QA-LSD

Quality Assessment
Enzyme Analysis for Lysosomal Storage Diseases

Erasmus MC

Pilots "large scale"

2006 (36)

2007 (46)

2008 (55)

2009 (59)

2010

European Study Group on Lysosomal Diseases

ERNDIM European Research Network Inherited Disorders of Metabolism

QA-pilots for LSD's
What is it all about?

- Constant supply of material
  - A constant difficulty for QA-schemes
- Detecting enzyme deficiencies
  - Material form LSD patients: EBV lymphoblasts
- Reproducibility
  - Duplicate, triplicate or quadruplicate samples

QA-pilots for LSD's
The 2008 and 2009 pilots

- Participants
  - pilot 2008 : 55 participants (Protein- & 4MU-standards)
  - pilot 2009-1: 59 participants (Leuko's, EBV lympho's)
  - pilot 2009-2: 27 participant (DBS)

  6 samples & 8 enzymes, LSD patients included

- Result have been presented at the ESGLD Workshop 2009
  September 10-13, 2009, Bad Honnef

- A paper on 4 years "large scale" QA-LSD (to be published in 2010)
  Authors from ESGLD, SKML and ERNDIM

Protein- and 4MU-standards, 4 duplicates
(QA-ESGLD/ERNDIM 2008)

Unintended,
EBV cell cultures were lost by fungus infection

Confusing
I did not double-check the samples

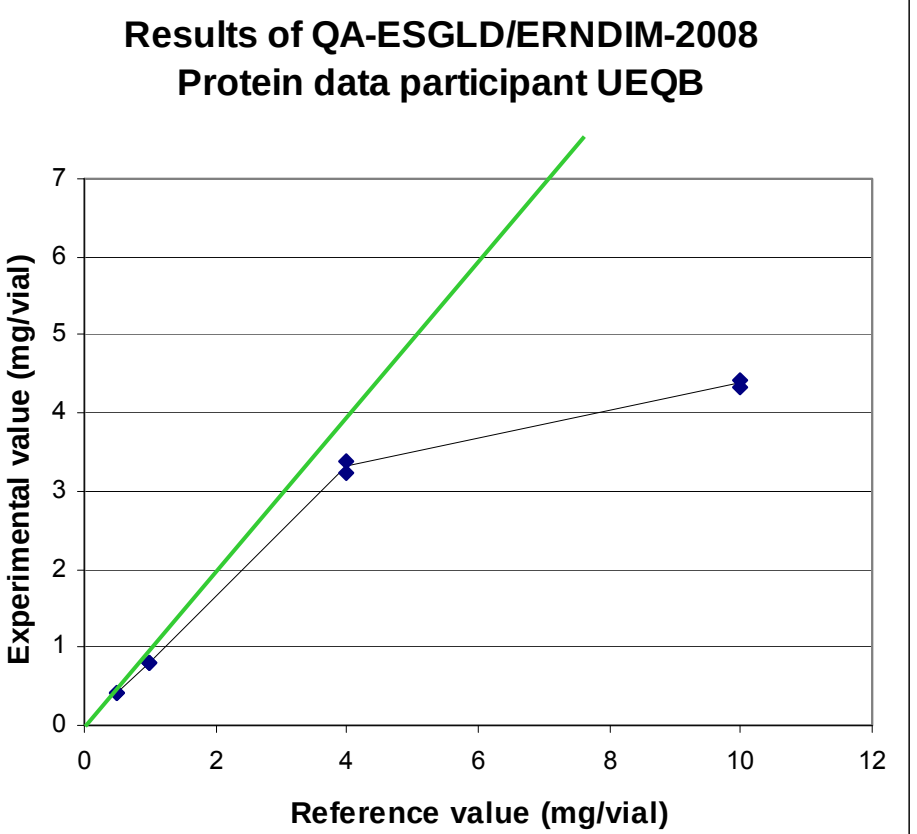
Informative
The identical samples emphasised the problem of reconstitution of lyophilised samples

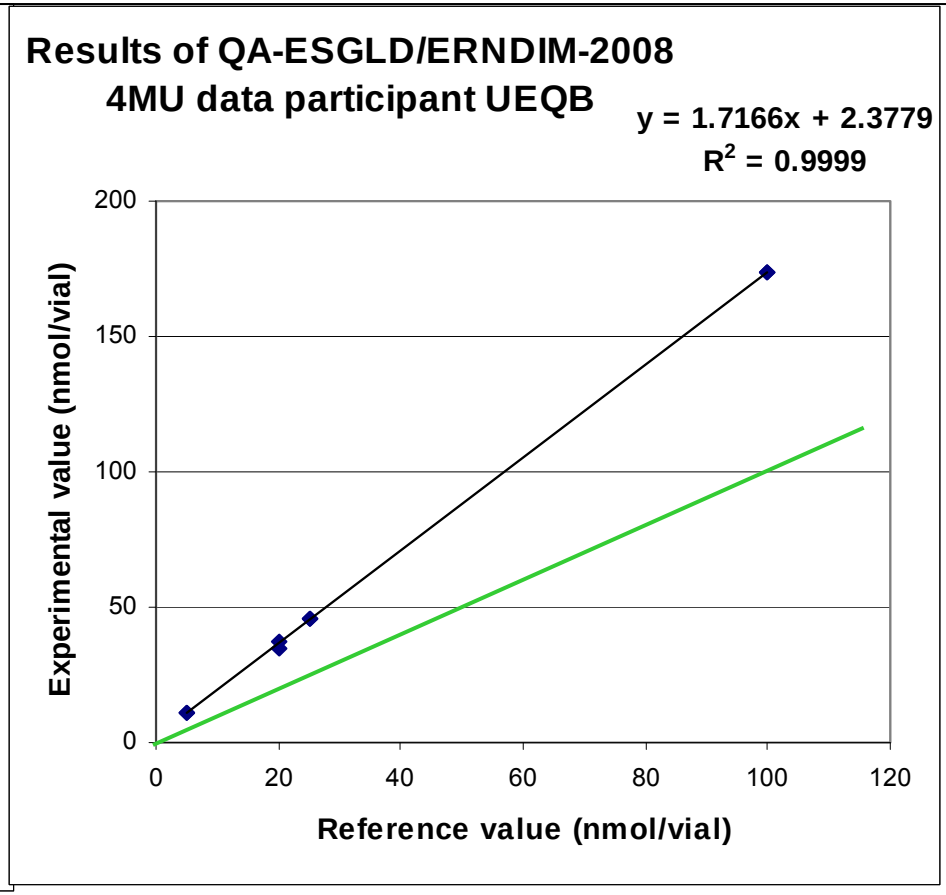
Protein- and 4MU-standards, 4 duplicates
(QA-ESGLD/ERNDIM 2008)

