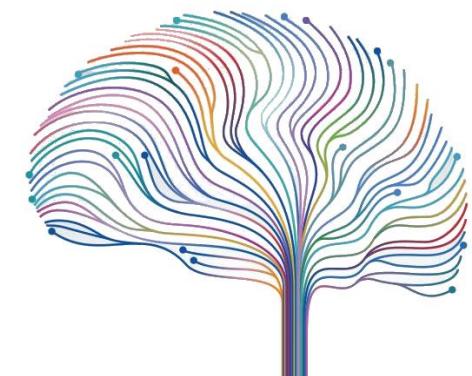


**CO**RATA Belgique  
19-20/09/18 - Rouen

# Neurochimie : *Intérêts nuancés de l'automatisation & perspectives d'avenir*

Phn. Jean-Louis Bayart  
Candidat spécialiste en biologique clinique  
2<sup>ème</sup> année



## Neurological biomarkers : a growing market

➤ Expected annual global growth of 10% until 2025, mainly due to population ageing.

➤ Increasing spectrum of characterized neuro-immunological disorders

eg : Anti-NMDA encephalitis (2007)

➤ (re)Introduction of markers in clinical practice guidelines

eg : CSF Oligoclonal bands as an alternative to dissemination in space to establish MS in patients presenting a Clinically Isolated Syndrome (McDonald criteria 2017)

➤ Shift from laborious manual techniques to automation

➤ Shift from CSF- to blood-based biomarkers

➤ Numerous promising fields :

Traumatic brain injury diagnosis

Parkinson disease

Frontotemporal dementia

Lewy body dementia

...

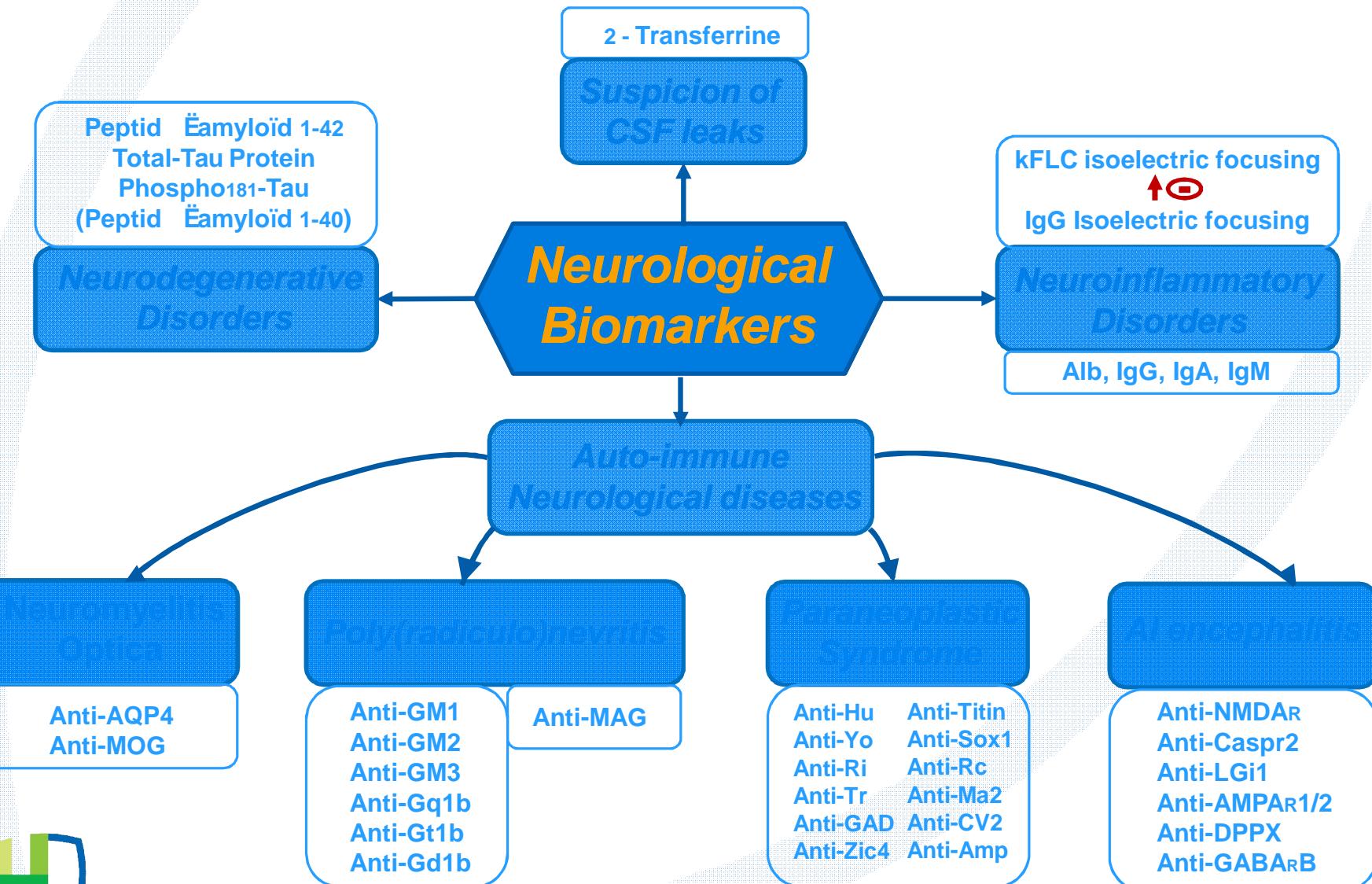


Kewal K. et al. Applications of Biotechnology in Neurology, 49-153, 2012.  
Business wire . The neurological biomarkers market 2018-25.

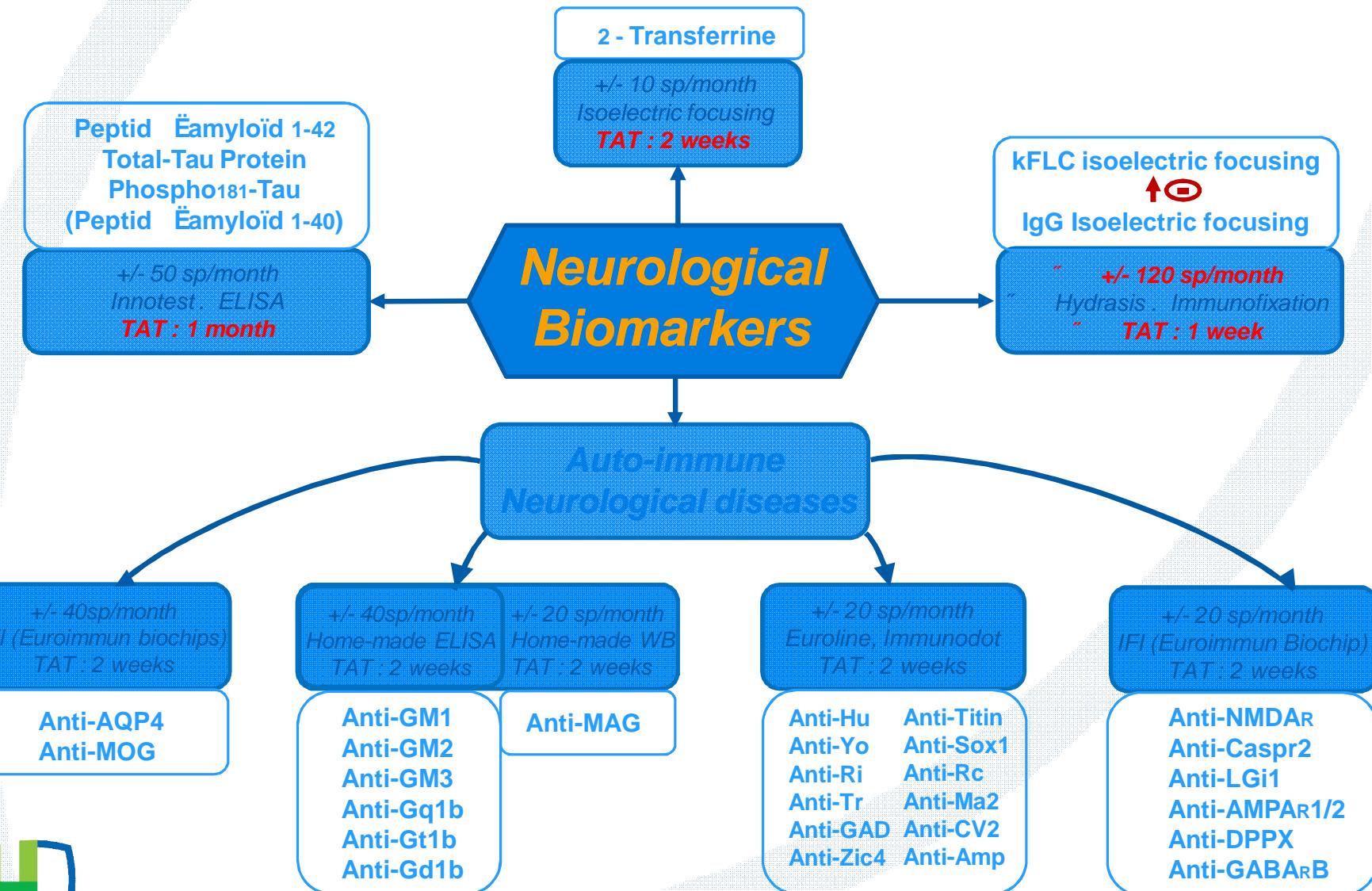
Dalmau J. et al. Lancet Neurology, 7, 1091-98, 2008  
Thompson et al. Lancet Neurology, 17, 162-73, 2018



