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

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Translational Medicine and Early Development
Biomarkers & Clinical Bioanalysis

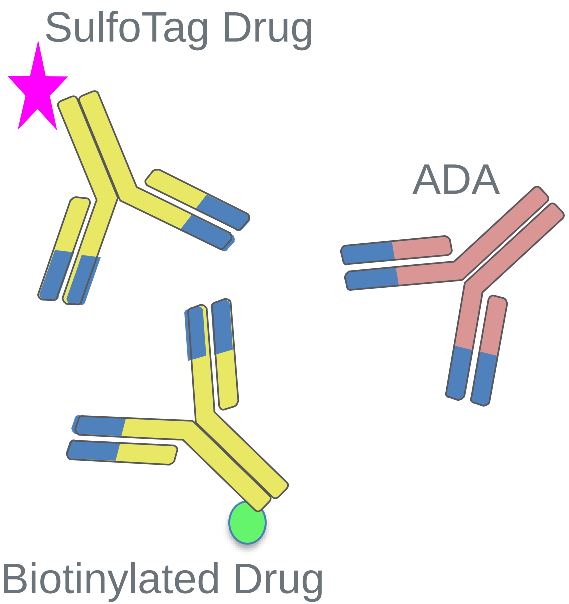
ADA Assay method : classical bridging format

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

Mix solution



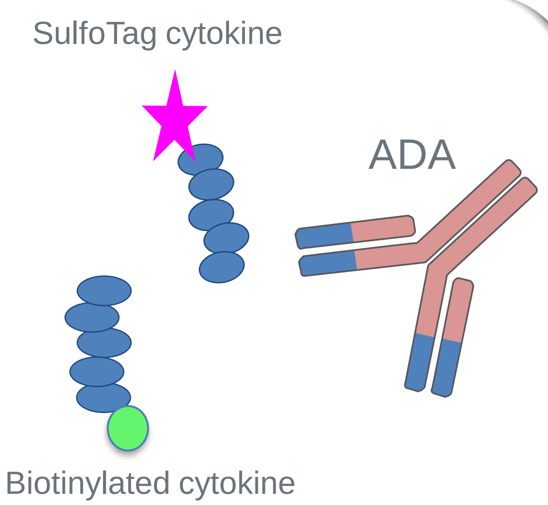
Assay method for ADA detection directed against cytokines: new project for a clinical study

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

Need to develop assay three methods for ADA detection

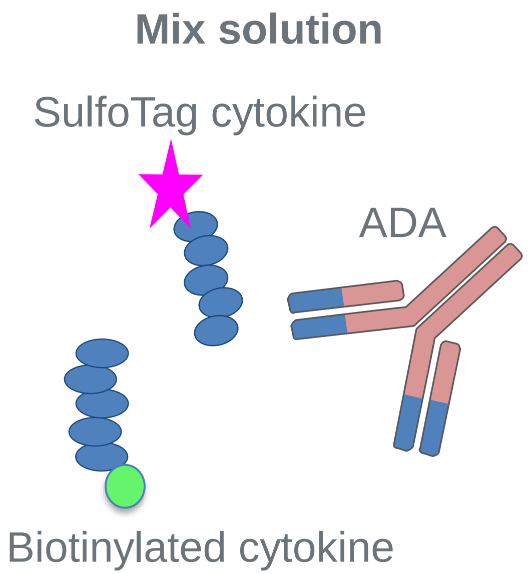
Difficulties related to cytokines :

