

Quality Assessment Enzyme Analysis for Lysosomal Storage Diseases

ERNDIM / EUGT meeting 5-6 October 2006, Prague

Results of 1st “large scale” pilot



Quality Assessment Enzyme Analysis for Lysosomal Storage Diseases



European
Study
Group
on
Lysosomal
Diseases



Laboratories
Rotterdam

Hamburg/Heidelberg
Dr, Zoltan Lukacs/Dr. Friederike Bürger



QA-pilot for LSD's

the aims

- **Inter-laboratory variation**
 - Many participants: > 30
- **Intra-laboratory variation**
 - Analyse two pairs of identical control samples (blind)
- **Proficiency testing of enzyme deficiencies**
 - Which samples are best?
 - Blood samples: most widely used, but impracticable
 - Fibroblasts: good but laborious
 - EBV lymphoblasts: easy to obtain, not widely used

QA-pilot for LSD's

the set up

- **Samples, without clinical information**
 - Leukocytes
 - EBV lymphoblasts
 - Fibroblasts
- **Enzymes: easy ones**
 - 4MU-substrates
 - Simple colorimetric assays
- **Shipping: economic**
 - Send at room temp., postal service
 - Lyophilised enzymes, stable at room temp. for 5 days
 - Fibroblasts
- **Data entry through existing ERNDIM programmes**
 - “Metabolite presentation” (www.erndimqa.nl → Lysosomal Enzymes)

QA-pilot for LSD's the participants

European
Study
Group
on
Lysosomal
Diseases



- **Questionnaire to members** (85 from 21 countries)
 - 40 labs want to join a QA-pilot
 - 65% want to include DBS in future QA-schemes
- **Samples sent, data returned**
 - 40 labs received samples
 - 36 labs entered data on www.erndimqa.nl

QA-pilot for LSD's the samples

- **10 samples**
 - 4 leukocytes (two duplicate samples)
 - 4 EBV lymphoblasts
 - 2 fibroblasts
- **10 easy enzymes**
 - specific enzyme activity (e.g.. nmol/h/mg)
 - normalise to % of mean control
- **2 separate experiments**
 - test reproducibility

QA-pilot for LSD's

Control fibroblasts, specific enzyme activity

Cycle: 2006 Sample Nr: LE Fibroblast 1 Analyte: L10. beta-Glucosidase (nmol/h/mg) Change

LE Fibroblast 1					
L10. beta-Glucosidase (nmol/h/mg)					
Method		Home made			
Selections for Report		Statistical Results			
Hospital Name	Erasmus MC, Ee 2402	Parameter	Your Lab	Method	All Labs
Department	Dpt. of Clinical Genetics	n	1	18	18
Contact Person		Mean	279	258	237
Deadline	01-07-2006 23:59	Median	279	272	265
Unit	nmol/h/mg	SD		119	108

Scale Standard Deviations	Scale nmol/h/mg				
>3SD	> 562				
2-3SD	454 - 562	■			
1.5 - 2.0SD	400 - 453				
1.0 - 1.5SD	346 - 399	■ ■ ■			
0.5 - 1.0SD	292 - 345	■ ■ ■ ■			
0.0 - 0.5SD	237 - 291	■ ■ ■ ■ ■ X ■ ■ ■ ■			
-0.5 - 0.0SD	183 - 236	■ ■ ■ ■ ■			
-1.0 - -0.5SD	129 - 182	■ ■ ■ ■			
-1.5 - -1.0SD	75 - 128	■ ■ ■			
-2 - -1.5SD	21 - 74	■ ■ ■			
-3 - -2SD	< 20	■ ■ ■ ← Deficient?			
<-3SD					

X Your lab ■ Home made

QA-pilot for LSD's

Control fibroblasts, specific enzyme activity

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-1.5 - -1.0SD	75 - 128	■ ■ ■ ■ ■	
-2 - -1.5SD	21 - 74		
-3 - -2SD	< 20	■ ■ ■	← Deficient?
<-3SD			