Best performers: 21 of 47 labs

Protein: CV's of 4 duplicates < 20%

4MU: $R^2 > 0.98$



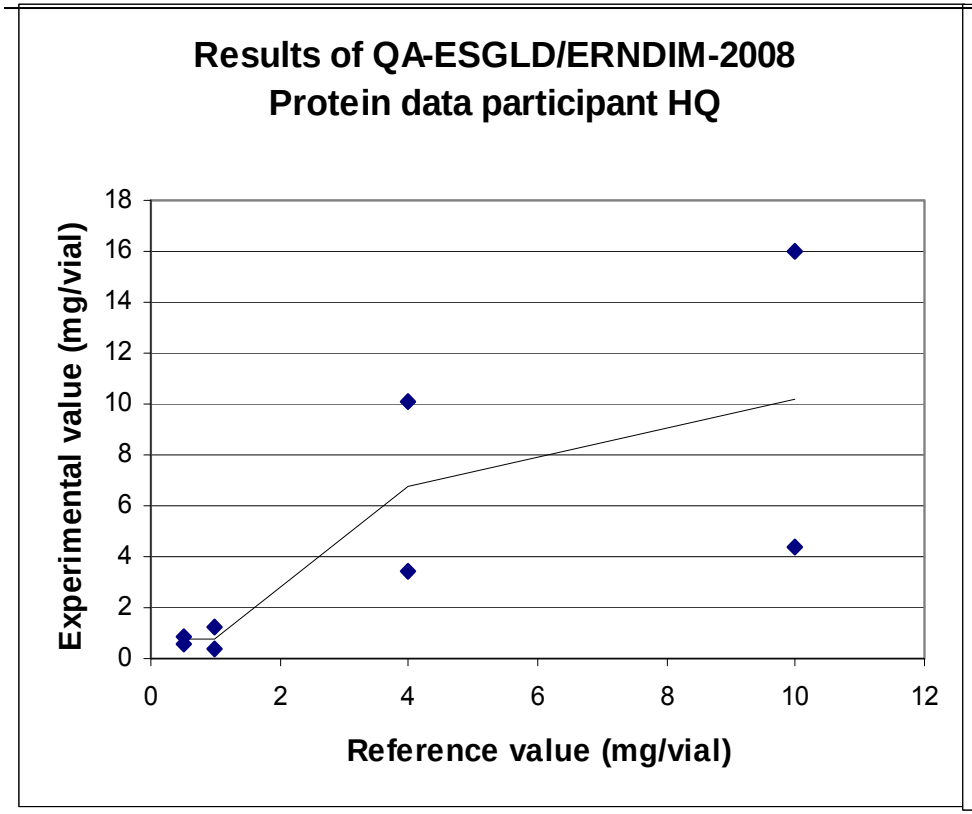


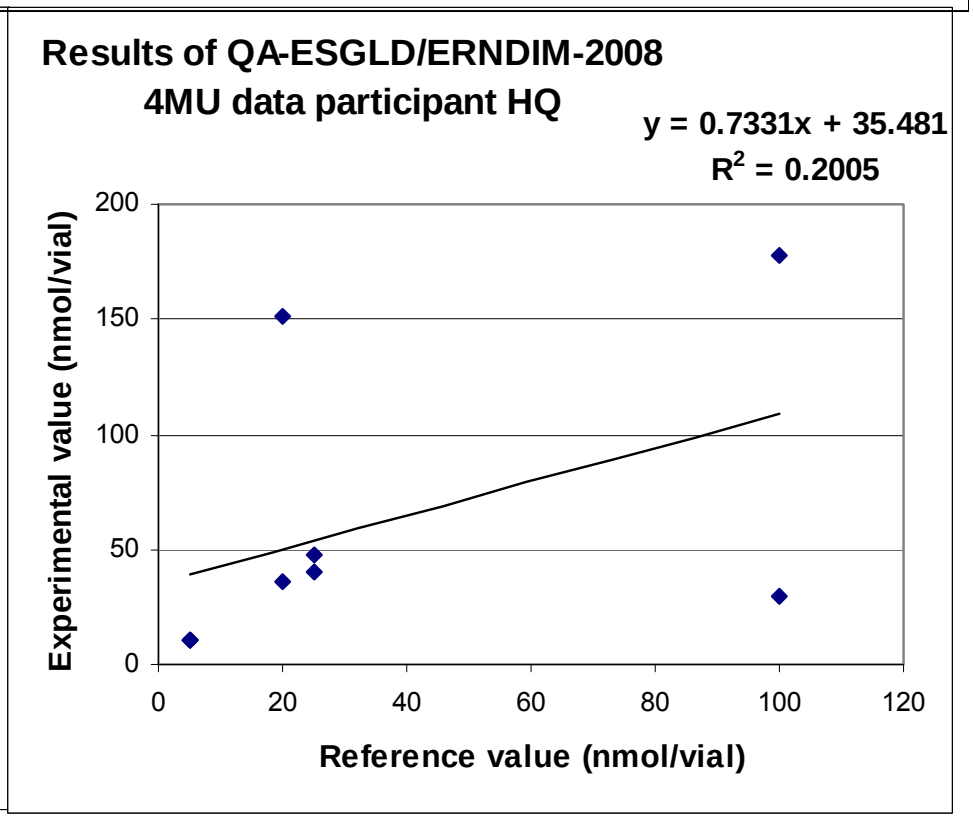
Protein- and 4MU-standards, 4 duplicates
(QA-ESGLD/ERNDIM 2008)

