

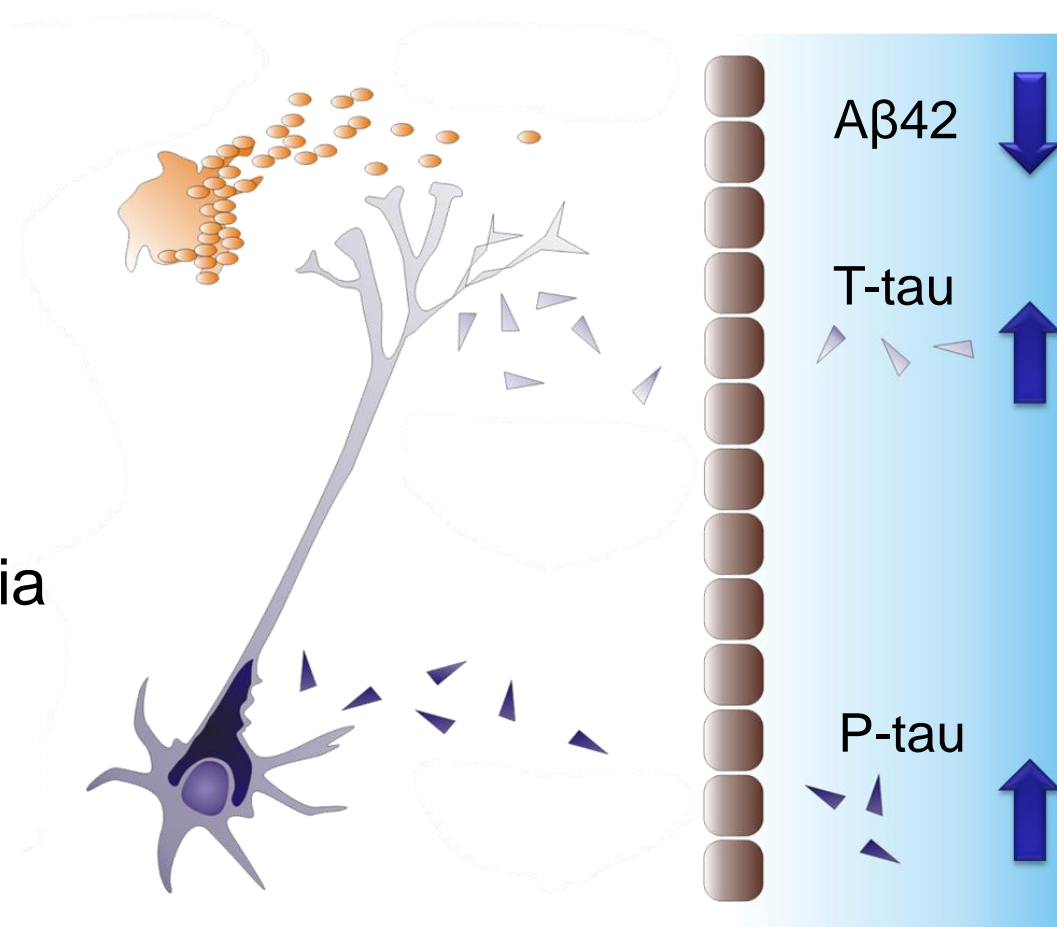
# Alzheimer's Association Quality Control Program

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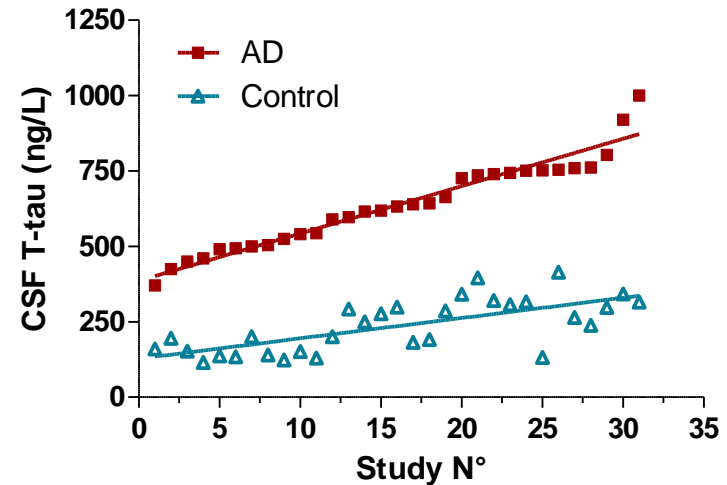
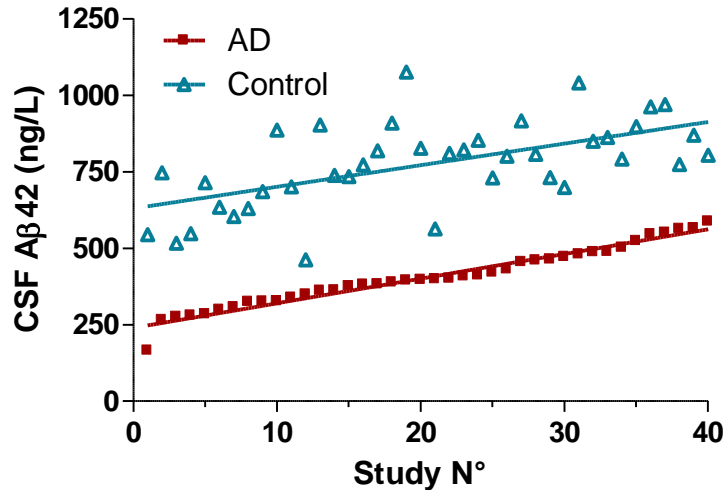
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# CSF AD biomarkers

