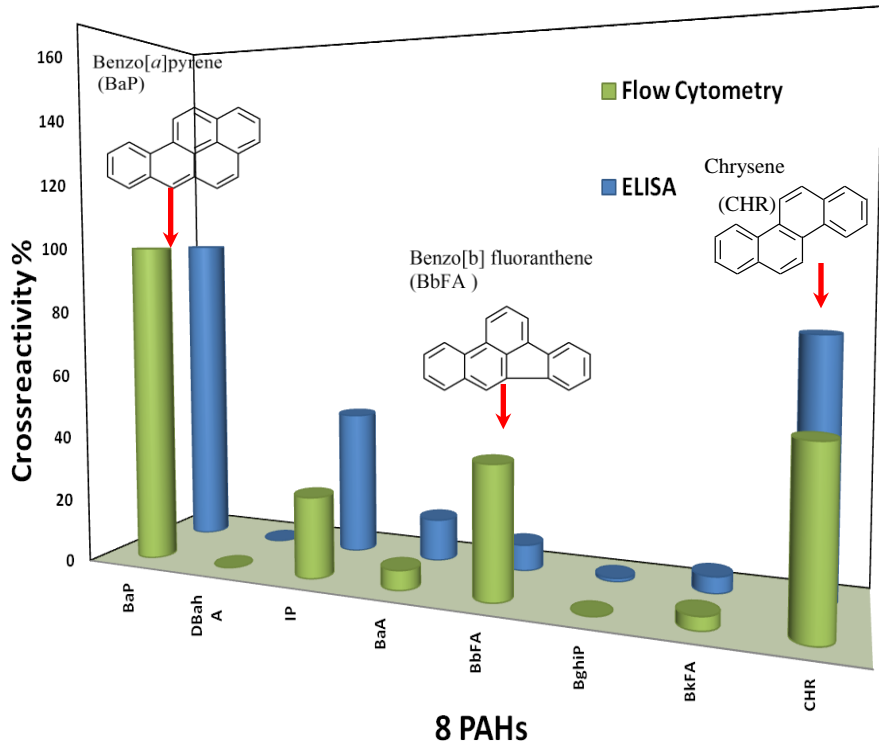
Benzo[a]pyrene (B[a]P) Maximum levels (MLs)

- 1 ppb cereal-based food for kids
- 10 ppb shellfish
- 5 ppb smoked fish ((EC)No1881/2006).

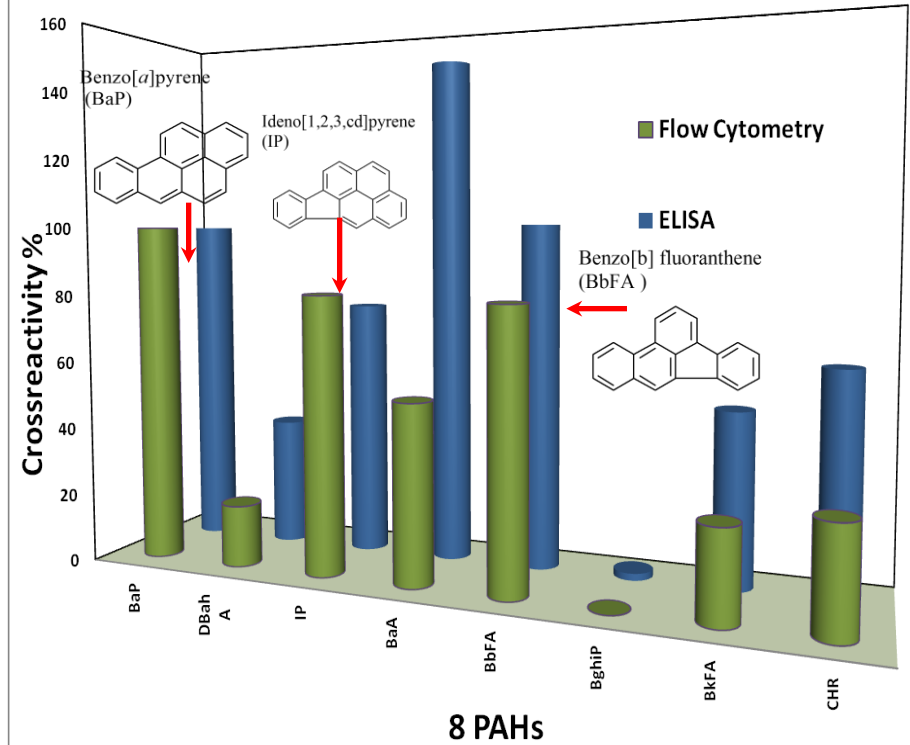


Cross-reactivities (8 PAHs)