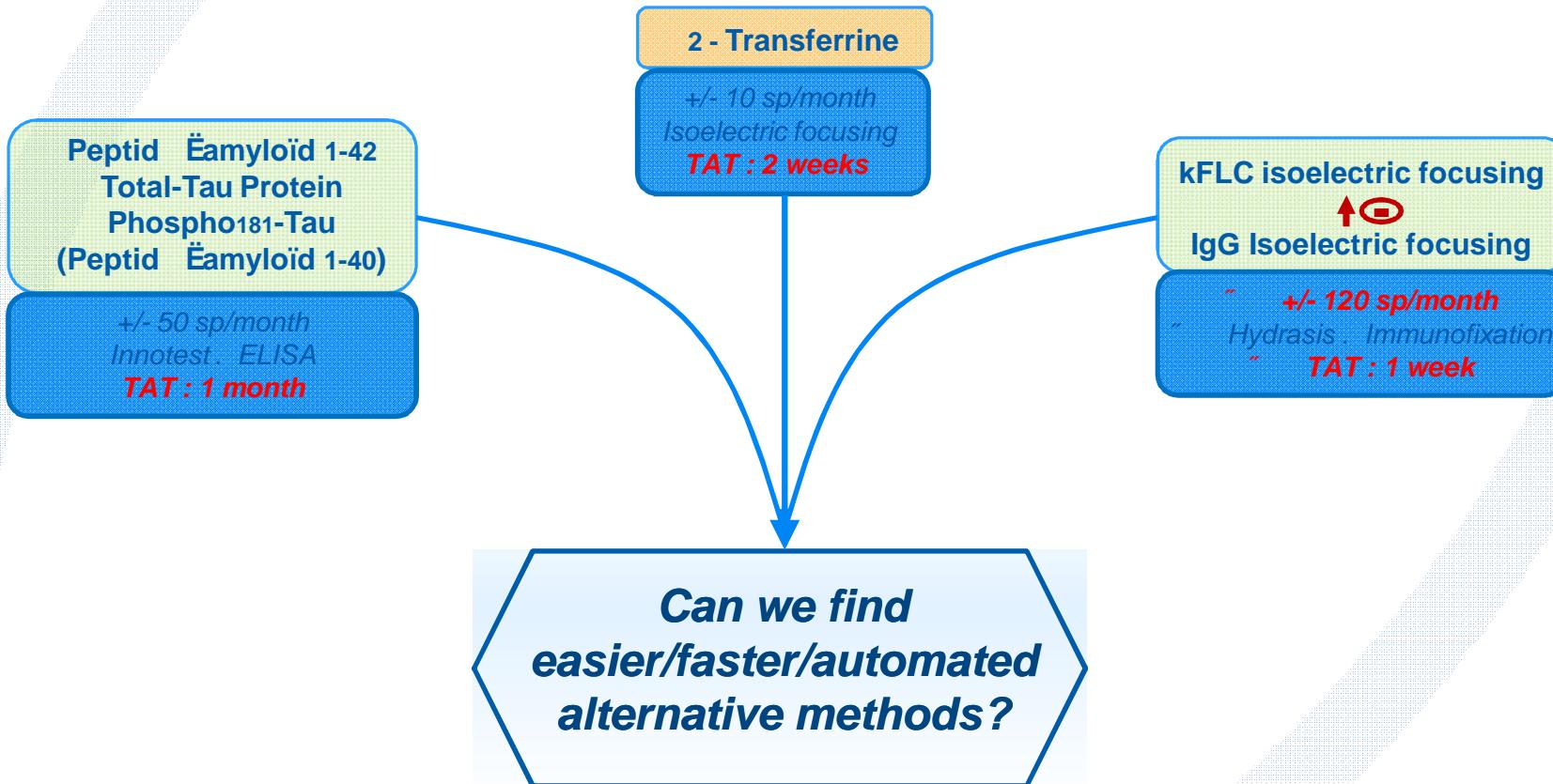
## Tests currently performed at St-Luc's Hospital

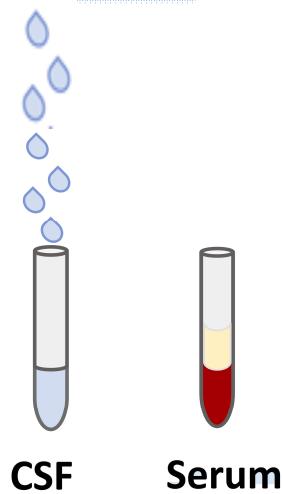


## Tests currently performed at St-Luc's Hospital



## Test currently performed at St-Luc's Hospital



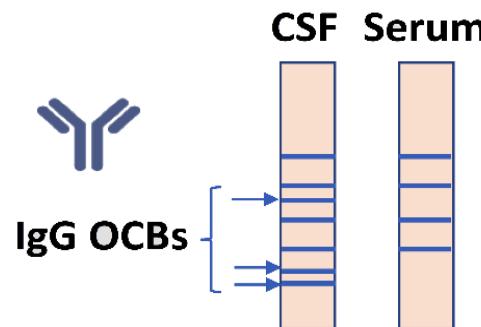


## CSF IgG Isoelectric focusing (Oligoclonal Bands (OCB))

**First step :**  
**Measure Serum and CSF IgG**  
**(+Alb, IgM, IgA) on the same analyzer**



**Second step :**  
**Dilute Serum samples to have same [IgG] as [IgG]<sub>CSF</sub>**



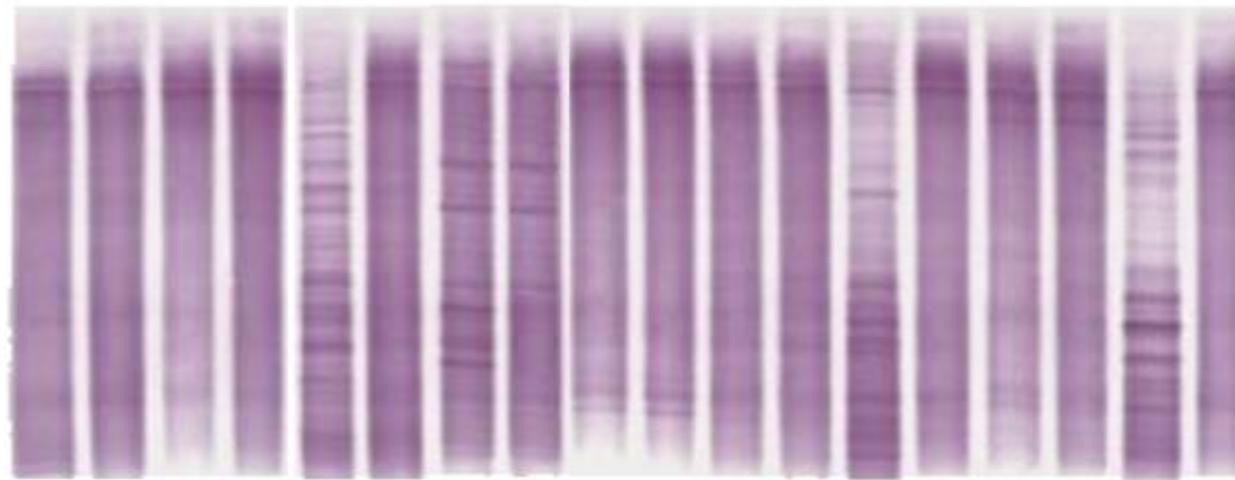
**Third step :**  
**Perform Isoelectric focalisation  
for both CSF and Serum**



## HYDRAGEL 9 CSF ISOFOCUSING

sebia

1 1' 2 2' 3 3' 4 4' 5 5' 6 6' 7 7' 8 8' 9 9'



1  
2  
3  
4

1 : Negative pattern

2 : Pure positive pattern → IgG intrathecal synthesis\*

3 : Mixed pattern

4 : Mirror pattern : Peripheral inflammatory process ?