Can be improved: 26 of 47 labs

Protein: CV of 1 or more duplicates > 20%

4MU: $R^2 < 0.98$





Reproducibility of protein/vial 2009

duplicate samples leukocytes

Lab No	CV Protein
24	0.0
39	0.0
30	0.8
37	1.4
46	1.4
32	1.4
9	1.5
43	1.8
54	2.0
18	2.5
51	2.7
7	3.2
19	4.6
12	5.0
20	5.1
1	5.9
52	6.1
28	6.1
15	6.2
13	6.2
3	7.4
16	8.0
11	8.4
34	8.6
22	8.7
36	9.2
25	9.6
44	10.0
31	11.0
61	11.5
48	12.3
2	12.8
4	13.9
38	18.2
10	18.5
21	21.0
27	21.4
57	24.4
60	27.5
5	27.9
59	28.1
47	50.6
17	155.2

43 Labs

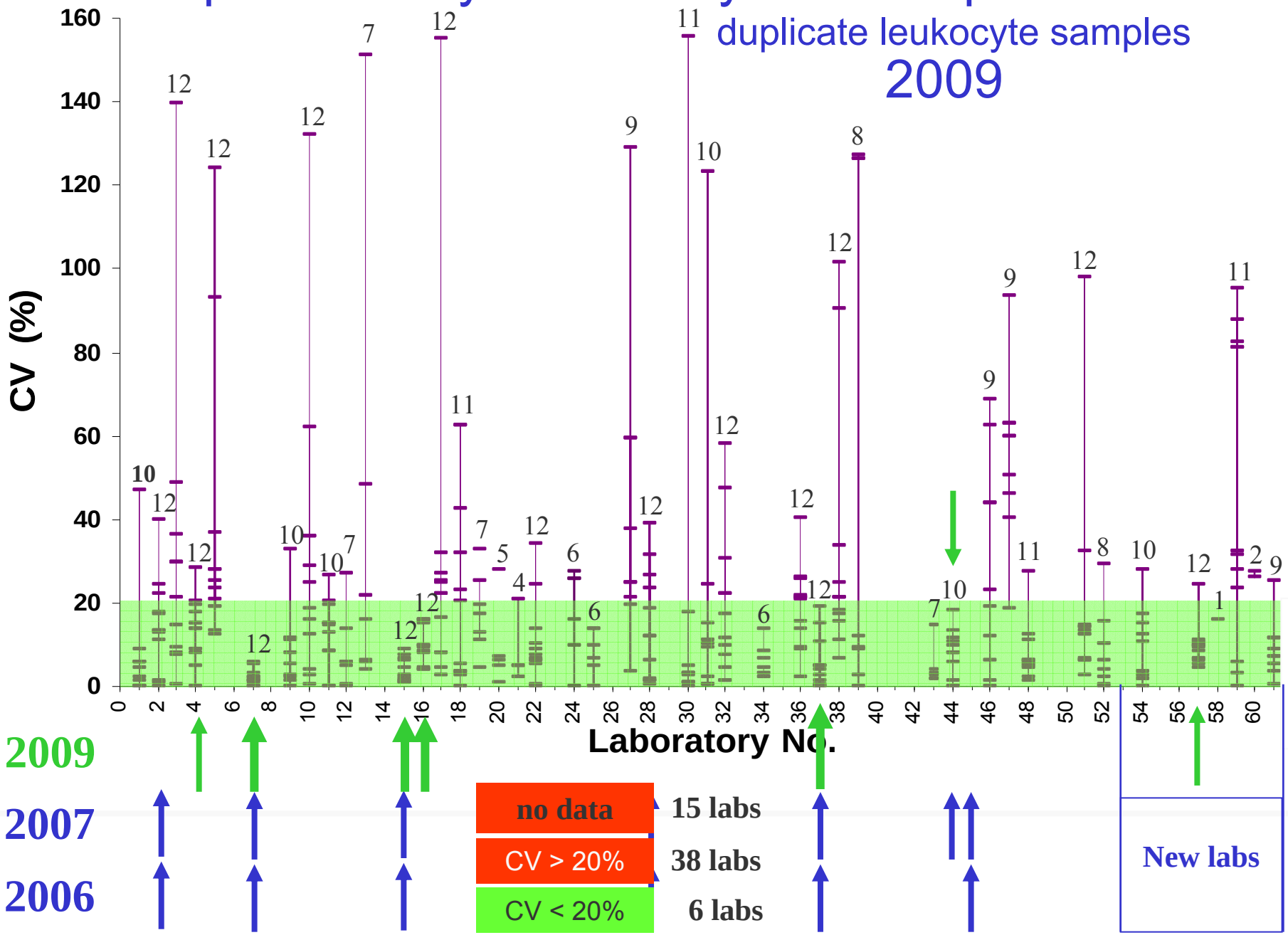
59 “participants”

no data	16 labs
CV > 28%	2 labs
20%<CV < 28%	6 labs
CV < 20%	35 labs

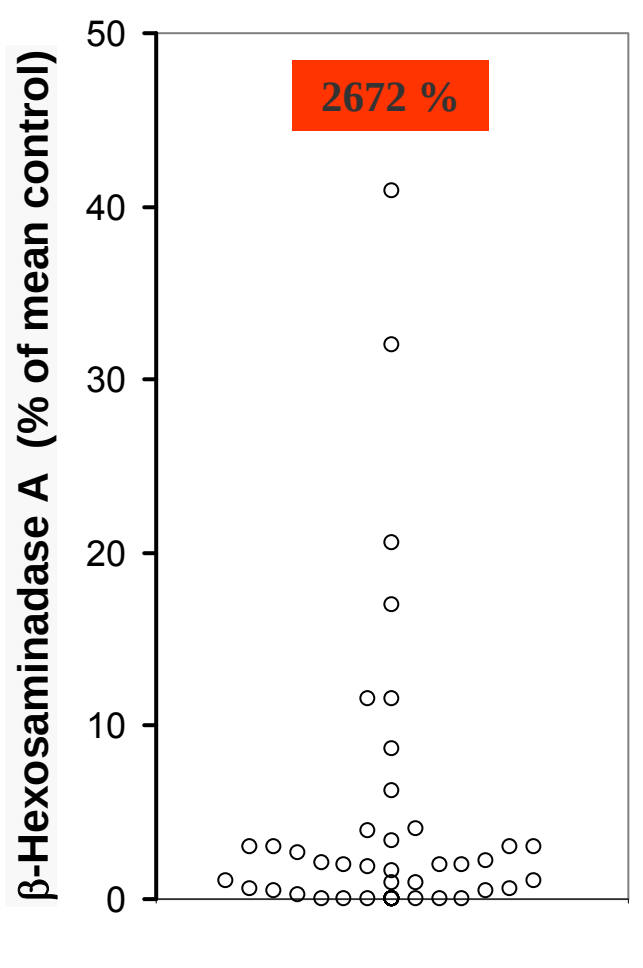
Reproducibility of 11 enzymes and protein