Range of 18 labs: 4 – 460 nmol/h/mg

X Your lab ■ Home made

QA-pilot for LSD's

Control fibroblasts, % of *participants* mean control

Cycle: 2006 Sample Nr: LE Fibroblast 1 Analyte: L11. beta-Glucosidase (% mean control)

LE Fibroblast 1					
L11. beta-Glucosidase (% mean control)					
Method		Home made			
Selections for Report		Statistical Results			
Hospital Name	Erasmus MC, Ee 2402	Parameter	Your Lab	Method	All Labs
Department	Dpt. of Clinical Genetics	n	1	18	18
Contact Person		Mean	74	102	96.0
Deadline	01-07-2006 23:59	Median	74	102	97.0
Unit	% mean control	SD		31.1	31.7

Scale Standard Deviations	Scale % mean control		
>3SD	> 191,0		
2-3SD	159,3 - 191,0		
1.5 - 2.0SD	143,5 - 159,2		
1.0 - 1.5SD	127,7 - 143,4		
0.5 - 1.0SD	111,8 - 127,6	■	■
0.0 - 0.5SD	96,0 - 111,7	■	■
-0.5 - 0.0SD	80,2 - 95,9	■	■
-1.0 - -0.5SD	64,4 - 80,1	■	■
-1.5 - -1.0SD	48,5 - 64,3	■	■
-2 - -1.5SD	32,7 - 48,4	■	■
-3 - -2SD	1,0 - 32,6		
<-3SD	< 1,0		

Range of 18 labs: 40 – 127 % of Mean

Your lab Home made

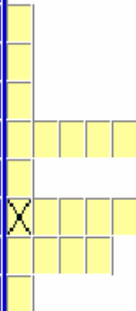
QA-pilot for LSD's

Gaucher fibroblasts, specific enzyme activity

Cycle: 2006 Sample Nr: LE Fibroblast 2 Analyte: L10. beta-Glucosidase (nmol/h/mg)

LE Fibroblast 2					
L10. beta-Glucosidase (nmol/h/mg)					
Method		Home made			
Selections for Report		Statistical Results			
Hospital Name	Erasmus MC, Ee 2402	Parameter	Your Lab	Method	All Labs
Department	Dpt. of Clinical Genetics	n	1	19	19
Contact Person		Mean	2	2.84	2.39
Deadline	01-07-2006 23:59	Median	2	2.60	2.10
Unit	nmol/h/mg	SD		2.09	2.19

Scale Standard Deviations	Scale nmol/h/mg
>3SD	> 8,97
2-3SD	6,78 - 8,97
1.5 - 2.0SD	5,68 - 6,77
1.0 - 1.5SD	4,59 - 5,67
0.5 - 1.0SD	3,49 - 4,58
0.0 - 0.5SD	2,39 - 3,48
-0.5 - 0.0SD	1,29 - 2,38
-1.0 - -0.5SD	0,20 - 1,28
-1.5 - -1.0SD	< 0,19
-2 - -1.5SD	
-3 - -2SD	
<-3SD	



How do these values relate to the control range of the participant?

Your lab Home made

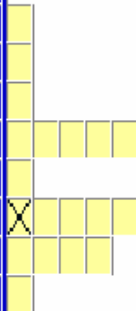
QA-pilot for LSD's

Gaucher fibroblasts, specific enzyme activity

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-1.5 - -1.0SD	< 0,19
-2 - -1.5SD	
-3 - -2SD	
<-3SD	



SD scores (nmol/h/mg) not useful for enzyme deficiencies










Your lab Home made

QA-pilot for LSD's

Gaucher fibroblasts, % of *participants* mean control

Cycle: 2006 Sample Nr: LE Fibroblast 2 Analyte: L11. beta-Glucosidase (% mean control) Change

