Accurate Results for Patient Care Workshop 2017
A JCTLM Members' and Stakeholders' meeting
4-5 December 2017, BIPM

JCTLM
Accurate results for patient care

Session 5a: Clinical Challenge – Biomarkers in neurodegenerative disease. Chair: Graham Beastall (IFCC) 09:00 - 11:00

Preanalytical and analytical aspects of CSF biomarkers assay

Dr Armand Perret-Liaudet
Chef du Service de Biochimie & Biologie Moléculaire Grand Est
Neurobiologie CHU Lyon – France
Centre de recherche en Neurosciences de Lyon, BioRan

Bureau International des Poids et Mesures
ifcc International Federation of Clinical Chemistry and Laboratory Medicine
ilac
EFLM European Federation of Clinical Chemistry and Laboratory Medicine
Hospices Civils de Lyon

Accurate Results for Patient Care Workshop 2017

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JCTLM
Accurate results for patient care

• **Potential clinical applications of biomarkers of neurodegenerative disease**	Sergio Bernardini (University of Rome Tor Vergata)	09.00
• **Preanalytical and analytical aspects of CSF biomarker assays**	Armand Perret-Liaudet (Lyon University Hospital)	09.30
• **Reference method for b-amyloid in CSF**	Josef Pannee (Inst. of Neuroscience & Physiology, Sweden)	10.00
• **Role of metals and metal containing biomolecules in neurodegenerative diseases such as Alzheimer's disease**	Claudia Swart (PTB)	10.30

NO CONFLICT OF INTEREST

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ifcc International Federation of Clinical Chemistry and Laboratory Medicine

ilac

EFLM European Federation of Clinical Chemistry and Laboratory Medicine

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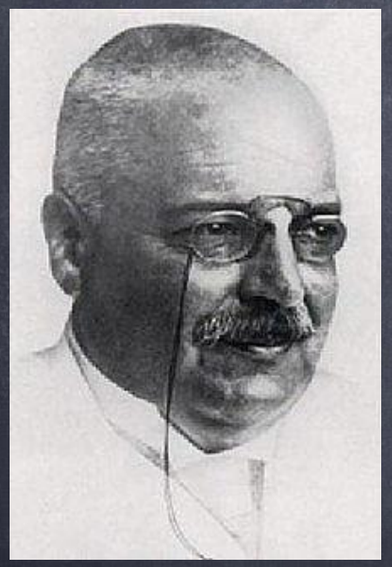
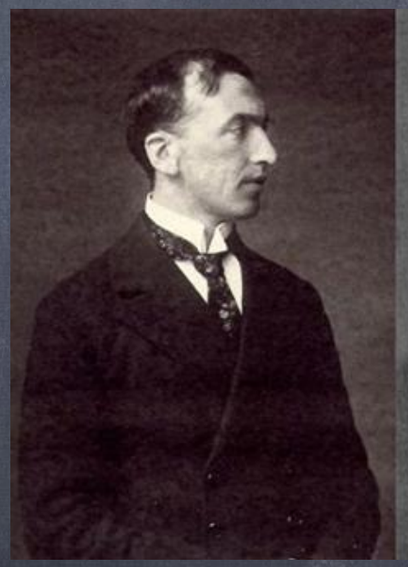
Currently used biomarkers

Alzheimer (AD) and Creutzfeldt-Jakob (CJD) Diseases





WHO criteria : possible CJD + positivity of p1433 → probable CJD

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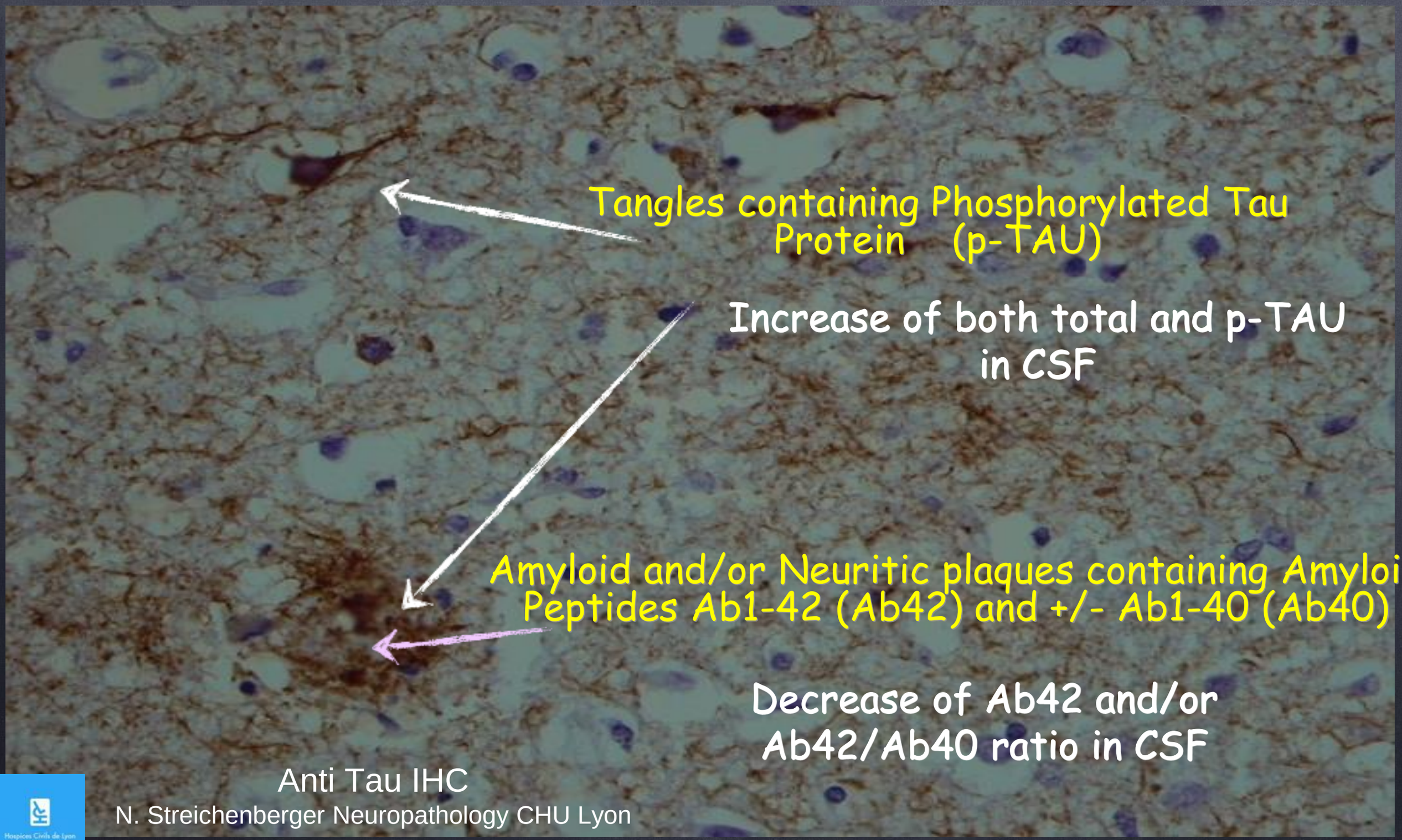
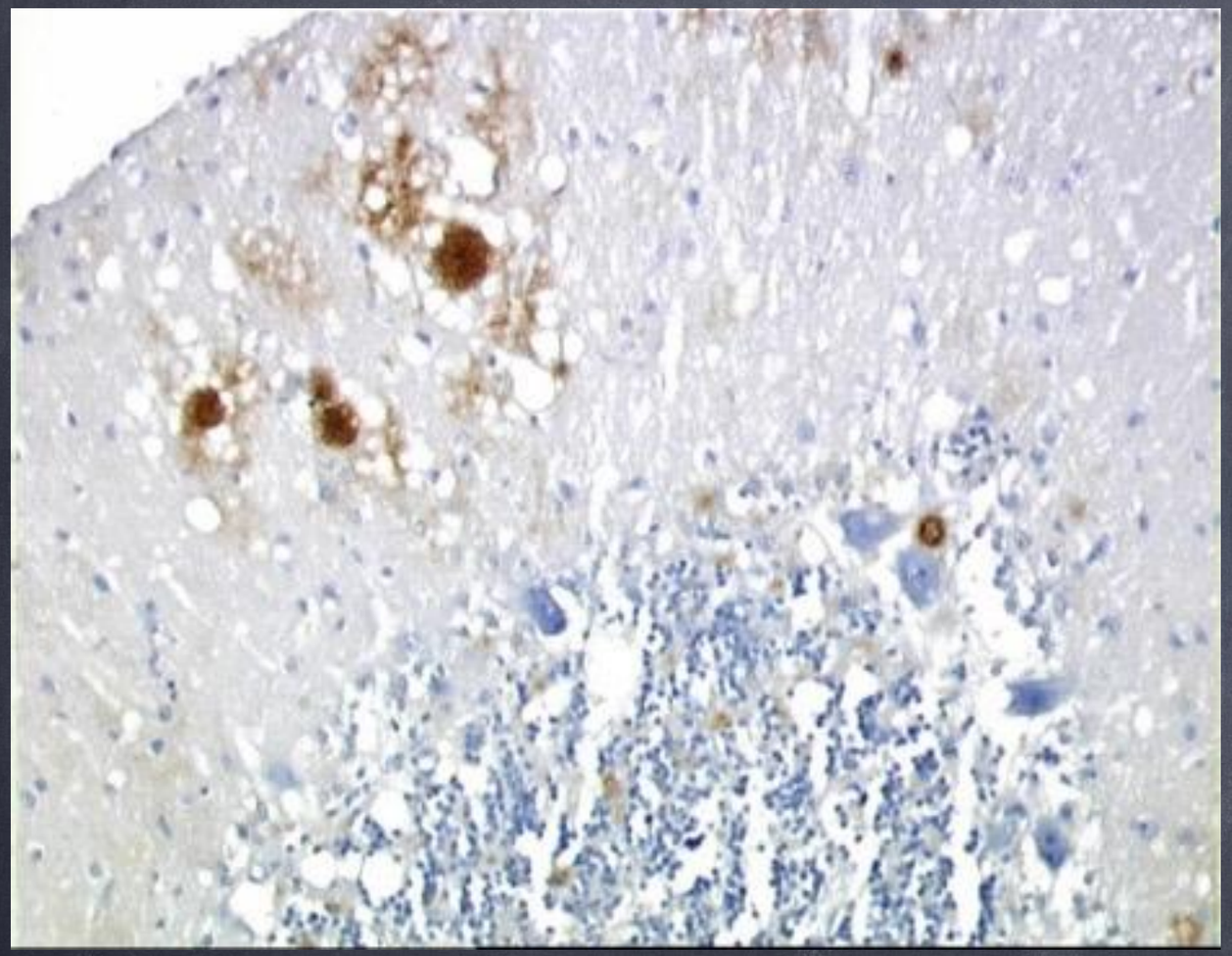
Neuropathological deposits in AD brain patients