duplicate leukocyte samples

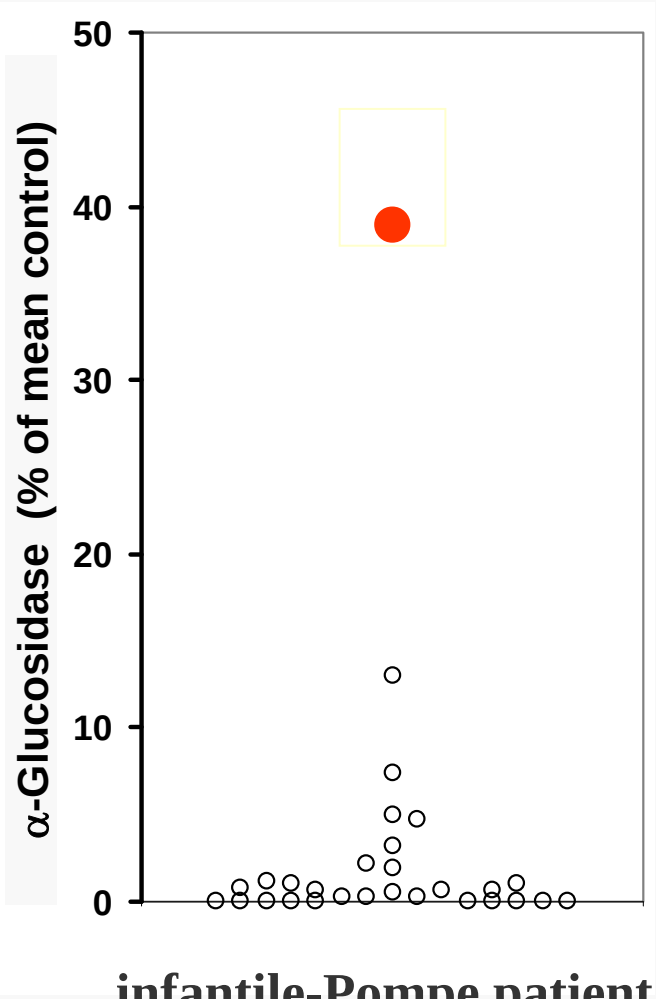
2009



Proficiency testing enzyme analysis in EBV lympho's 2009

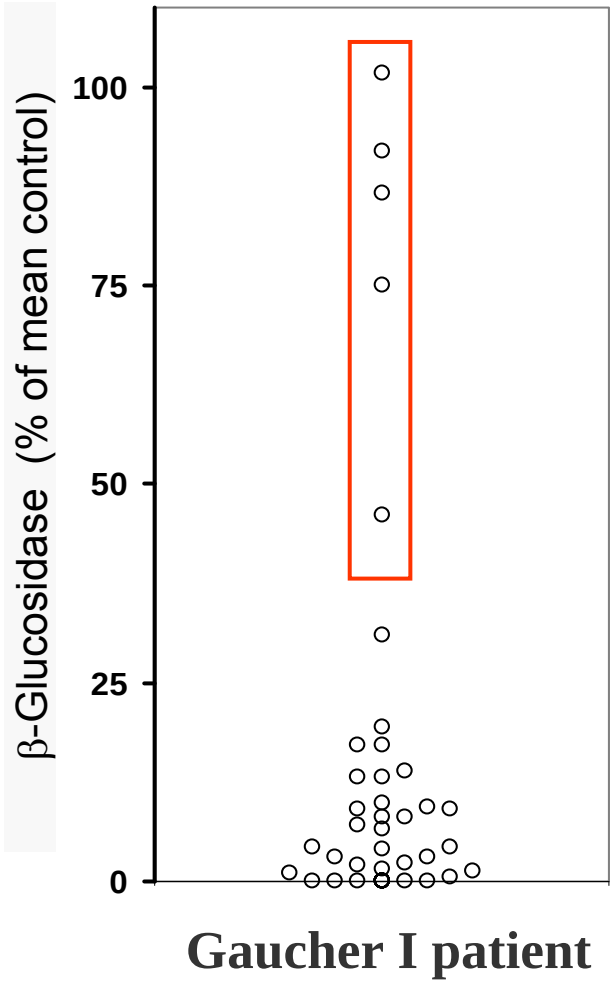


Tay-Sachs patient

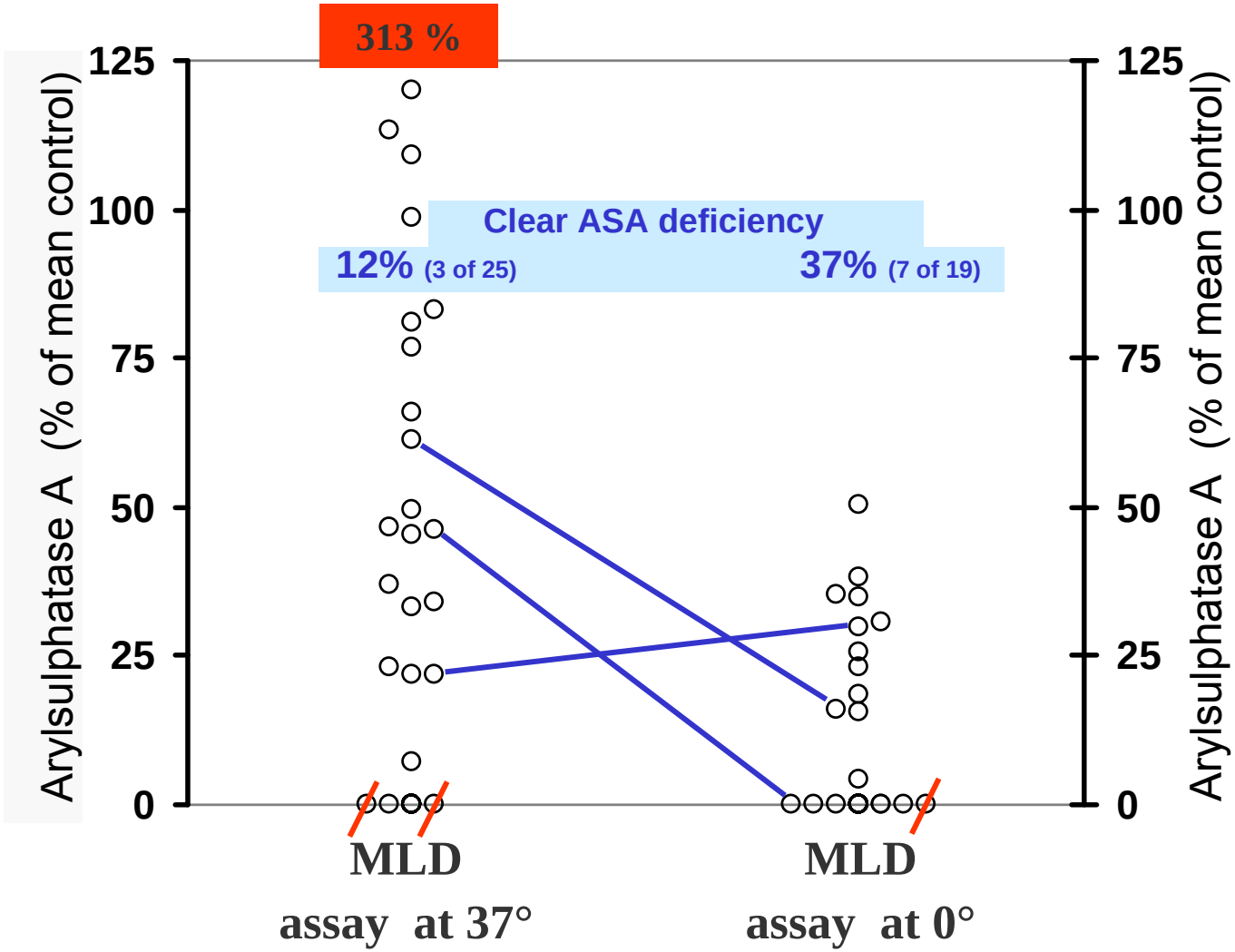


