

SMC™ : an alternative to other technologies for development of immunogenicity assay for cytokines ADA

**EIP Lisbon– 17-19 February 2020
Eric Guillemare**



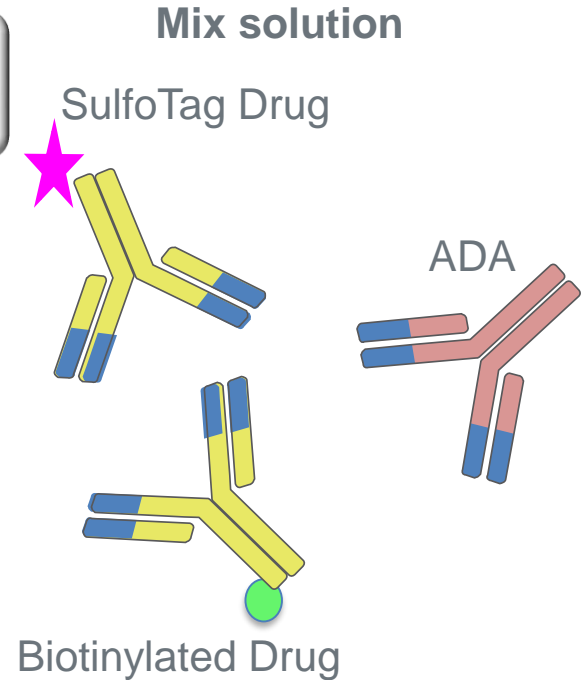
**Translational Medicine and Early Development
Biomarkers & Clinical Bioanalysis**

ADA Assay method : classical bridging format

Classical format : bridged complex ADA-Drug in solution
Drug labeled with biotin or SulfoTag

Drug : large molecule i.e. therapeutic antibody

- MW ADA ~ MW drug ~ 150 kDa
- Diversity of epitopes recognized by ADA
- Diversity of site for drug labeling without downgrading functionality



Assay method for ADA detection directed against cytokines: new project for a clinical study

Three cytokines in human samples with LOW/MEDIUM risks of immunogenicity :
CytA, CytB and CytC

Need to develop assay three methods for ADA detection

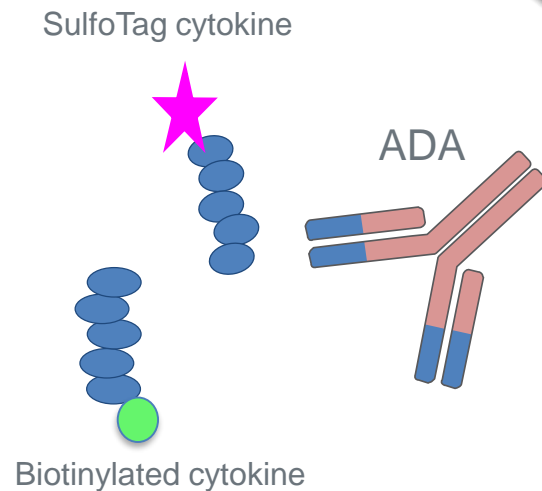
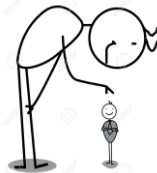
Difficulties related to cytokines :

- MW ADA ~ 150 kDa
- MW drug ~ 10-20 kDa
- Low number of epitopes recognized by ADA
- drug labeling risks to downgrade functionality
- Behavior of cytokines:

CytA : contains a membrane subunit

CytB: risk of aggregation or fixation to plasmatic protein

CytC: is a heterodimer

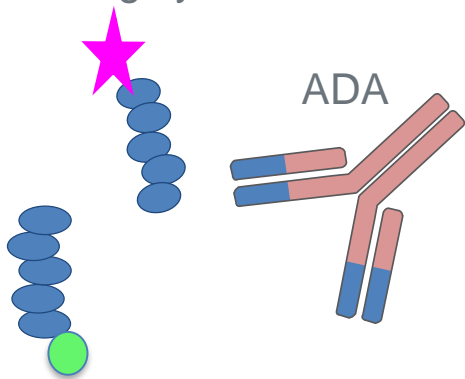


Assay method for ADA detection directed against cytokines: classical bridging format