- 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



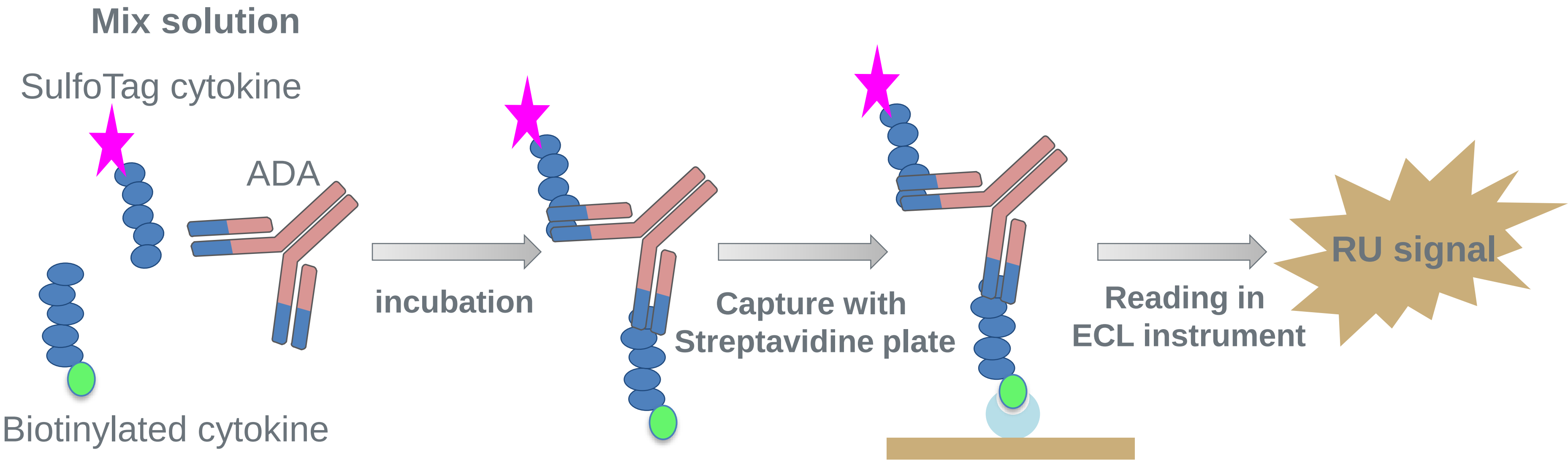
Assay method for ADA detection directed against cytokines: classical bridging format

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



- 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)