\*OCB-Positive = Min. 2 CSF-specific bands<sup>1</sup>

1 : Gastaldi et al. Neurological Sciences, 38, 217-24, 2017

# CSF Isoelectric focusing (IEF)

**! CSF Oligoclonal Bands ≠ Multiple Sclerosis !**



Disease	Prevalence
<i>Multiple sclerosis</i>	85-90% (Vary with latitude)
<i>Clinically Isolated Syndrome</i>	65-70%
<i>Paraneoplastic syndrome</i>	65-75%
<i>Neuroborreliosis</i>	55-75%
<i>Neurolupus</i>	20-40%
<i>ADEM</i>	0-20% (> 85% mirror patterns)
<i>Non-AI &amp; Non-inflammatory Neurological disorders</i>	0-15%



***There are no absolute rules for IgG Isoelectric focusing***



Bednarova et al. Folia Microbiologica, 51, 599-603, 2006  
Djukic et al. Journal of Neurology, 259, 630-636, 2012  
Hegen et al. CCLM, 56, 1383-91, 2018  
McLean et al. J Neurosurg Psychiatry, 58, 548-54, 1995  
Psimaras et al. J Neurol Neurosurg Psychiatry, 81, 42-45, 2010  
Kidd et al. J Neurol, 265, 1906-12, 2018.

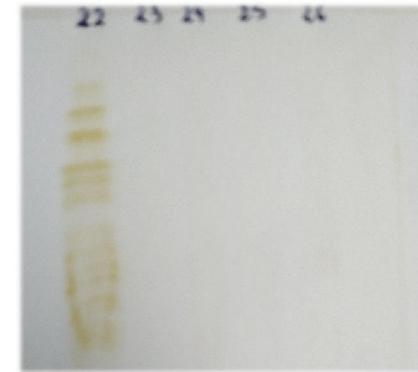
Cliniques universitaires Saint-Luc – Nom de l'orateur

Dobson et al. J Neurol Neurosurg Psychiatry, 84, 909-14, 2013  
Gultekin et al. Brain, 123, 1481-94, 2000  
Psimaras et al. J Neurol Neurosurg Psychiatry, 81, 42-45, 2010  
Franciotta et al. J of Neuroimmun, 200, 125-28, 2008  
Joseph et al. Neurology, 69, 644-54, 2007  
Falip et al. Rev Neurol, 32, 1120-4, 2001

## When do we actually need Kappa Isoelectric Focusing (IEF) ?

### 1 High MS suspicion but negative OCB result

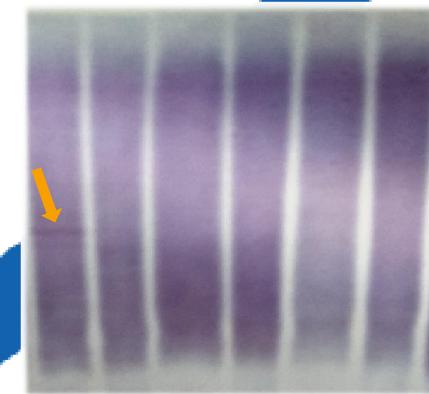
- 5-10% MS patients are OCB-negative
- Among them, nearly 50% are positive for Kappa IEF.



CSF Sample with  
oligoclonal free Kappa bands

### 2 Only one CSF-specific band visualized on IgG IEF

- Considered to be negative for actual guidelines
- Artifact or real intrathecal synthesis ?



CSF Sample presenting  
one specific IgG band

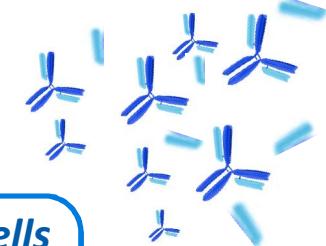
Gastaldi et al. Neurological Sciences, 38, 217-24, 2017

Djukic et al. Journal of Neurology, 259, 630-636, 2012

C Sindic et al. Journ of Neuroimmun, 33, 63-72, 1991

Gofette et al. J Neur, Neurosurg Psychiatry e, 75, 308-10, 2004

## CSF Free Light Chains (FLC) quantification



*In (neuro-)inflammatory disorders, FLC are produced in excess by B-cells*



*Surrogate marker of IgG intrathecal synthesis*

» *Same method (and now kits) as Serum FLC quantification*

N Latex FLC®  
Siemens  
Monoclonal antibodies



Freelite®  
The Binding Site  
Polyclonal antibodies

» *Various reporting units (Lack of consensus) :*

Absolute  
intrathecal value  
(mg/L)

FLC ratio :  
 $\frac{\text{FLC}_{\text{CSF}}}{\text{FLC}_{\text{Serum}}}$

FLC index :  
[  $\text{FLC}_{\text{CSF}} / \text{FLC}_{\text{Serum}}$  ]  
[  $\text{Alb}_{\text{CSF}} / \text{Alb}_{\text{Serum}}$  ]

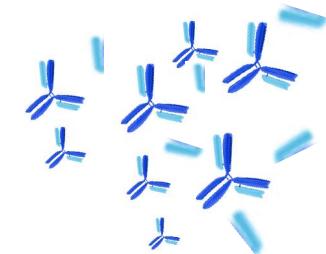
= Qalb

» *Kappa FLC (kFLC) seems to perform better than Lambda FLC for MS diagnosis.*



Duranti et al. Journal of Neuroimmun, 263, 116-20, 2013  
Presslauer et al. J Neurol, 255, 1508-14, 2008  
M. Christiansen et al. CCLM, 2018

## *Free kappa light chains in neuro-inflammatory disorders : Real substitute or just a complement method ?*



### Background :

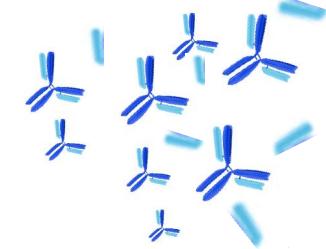
- » Numerous studies have demonstrated the added value of kFLC quantification vs OCB detection
- » However, most studies include few other neuroinflammatory diseases (INDC) & MS OCB- cases, leading to an overestimation of kFLC diagnostic performance for MS diagnosis.

### Objectives :

- » To challenge the diagnostic performance of kFLC quantification in a cohort including :
  - Numerous OCB-negative MS patients ➔ Higher sensitivity for MS diagnosis ?
  - Numerous other ( OCB + & - ) neuroinflammatory disorders (INDC)
    - ➡ Can we define a kFLC cutoff value to discriminate MS patients from INDC ?

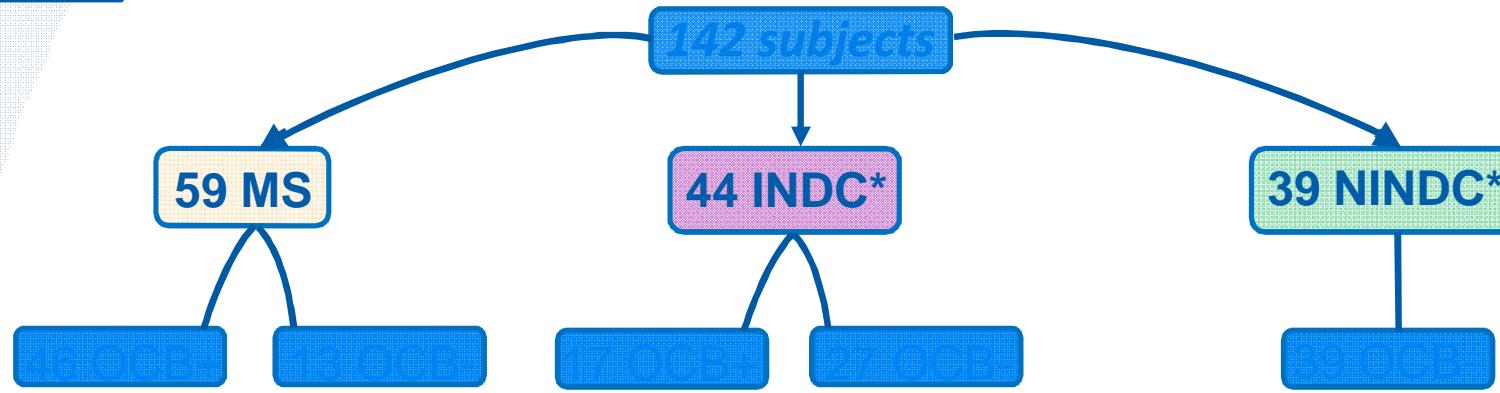


# *Free kappa light chains in neuro-inflammatory disorders : Real substitute or just a complement method ?*



## Material & Methods :

### **Study subjects**



**INDC** : Inflammatory disease control (eg : AI encephalitis, NMO, ADEM, Neuroborreliosis, Neurosarcoidosis, etc )  
**NINDC** : Non-inflammatory disease control (eg : Dementia, myelopathy, stroke, non-AI epilepsy, etc )