- Research
- Clinical trials
- Clinical practice
- New diagnostic criteria



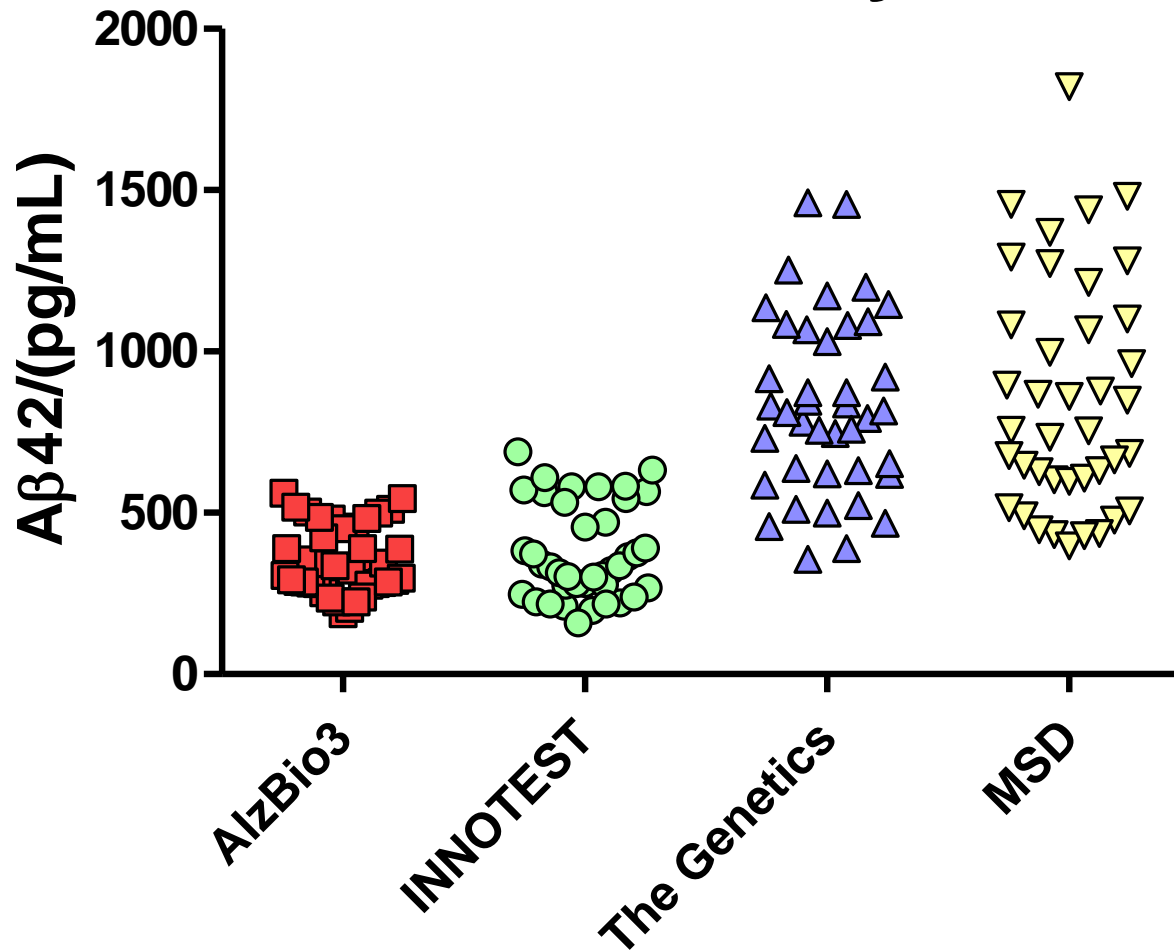
# Biomarker variability – across studies



- **Varying absolute levels** between studies (same assays)
- **Stable relative differences** between patients and controls
- Problem for **universal cut-offs and reference ranges**

# Biomarker variability

## - across assays



Modified from Bjerke, M. et al (2010) Int J Alzheimers Dis, Article ID 986310

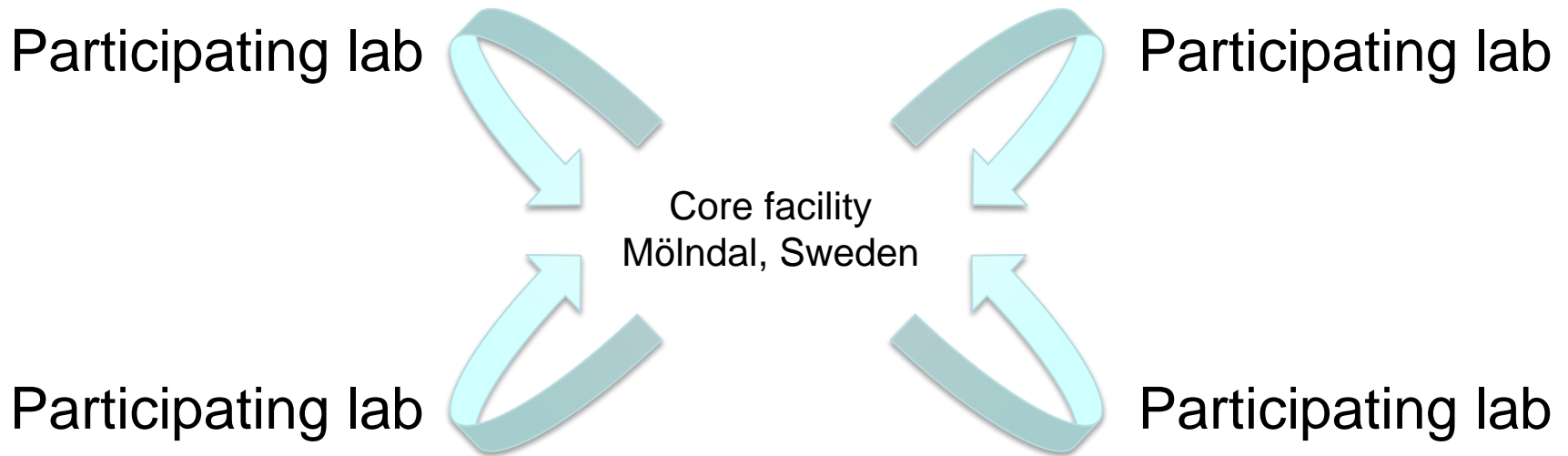
# The Alzheimer's Association external quality control program