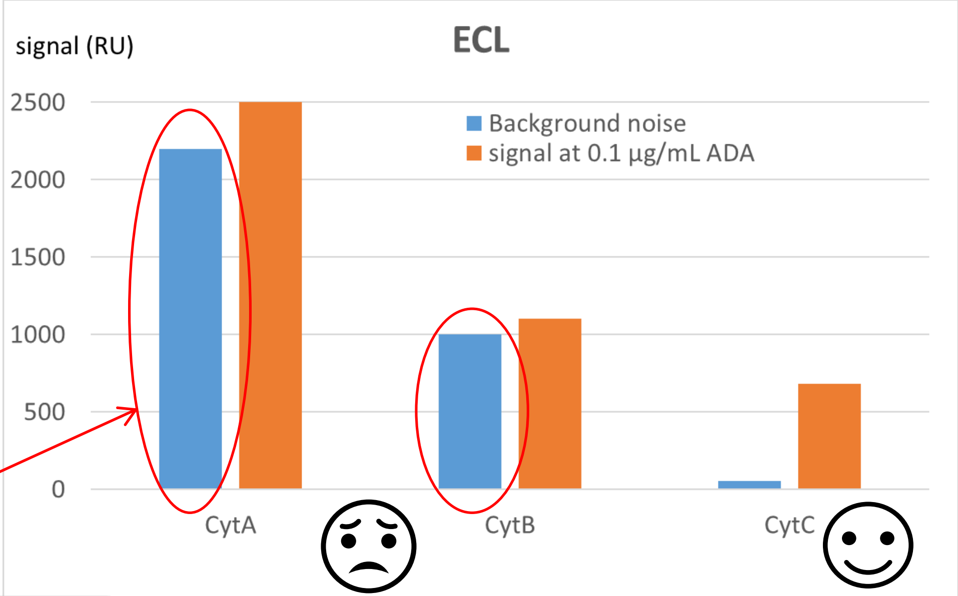


Method developments of ADA against cytokine using ECL

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 µg/mL ADA



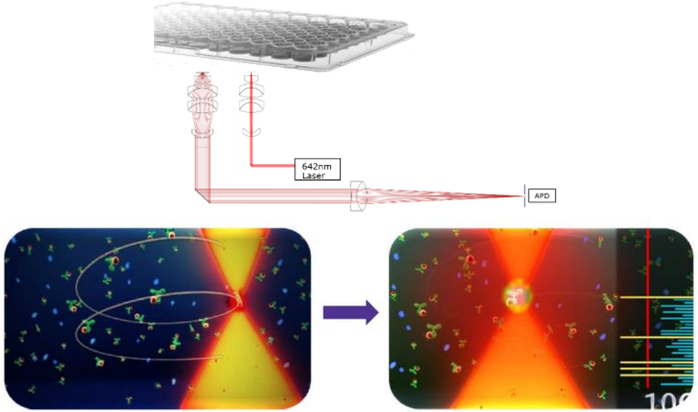
Too large background signal

- 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 µg/mL

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

Single Molecule Counting (SMC™) technology

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



Rotating objective moves laser spot at 50mm/sec through eluted analyte to scan.

Digital counting of molecules as they pass through interrogation space: Low level background "thresholded" out

- 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

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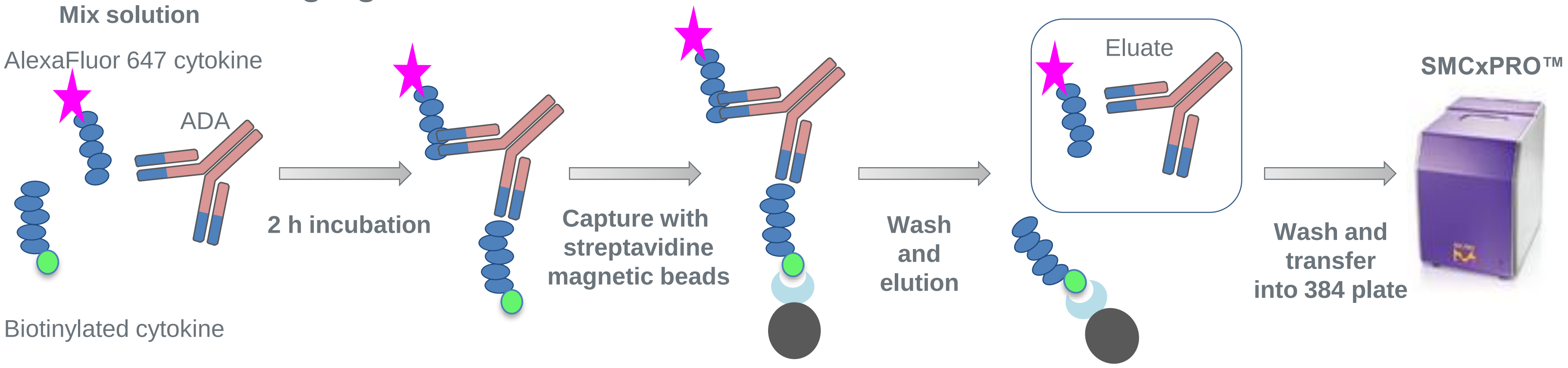
Method development of ADA using SMC™ technology