LE Fibroblast 2					
L11. beta-Glucosidase (% mean control)					
Method		Home made			
Selections for Report		Statistical Results			
Hospital Name	Erasmus MC, Ee 2402	Parameter	Your Lab	Method	All Labs
Department	Dpt. of Clinical Genetics	n	1	19	19
Contact Person		Mean	1	1.34	1.21
Deadline	01-07-2006 23:59	Median	1	1.10	1.06
Unit	% mean control	SD		0.964	0.749

Scale Standard Deviations	Scale % mean control	
>3SD	> 3,46	 >3SD: 4 or 400 % of Mean? → deficient or normal?
2-3SD	2,71 - 3,46	
1.5 - 2.0SD	2,34 - 2,70	
1.0 - 1.5SD	1,96 - 2,33	
0.5 - 1.0SD	1,59 - 1,95	
0.0 - 0.5SD	1,21 - 1,58	
-0.5 - 0.0SD	0,84 - 1,20	 
-1.0 - -0.5SD	0,46 - 0,83	
-1.5 - -1.0SD	0,09 - 0,45	
-2 - -1.5SD	< 0,08	
-3 - -2SD		
<-3SD		

 Your lab  Home made

QA-pilot for LSD's

Gaucher fibroblasts, % of participants mean control

Cycle: 2006 Sample Nr: LE Fibroblast 2 Analyte: L11. beta-Glucosidase (% mean control) Change

LE Fibroblast 2					
L11. beta-Glucosidase (% mean control)					
Method		Home made			
Selections for Report		Statistical Results			
Hospital Name	Erasmus MC, Ee 2402	Parameter	Your Lab	Method	All Labs
Department	Dpt. of Clinical Genetics	n	1	19	19
Contact Person		Mean	1	1.34	1.21
Deadline	01-07-2006 23:59	Median	1	1.10	1.06
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>3SD	> 3,46	>3SD: 4 or 400 % of Mean? → deficient or normal?
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1.5 - 2.0SD	2,34 - 2,70	
1.0 - 1.5SD	1,96 - 2,33	
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0.0 - 0.5SD	1,21 - 1,58	
-0.5 - 0.0SD	0,84 - 1,20	
-1.0 - -0.5SD	0,46 - 0,83	
-1.5 - -1.0SD	0,09 - 0,45	
-2 - -1.5SD	< 0,08	
-3 - -2SD		
<-3SD		