- Identify and monitor differences among labs
- Facilitate standardization of measurements
- Facilitate implementation of AD biomarkers to support optimal patient management

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the compassion to care, the leadership to conquer

# Program overview



2009-1A  
2009-1B

2010-2A  
2010-2B

QC-L

QC-L

**2010-3   2010-4   2011-5   2011-6   2011-7   2012-8**

Rounds 1-2: Mattsson N et al, **The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers.** *Alzheimer's & Dementia* 2011;7:386-395

Rounds 3-8: manuscript in preparation

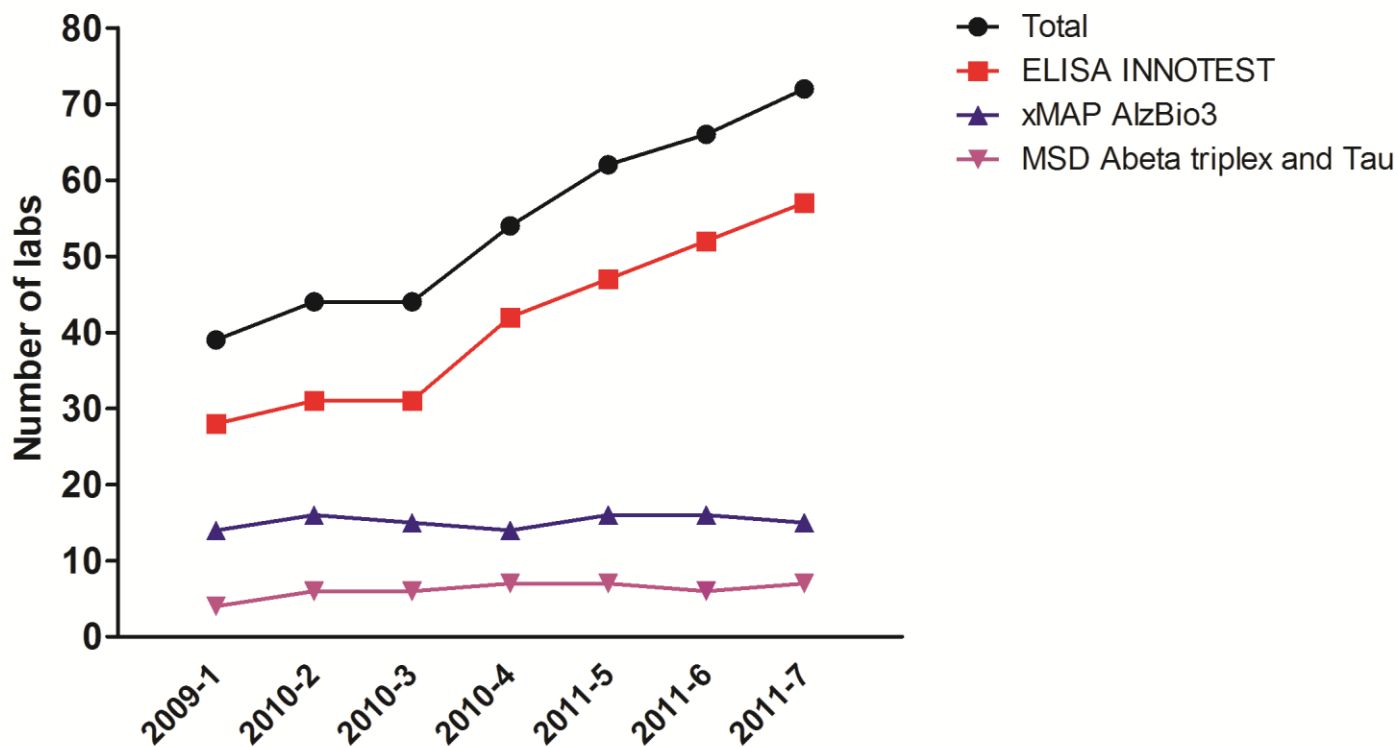
# Program participators



>85 laboratories  
>20 countries

# Labs and techniques

## - rounds 1 to 7



# A $\beta$ 42

## Alzheimer's Association QC program for CSF

### Longitudinal evaluations

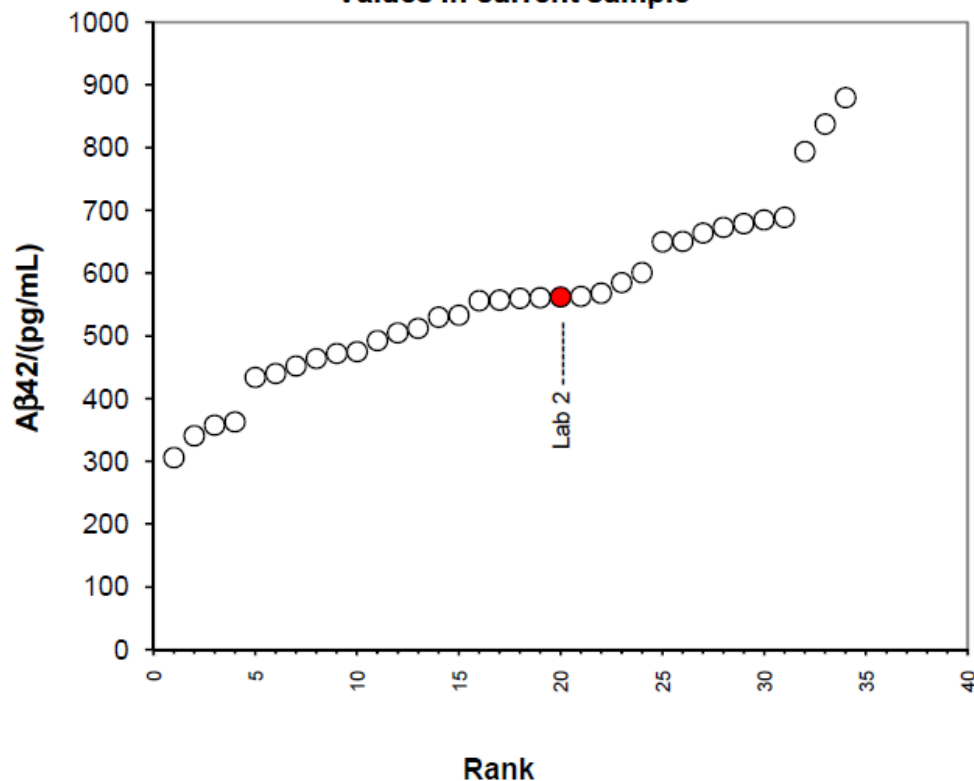
#### Göteborg (Lab 2)