Bridging format



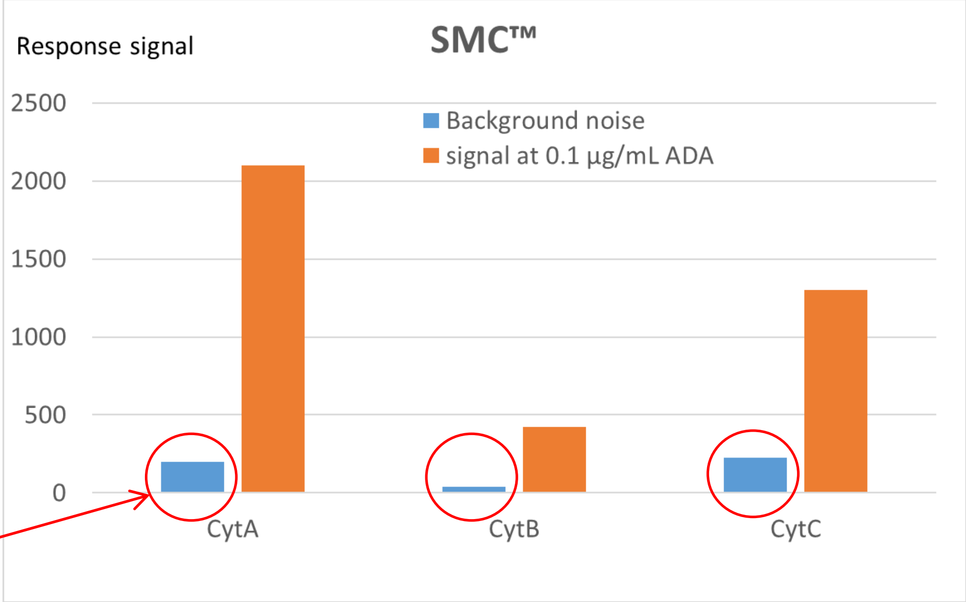
Positive elements:

- 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

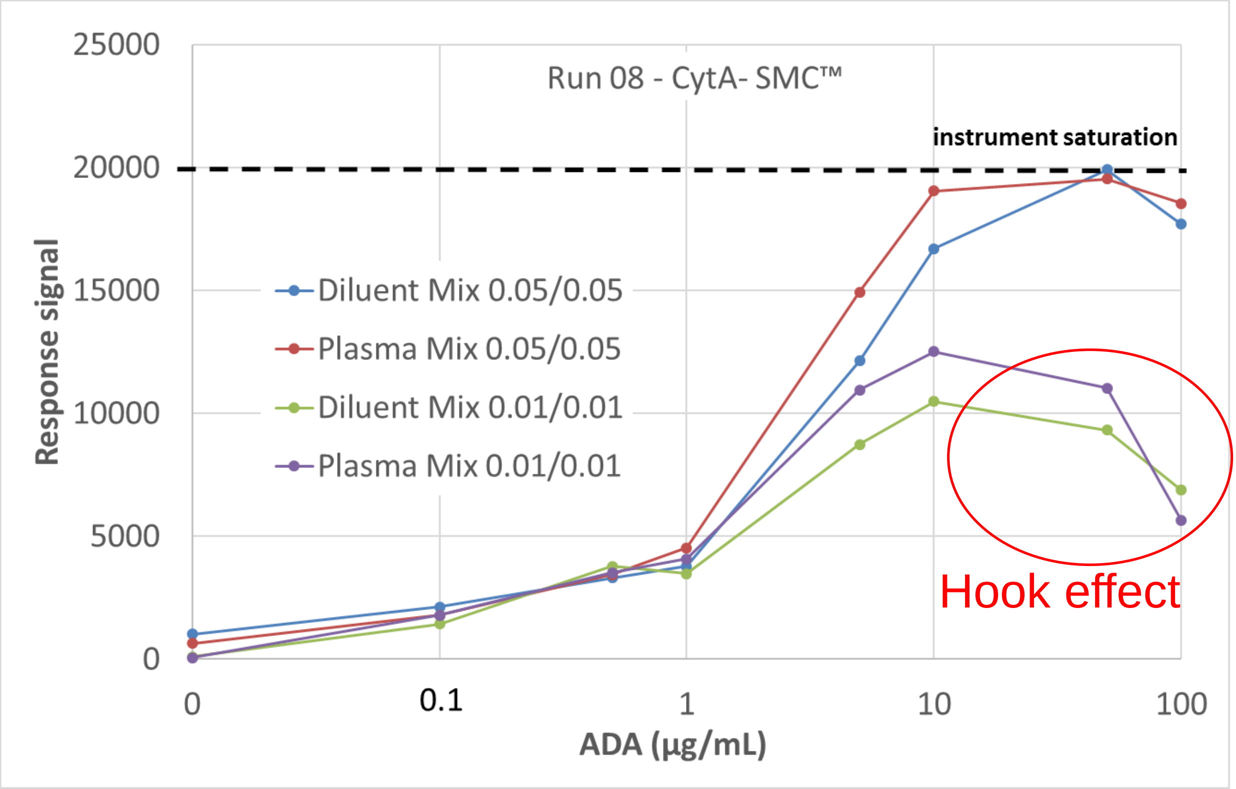
ADA method developments on **CytA,** CytB and CytC can be done with SMC™