infantile-Pompe patient

Proficiency testing enzyme analysis in EBV lympho's 2009



Proficiency testing enzyme analysis in EBV lympho's 2009

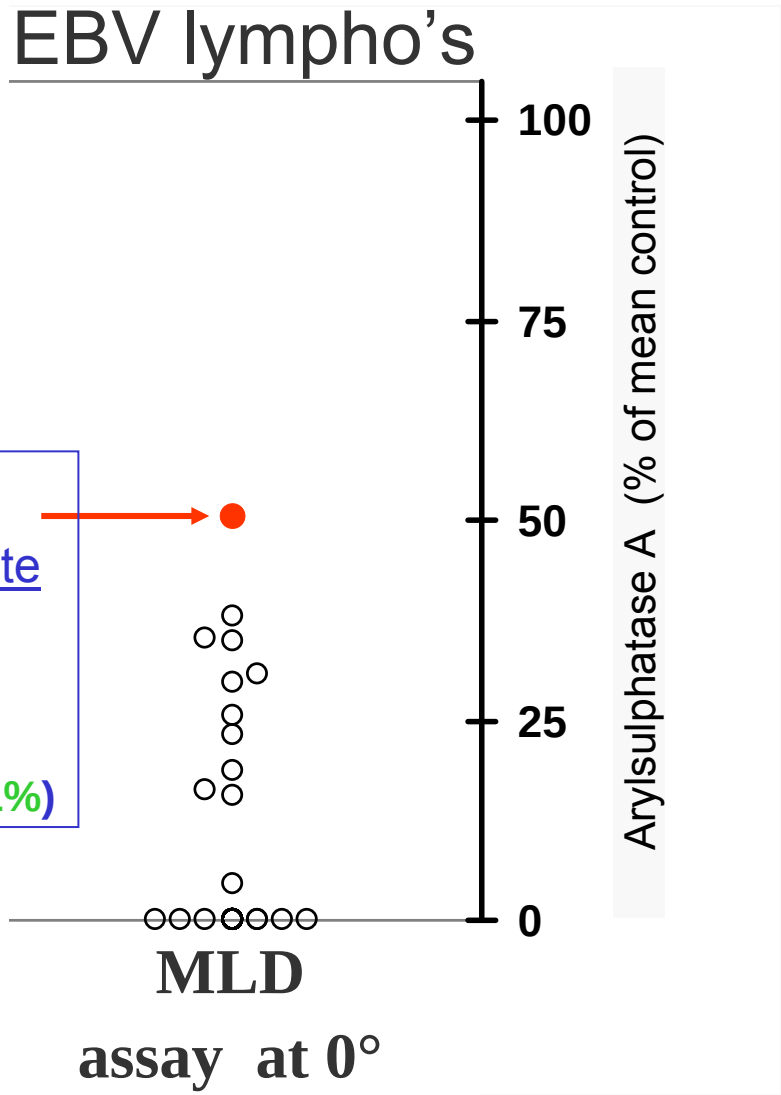


Why was ASA deficiency missed?