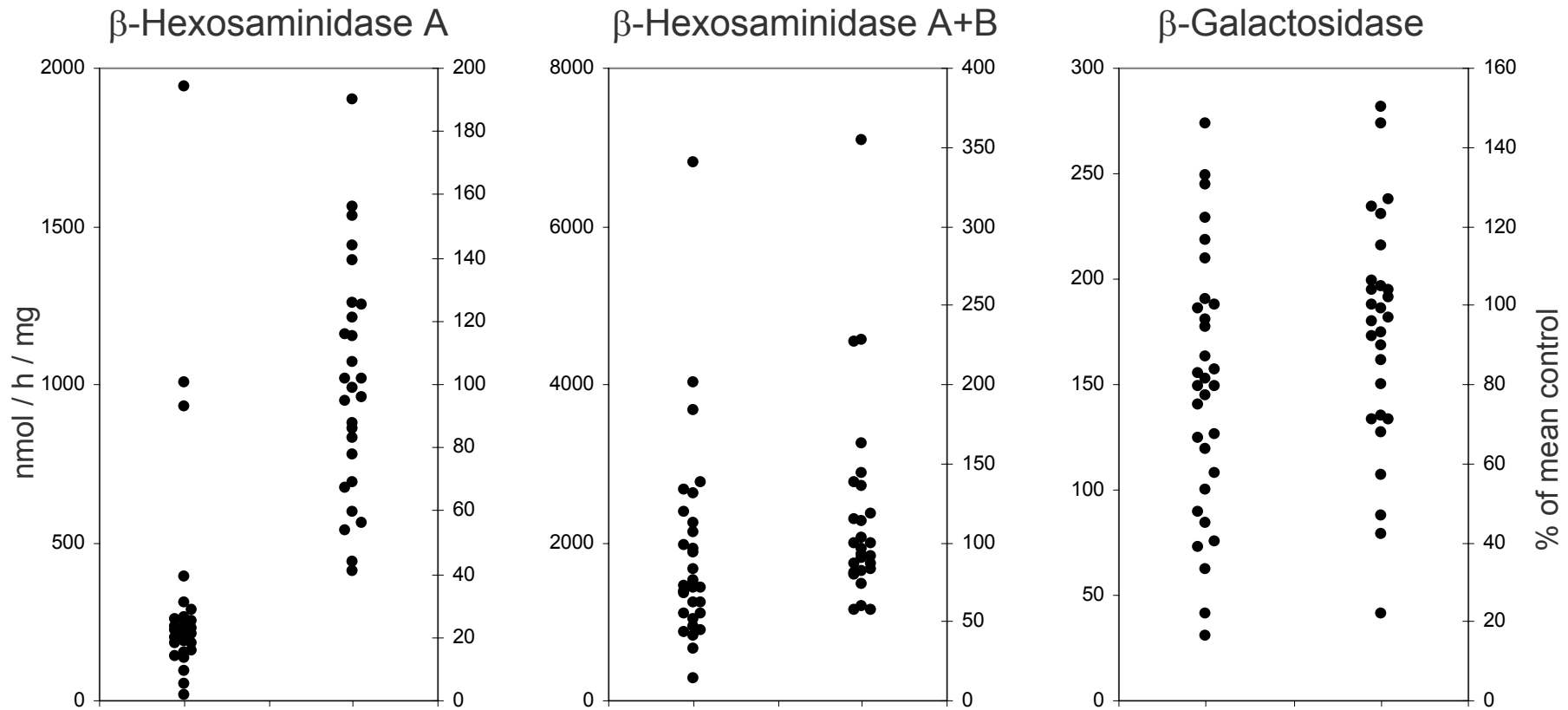
Range of 18 labs: 0.1 – 11 % of Mean

SD scores (% mean control) with cut-off not useful for enzyme deficiencies