Etiological AD markers in the CSF



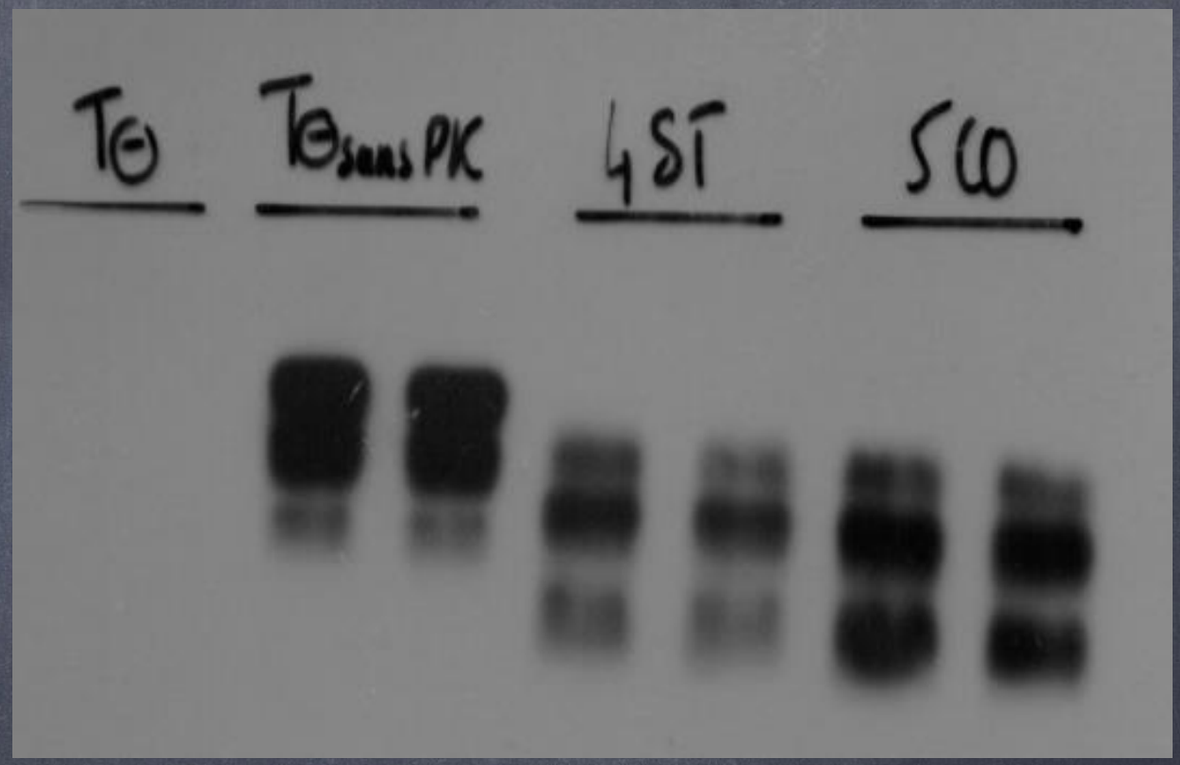
Neuropathological criteria of CJD

1/ Pathological Prion deposits



Anti PrP IHC

N. Streichenberger Neuropathology CHU Lyon



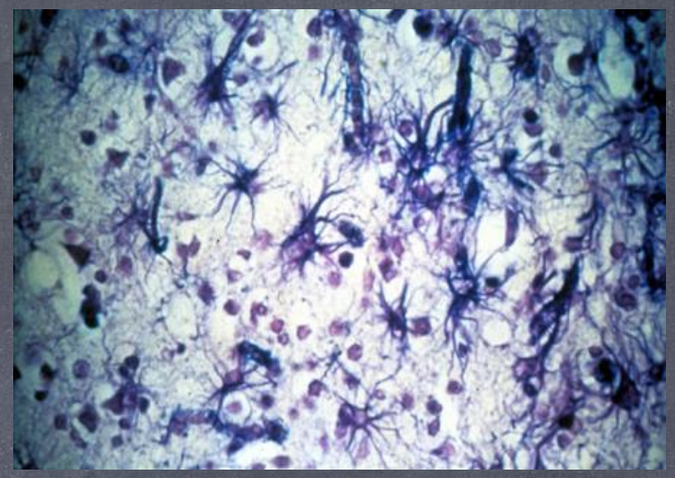
Anti PrP WB

A. Perret-Liaudet CHU Lyon

CSF total PrP by ELISA and CSF PrPsc by PMCA/RT-QUICK → Candidates biomarkers → outside this topic

Neuropathological criteria of CJD

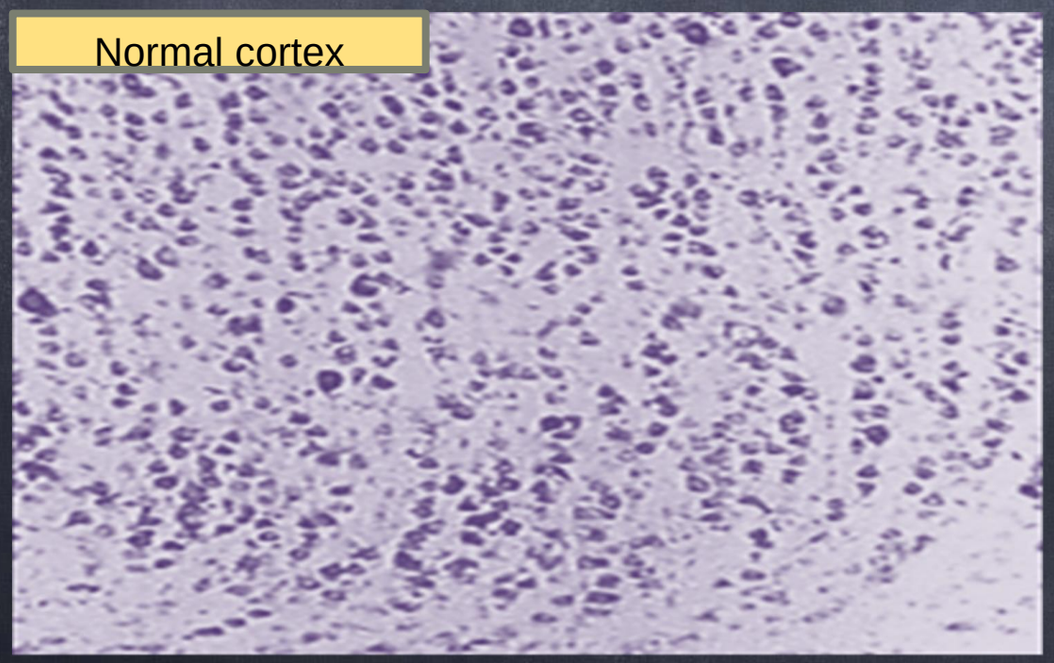
2/ Astrogliocytosis

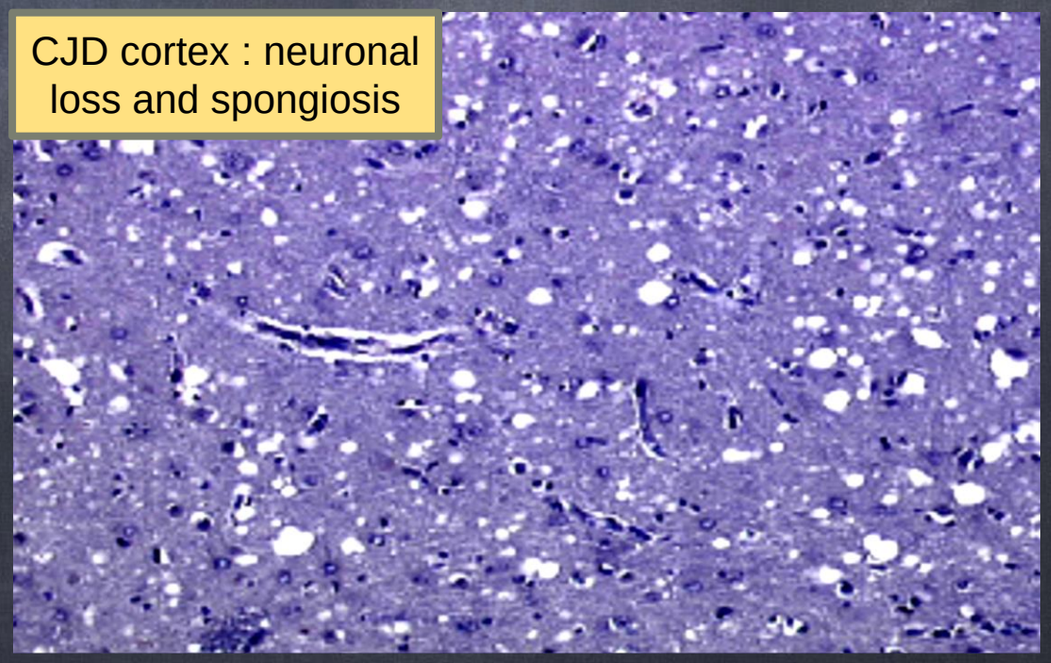


Measure of csf GFAP/S100-B : not included for CJD diagnosis criteria

3/ Spongiosis + Neuronal lysis

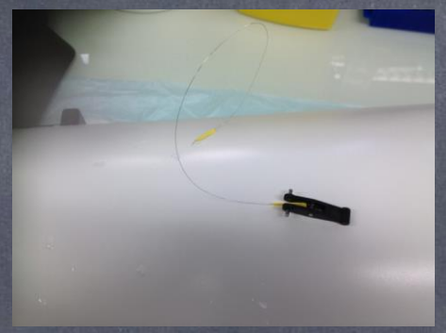
Detection of 14.3.3 protein in the CSF of CJD patients





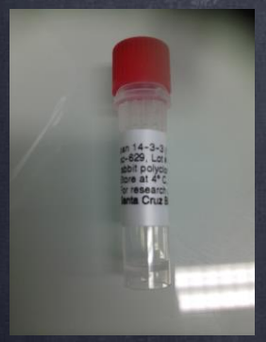
HE x 20 N. Streichenberger Neuropathology CHU Lyon

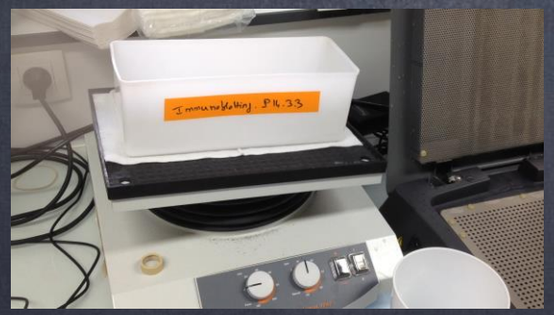
Manual WB for p1433

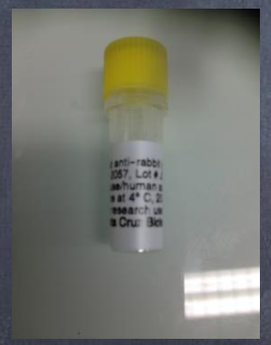


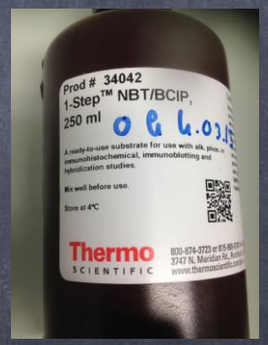
9h00

15h00



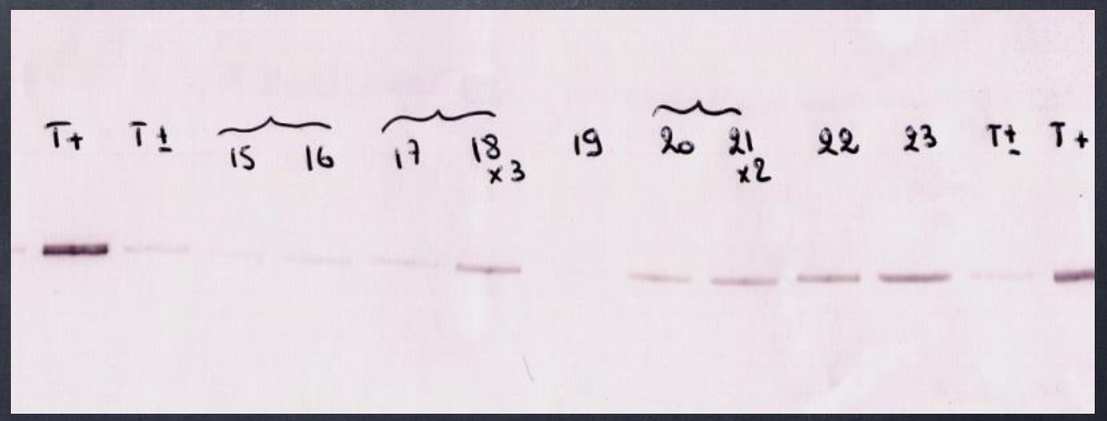






9h00

14h00



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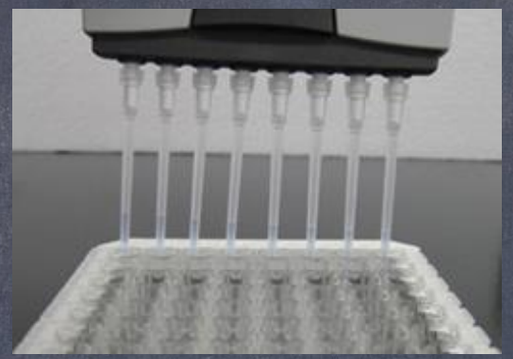
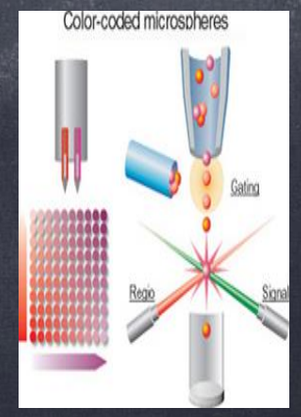
Immunometric assays for csf AD biomarkers

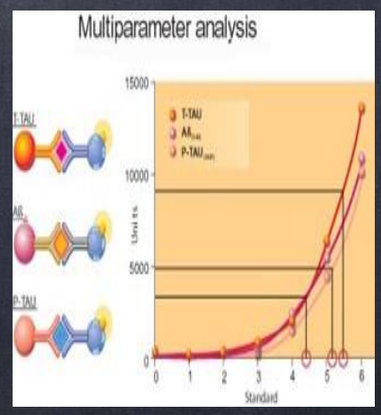
Open Classical ELISA assays

Manual or Semi-automated ELISA



Multiplex ELISA assays





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Significant variability in AD CSF biomarkers values

—> variability in diagnostic accuracy (grey zone around the clinical cutoff) and in clinical cutoffs

For p1433, high analytical variability (intra and intercentre)

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- Variability was reported mainly linked to:

Analytical variability

Existence of pre-analytical confounders

Preanalytical confounders

- Recommendations reports in the field of AD CSF biomarkers
  - Vanderstichele H. et al. 2011; Del Campo M. et al , 2012…
  - Fourier A. et al. 2015:

Clinica Chimica Acta 449 (2015) 9–15

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journal homepage: www.elsevier.com/locate/clinchim

Invited critical review

Pre-analytical and analytical factors influencing Alzheimer's disease cerebrospinal fluid biomarker variability

Anthony Fourier [a,b], Erik Portelius [d], Henrik Zetterberg [d,e], Kaj Blennow [d], Isabelle Quadrio [a,b], Armand Perret-Liaudet [a,b,c,*]