### **Lab analysis**



**Albumin & IgG  
quantification**  
**Immage (Beckman)**

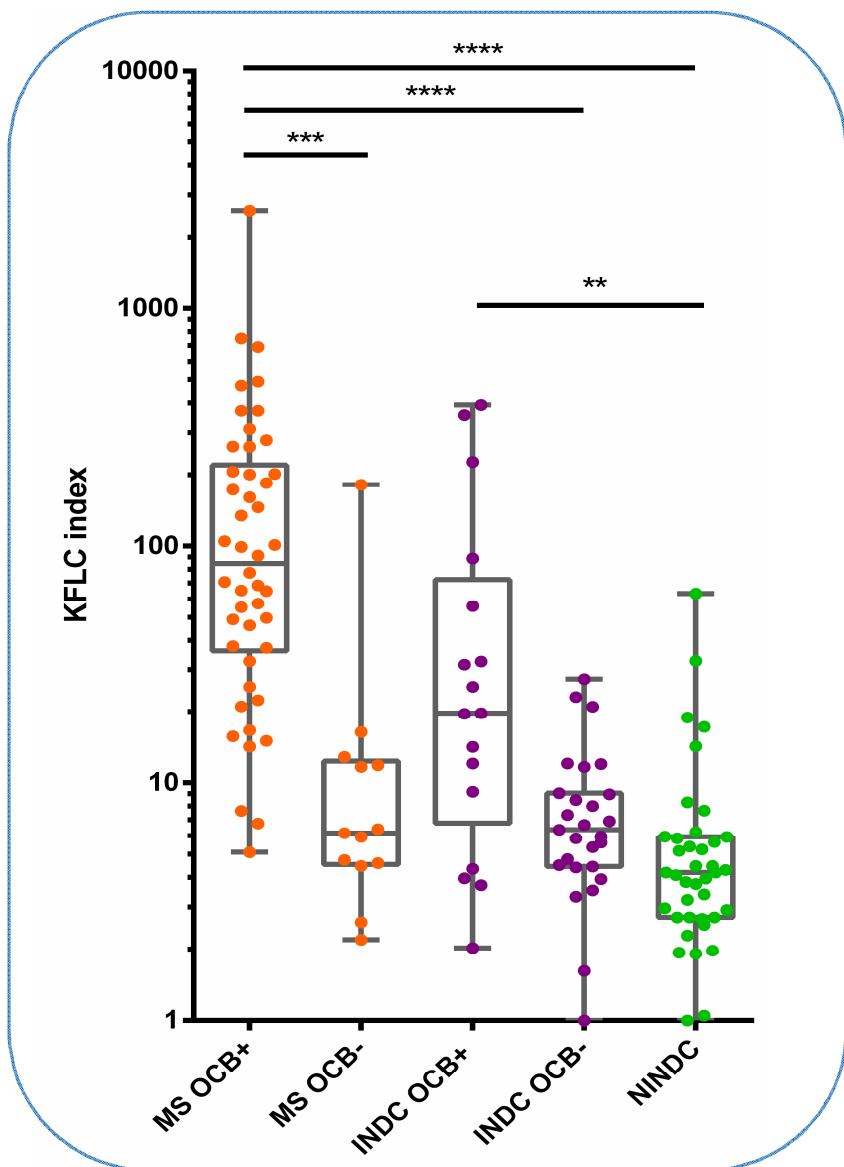


**IgG IEF & IF  
Hydrasis**  
**(Sebia)**



**kFLC quantification  
SPAplus**  
**(The Binding Site)**

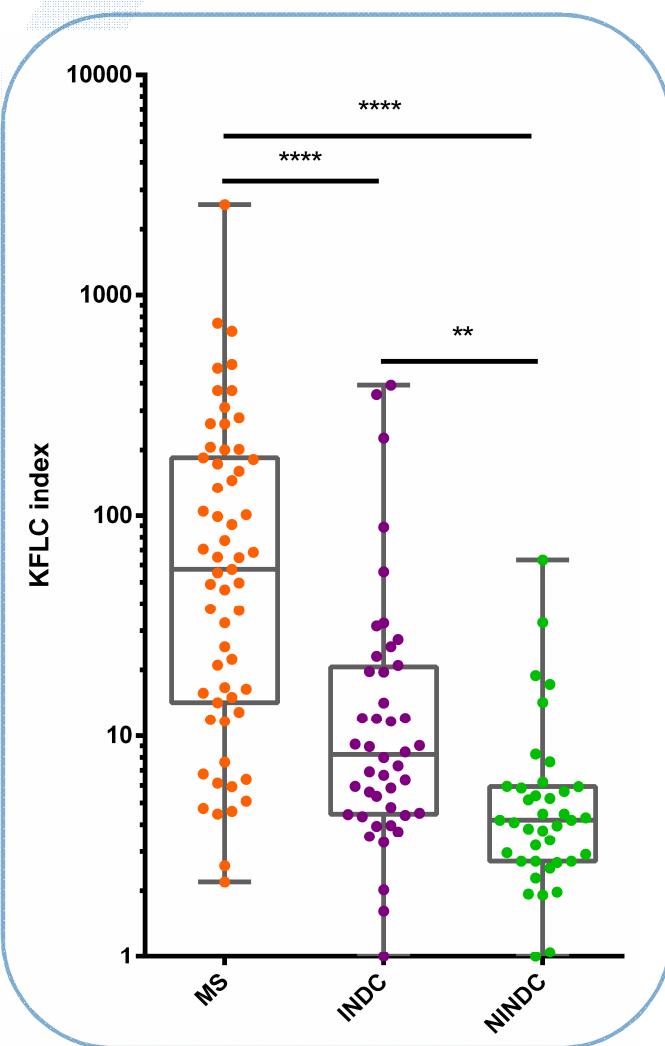
## Results :



**FLC indexes in OCB+ & OCB-, MS, IND and NIND patients.**  
Kruskal-Wallis test followed by Dunn's multiple comparison tests were performed.  
\*\*, \*\* and \*\*\* indicate p-values of  $\leq 0.01$ ,  $\leq 0.001$  and  $\leq 0.0001$ , respectively.

- » *kFLC values were above the LOQ in 94.3% of the CSF samples. (1 MS OCB-, 1 INDC OCB- and 6 NINDC were below the LOQ)*
- » *kFLC index was significantly increased in OCB-positive MS and INDC patients versus NINDC patients.*
- » *kFLC index of OCB-negative MS patients was comparable to that of OCB-negative INDC or NINDC subjects.*
- » *No statistically significant difference between OCB+ MS patients and OCB+ INDC patients was observed.*

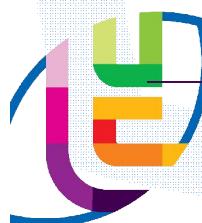
## Results :



	MS	INDC	NINDC
<b>Number</b>	59	44	39
<b>Mean age (years <math>\pm</math> SD)</b>	$45.8 \pm 14.3$	$46.7 \pm 16.0$	$48.7 \pm 14.2$
<b>Positive OCB n= (%)</b>	46 (78.0)	17 (38.6)	0 (0)
<b>Median Qalb*<math>10^3</math></b> Interquartile range	5.3 [3.5-7.5]	5.6 [3.6-9.8]	4.5 [3.6-7.2]
<b>Median kFLC<sub>Index</sub></b> Interquartile range	57.36 [14.2-184.4]	8.25 [4.46-20.63]	4.19 [2.72-5.92]
<b>Median csFkFLC<sub>(mg/L)</sub></b> Interquartile range	2.68 [0.74-10.92]	0.48 [0.18-1.65]	0.17 [0.11-0.25]
<b>Median serumkFLC<sub>(mg/L)</sub></b> Interquartile range	9.36 [7.17-12.63]	8.22 [6.16-13.87]	8.05 [5.55-10.75]

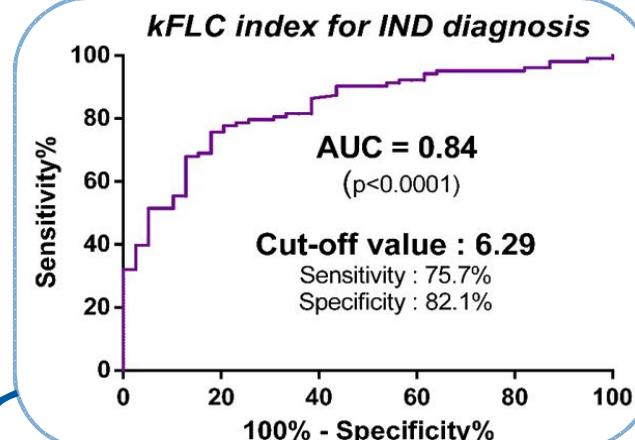
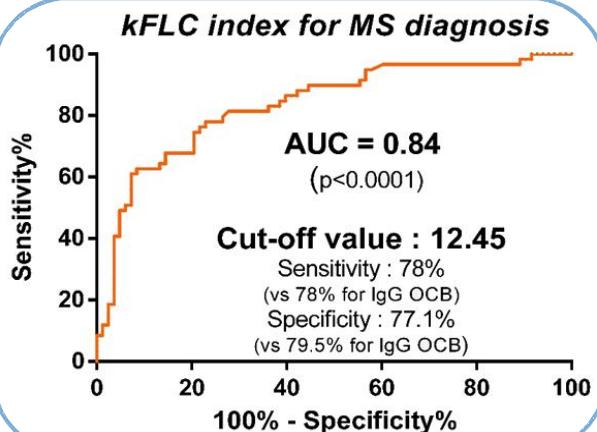
*Demographic and paraclinical characteristics of patient groups.*

► When omitting OCB status, a statistically significant difference was observed between MS and INDC groups ( $p<0.0001$ ).