Round:	2010:4 QC-L
Result:	562 pg/mL
Method:	INNOTEST

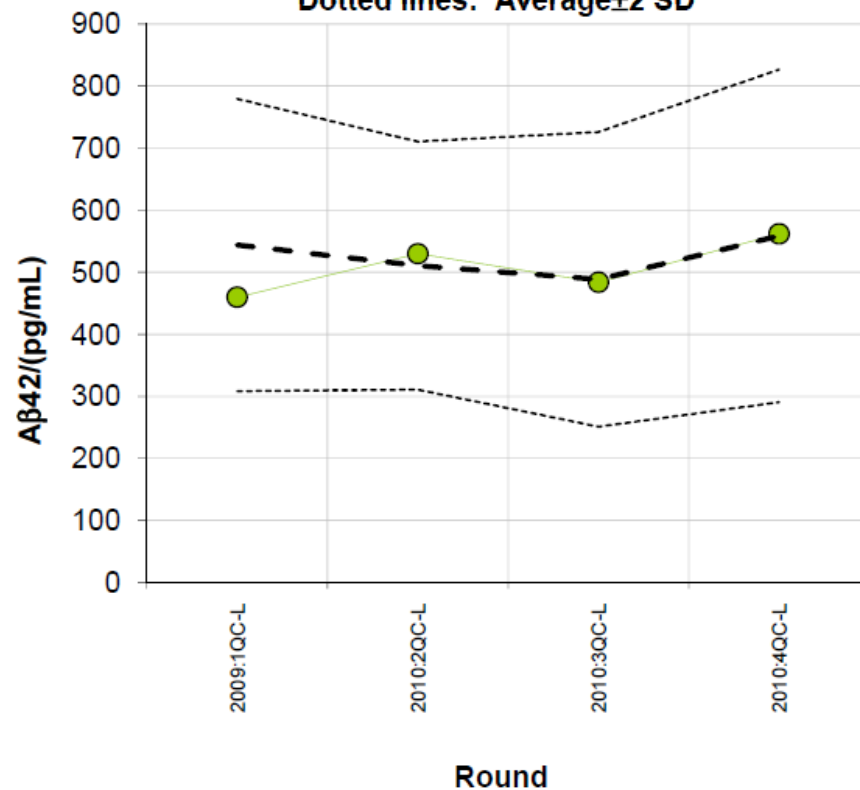
#### All 34 labs in this round

Mean:	559 pg/mL
SD:	134 pg/mL
CV:	24%

Values in current sample

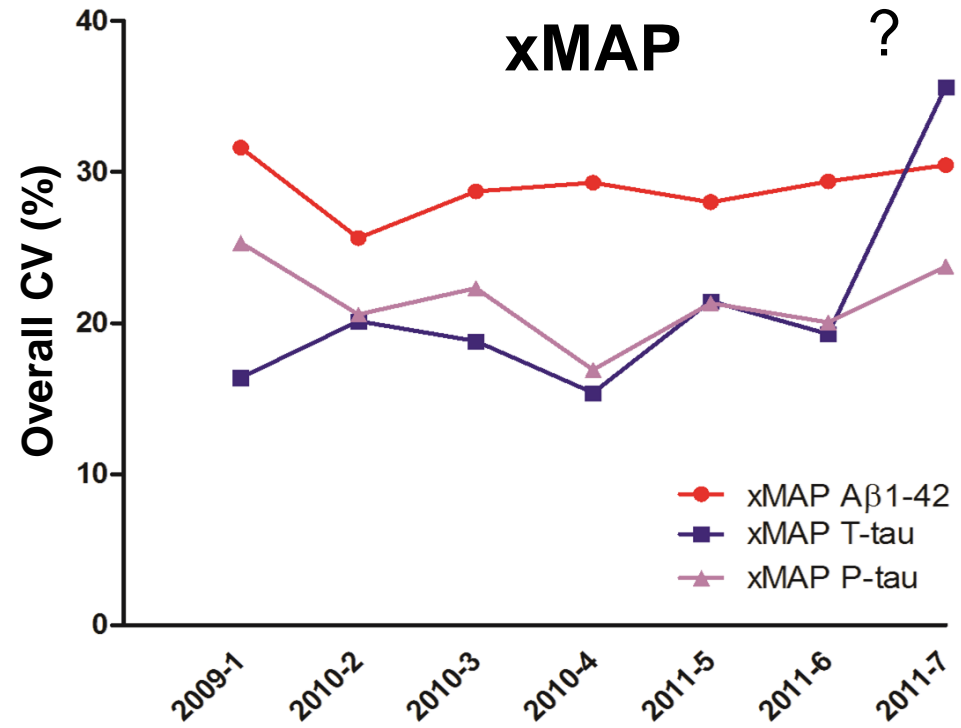