Mix solution

Format : bridged complex ADA-Drug in solution
Drug : cytokine labeled with biotin or SulfoTag

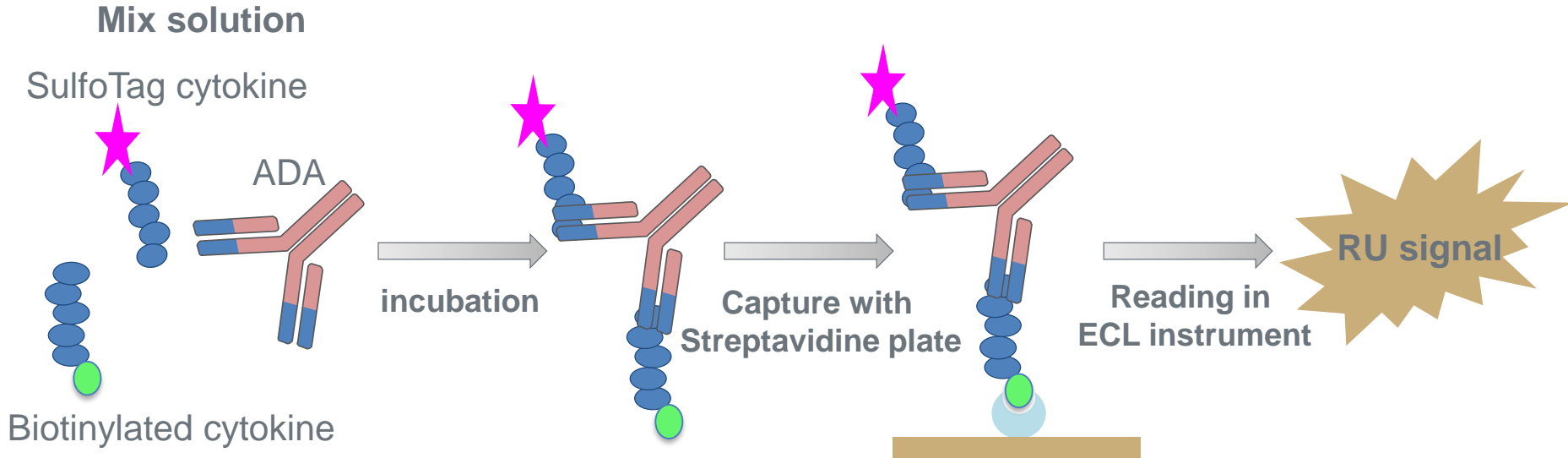
SulfoTag cytokine



Biotinylated cytokine

- Need to adapt rate of labeling on each cytokine
- Need to optimize quantity of labeled cytokines in Mix solution

Assay method for ADA detection directed against cytokines: classical bridging format using Electrochimiluminescence (ECL)

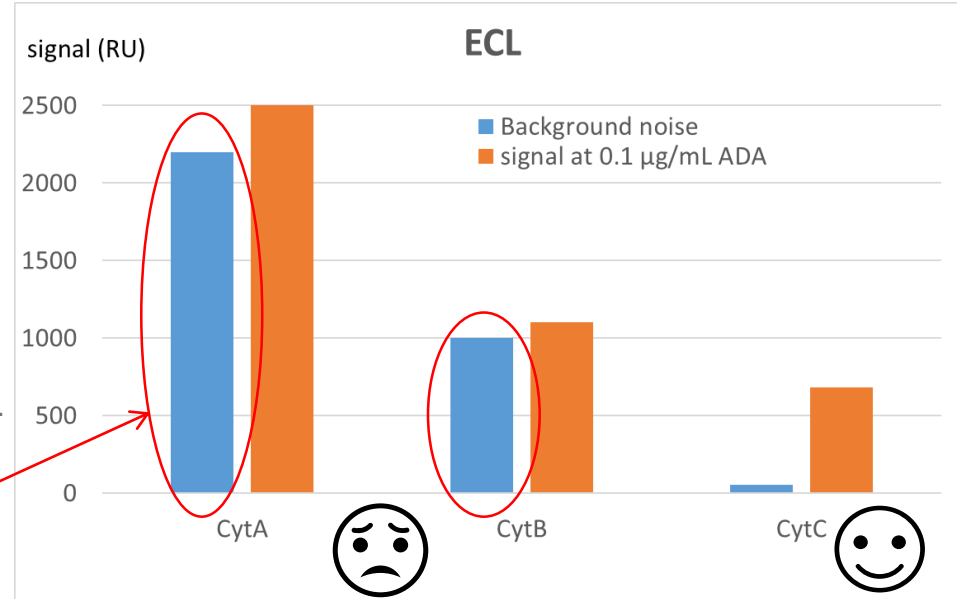


Method developments of ADA against cytokine using ECL

Results for three cytokines using ECL and after optimization !