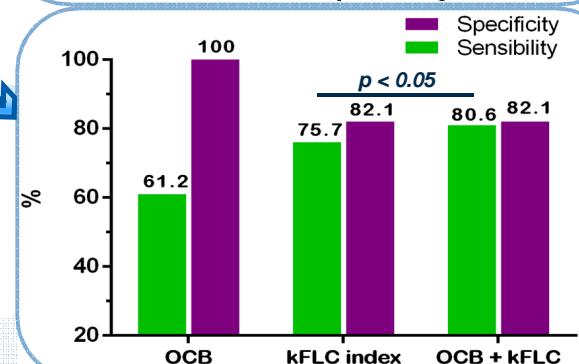


## Results :

**ROC curve analysis  
on 142 kFLC index values**



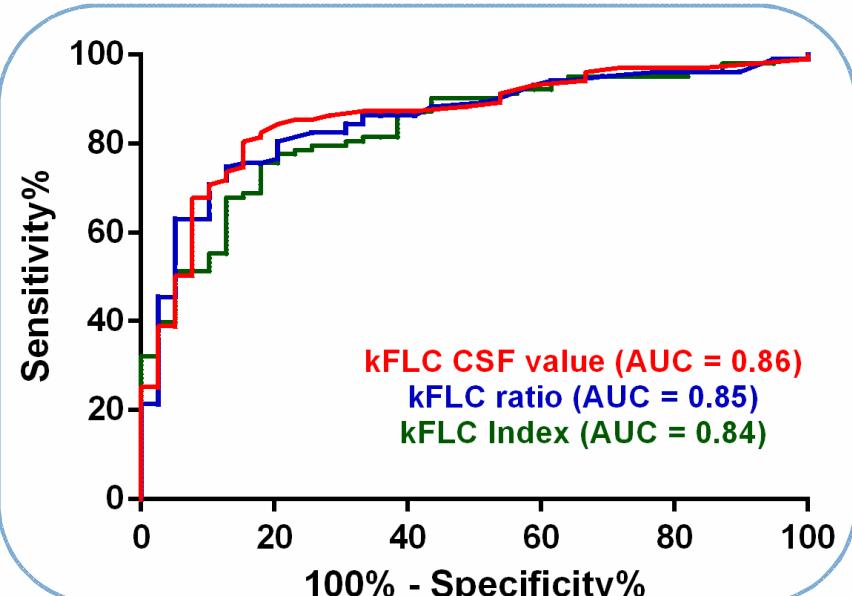
- » kFLC performs as well as IgG OCB for MS diagnosis
- » « Good » agreement with IgG OCB (Cohen's Kappa value : 0.77 )



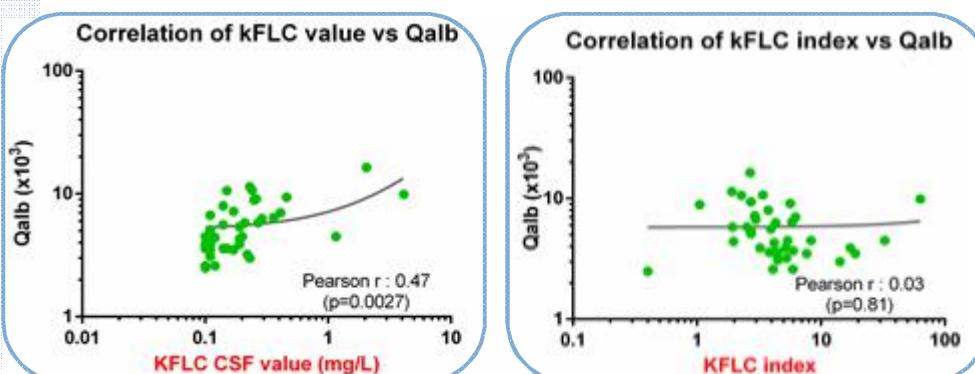
Sensitivity and specificity of OCB, kFLC index or mixed approach (OCB positivity or kFLC index  $> 6.29$ ) for IND diagnosis.



## Results :



ROC curves for IND diagnosis using  
FLC CSF value, FLC ratio or FLC index.



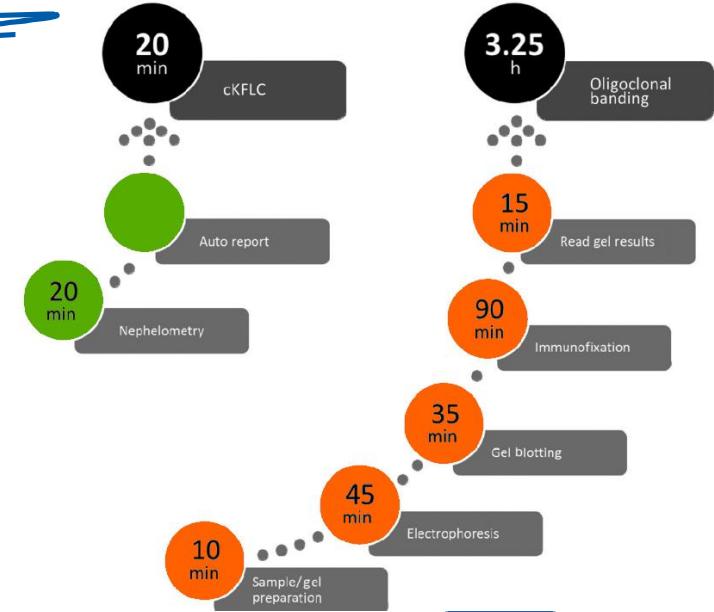
- » No consensus yet on reporting units
- » Interestingly, we observed close diagnostic performance for IND diagnosis when using CSF « gross » values (mg/L) or Ratio / Index.

» Significant correlation  
between kFLC values and Qalb

» Avoid using kFLC « gross » value when  
there is a blood-CSF barrier breakdown  
(False positive cases)

## kFLC : pro & cons

- Quantitative result (*No subjective interpretation*)
- Fully automated : reduced cost & TAT
- Same reagent kit as serum dosage
- Broader access for « non-specialized » laboratories
  
- OCB detection is incorporated in McDonald MS criteria Å What about kFLC ?
- Specific wash process needed on SPAplus analyzer
- Belgium : Not reimbursed Å & OCB detection reimbursement is satisfying (B 4000)



Thompson et al. Lancet Neurology, 17, 162-73, 2018  
<https://ondpanon.riziv.fgov.be/nomen/fr/544132>