[a] Neurobiology Laboratory, Biochemistry and Molecular Biology Department, Hôpitaux de Lyon, Lyon, France
[b] University of Lyon 1, CNRS UMR5292, INSERM U1028, BioRan, Lyon, France
[c] Société Française de Biologie Clinique (SFBC), Alzheimer Biomarkers group co-coordination, France
[d] Clinical, Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Sweden
[e] Department of Molecular Neuroscience, UCL Institute of Neurology, London, UK

CSF Sampling

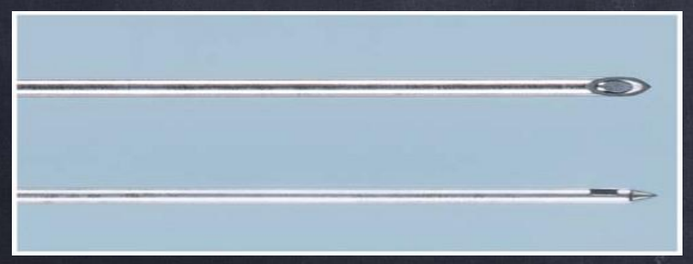
AD biomarkers

p1433

- **Needle diameter and type**

  - Aim 1: decreasing the % of side effects linked to CSF puncture

  - Inverse correlation between the % of side effects and the inner diameter of the needle and / or its atraumatic behaviour

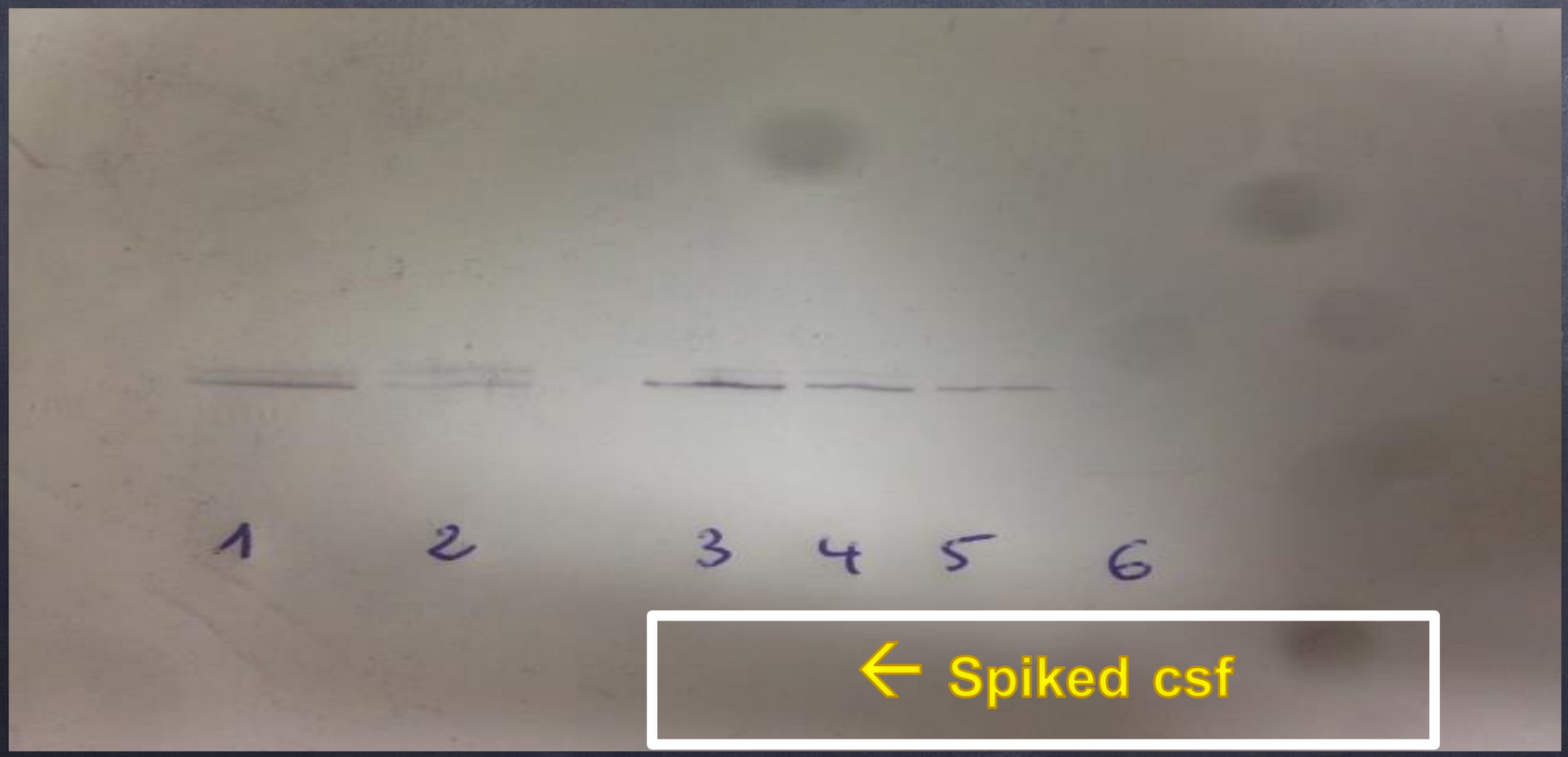


CSF Sampling

p1433

- **Needle diameter and type**
  - Aim 2: avoiding hemorragic puncture
  - **High content of p1433 in erythrocytes and/or in blood: high impact of haemolysis on csf levels**

Confirmed in Hamlin C 2012



← Spiked csf

CSF Sampling

- **Needle diameter and type**

AD biomarkers

  - Aim 2: avoiding hemorragic puncture
  - **Significant impact on csf Ab42 csf in case of hemolysis → levels decrease by 10-30%**
  - **Significant impact when Total protein**
    - **> 2,5g/L → t-Tau and p-tau**
    - **> 1 g/L → Ab42 levels decrease by 20-40 %**

Using an atraumatic needle with low inner diameter is recommended

Reject the haemolysed csf for these different biomarkers

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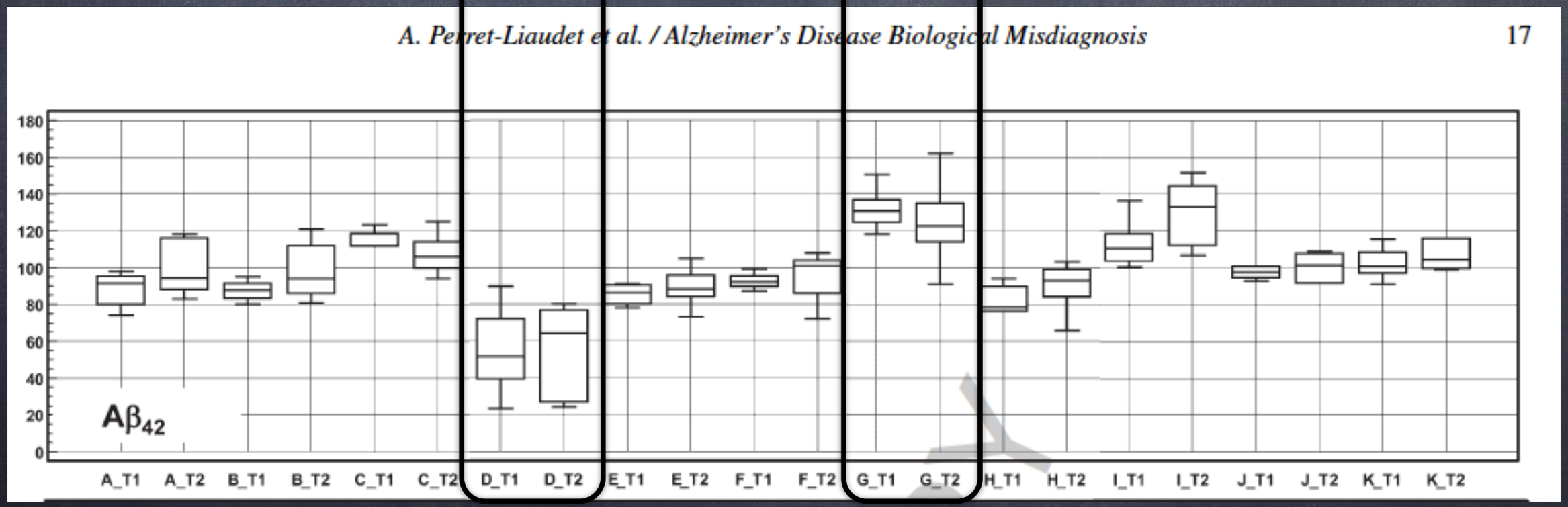
CSF Sampling: the tube



Ab42csf variability linked to PP tubes

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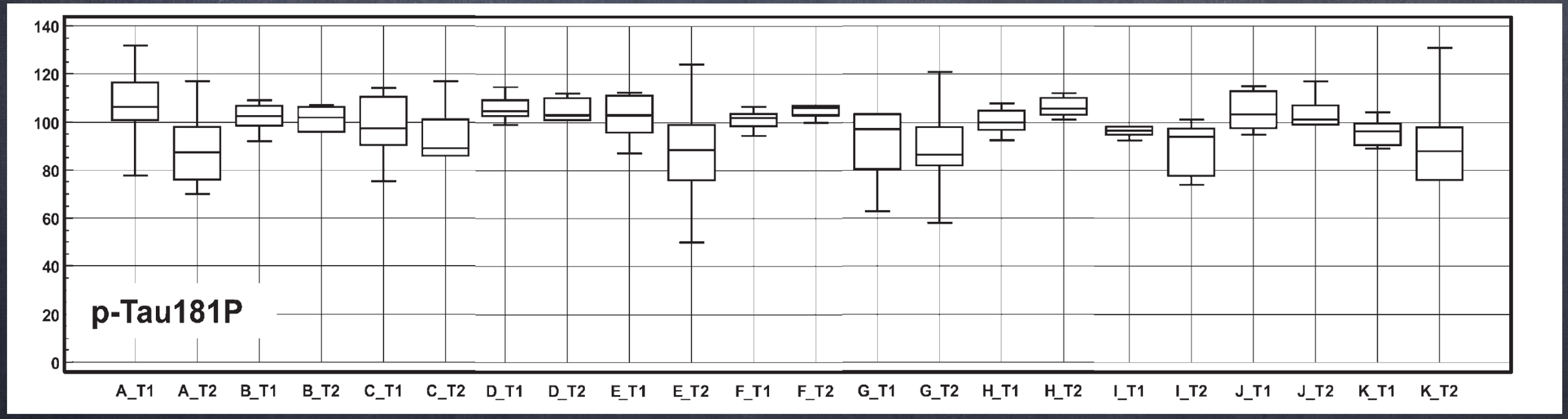
CSF Sampling: the tube



P-tau csf variability near the analytical CV

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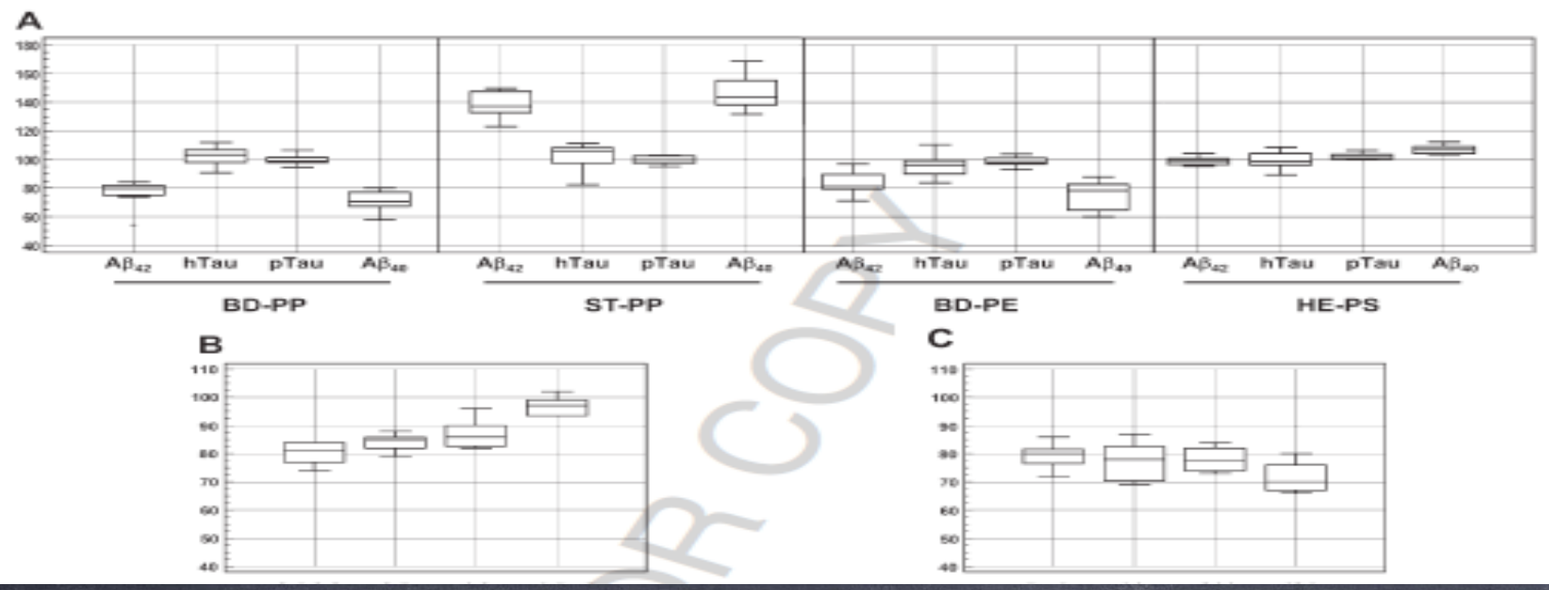
CSF Sampling: multicenter study in the frame of SFBC task force