Reagents :

- **Matrix** : Human plasma
- **Standards** : ADA obtained by injection of cytokines CytA, CytB or CytC in rabbit
- **Low PC** : spiked at the expected sensitivity: 0.1 $\mu\text{g/mL}$ ADA

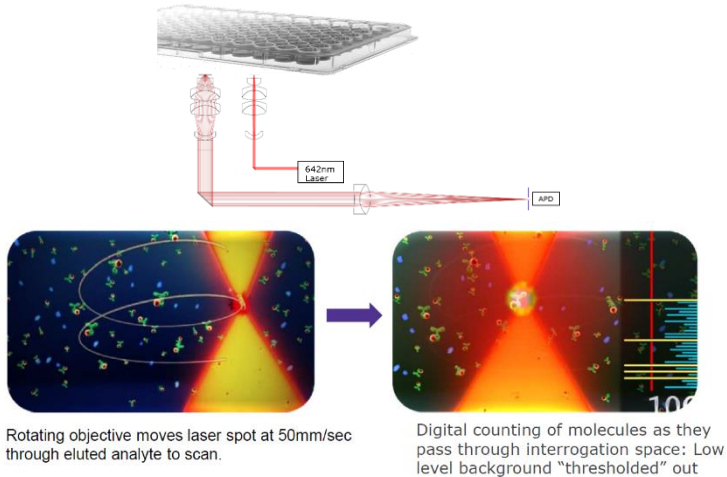


- For CytA and CytB : in spite of the optimization of the experimental conditions, the background signal was still too strong
- For CytC : sensitivity at 0.1 $\mu\text{g/mL}$

Need to test another technology : **SMC™**

Single Molecule Counting (SMC™) technology

Merck's propriety Single Molecule Counting (SMC™) technology :



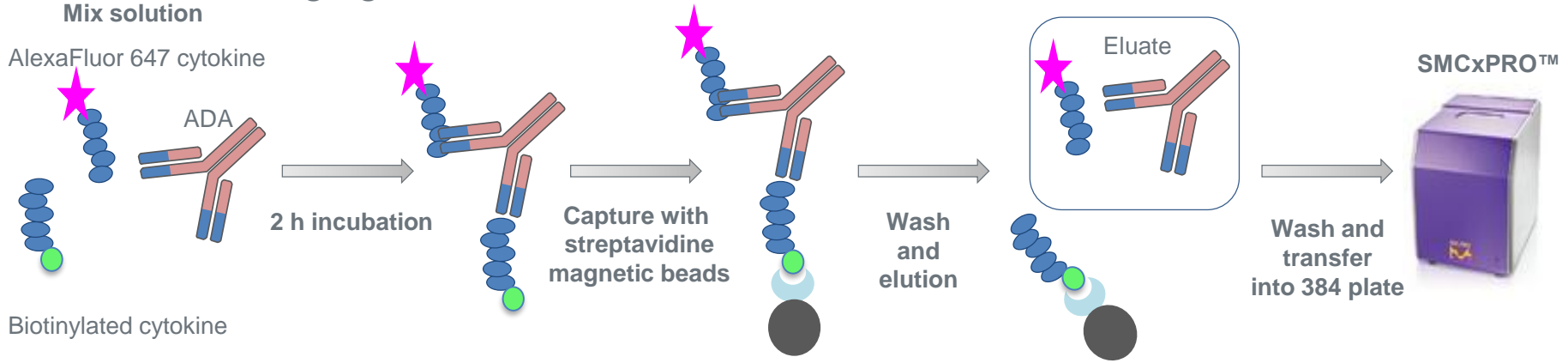
- Rotating objective scans laser spot through analyte.
- 642 nm laser focused through plate
- Single fluorochromes excite and emit fluorescence : labeling with AlexaFluor 647
- Signal output = Response signal