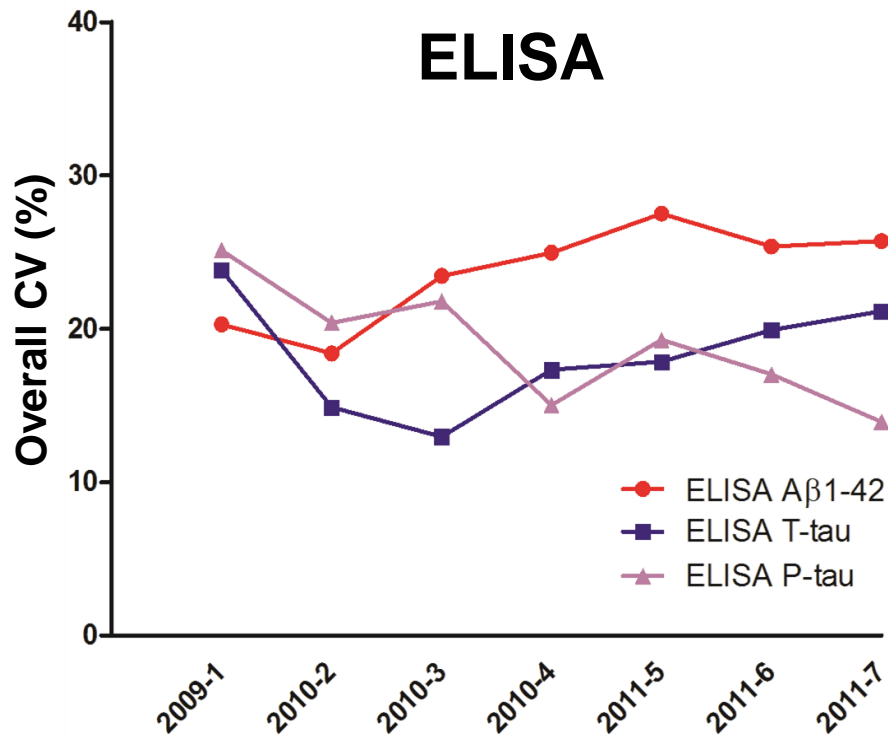


Longitudinal QC-sample  
Dotted lines: Average  $\pm$  2 SD



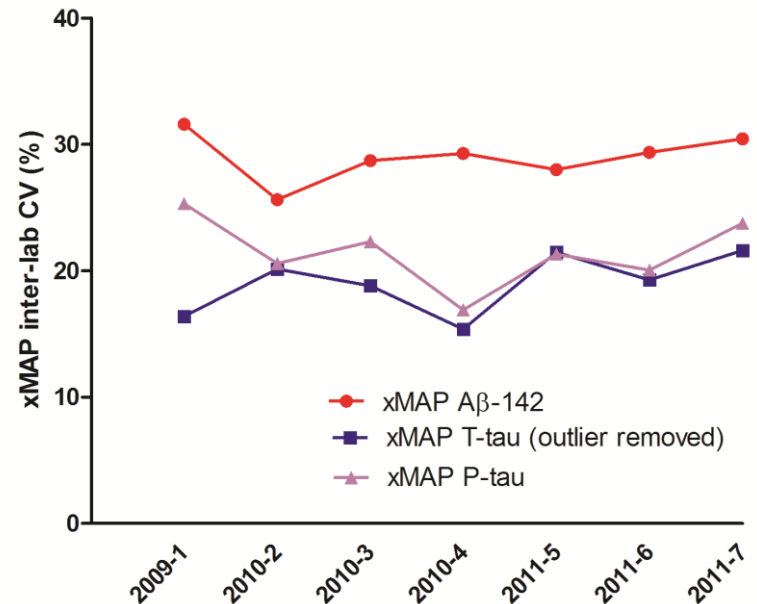
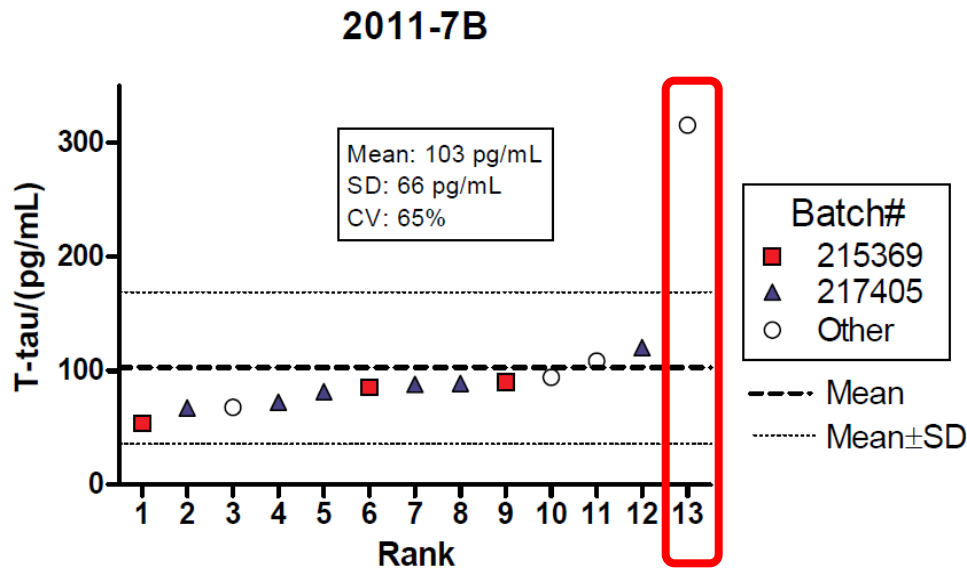
# Overall variability