Risk of Alzheimer's Disease Biological Misdiagnosis Linked to Cerebrospinal Collection Tubes

Journal of Alzheimer's Disease 31 (2012) 13–20



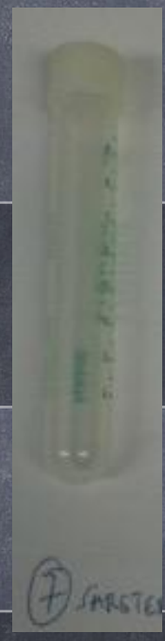
- confirmation of the data obtained in the monocentric study

Proposition to standardise the sampling tube

Shifting from local tubes to consensual tube (Lyon, Lille and Montpellier): consequences for Ab42csf

* defined by YOUDEN index

AD Cutoff* (csf Ab42)	local tubes	new tube
Lyon	475	669
Lille	380	746
Montpellier	572	831
between lab CV	20,1 %	10,8 %



- Clear data showing a significant improvement of variability

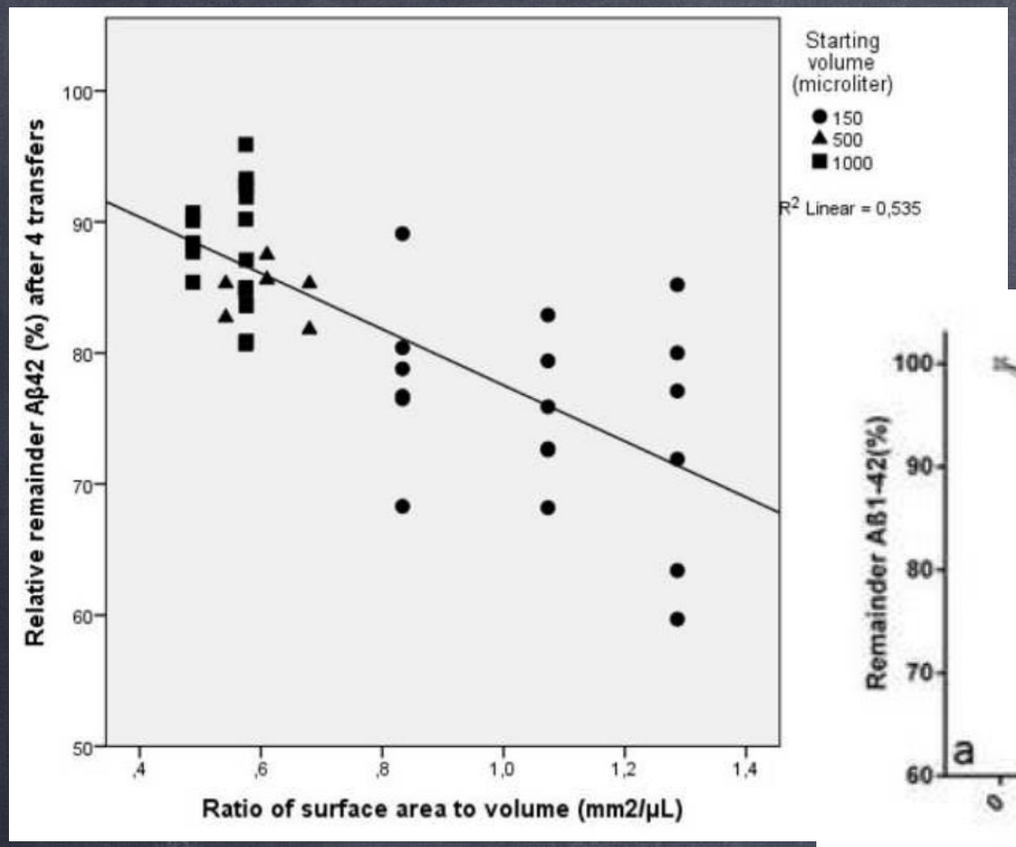
Alzheimer's & Dementia ■ (2013) 1–7

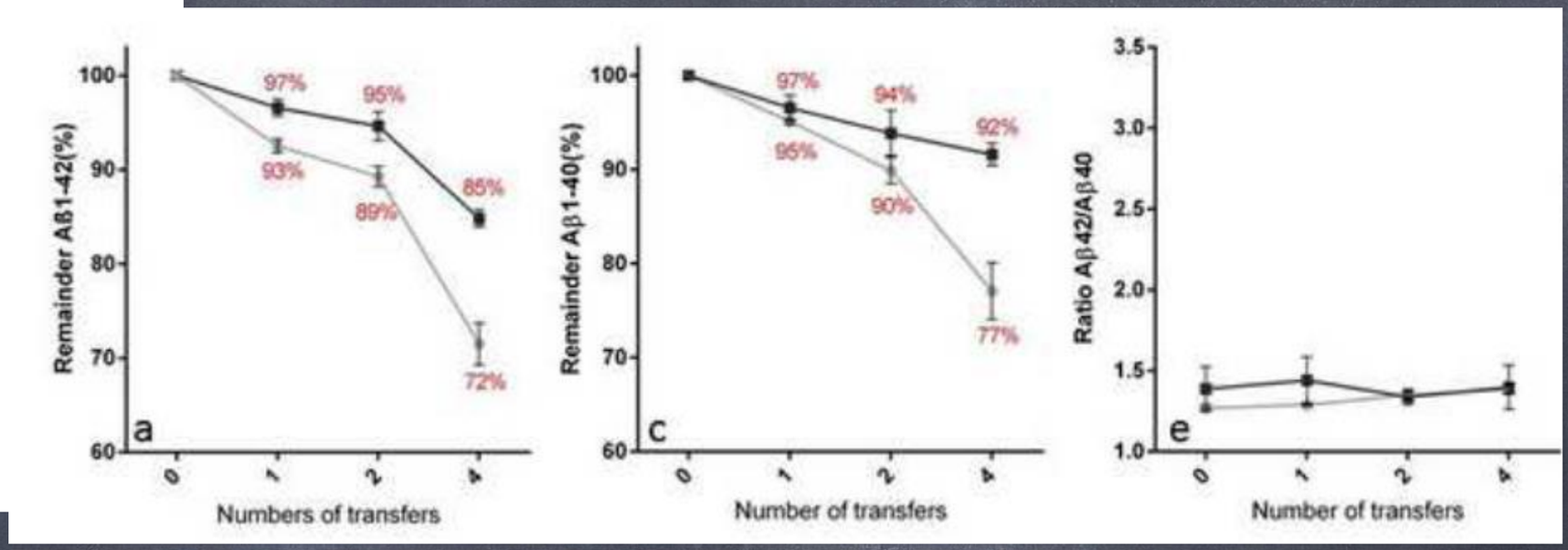
Research Article

Impact of harmonization of collection tubes on Alzheimer's disease diagnosis

Sylvain Lehmann[a,*], Susanna Schraen[b], Isabelle Quadrio[c], Claire Paquet[d,e], Stéphanie Bombois[f], Constance Delaby[a,g], Aline Dorey[h], Julien Dumurgier[d], Christophe Hirtz[a], Pierre Krolak-Salmon[h], Jean-Louis Laplanche[i], Olivier Moreaud[j], Katell Peoc'h[i], Olivier Rouaud[k], Bernard Sablonnière[b], Eric Thouvenot[l], Jacques Touchon[f], Olivier Vercruysse[f], Jacques Hugon[d,e], Audrey Gabelle[f], Françoise Pasquier[f], Armand Perret-Liaudet[c]

The ratio Ab42/40 minimizes the samping tube adsorption effect





Willemse E. Alz and Dem 2017

Using the consensual reported tube is recommended

Potentially

Ratio 42/40 could be used in case of another PP tube

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Centrifugation of samples

AD biomarkers

p1433

- Centrifugation or not ?
  - Yes , to eliminate the possible hemolysis whatever the visual aspect of CSF
- Spinning conditions (done for AD csf panel and p1433)
  - last results : spinning conditions are not a confounder factor
  - For AD biomarkers, the recommendation done : 2000g 10min with controlled temperature is enough to ensure stability of CSF AD biomarkers levels

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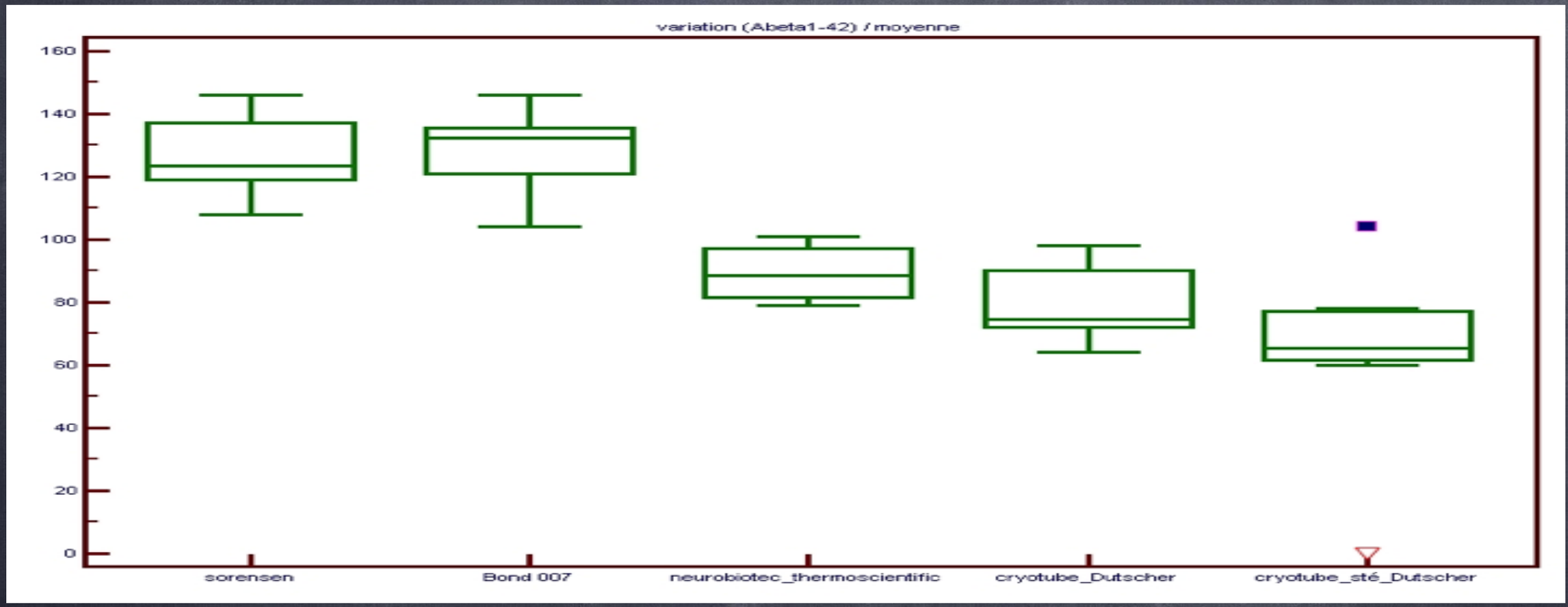
Storage and stability

AD biomarkers

Storage tube ... plastic Ab42 adsorption



- Values expressed as pourcentages of the mean values obtained in CSF Ab1-42 levels with all the tubes
- The problem is the same that for sampling tubes

Storage and stability

AD biomarkers

p1433

- Stability was proven:
  - 5 days at 4 °C : all the biomarkers are stable (supernatant or clear csf without cells)
  - Up to 2 years at -80°c, aliquoted in small tubes adapted to 500 ul of CSF (for AD and p1433 biomarkers)
  - Number of cycles of freezing/thawing
    - p1433 is stable (up to 5 cycles)
    - AD biomarkers: variablity upon markers (p-Tau>Ab42)

Current Recommendation for AD csf biomarkers:

Maximum of 2-3 cycles

Freeze at -80°C supernatants stored at 4°C (max 5 days)

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Analytical variability

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Analytical variability

- French External Quality Assessment Program for csf 1433 protein:

Organized by the French network of prion Diseases
APHP Lariboisière 2007-2017 (Pr JL Laplanche)

--> In 2012, variability of results between 5 laboratories

Analyse du CQ national 2012 pour la détection de la protéine 14-3-3 dans le liquide céphalo rachidien

Laboratoire	1	2 R	3	4	5
CQ1	Positif	Négatif	Traces	Traces	Négatif
CQ2	Négatif	Négatif	Négatif	Négatif	Négatif
CQ3	Positif	Positif	Positif	Positif	Positif

Analyse des résultats rendus par les différents laboratoires sur la base des valeurs brutes

CQ1 : 60% de concordance sur + et traces
CQ2 : 100% de concordance.
CQ3 : 100% de concordance