A SMC™ Immunogenicity Assay Development Kit (Cat. No. 03-0175-00) has been available since last year

- Need feedback for ADA
- Assessment of SMC™ for development ADA methods for CytA, CytB and CytC using SMCxPro
- **Expected benefit:** Decrease background and a sensitivity at 0.1 µg/mL

Method development of ADA using SMC™ technology

Bridging format



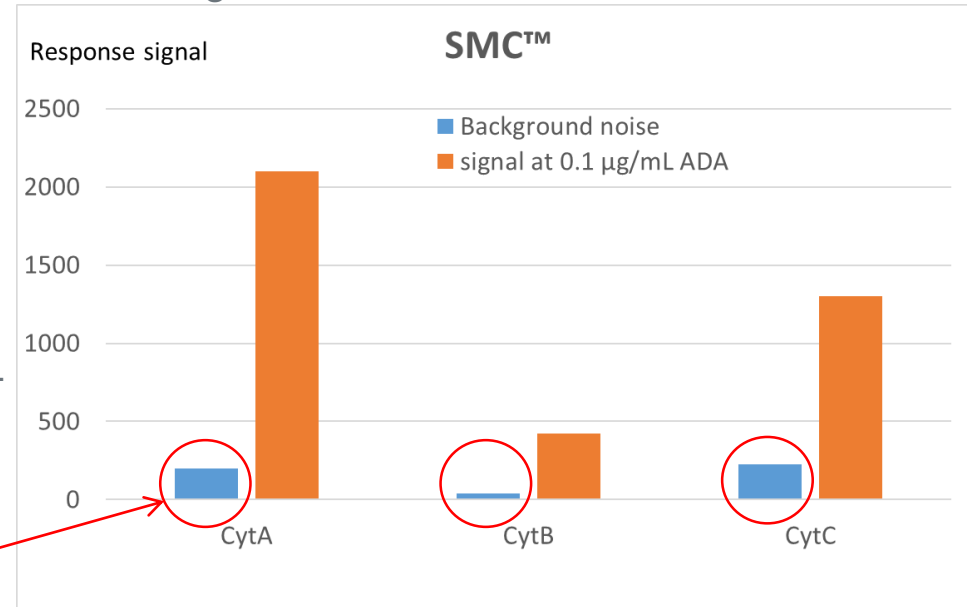
Positive elements:

- Capture with magnetic beads → best capture/presentation of epitope, washing efficiency
- Elution step → decrease non-specific signal
- SMC™ is described as an ultrasensitive technology for PK and BM purpose, but there are no publications about ADA.

Method developments of ADA against cytokine with SMC™

Results for three cytokines using SMC™

- **Matrix** : Human plasma
- **Standard** : ADA obtained by injection of cytokines CytA, CytB or CytC in rabbit
- **Low PC** : spiked at the expected sensitivity: 0.1 µg/mL ADA