Your lab Home made

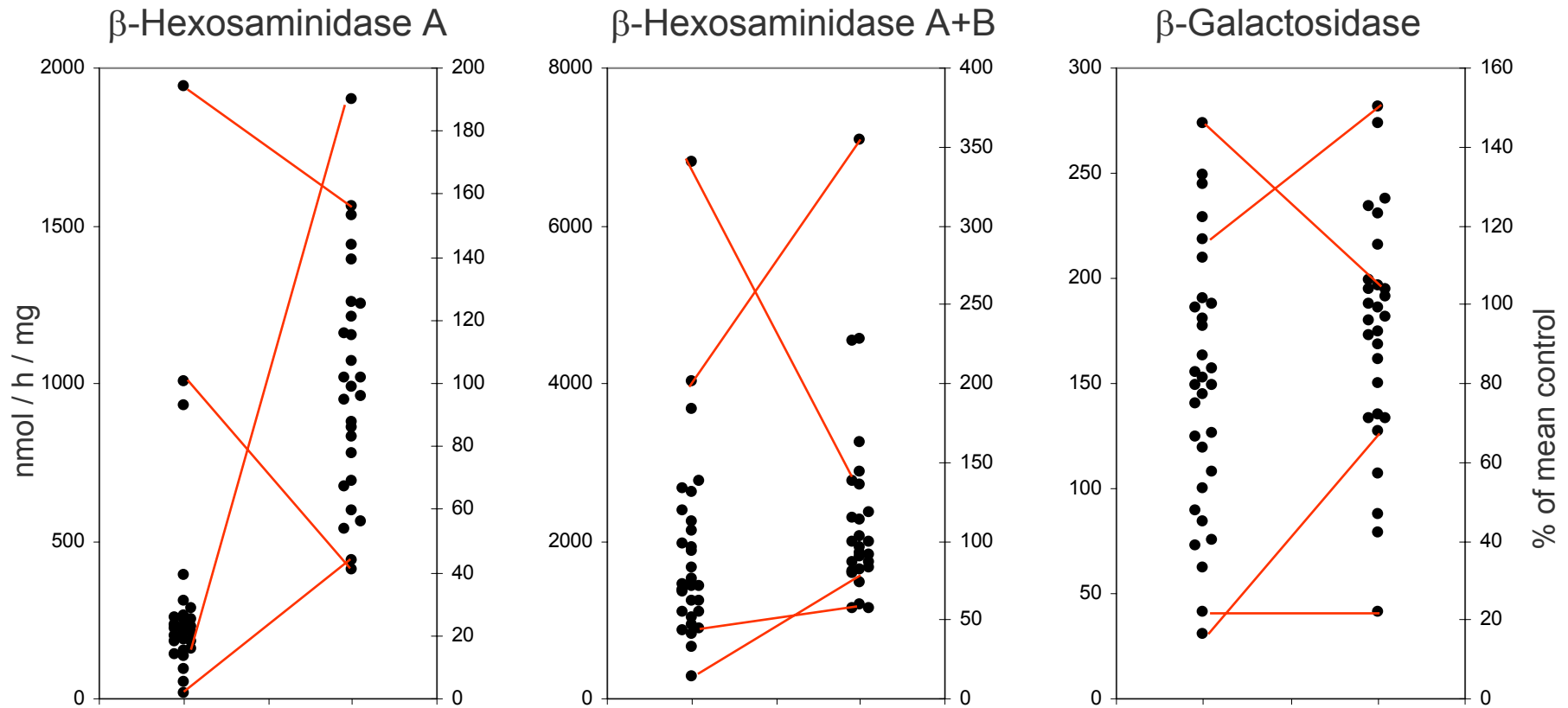
Control leukocytes

Specific enzyme activity (*left*) versus % of mean control (*right*)