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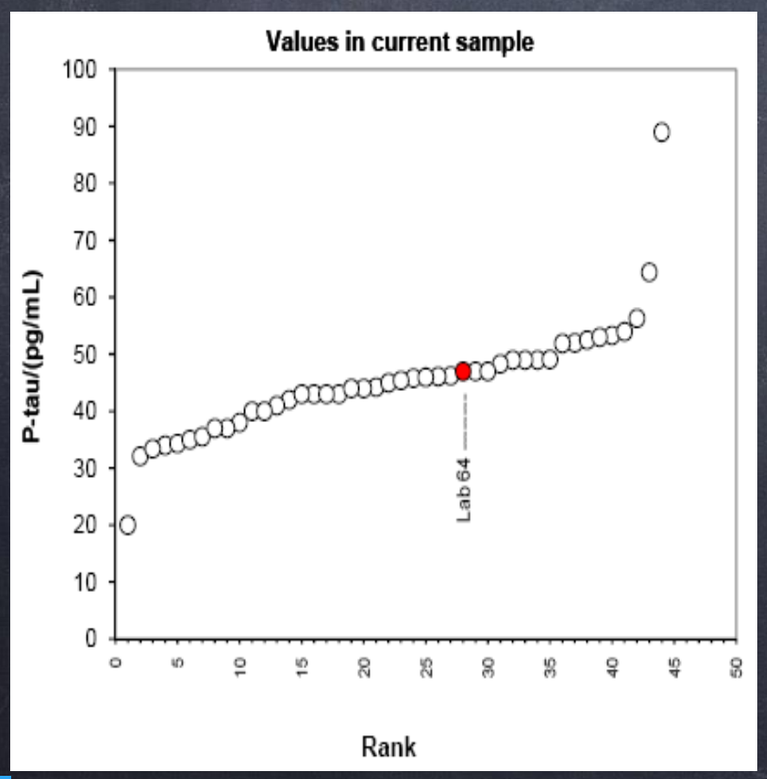
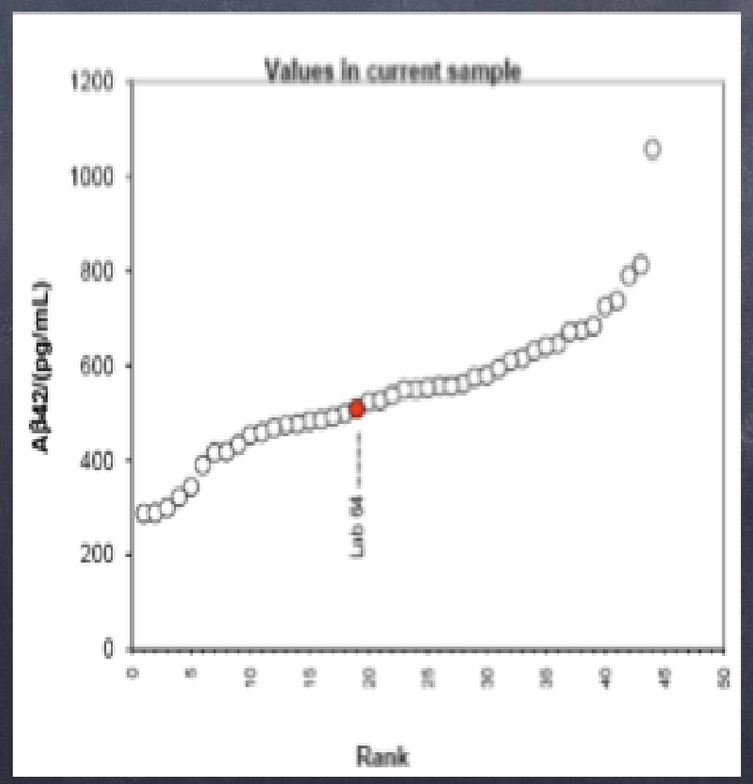
Analytical variability

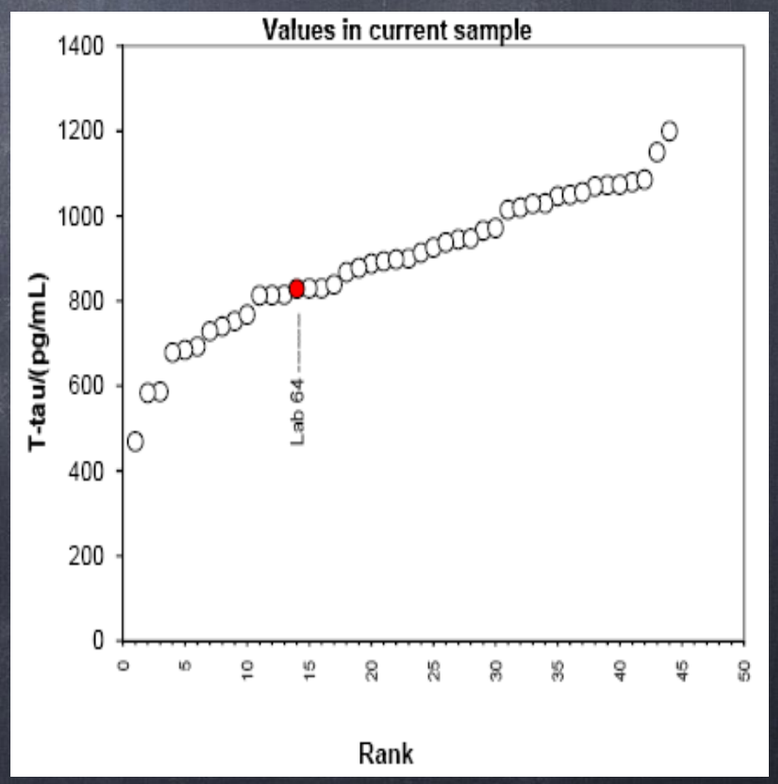
- External Quality Assessment Program:

The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers

--> In 2011, large variability of values between laboratories







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Variability linked to assays providers

Aβ40
IBL/Euroimmun

CSF Aβ40 :
IBL values = x 2 Euroimmun

CSF Aβ42/40 ratio:
cutoff is highly modified

1/ Differences between assays providers explained by

a/ difference of antibodies
b/ difference of calibrators
c/ calibration models…

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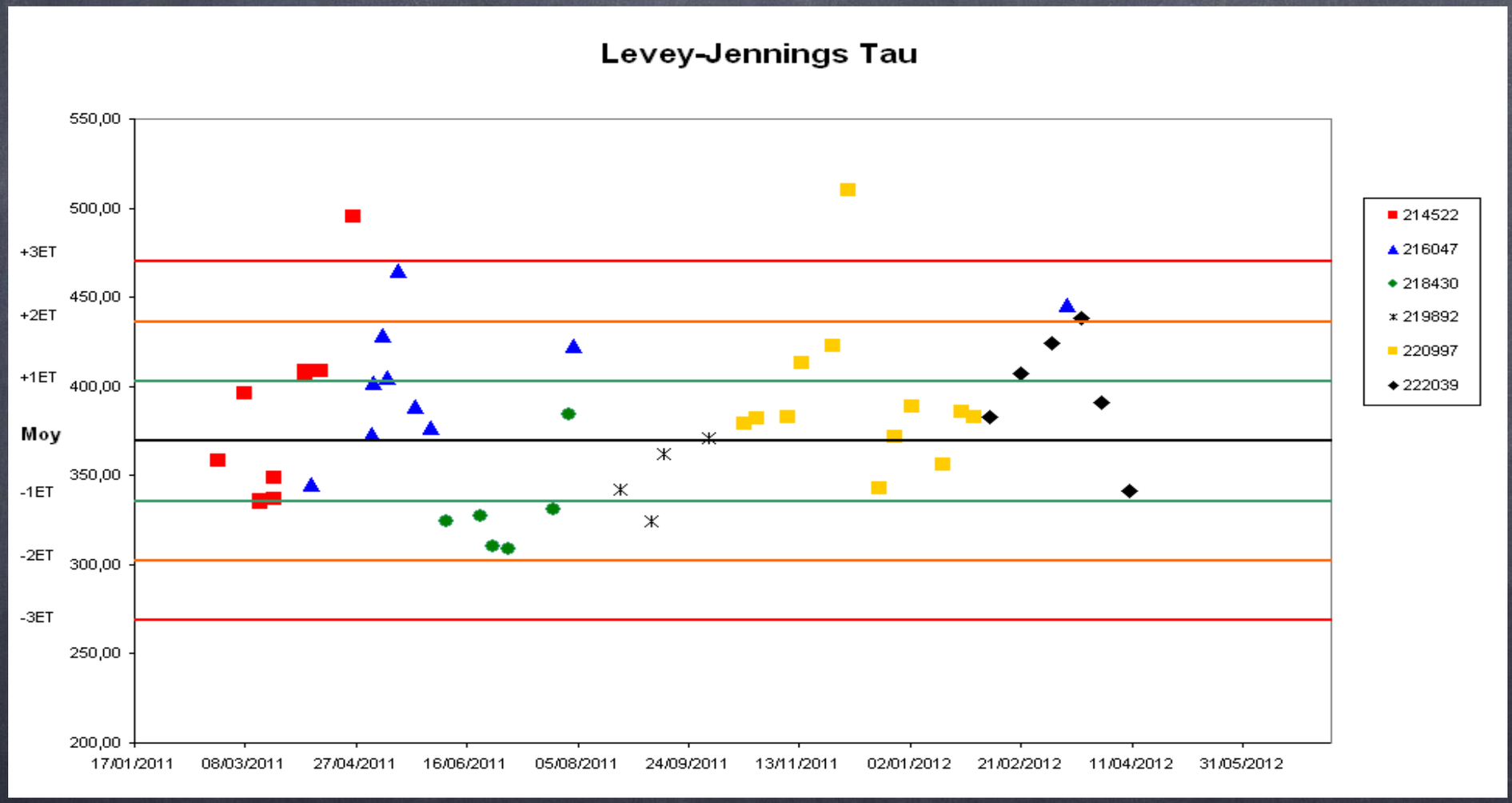
Variability linked to assays providers

2/ Lot to lot variability (example : t-TAU fujirebio 2011-12)



3/ Quality of Instructions For Use (IFU)

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Variability linked to kit users

1/ Non Compliance to the Instructions For Use
2/ Variability in handling lyophilized calibrators
(Solubilization, dilution … in plastic tubes…)
3/ different pipetting methodologies…
….

—> Lack of training
—> Lack of maintenance

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Solving inaccuracy

1/ Bias between different assays
2/ Variability inter lots

Inaccuracy —> Reference Methodes—> Certified Reference Material (CRM)

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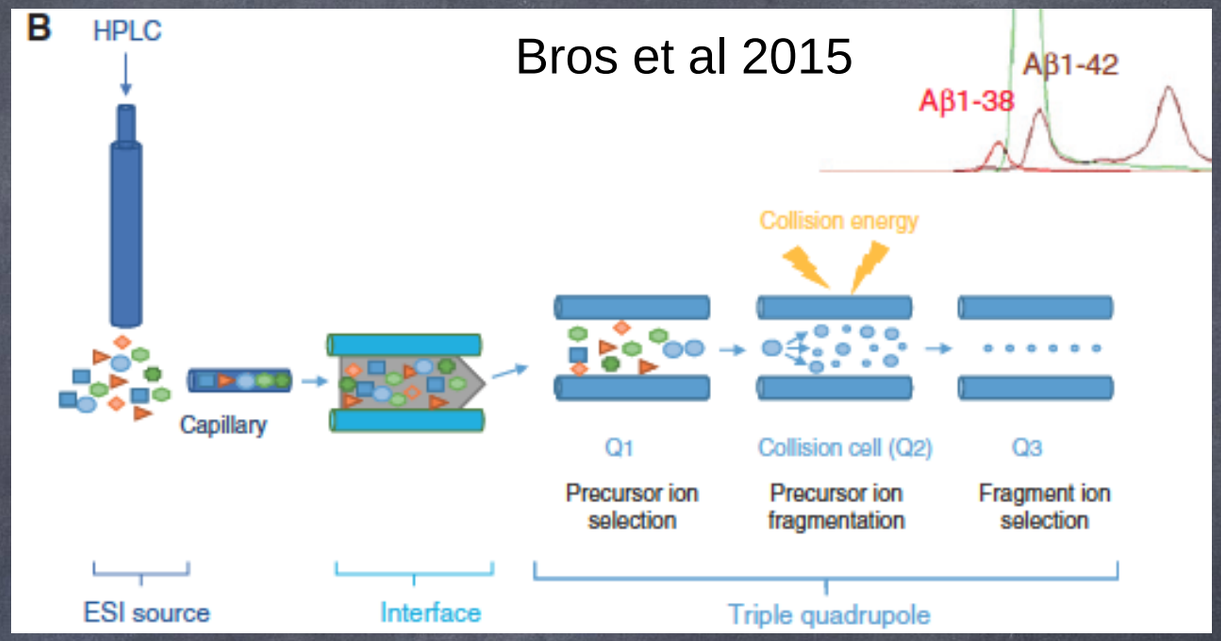
Reference Methods

- Mass Spectrometry
- 2 Validated methods for Aβ42
- Methods accepted by JCTLM
- CRM was prepared



Bros et al 2015

- The Ab42 immunological assays : traceability versus this CRM

IFCC group

  - Native csf was validated
  - Data reported on commutability : 33/36 Ok
  - Stability and homogeneity running