Low background signal

- Low background noise signal
- Sensitivity at 0.1 µg/mL



ADA method developments on
CytA, CytB and CytC can be done with SMC™

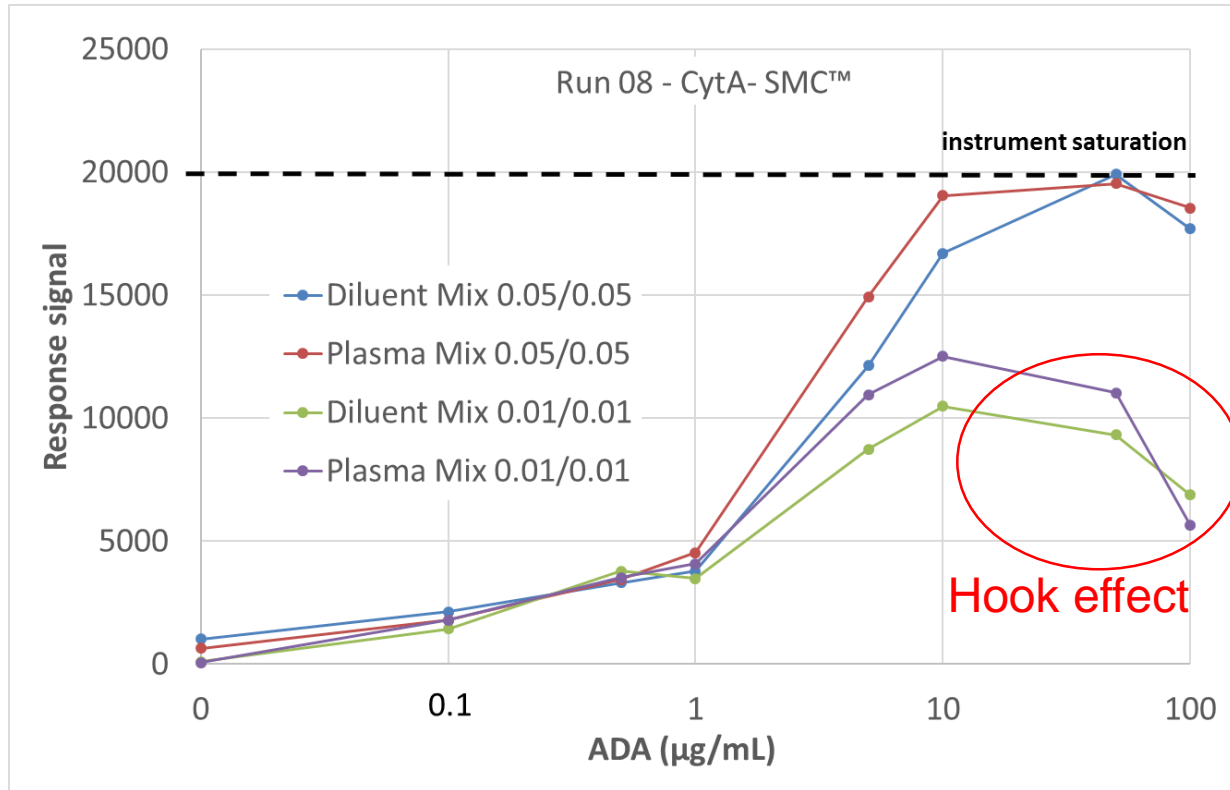
Method developments of ADA against CytA with SMC™

Instrument saturation at 20 000 response signal



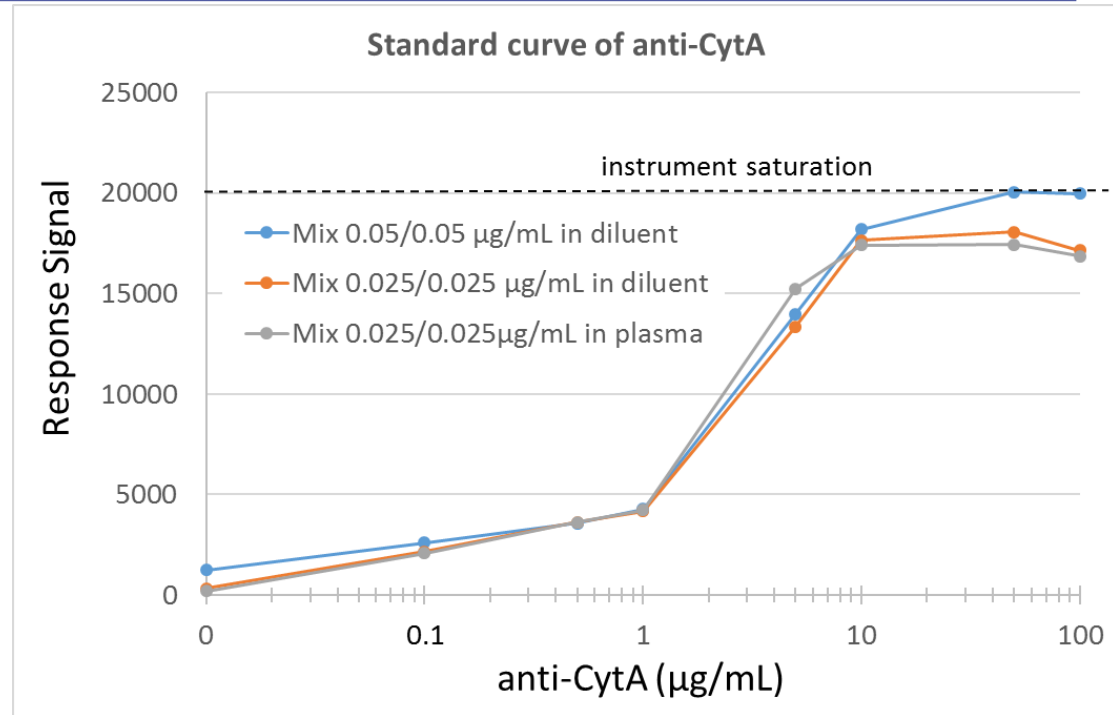
Need to optimize conditions in order to avoid Signal saturation:

- Decrease quantity of labeled cytokines : be careful to hook effect !
- Decrease MRD



Method developments of ADA against CytA with SMC™

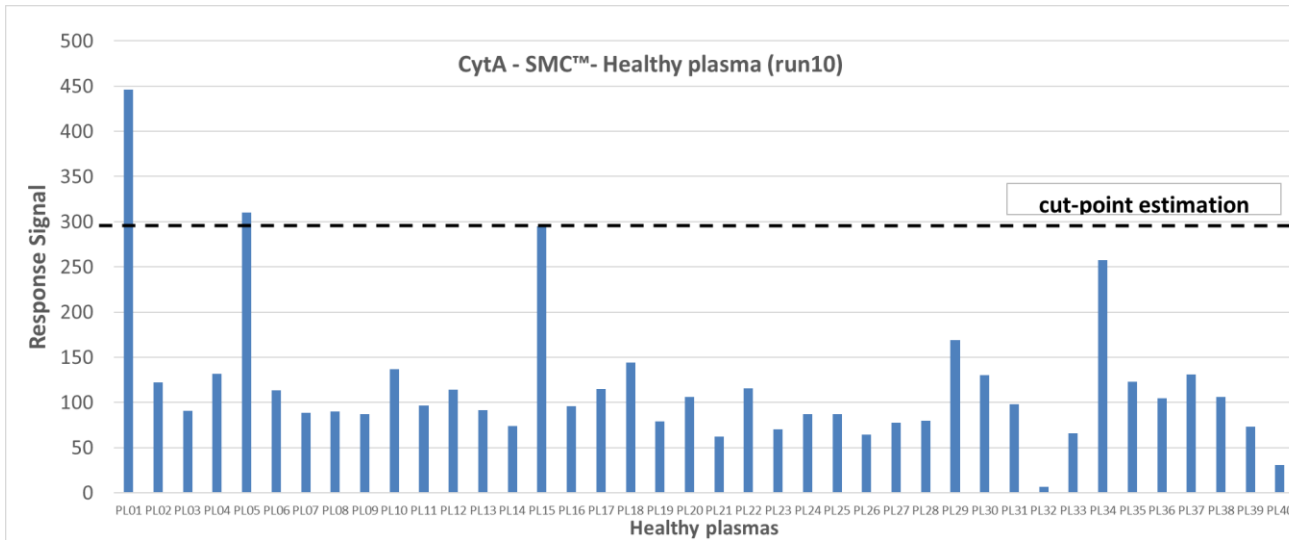
- **Mix solution** : 0.025 and 0.05 µg/mL
- **Standard ADA** : 0.1 to 100 µg/mL
- **Matrix**: diluent or plasma
- **MRD** : 1/60
- **Final conditions** :
MRD 1/60
Mix 0.025 µg/mL (labeled cytokines)



- Low background noise signal
- Sensitivity at 0.1 µg/mL
- No instrument saturation for high concentrations of ADA

Estimation of cutpoint with SMC™

For a first estimation of cut-point, 40 healthy plasmas were analyzed in screening condition MIX 0.025 µg/mL and MRD 1/60



Estimation of Ncut-point at 1.34 and cut-point at 295 response signal (based on 95 percentile)