Accepted: 23 May 2018

DOI: 10.1111/ane.12969



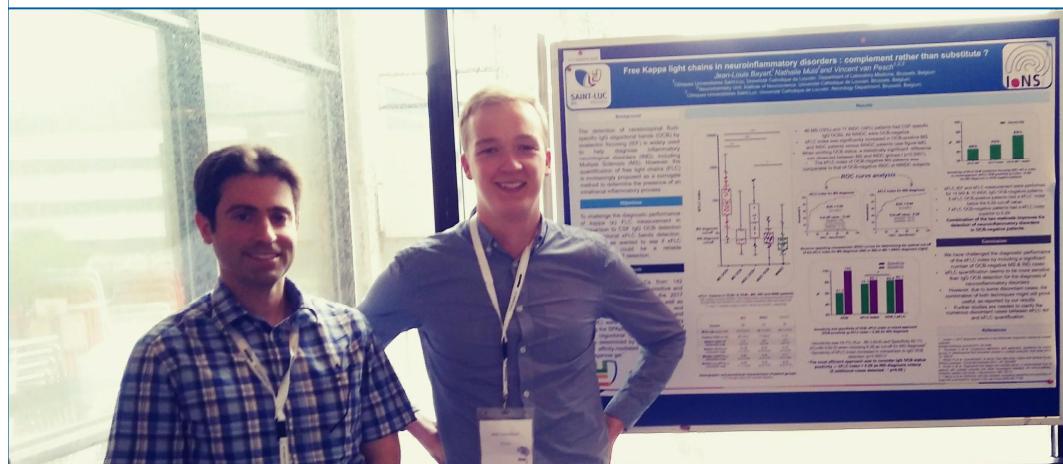
ORIGINAL ARTICLE

WILEY

Acta  
Neurologica  
Scandinavica

# Free Kappa light chains in neuroinflammatory disorders: Complement rather than substitute?

J.-L. Bayart<sup>1</sup> | N. Muls<sup>2</sup> | V. van Pesch<sup>1,2,3</sup>



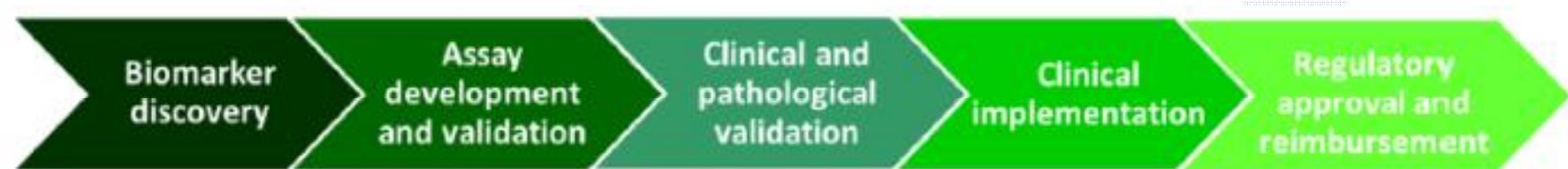
Society for CSF Analysis  
and Clinical Neurochemistry

2nd Meeting of the Society for CSF analysis  
and clinical neurochemistry

Amsterdam, June 7-8, 2018

# *Emerging trends in Neurochemistry : Illustration with the SIMOA® technology*

« *There are more than 4000 circulating proteins  
About one tenth (375) of these are reliably  
measurable by conventional technologies  
Of these, one half (171) has FDA clearance* »



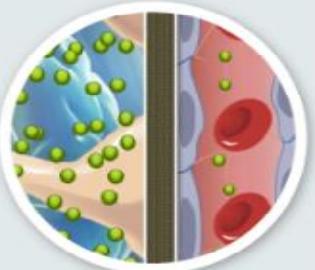
D. Wilson et al. Journal of Lab Automation, 21, 533-47, 2016  
Teunissen et al. Alzheimer's Research & Therapy, 10, 2018

## The SIMOA technology

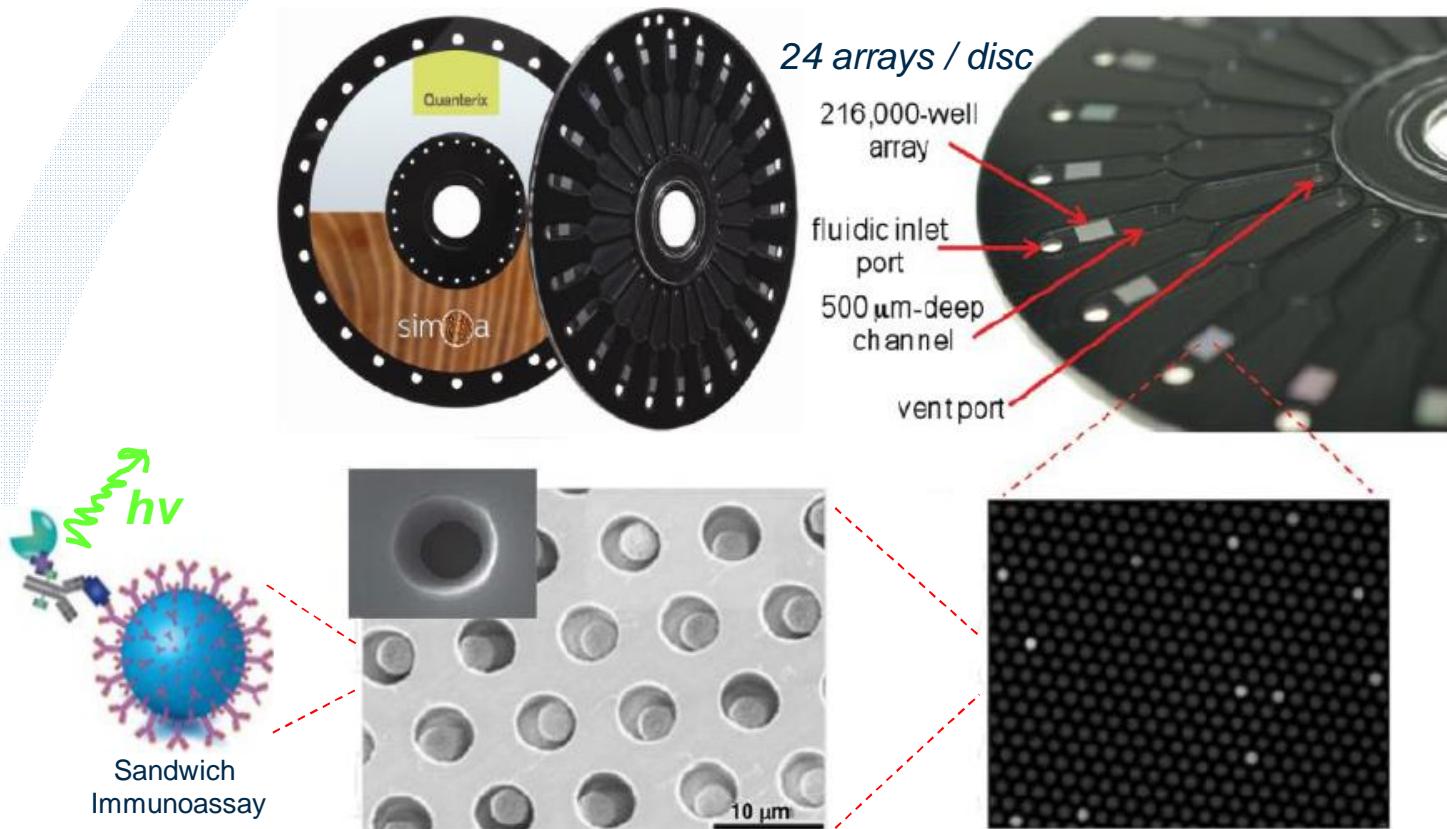
**SIMOA = SIngle Molecule Array**

- » **Digital Immunoassay technology**
- » **Improve detection sensitivity three logs, to attomolar range ( $10^{-16}M$ )**  
(vs femtomolar ( $10^{-13}M$ ) for conventional technology)
- » **Suitable for both life science research and clinical laboratories**  
(Future potential cost close to current technologies)
- » **250+ publications in research areas (> 75% related to Neurology)**

D. Wilson et al. Journal of Lab Automation, 21, 533-47, 2016  
Teunissen et al. Alzheimers Research & Therapy, 10, 2018

Neurology	Cardiology	Oncology	Inflammation	Infectious Disease
				

## *The SIMOA technology : The cousin of the digital PCR*



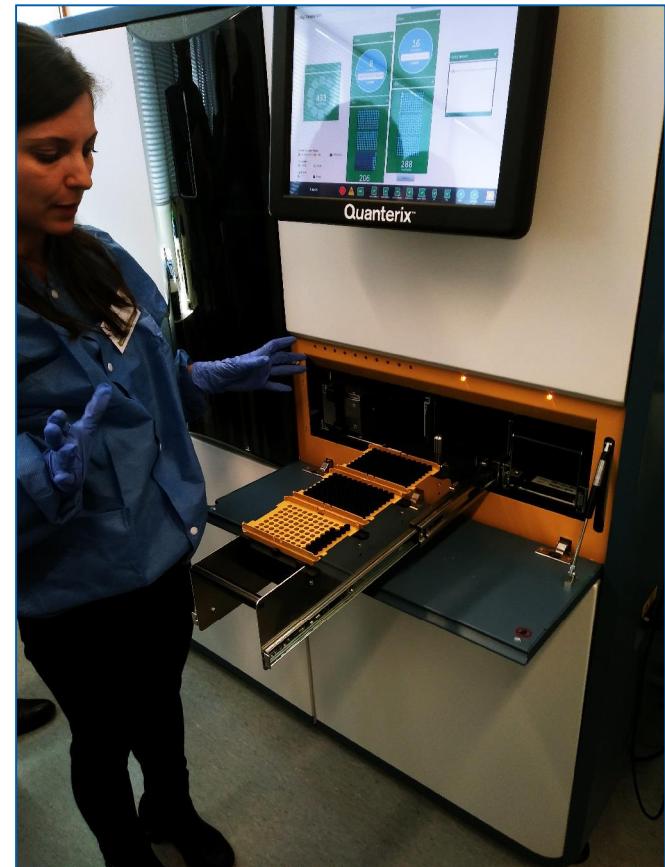
- » Each array contains 216.000 wells sized to receive one paramagnetic bead
- » Fluorescent product from the enzyme-substrate reaction is confined to each wells
- » All 216.000 wells are then verified simultaneously by a digital camera
  - » Multiplexing is also possible (Up to 10 proteins / array)