Rotterdam uses pNCS assay in 96 well plates

Marijke Boer: precipitate in the wells

EBV lympho's	Arylsulphatase A activity	
	With precipitate	No precipitate
Control (n=8)	50 – 71	27 – 40
MLD (n=2)	34; 35 (≈57%)	8.6; 5.5 (≈11%)



Feedback from Participants about ASA assay

Which SOP is used by best-performers ?

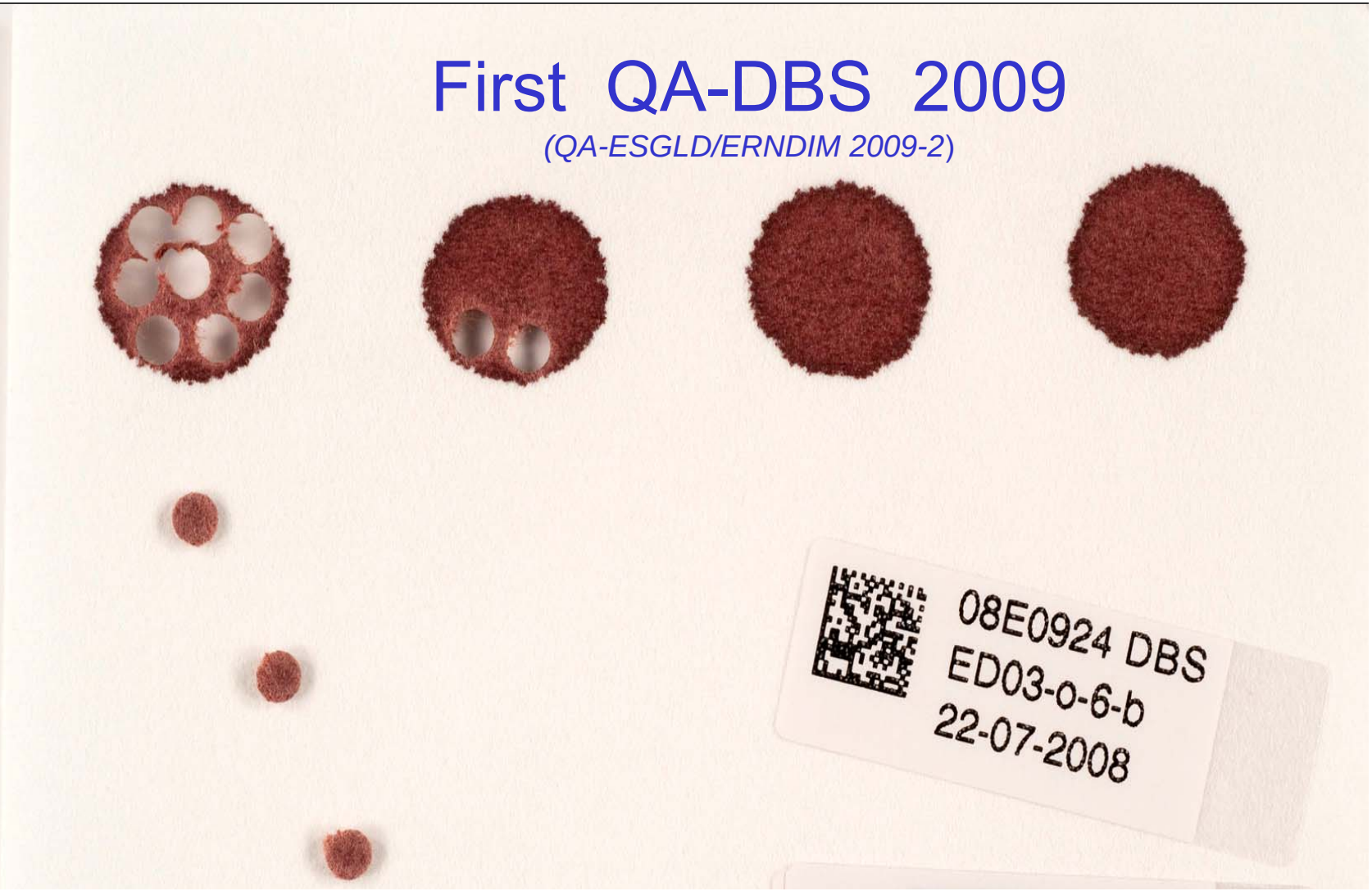
- Question: Which SOP is used by best performers?
  - Asked to the 9 best-performers

- Response: 1 of 9 participants

Dried
Blood
Spots

First QA-DBS 2009

(QA-ESGLD/ERNDIM 2009-2)



Eight 3-mm punches per 12 mm blood spot
60 μl blood / 12 mm spot

QA-DBS 2009 compliance of data entry

(QA-ESGLD/ERNDIM 2009-2)

27 participants

6 samples / lab

2 spots (12 mm) / sample

8 enzymes

Chitotriosidase

α-Galactosidase

β-Galactosidase

α-Glucosidase

β-Glucosidase

β-Hexosaminidase A

β-Hexosaminidase (A+B)