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



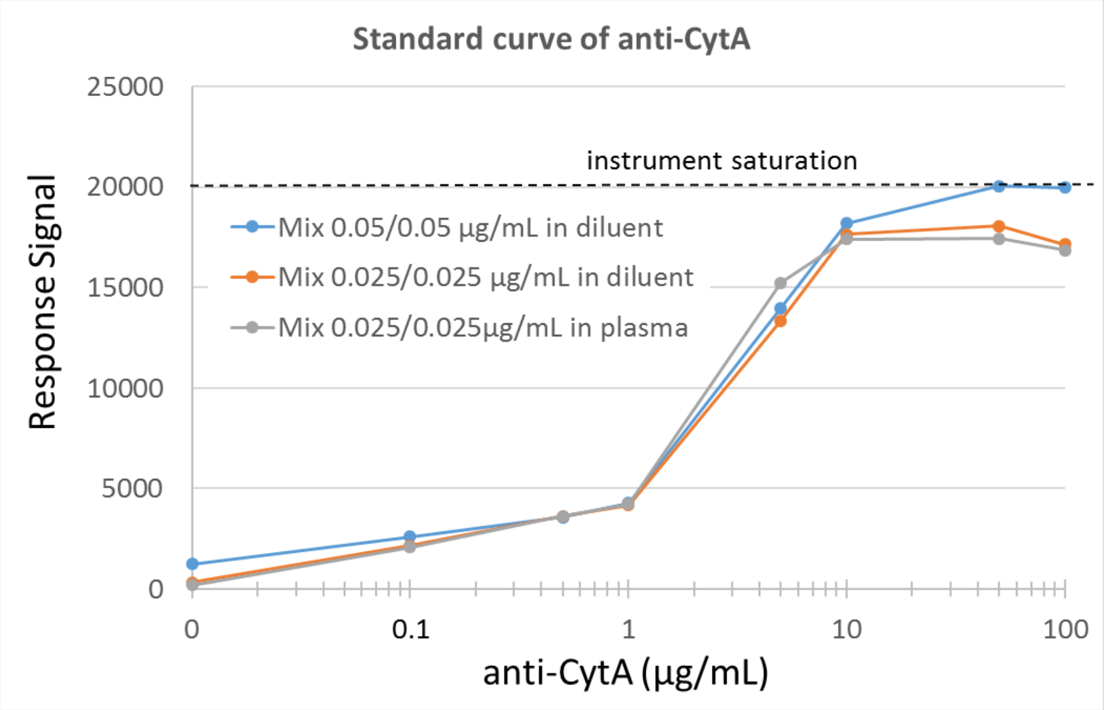
Method developments of ADA against CytA with SMC™