## - rounds 1 to 7



Mean CVs for A, B and QCL samples

# Overall variability - influence of outliers

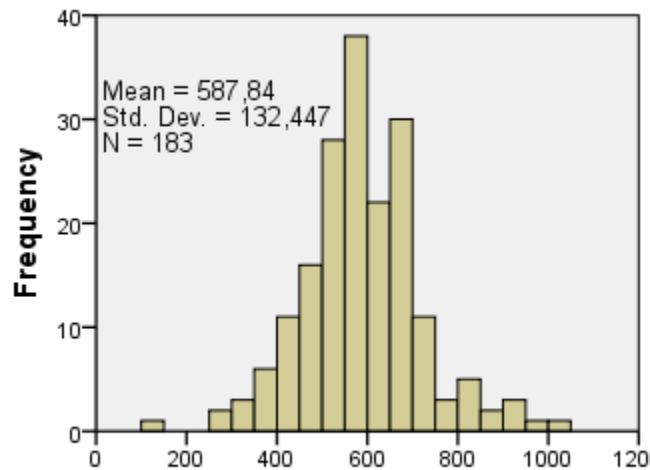


# Causes of variability

<b>Preanalytical</b>	Sample handling
	Assay kit handling and storage
<b>Analytical</b>	Laboratory Equipment <ul style="list-style-type: none"> <li>• Calibration</li> <li>• Detection instrument</li> <li>• Pipetting</li> </ul>
	Analyst <ul style="list-style-type: none"> <li>• Competency</li> <li>• Familiarization with the Method</li> <li>• Forward/Reverse Pipetting</li> <li>• Reagent handling</li> </ul>

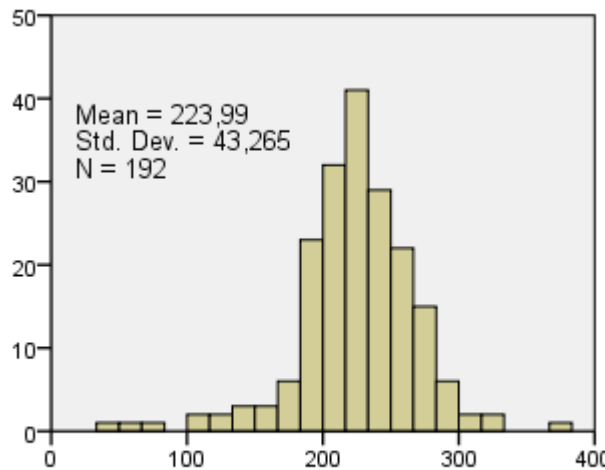
<b>Postanalytical</b>	Data handling <ul style="list-style-type: none"> <li>• Analyzing singlets/duplicates</li> <li>• Decisions for rejecting data</li> <li>• Type of curve fitting used</li> <li>• Software for data calculation</li> </ul>
	<b>Kit Manufacturing</b> Documentation <ul style="list-style-type: none"> <li>• Test Procedure Instructions</li> <li>• Minimal Method Optimization</li> </ul> Reagents <ul style="list-style-type: none"> <li>• Source of reference standard</li> <li>• Buffer-composition</li> <li>• Lot-lot variability</li> <li>• Vendor-vendor variability</li> <li>• Quality controls</li> </ul>