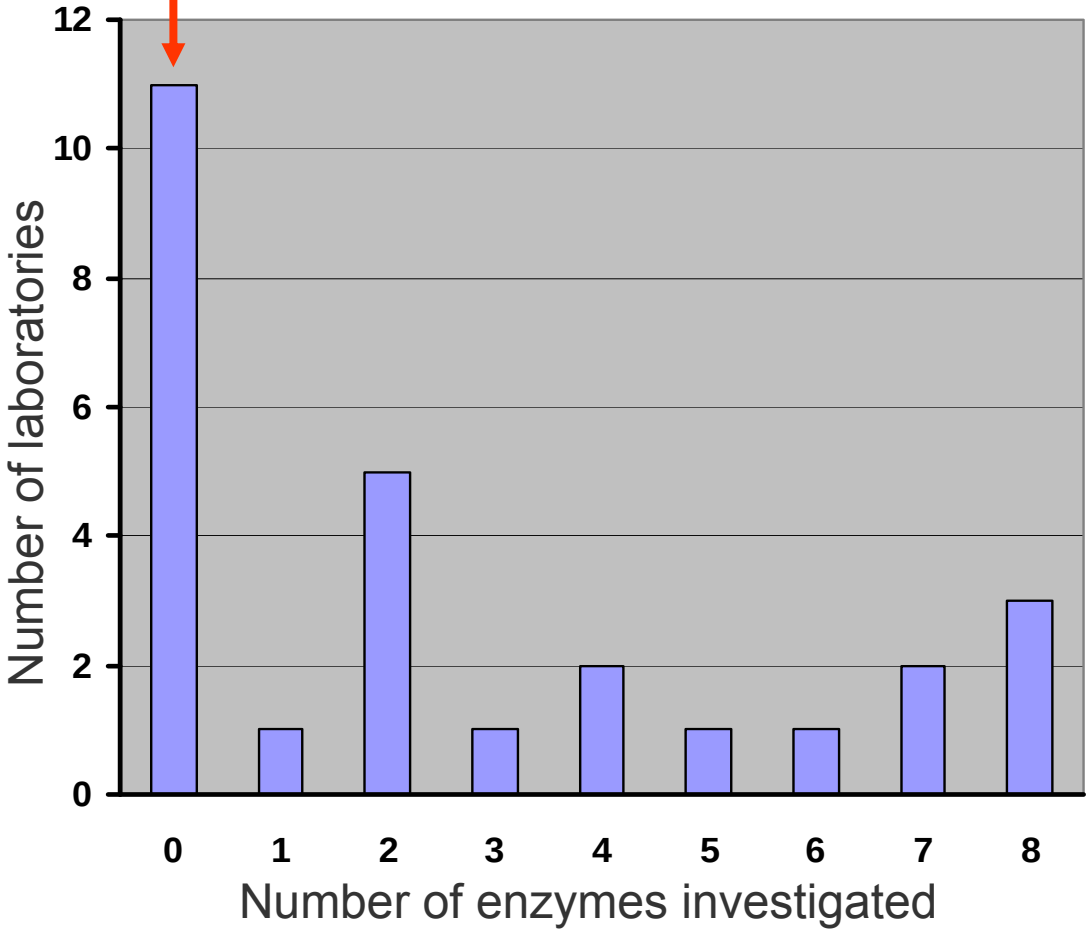
α-Iduronidase

41 %

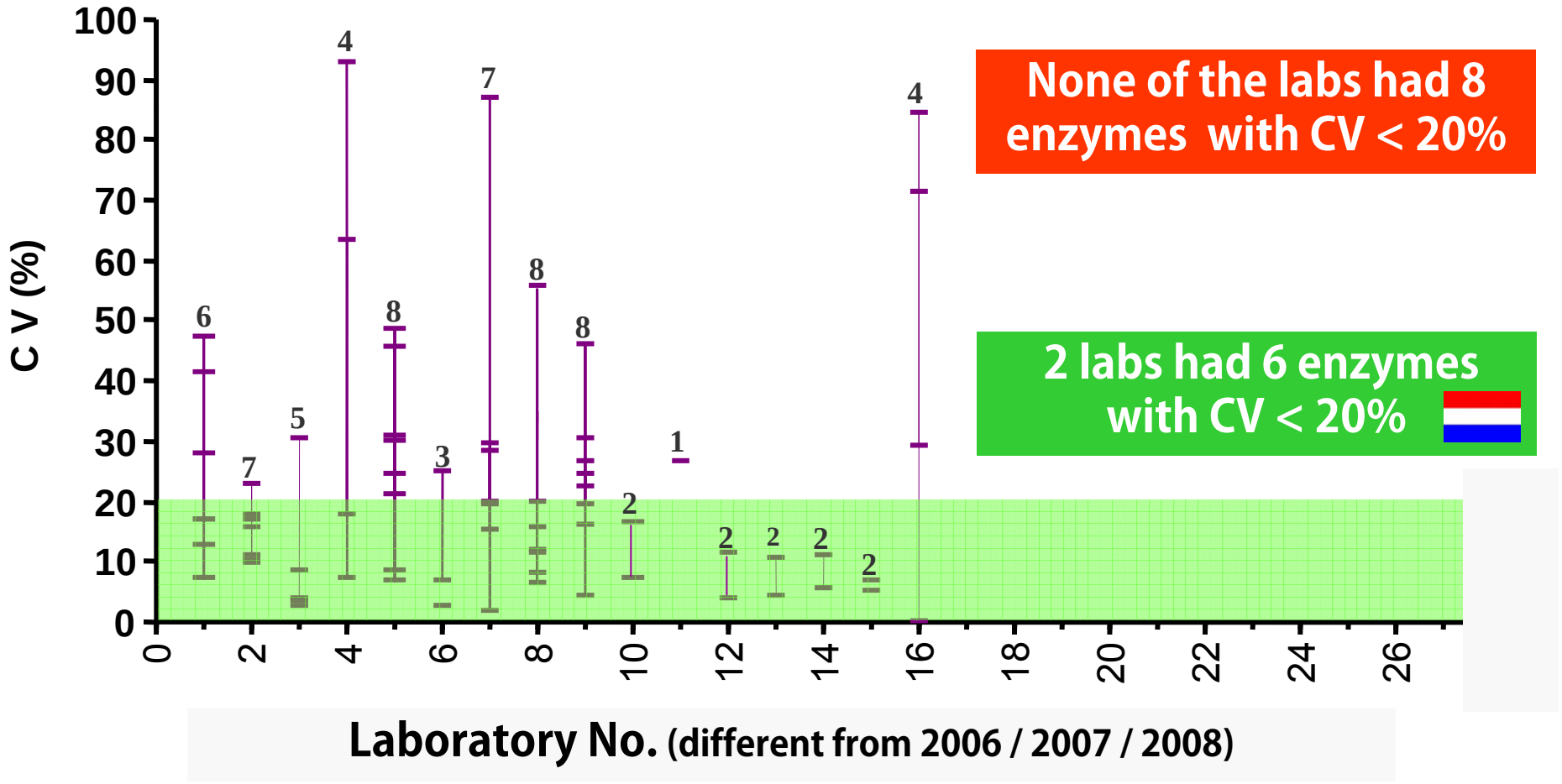
Non-responders

10–11 weeks for 3 experiments

Insufficient time?