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Solving imprecision

Decrease analytical CV
by the way of automatization

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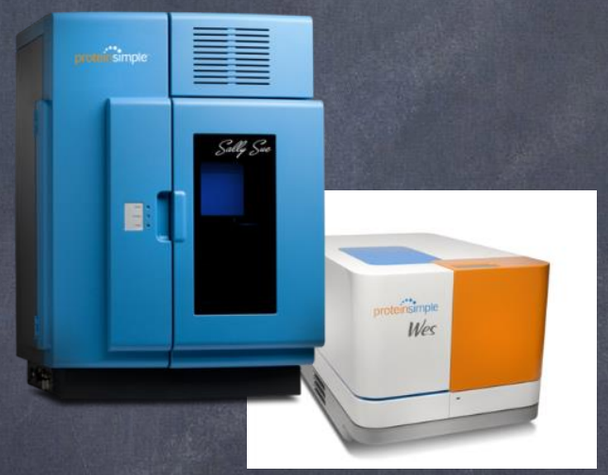
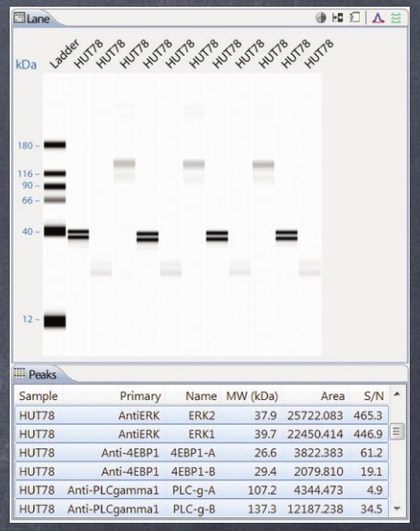
Shifting from Manual WB to automatized Size separation (Simple Western)

Pipet Samples



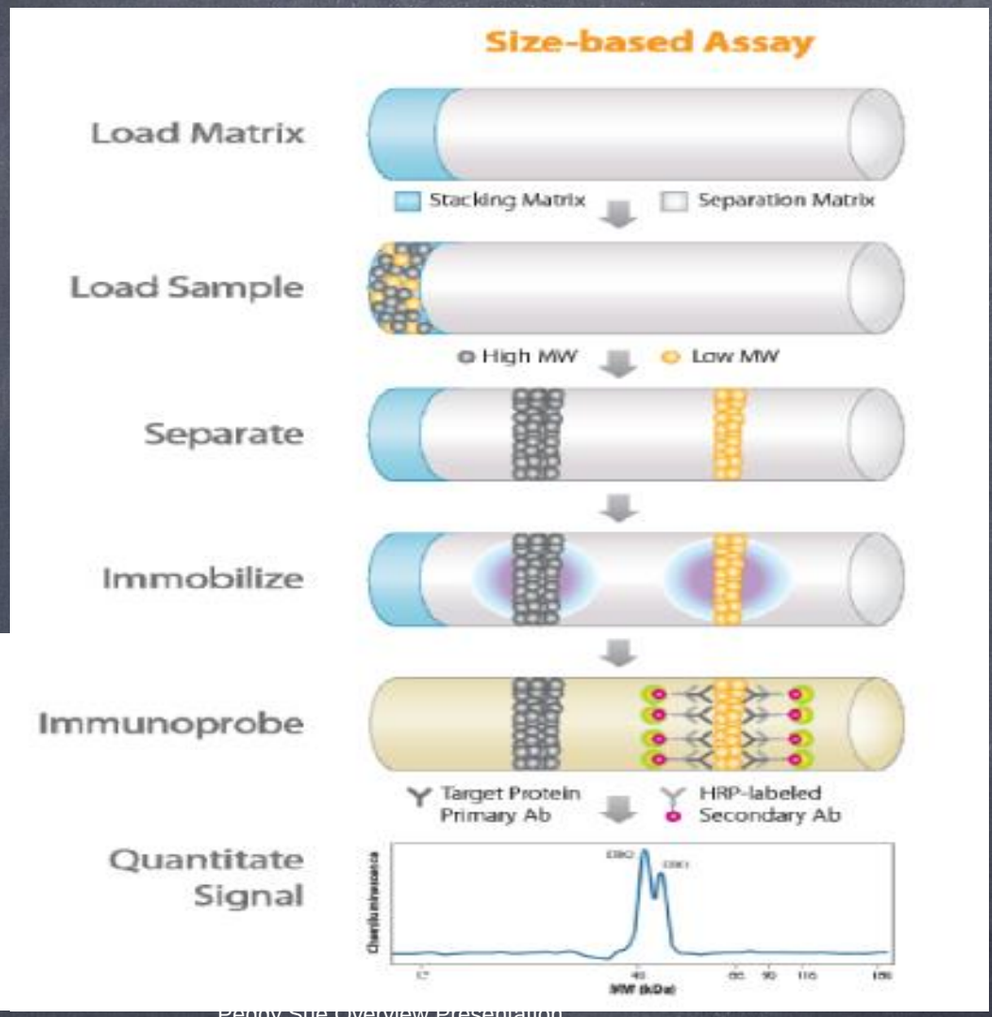
Press Start



Sample	Primary	Name	MW (kDa)	Area	S/N
HUT78	AntiERK	ERK2	37.9	25722.083	465.3
HUT78	AntiERK	ERK1	39.7	22450.414	446.9
HUT78	Anti-4EBP1	4EBP1-A	26.6	3822.383	61.2
HUT78	Anti-4EBP1	4EBP1-B	29.4	2079.810	19.1
HUT78	Anti-PLCgamma1	PLC-g-A	107.2	4344.473	4.9
HUT78	Anti-PLCgamma1	PLC-g-B	137.3	12187.238	34.5

Results Automatically

Shifting from Manual WB to automatized Size separation (Simple Western)



Peggy Sue Overview Presentation

Detection of CSF 14-3-3 Protein in Sporadic Creutzfeldt-Jakob Disease Patients Using a New Automated Capillary Western Assay

A. Fourier[1,2] · A. Dorey[1,3] · A. Perret-Liaudet[1,2,3] · I. Quadrio[1,2,3]

Mol Neurobiol
DOI 10.1007/s12035-017-0607-2

Received: 14 March 2017 /Accepted: 8 May 2017

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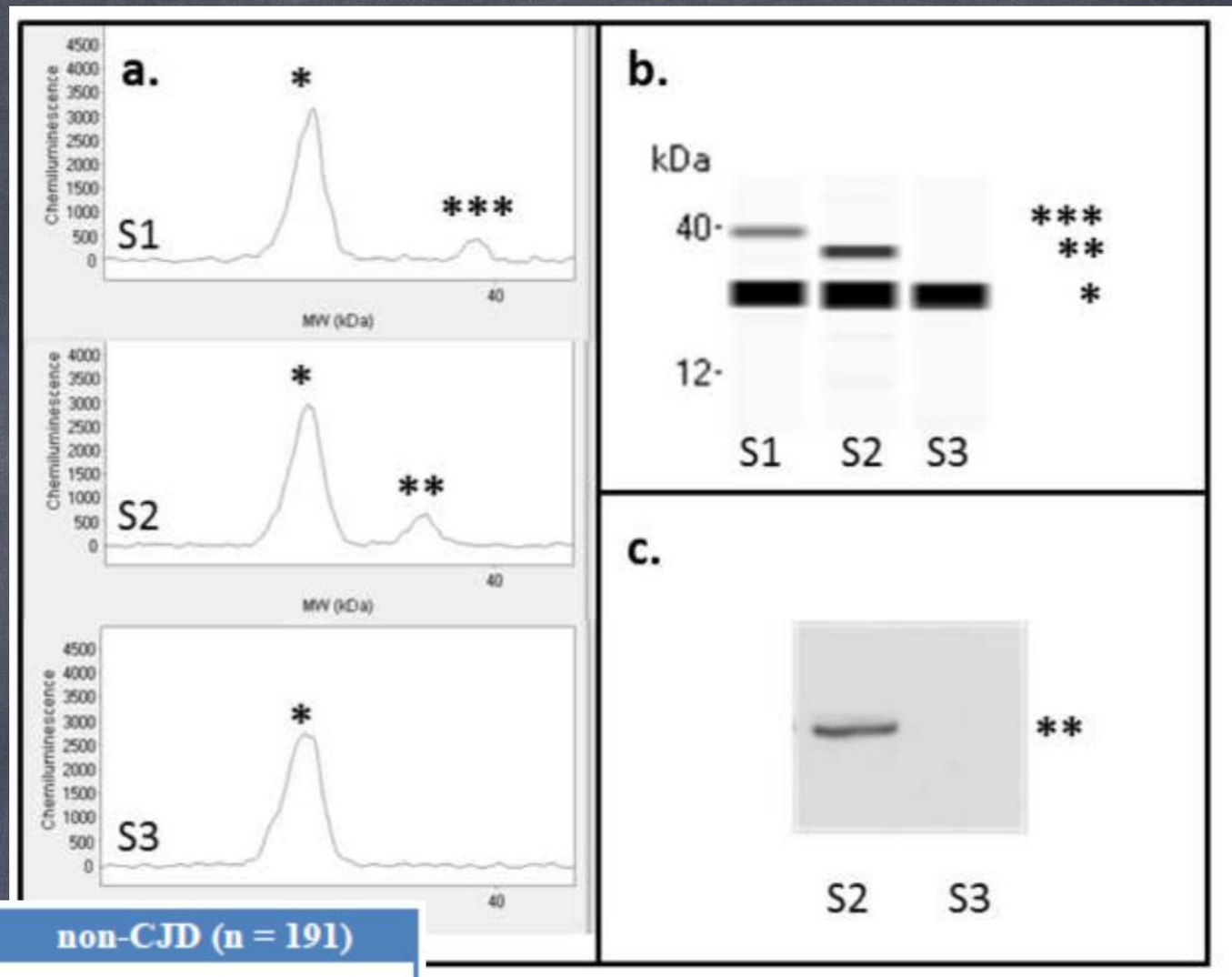
Raw data obtained from SW

- Visual separation size



protein *** His-Tag recombinant 14-3-3 protein

	sCJD (n = 77)	non-CJD (n = 191)
Demography		
Gender, M/F	31/46	96/95
Age, years	71.0 (52.4-86.7)	72.0 (54.1-86.0)
CSF samples		
Total proteins, g/L	0.39 (0.26-0.70)	0.40 (0.23-0.68)
Automated capillary Western results		
Area 14-3-3	7259 (1723-26744) *	411 (0-2040)
Height 14-3-3	512 (99-1977) *	32 (0-199)
S/N 14-3-3	13.2 (2.8-40.0) *	2.9 (1.1-7.3)
Areas Ratio	872 (195-3149) *	60 (0-234)

P1433 Specificity is increased from 85% with Western blot to 95% with simple western

Significant Improvement of both repeatibility and intermediate fidelity CVs

	WB assay	SW assay	
		Area 14-3-3	Areas Ratio
Intra-assay variability (n = 5 repeats)			
Positive CSF QC1 (strong 14-3-3 band)	10%	6%	7%
Positive CSF QC2 (faint 14-3-3 band)	30%	13%	12%
Inter-assay variability (n = 20 repeats)			
Positive CSF QC1 (strong 14-3-3 band)	34%	22%	17%
Positive CSF QC2 (faint 14-3-3 band)	39%	29%	25%

Table 3: SW and WB variability analyzing 2 CSF positive quality controls
(QC1 with a strong 14-3-3 protein band and QC2 with a faint 14-3-3 protein band using WB)

Influence of hemoglobin and proteinorachia is confirmed

CSF sample	Size (kDa)	Areas Ratio	Interpretation*
Hemolysis			
Neat CSF (limpid)	35	118	Negative
Likely-visible hemolysis (hemoglobin 0.28 g/L)	35	138	Negative
Strongly-visible hemolysis (hemoglobin 1.42 g/L)	35	919	Positive
Total protein concentration			
Neat CSF (0.43 g/L)	35	118	Negative
Protein-spiked CSF (1 g/L final)	35	102	Negative
Protein-spiked CSF (1.5 g/L final)	33	1109	Positive
Protein-spiked CSF (2.5 g/L final)	33	1560	Positive
Protein-spiked CSF (4 g/L final)	33	3751	Positive

Table 5: CSF hemolysis and protein spiking assays

*Positive 14-3-3 protein result if Areas Ratio > 235; Negative 14-3-3 protein result if Areas Ratio ≤ 235

Shifting from manual ELISA to others platforms

Semi-automated ELISA

Random Access platforms

Semi-automated ELISA versus manual ELISA

Repeatability

Performance	Aβ42	Aβ40	
Manual ELISA	5.9% Fujirebio	3.9-6.1% IBL	
Semi-automated ELISA old ref Fujirebio (2014)	2.9-3.3% Fujirebio	3.4-4.3% IBL	

Significant decrease of repeatability CV

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Solutions found by collaboration between academics and company (JPND)