D. Wilson et al. Journal of Lab Automation, 21, 533-47, 2016  
A. Given et al. The AAPS Journal, 2017



# SIMOA HD-1

## Fully automated Analyzer

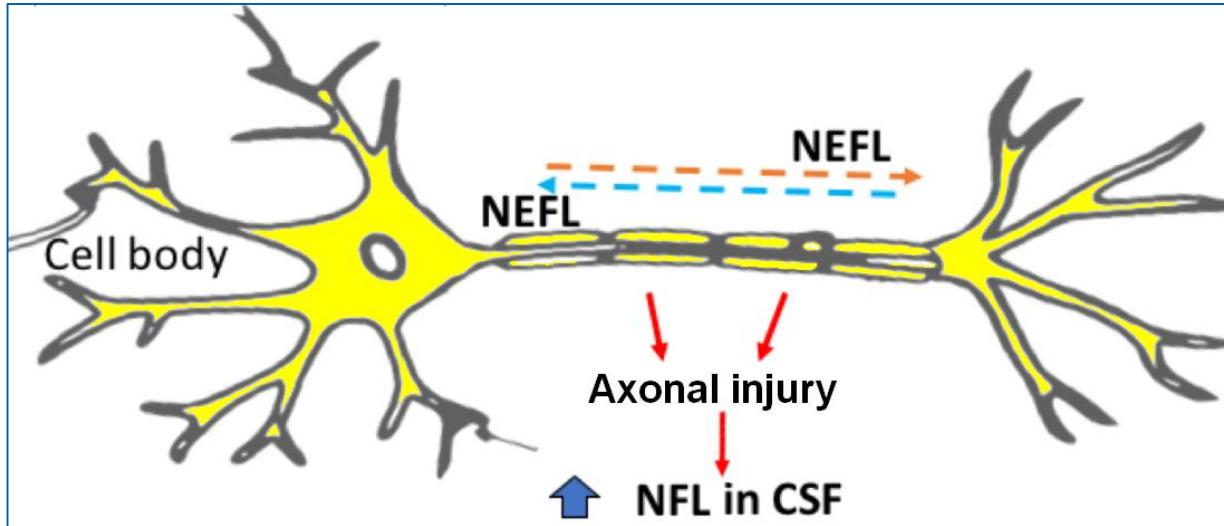


Assay	Sample Volume, $\mu\text{L}$	Upper Range, pg/mL	Time-to-First-Result, min	Sample	Mean, pg/mL	Within-Run CV, %	Between-Run CV, %	Between-Day CV, %	LoD, pg/mL	LoQ, pg/mL
PSA	25	400	62	Low	3.04	6.6	8.8	0.0	0.020	0.037
				High	60.2	5.0	4.1	6.9		
$\text{A}\beta 42$	25	400	77	Low	2.37	3.9	10.7	3.8	0.034	ND
				High	51.8	5.7	6.7	2.3		
Tau	38	400	77	Low	2.28	6.0	14.0	0.0	0.019	0.023
				High	109	3.8	9.1	0.0		
$\text{TNF}\alpha$	25	200	77	Low	2.77	3.4	4.1	0.0	0.014	ND
				High	29.9	2.8	4.8	0.0		

## Added value of SIMOA technology : Illustration with NFL

### **NeuroFilament Light (NFL) :**

*Protein of the cytoskeleton providing structural support for axons and regulating their diameter*



- » In MS patients, CSF [NFL] increases during relapse
- » CSF NFL correlates very well with contrast-enhancing lesions on MRI
- » CSF [NFL] decreases by effective treatment

**Ideal biomarker for disease monitoring** Å  
Å but, unfortunately, invasive (repetitive lumbar punctures)

L. Novakova et al. Neurology, 89, 2017

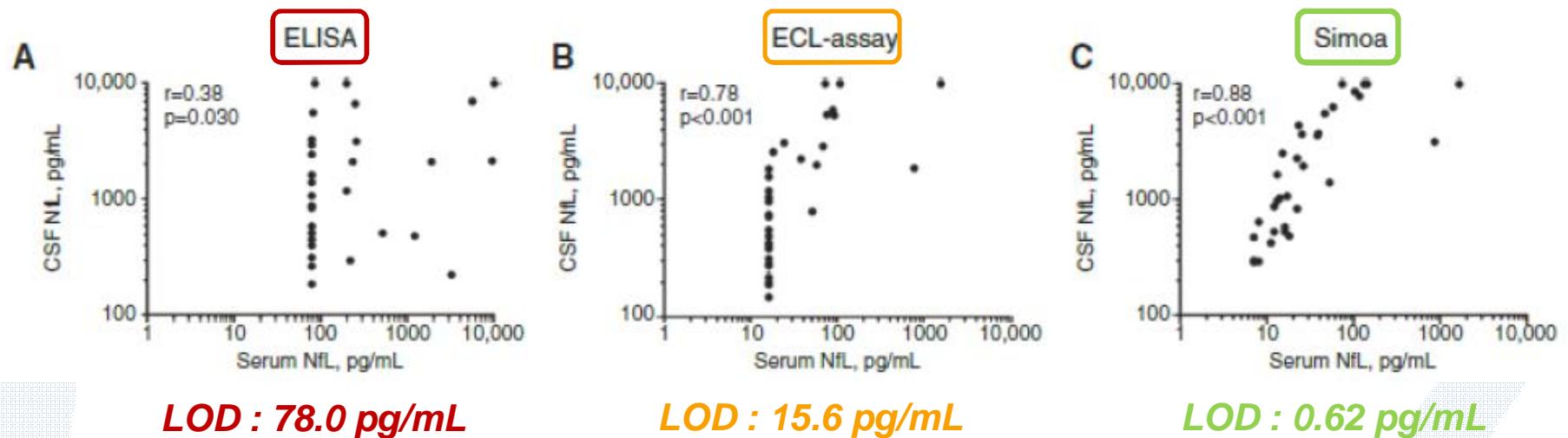
G. Disanto et al. Annals of Neurology, 81, 857-70, 2017

K. Varhaug et al. Neurology, 95, 2018



## Added value of SIMOA technology : Illustration with NFL

Correlation between Serum NF-L and CSF NF-L as measured on multiple platforms

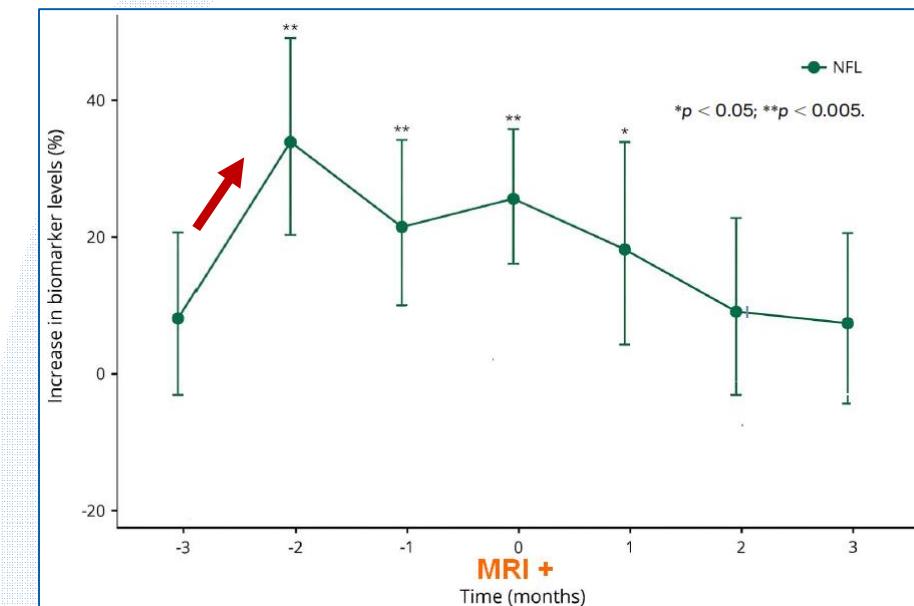


- » [NFL]<sub>serum</sub> correlates well with [NFL]<sub>CSF</sub>
- » Less invasive procedure makes longitudinal studies feasible