# Causes of variability between-labs or between-batches?



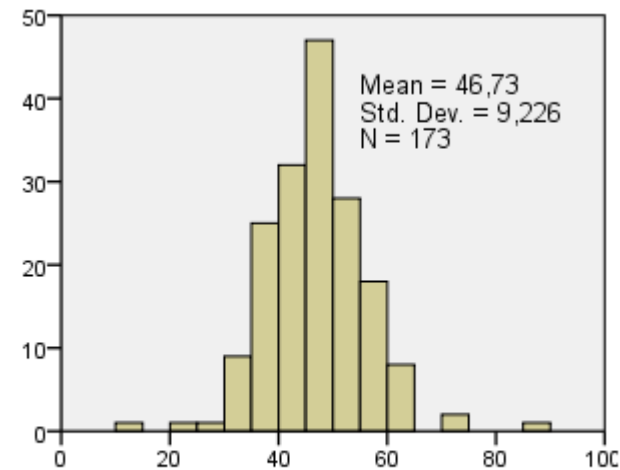
QC-L Ab42 (ng/L) ELISA

**CV=23%**



QC-L T-tau (ng/L) ELISA

**CV=19%**

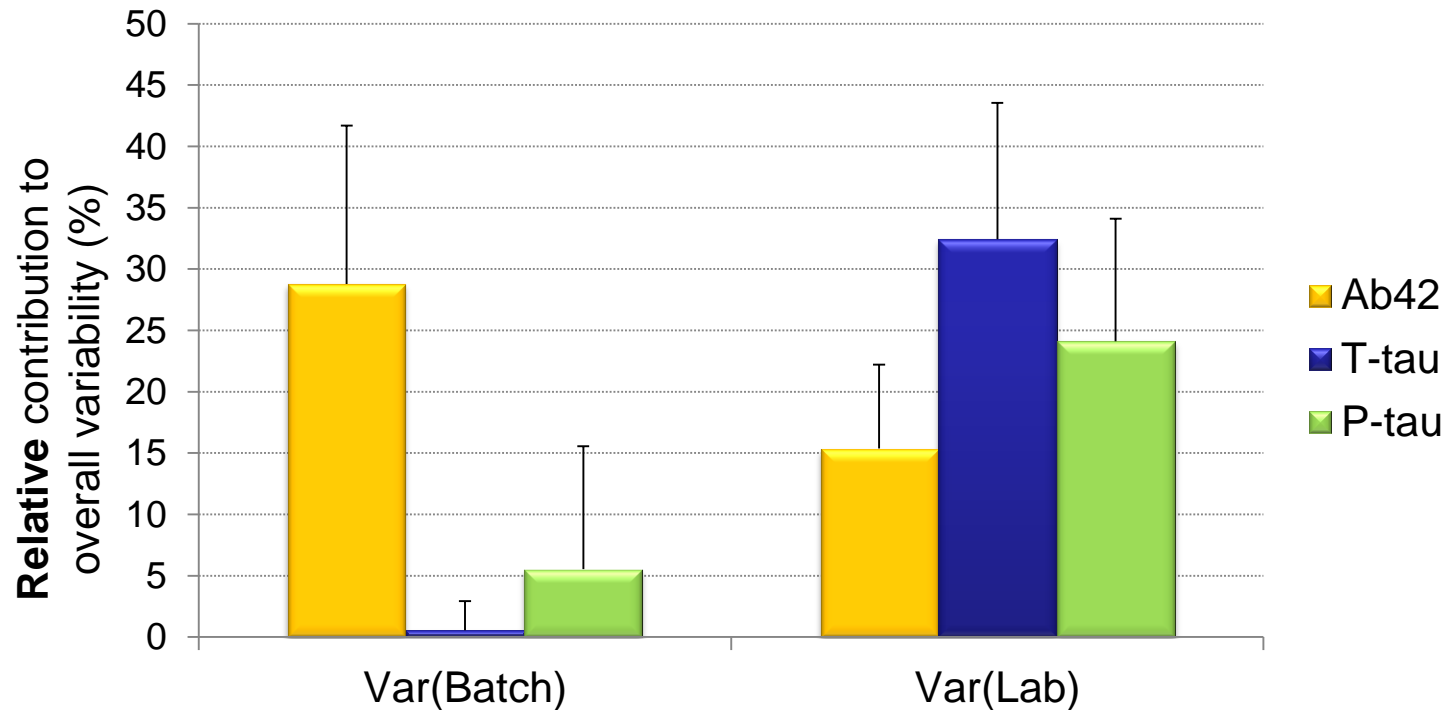


QC-L P-tau (ng/L) ELISA

**CV=20%**

QC-L results from batches used  $\geq 4$  times and labs reporting  $\geq 4$  results

# Causes of variability between-labs or between-batches?



Variance Component Analysis (REML)