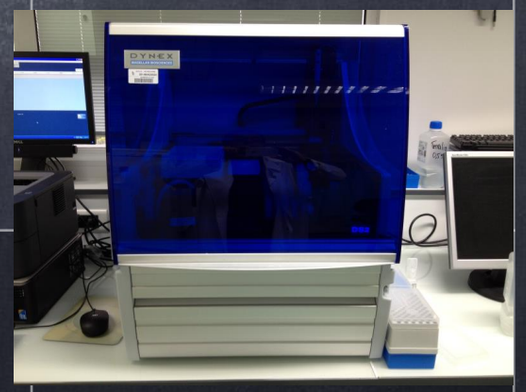
Consortium BIOMARKAPD , JPND

Before : Lyophilised Calibrants to be diluted
RT (18-26°C)

Optimization of SOPs

Ready to use Calibrants
Temperature fixed at 25+0,5°C

Effect on intermediate Fidelity

Performance Aβ42 Fujirebio	CV	Grey zone 700 ng/L	
Semi-automated ELISA old ref Fujirebio (2014)	10.2-10.3%	630-770	
Semi-automated ELISA new ref Fujirebio (2015)	4.2-8%	660-740	

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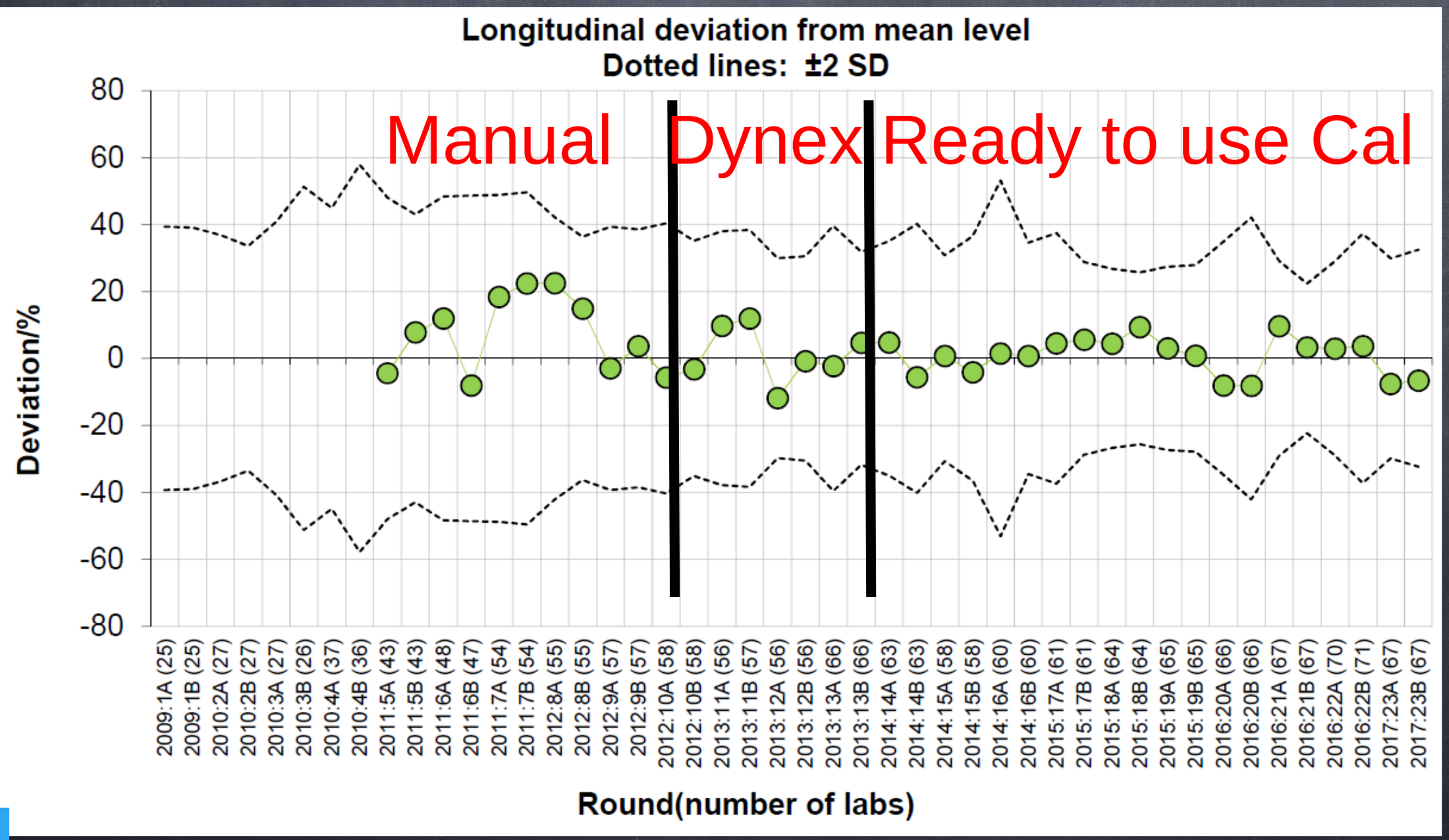
Improvement of precision

- External Quality Assessment Program:

-->Longitudinal Aβ42 CQ 2011-2017 (Lyon)



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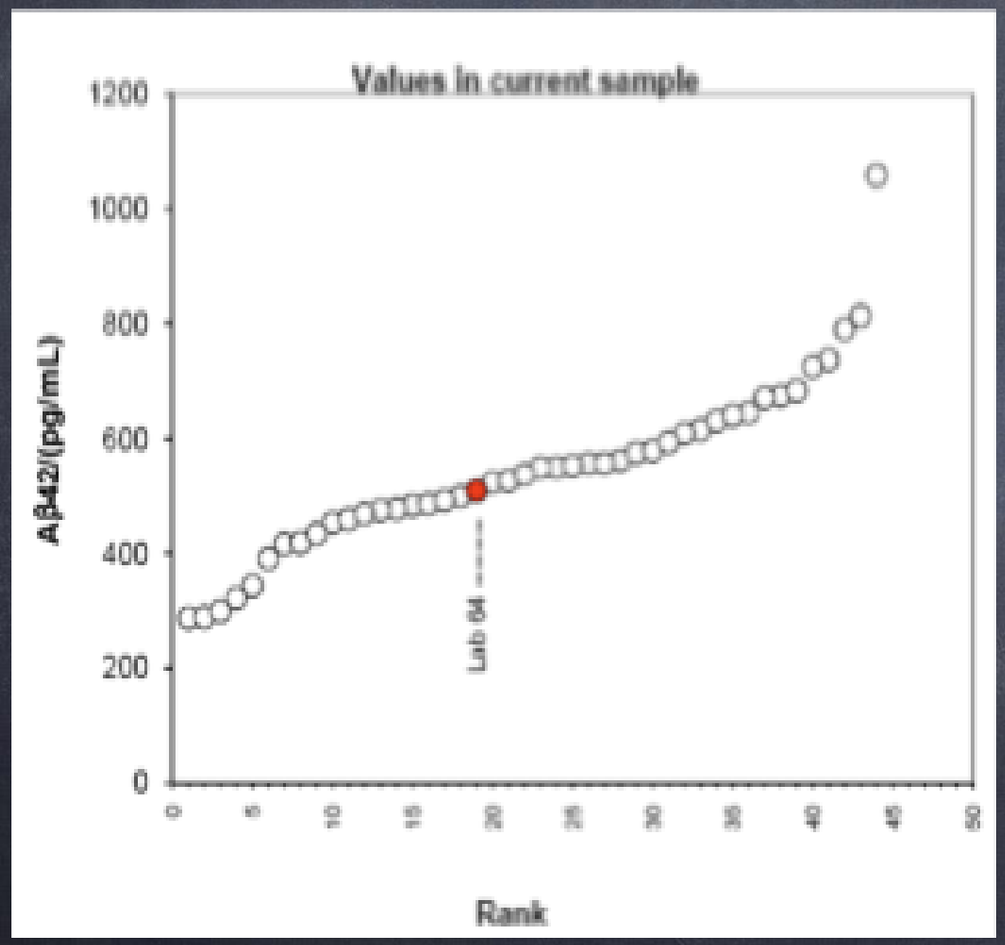
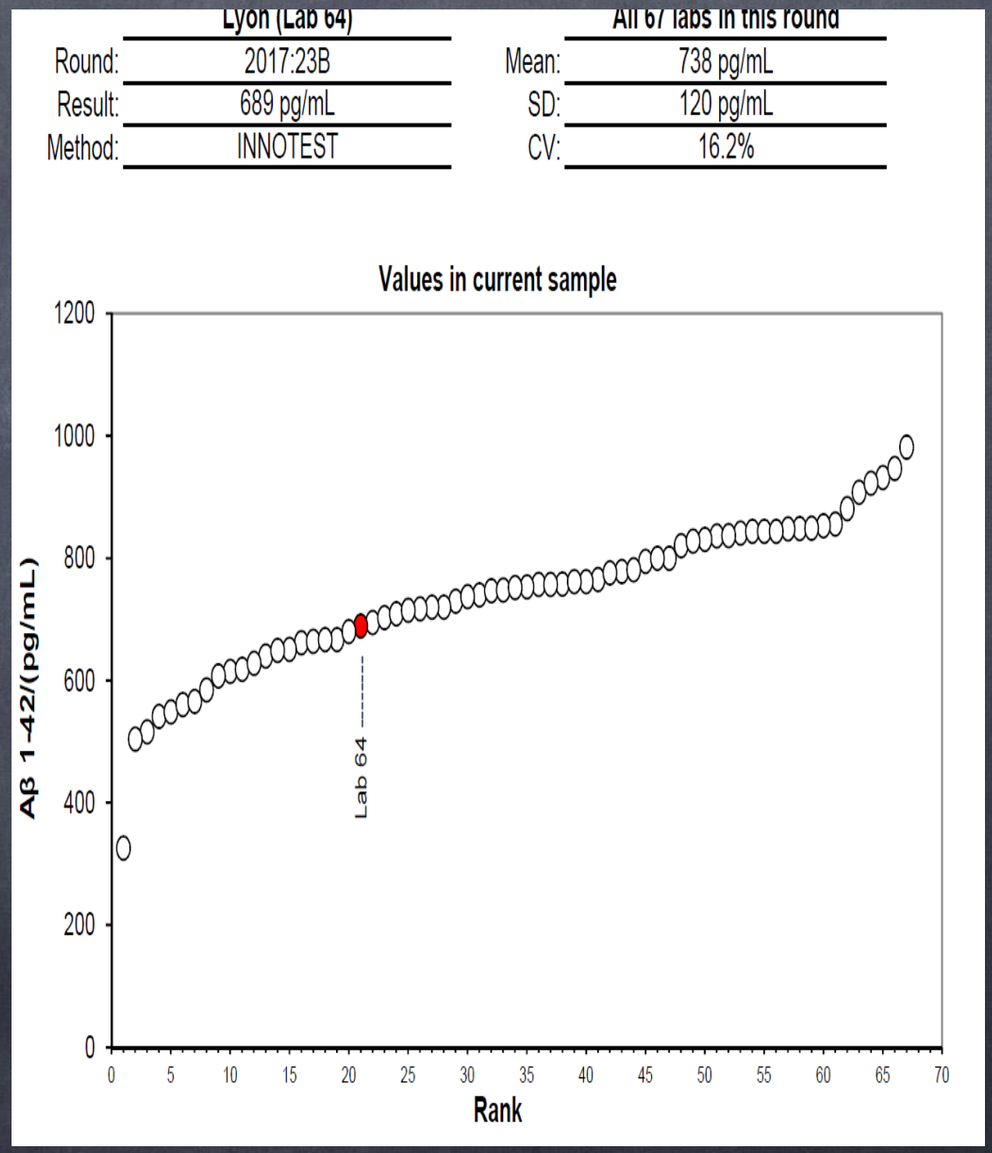
Improvement of precision

- External Quality Assessment Program:

--> Comparaison intercentres 2011-2017,





- CV around 30% in 2011 → **15% in 2017**

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Fully automatized Plateforms

Electrochemiluminescence

CSF Aβ42

Aβ42 Performance	CV
Repetabilité	1 - 1.6%
Inter-module	1.1 - 3.9%
Fidélité intermédiaire	1.9 - 4%

Aβ42 Performance	CV
Repetabilité	1.2 - 4.6%
Inter-module	1.2 - 3.7%
Fidélité intermédiaire	

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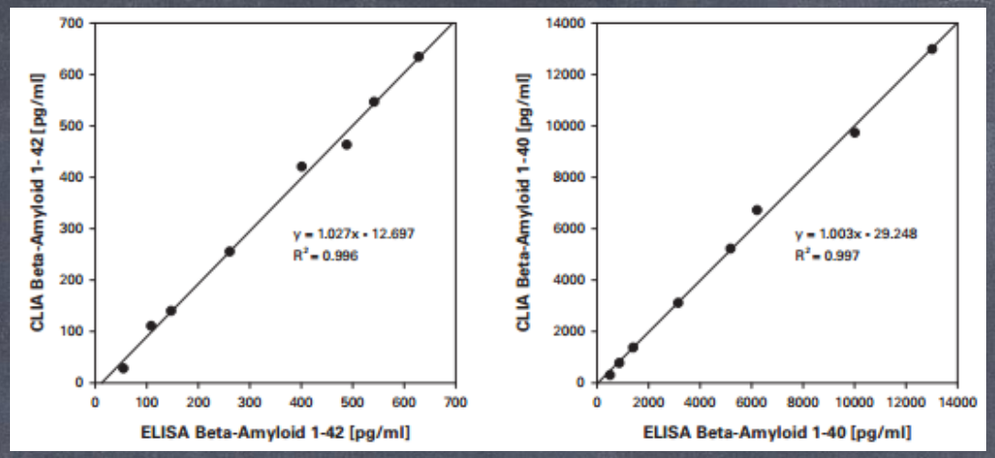
ELISA Manual testing versus random access (internal data of Euroimmun presented in AD/PD)