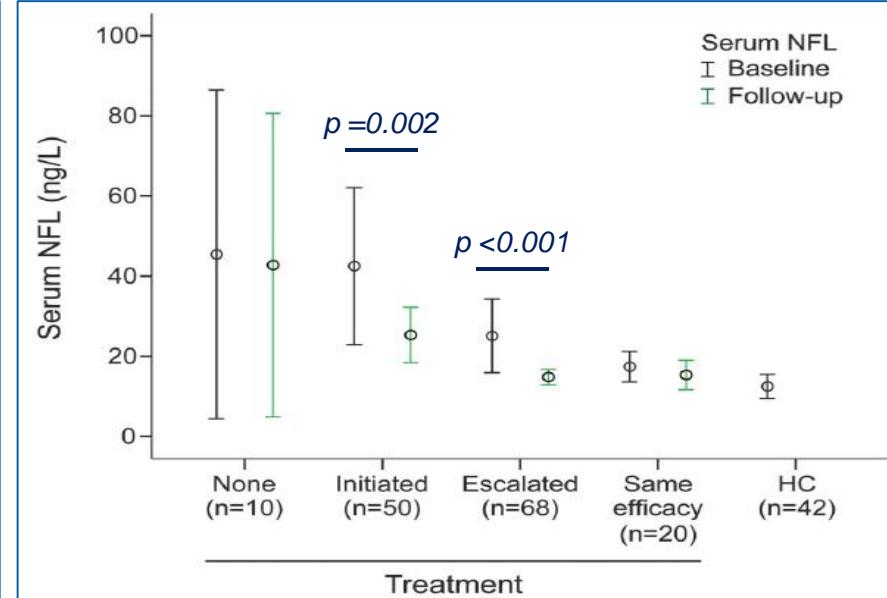


## Added value of SIMOA technology : Illustration with NFL

### Serum [NFL] reflects disease activity



Relationship between new T1 gadolinium-enhanced lesions and NFL measurement in RRMS patients ( $n = 85$ )



Serum NFL in patients with MS at baseline and follow-up and in Healthy controls (HC)

Increase up to two months earlier than MRI lesions



MRI trigger ?

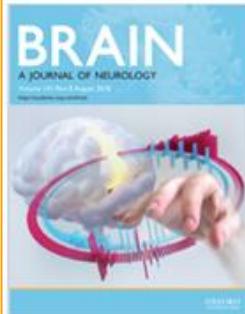


Potential for treatment monitoring



L. Novakova et al. Neurology, 89, 2017  
K. Varhaug et al. Neurology, 5, 2018





Volume 141, Issue 8  
August 2018

## Peripheral blood neurofilament light chain levels: the neurologist's C-reactive protein?

Gavin Giovannoni, MBBCh, PhD ✉

*Brain*, Volume 141, Issue 8, 1 August 2018, Pages 2235–2237,  
<https://doi.org/10.1093/brain/awy200>

Published: 24 July 2018

## Neurofilament light chain

A prognostic biomarker in amyotrophic lateral sclerosis

OPEN

JAMA Neurology | Original Investigation

Association of Plasma Neurofilament Light

Editorial

With Neurodegeneration in Patients With Alzheimer Disease

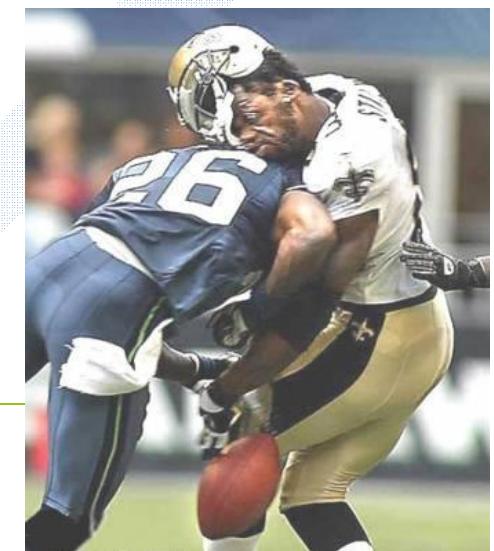
Serum neurofilament light as diagnostic biomarker for acute cerebral ischemia: a promising tool

Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports

*Journal of Neurotrauma*, Vol. 33, No. 19 | Original Articles

Serum Neurofilament Light in American Football Athletes over the Course of a Season

Serum and cerebrospinal neurofilament light chain levels in patients with acquired peripheral neuropathies

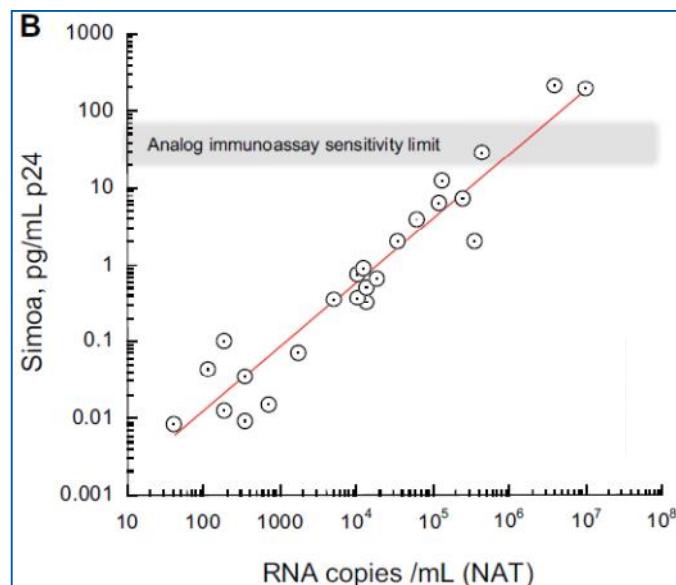


RESEARCH REPORT

## SIMOA technology : Others applications...

### HIV 5th generation assay ?

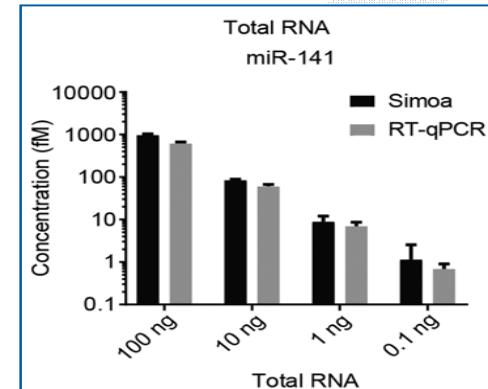
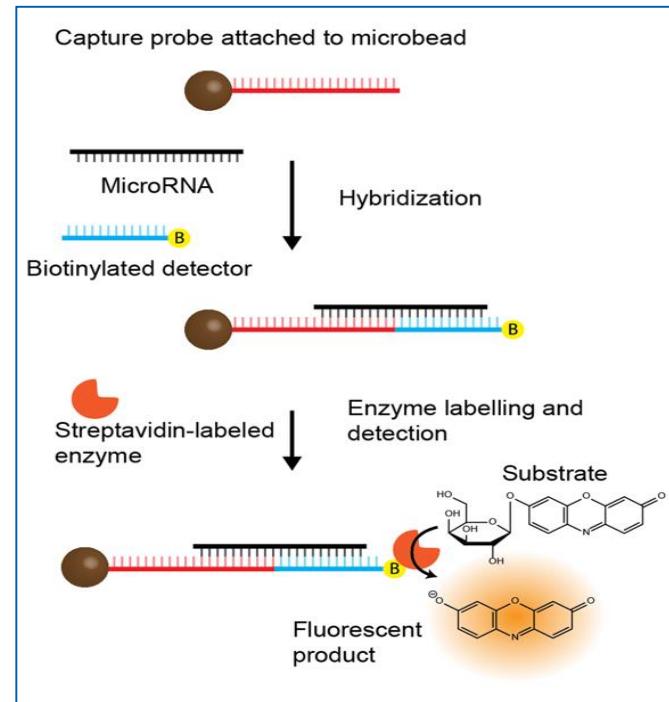
Assay category	Assay	Proportion positive on performance panel ( <i>n</i> = 300)
Fourth-generation HIV Ag/Ab	Bio-Rad GS HIV combo Ag/Ab EIA	0.40
	Vitros HIV combo assay	0.33
POC rapid assays	Alere Determine HIV-1/2 Ag/Ab combo assay	0.32
	SD Bioline HIV Ag/Ab combo assay	0



**Sensitivity :**

RT-PCR (Abbott m2000) : 40 RNA copies/mL  
Simoa : 60 RNA copies/mL

### miRNA quantification without RT-PCR



M. Stone et al. Journ of clin Microbiology, 56, 2018  
Given et al. The AAPS Journal, 2017  
L. Cohen et al. Nucleic acids Research, 2017

***Thank You  
For your attention !***

**29 november @ St-Luc :  
Alzheimer disease biomarkers**

**From manual ELISA to automation  
((pre-)Analytical considerations & clinical interpretation)**  
*In association with the Neurology Unit*