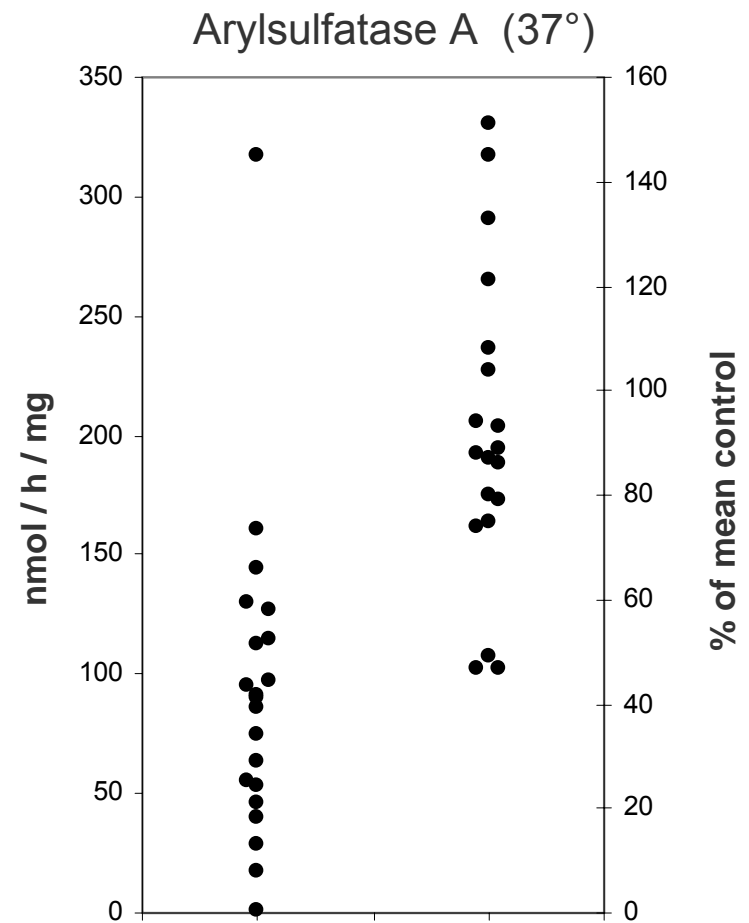
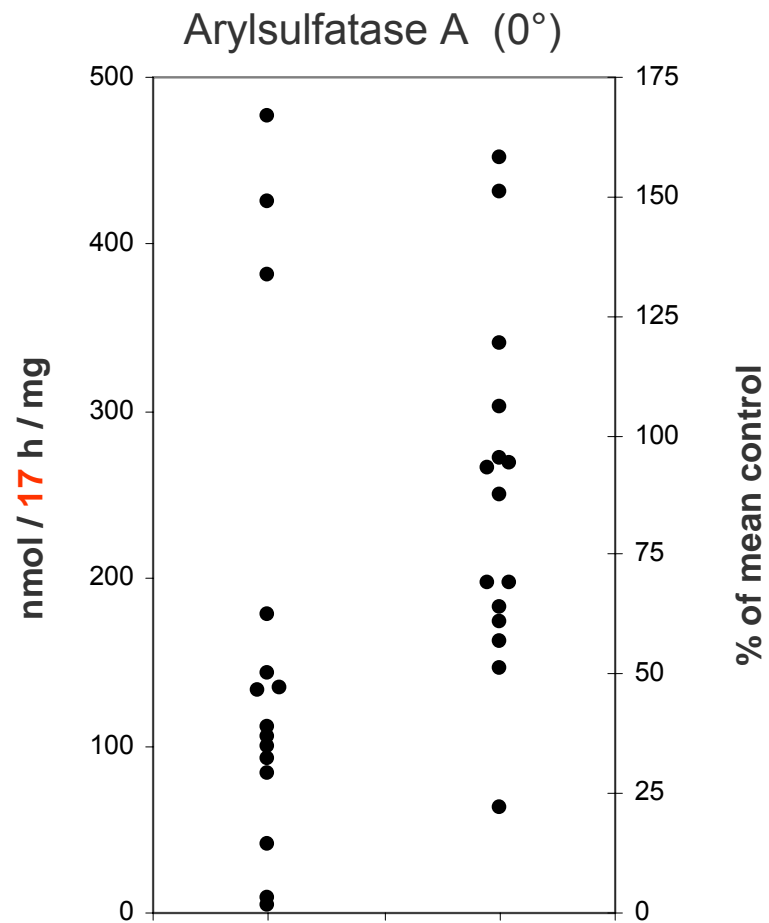
Control leukocytes

Specific enzyme activity (*left*) versus % of mean control (*right*)