- **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

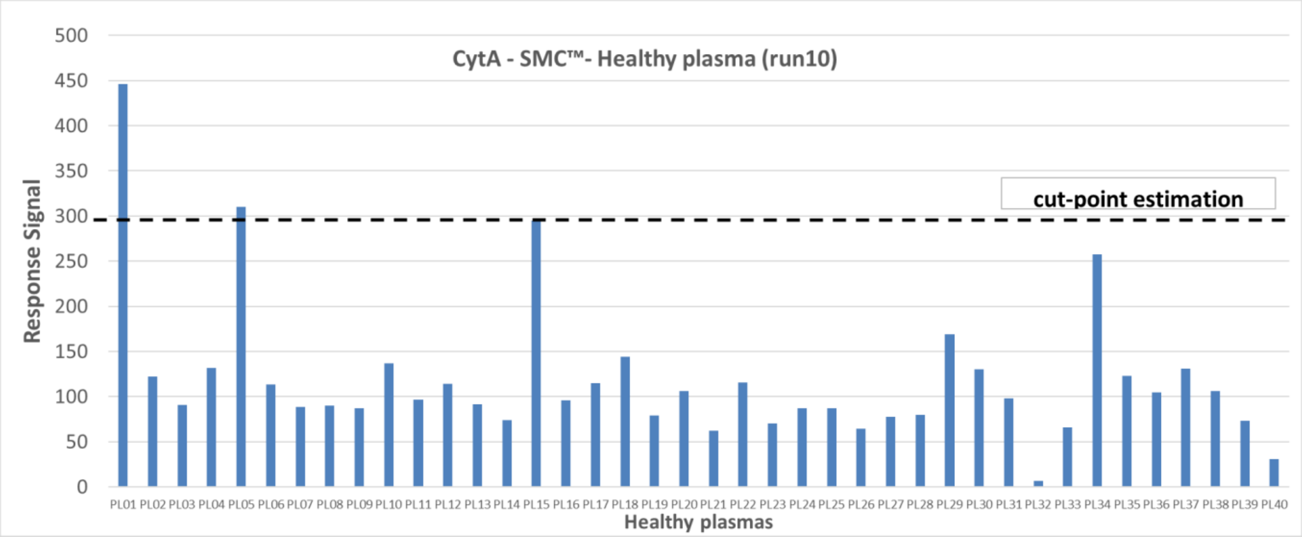
Mix 0.025 µg/mL (labeled cytokines)



➢ 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



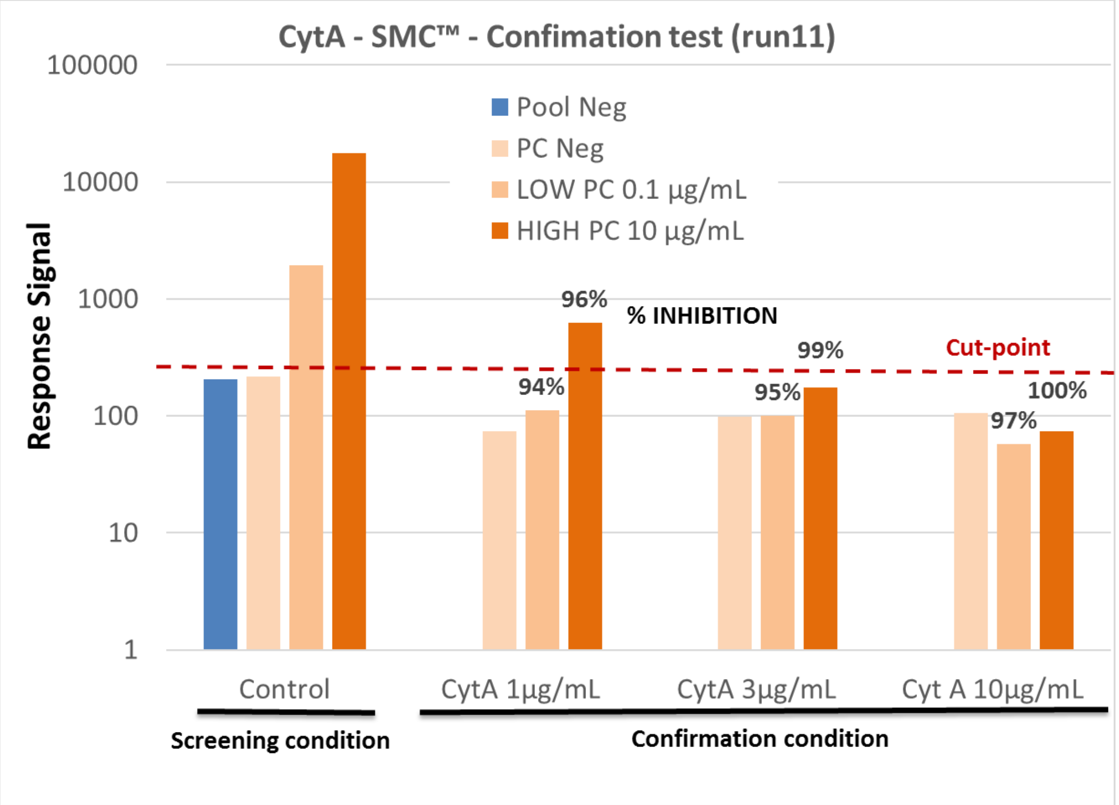
Estimation of Ncut-point at 1.34 and cut-point at 295 response signal (based on 95 percentile)