Sample	n	Beta-amyloid 1-42		Beta-amyloid 1-40	
		[pg/ml]	CV [%]	[pg/ml]	CV [%]
Calibrators	30	89	8.6	112	6.2
	30	805	3.5	10301	4.9
Controls	30	170	4.5	192	4.4
	30	950	4.1	13831	7.8
QC1	9	116	7.7	311	1.5
QC2	9	143	4.5	802	3.4
QC3	9	251	9.6	1430	2.8
QC4	9	409	3.0	3239	4.6
QC5	9	449	6.4	5437	2.9
QC6	9	529	2.5	7003	3.1
QC7	9	613	4.5	13553	6.7

Determination of inter-assay precision (triplicates)

New automated chemiluminescence immunoassays for CSF beta-amyloid determination

V. Herbst, B. Brix, and M. Block

Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany

Poster presented at AD/PD, Wien, May 2017

Aβ42 → Median CV : 4.5%
Aβ40 → Median CV : 3,1%

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Conclusion : analytical aspects

- The analytical standardization is running:
  - Standardization of SOPs, Ready to use calibrators….
  - Semi-automatization …

    → Precision was improved
  - Full Random Access
  - RPM + CRM (for Ab42, Ab40…)

    → Analytical Accuracy and Precision will be improved

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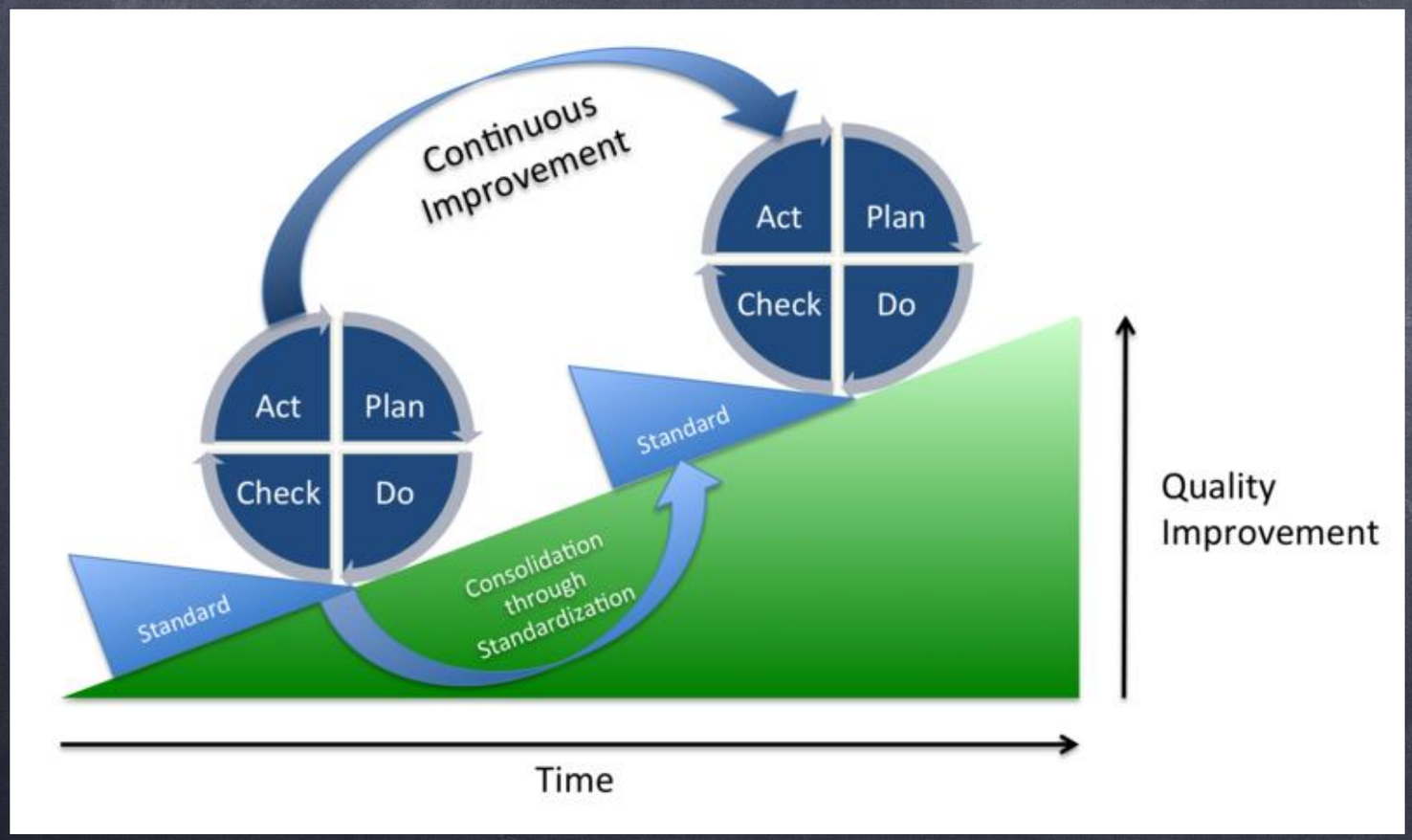
Preanalytical aspects : not finished !

- Main problems **seemed** to be over
  - Plastic of sampling and storage tubes
  - In fact, with the very promising results in repeatability CV obtained with full random acess

  → Some points need to be revisited …



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Preanalytical aspects revisited with Full random access machine

Assays in Lyon, Sep 2017

Our Storage tube not tested by the company
→ alternatively manual transfer to Hitachi cupule

Repetability Aβ42	CV range (2 levels of concentration)
0.5 ml Sdt	0.7 % - 1 %
2 ml Sdt	0.4 % - 0.6 %
Cupule of company	0.4 % - 0.6 %
Overall	0.1 % -1.6 % -

CSF Aβ42

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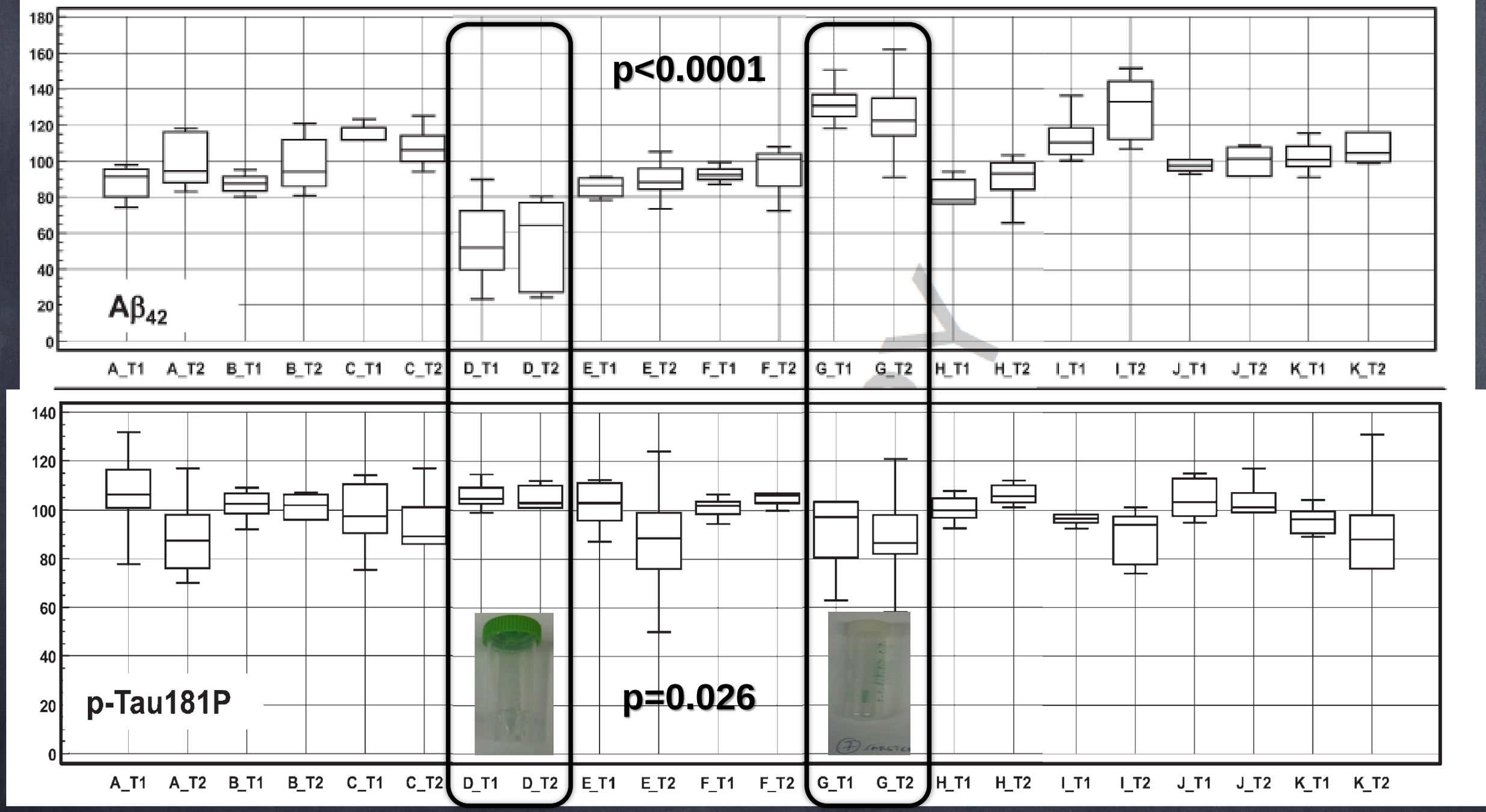
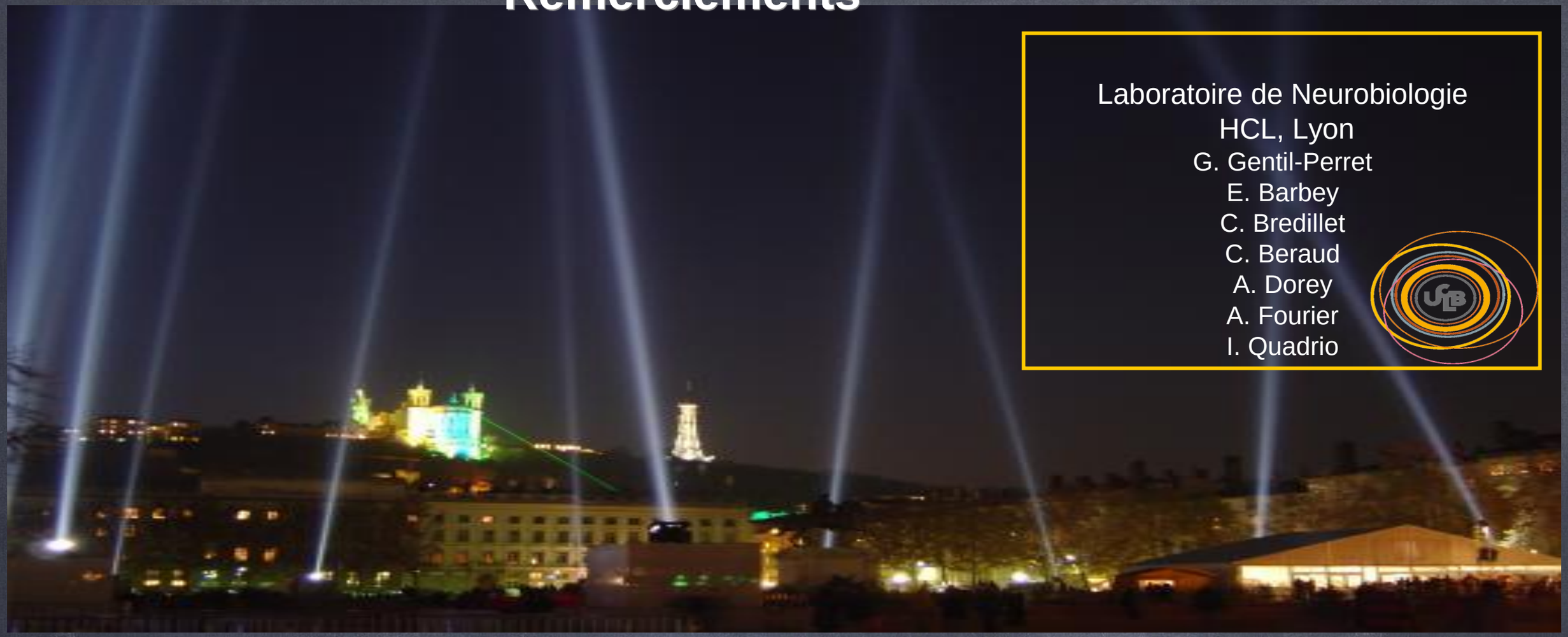


- Between tubes, 10 to 15 % Difference of mean of p-Tau :

-->need to be revisited with machine at 1-2% repetability CV

Significativity at 2.8 x CV

Remerciements



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