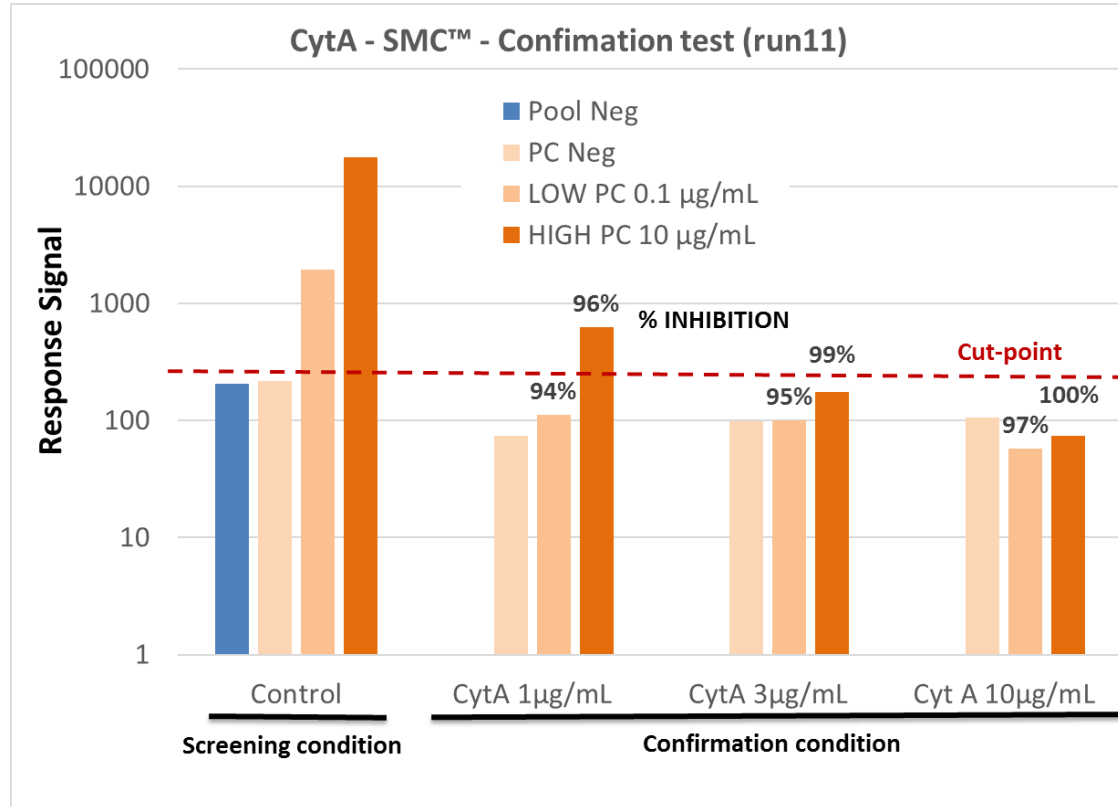
Assessment of confirmation condition with SMC™

- **Confirmation test** : competition with CytA at 1, 3 and 10 µg/mL
- **Low PC** : 0.1 µg/mL standard ADA
- **High PC** : 10 µg/mL standard ADA



3 and 10 µg/mL of CytA were sufficient to reach cut-point level

Be careful: too high CytA quantity could be unbalance the complex ADA-labeled cytokine and act as protein effect.



Conclusions on these ADA methods

- Only CytC ADA method could be developed with ECL (low background signal only for this cytokine)
- SMC™ technology allows to decrease background signal and reach sensitivity at 0.1 µg/mL for CytA, CytB and CytC
- For CytA:
 - Experimental conditions optimized for screening and confirmation tests
 - Need to verify free drug tolerance, specificity and titration process
 - Next step : validation of method

Conclusions on SMC™ technology

SMC™ is an alternative to other technologies for development of immunogenicity assay

	Pros	Cons
SMC™ technology	Decrease background signal Increase sensitivity Magnetic beads allow to increase capture Reagents consumption can be reduced	require magnetic washer and robustness depends in part on the wash step optimization
Productivity (Number of samples per day for 1 equipment and 1 analyst)	around 40 samples / day more than 1 plate/day depending on magnetic washing equipment	
SMC™ Reagent	Immunogenicity Bead Based Assay Development Kit (967 €); possibility to buy buffers separately	
Robustness	yes but need to be validated for ADA method	
LIMS interface	yes	
IQ - OQ availability	yes	
Availability in CRO	not all CRO	
Multiplex		no

THANK YOU

Acknowledgment:

L. Diouloufet, P. Juhue, N. Bremond, L. Vermet, O. Fedeli
Sanofi Montpellier, France

And

Ludivine Grosjean , Daniel Garcia West
Merck KGaA Darmstadt, Germany