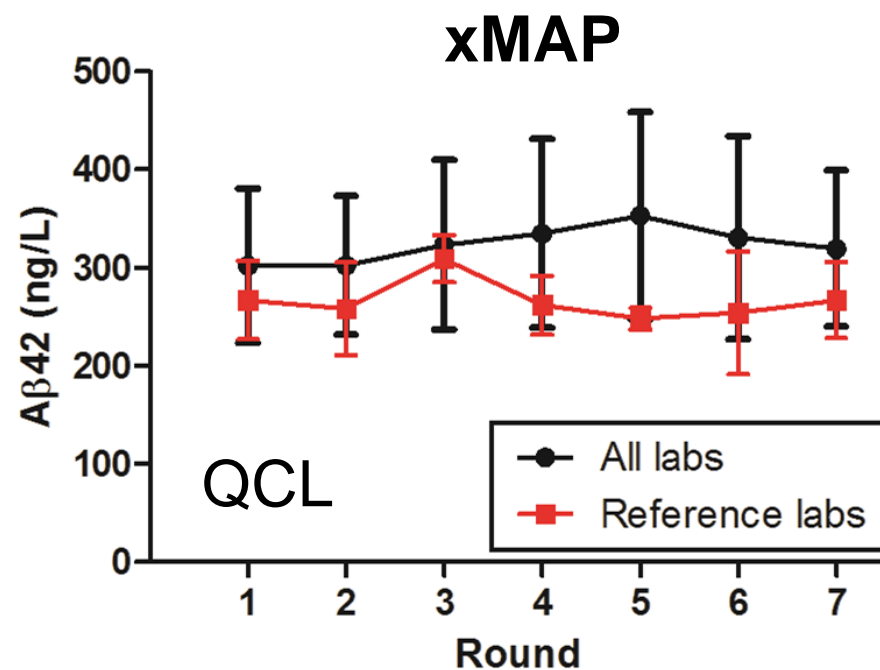
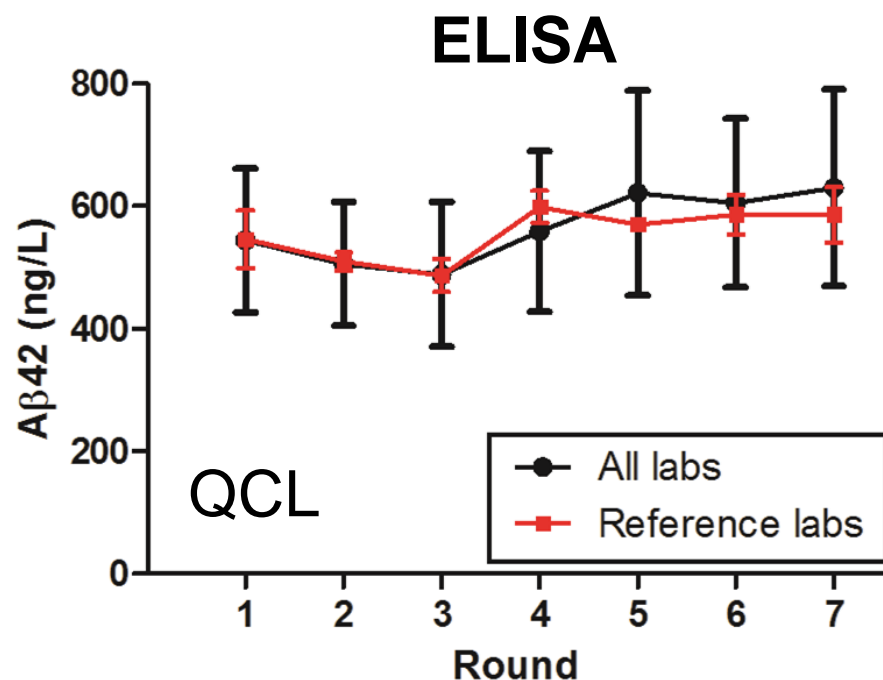
Calculated on results for the QC-L sample (INNOTEST ELISAs)

Batches used  $\geq 4$  times and labs reporting  $\geq 4$  results included

Error bars mark upper 95% CI

# Causes of variability

## influence of experience?



Low variability among *reference labs* ( $N=3$ ) for A $\beta$ 42

# QC program checklist

## INSTRUMENTATION, EQUIPMENT AND LAB TECHNICIANS

1	Preventive maintenance service on instrument performed within 6 months	Yes
2	Plate reader accuracy tested with calibrated plate within last year	Yes
3	Pipettes calibrated (within last 3 months)	Yes
4	Multichannel pipette used when appropriate	Yes
5	Automated plate washer used	Yes
6	Automated liquid handling (pipetting robot) used	Yes
7	Incubations done under controlled conditions (RT 20-25°C; cold 2-8°C)	Yes
8	Assays performed by qualified (specially trained) Lab technicians	Yes

## QC SAMPLES

1	QC samples stored at minus 70 - 80°C	
2	QC samples thawed on the bench at room temperature before analysis	Yes, e
3	QC samples vortexed before analysis	

## ASSAY: Reagents

1	Kits stored following kit insert: ELISA plate at +2-8°C; standards at < -20°C	
2	Assay components (standards, plate and reagents) only from the same kit box	
3	Kit used within expiry date given provided by the manufacturer	

## ASSAY: Standards (calibrators) and QC system

1	Calibrators diluted from stock in separate polypropylene tubes	
2	Calibrators (standards) and samples analyzed in duplicates	
3	Calibrators (including no. of calibrators) prepared according to kit insert	
4	Internal control samples used for quality control	Yes (pooled CSF) <input type="checkbox"/>

## ASSAY: Conditions

1	The test procedures in the kit inserts are followed without any deviation	
2	Polypropylene plates used for pre-incubation (Aß 1-42)	
3	If yes, polypropylene plate used for both standards and CSF samples	

## ASSAY: Data analysis and run acceptance

1	Plate reader settings: 450 nm, endpoint	Yes <input type="checkbox"/>
2	Standard curve calculated using the 4 parameter logistic equation	Yes <input type="checkbox"/>

No significant findings yet  
Too little data? (complex interactions?)  
Problem with self-reporting?

# Future prospects

- Further pinpoint error sources
- Alert outliers → revise procedures
- Alert kit producers → improve kit stability
  
- Implementation of SOPs by hands-on courses
- Certified reference materials and methods
- Novel assays for fully automated analytical platforms (reduce variation due to between-lab analytical procedures)

# Proposed role of the QC program in further CSF biomarker standardization

## Reference methods

GCSB  
IFCC working group

Use reference methods to set levels in **reference materials**

- Pilot batches by GCSB with partners including the IFCC working group
- Large-scale production by IRMM

**Supply** reference material to assay producers by IRMM (non-profit)

**Certification** of methods and materials by IFCC

**Collaboration** with other research groups

## QC program

Monitor and evaluate the progress of the global standardization efforts

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Institute for Reference  
Materials and Measurements

 **ifcc**  
International Federation  
of Clinical Chemistry  
and Laboratory Medicine



# Acknowledgments



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Staffan Persson, coordinator

Åsa Källén, laboratory technician

Monica Christianson, laboratory technician

Sara Hullberg, laboratory technician

Dzemila Secic, laboratory technician