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

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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

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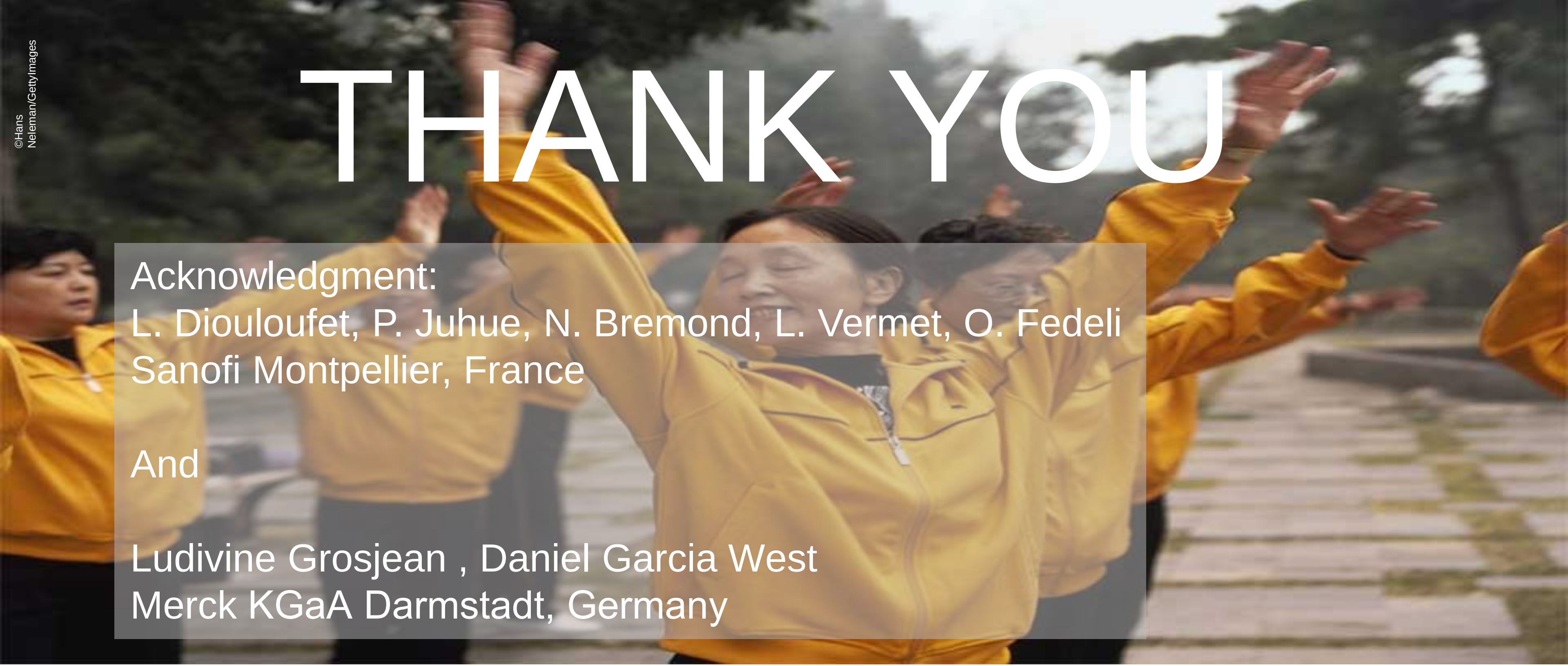

THANK YOU

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