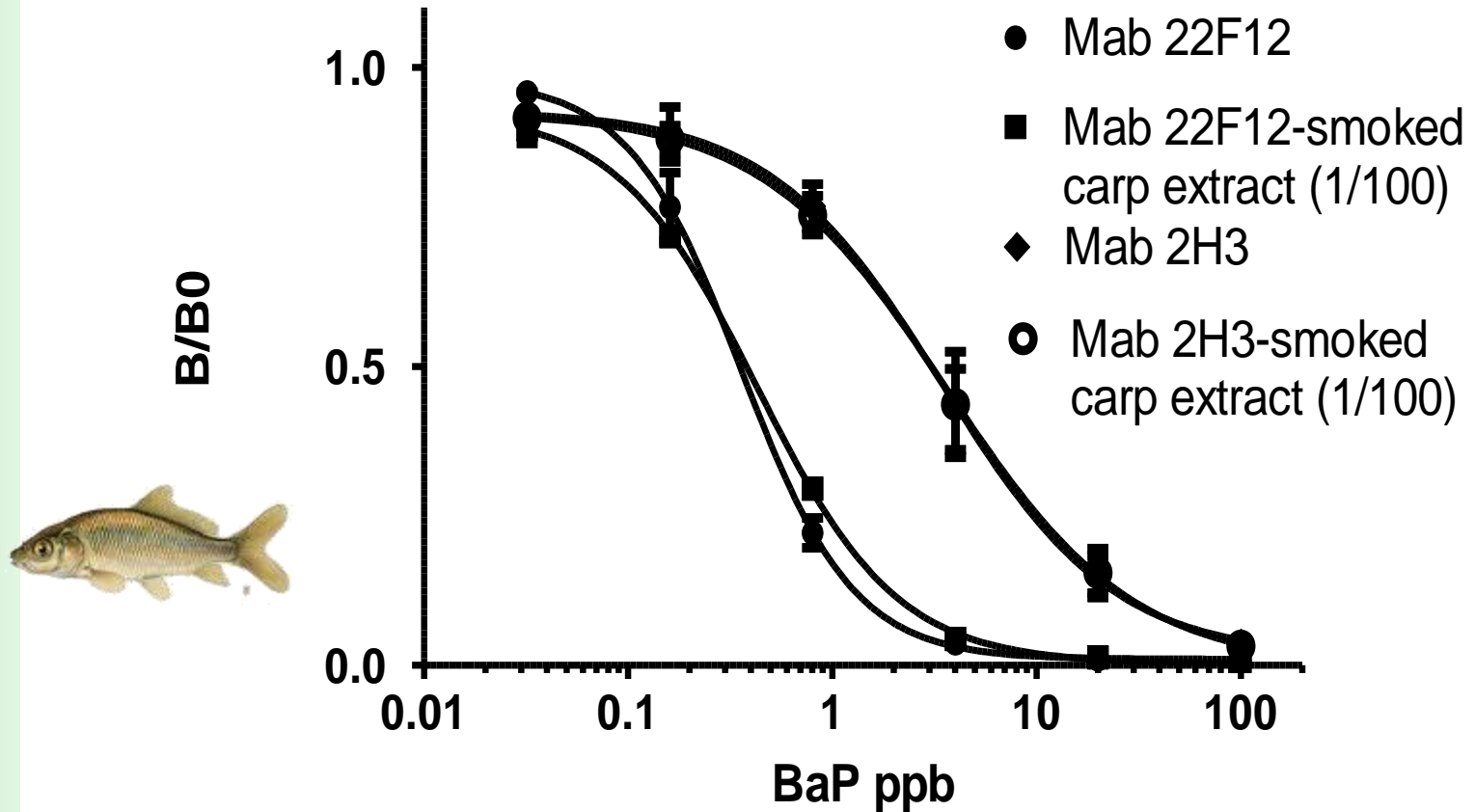
Mab 22F12



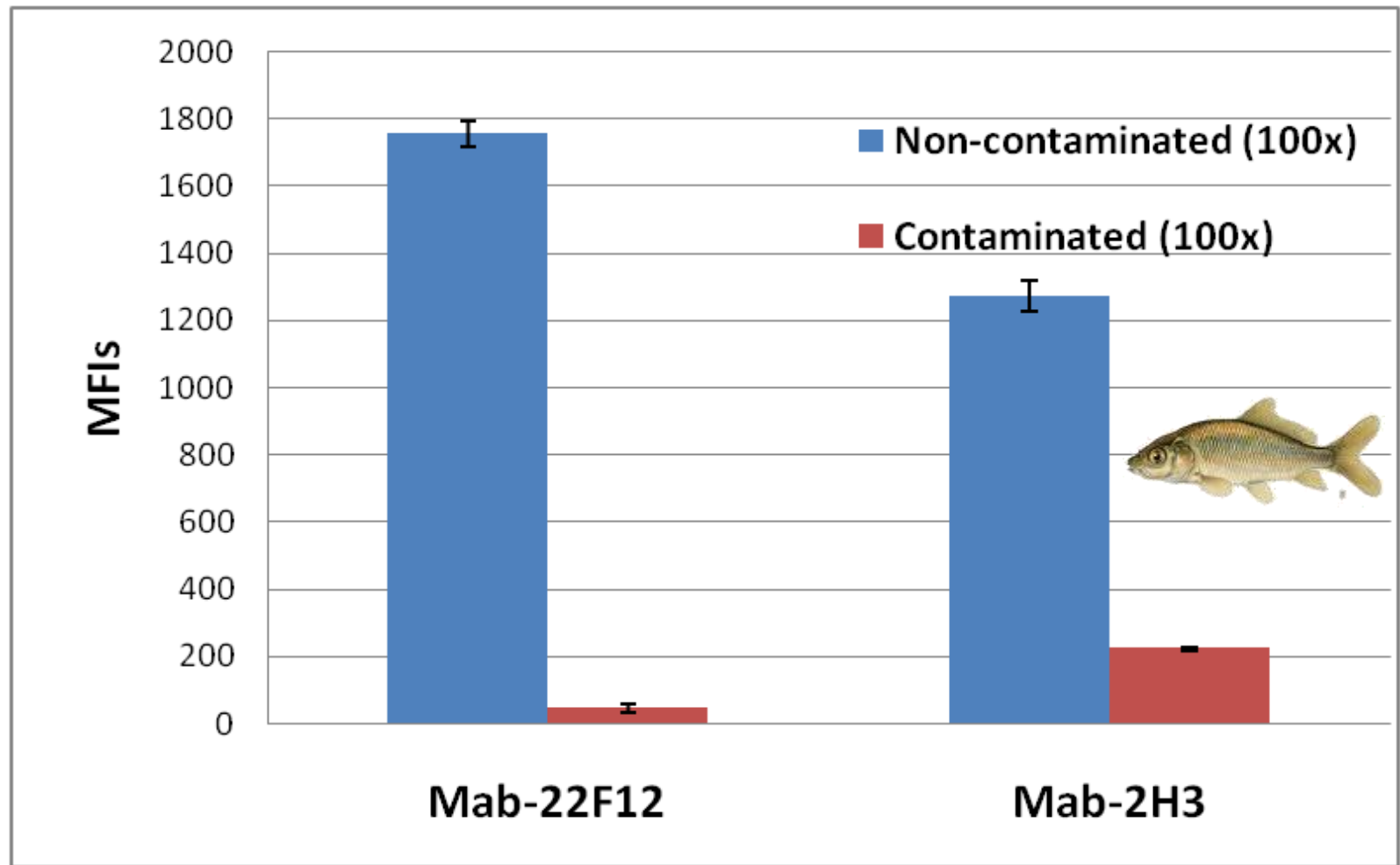
Mab 2H3



Food matrix effect



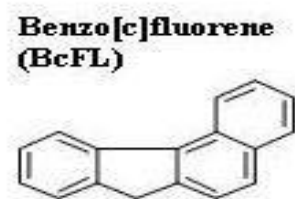
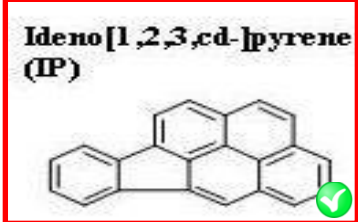
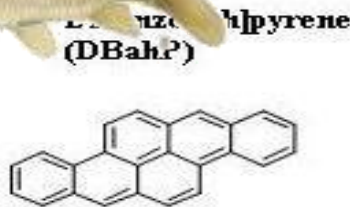
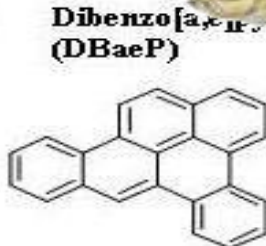
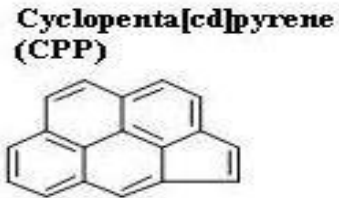
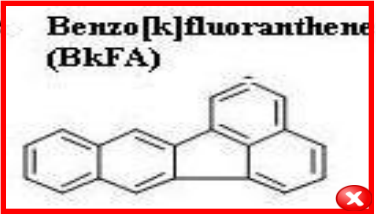
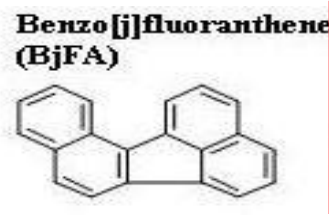
Food sample PAH's measurement



VSCHT



Conclusions



Thank you very much for your attention