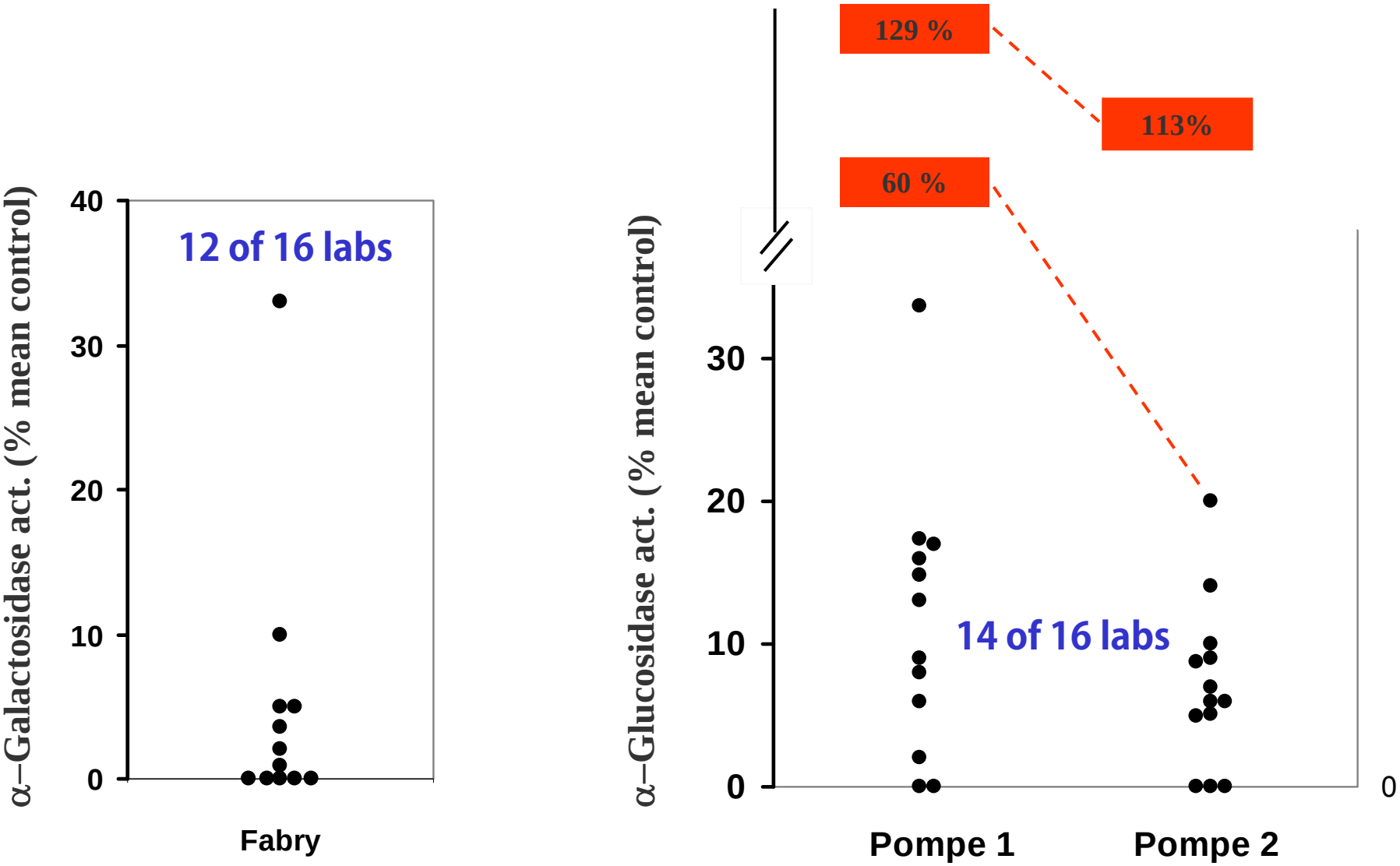


Coefficients of Variation enzyme analysis in DBS (n=3)
1–8 enzymes



Proficiency testing enzyme analysis in DBS

(QA-ESGLD/ERNDIM 2009-2)



Dried
Blood
Spots

Blood samples for DBS

Two 12 mm blood spots / sample / laboratory

- 8 enzymes in duplicate
- 40 participants → 5 ml blood / patient

→ Who can provide patients' blood?

How to prevent "wasting" patients' blood?

QA for LSD's
Cost estimate (50 participants)

- Cost break up:

  10 enzymes: 8 *"conventional"* samples + DBS / year

– EBV lympho's from LSD patients, 6 samples	€ 75
– Control leukocytes, 2 samples (Rottedam)	€ 0
– DBS samples (All participants)	€ 0
– Preparation of 8 homogenates (Rotterdam)	€ 50
– Freeze-drying, Shipping & Data Base managing	€ 150
– Transport of EBV cells & miscellaneous	€ 25
– Costs of ERNDIM	€ 100
Total cost / participant	€ 400

QA for LSD's, a new ERNDIM scheme Starting 2010

- Participants pay the costs
- Lyophilised homogenates of leuko's & EBV-lympho's from LSD-patients.
- DBS samples included, when available
- Website adaptations for Enzyme analysis
  - ”Error free” extraction of raw-data from the ERNDIM database
  - Graphical presentation (dot-plots)
  - Patient samples expressed as % of normal enzyme activity
- Certificate of Participations

QA for LSD's, a new ERNDIM scheme
What needs to be done

- Some thoughts about the Certificate of Participation:
  - score for detecting LSD patients (+)
  - score for reproducibility (+ to —)
  - score for detecting enzyme deficiencies in "normal" samples (—)
  - score "No show" on an ERNDIM DPT meeting (—)
  - Poor performance should have consequences

- Repetitive poor-performance should have severe consequences
  - We should ask our accrediting bodies to set more stringent rules
    Merely participating is not sufficient, the scores should get moer weight.

Enduring QA-programme for the LSD's