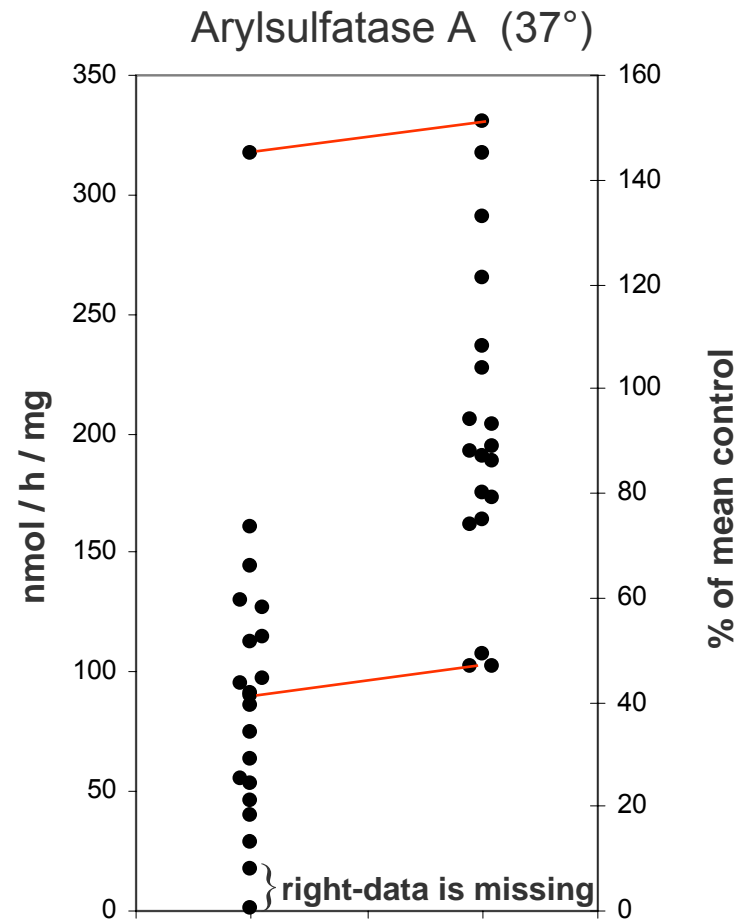
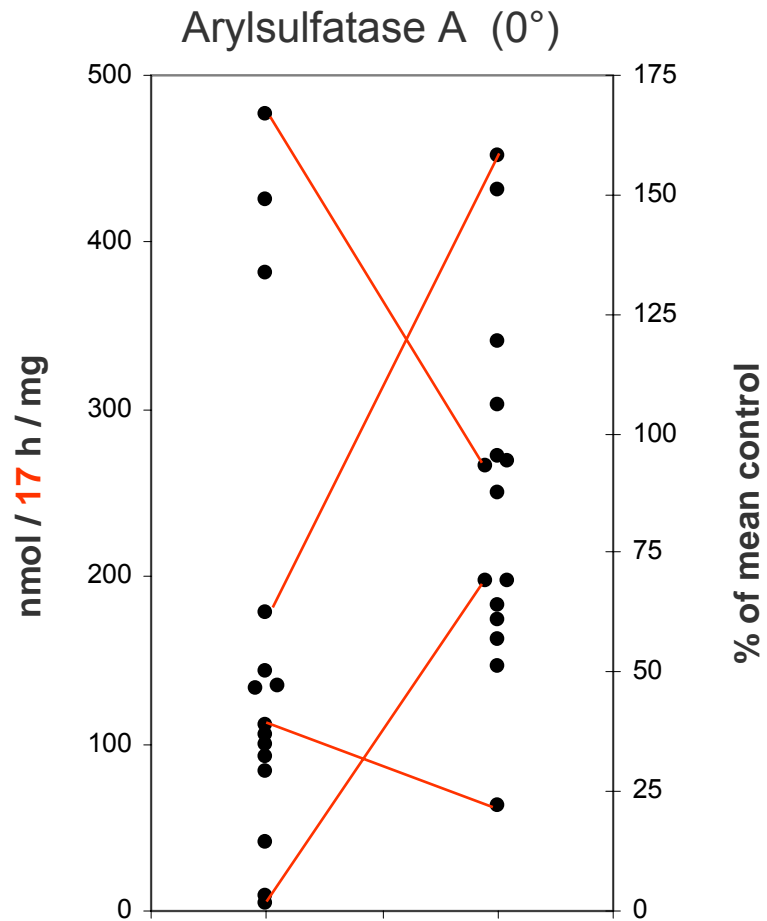
Control leukocytes

Specific enzyme activity (*left*) versus % of mean control (*right*)



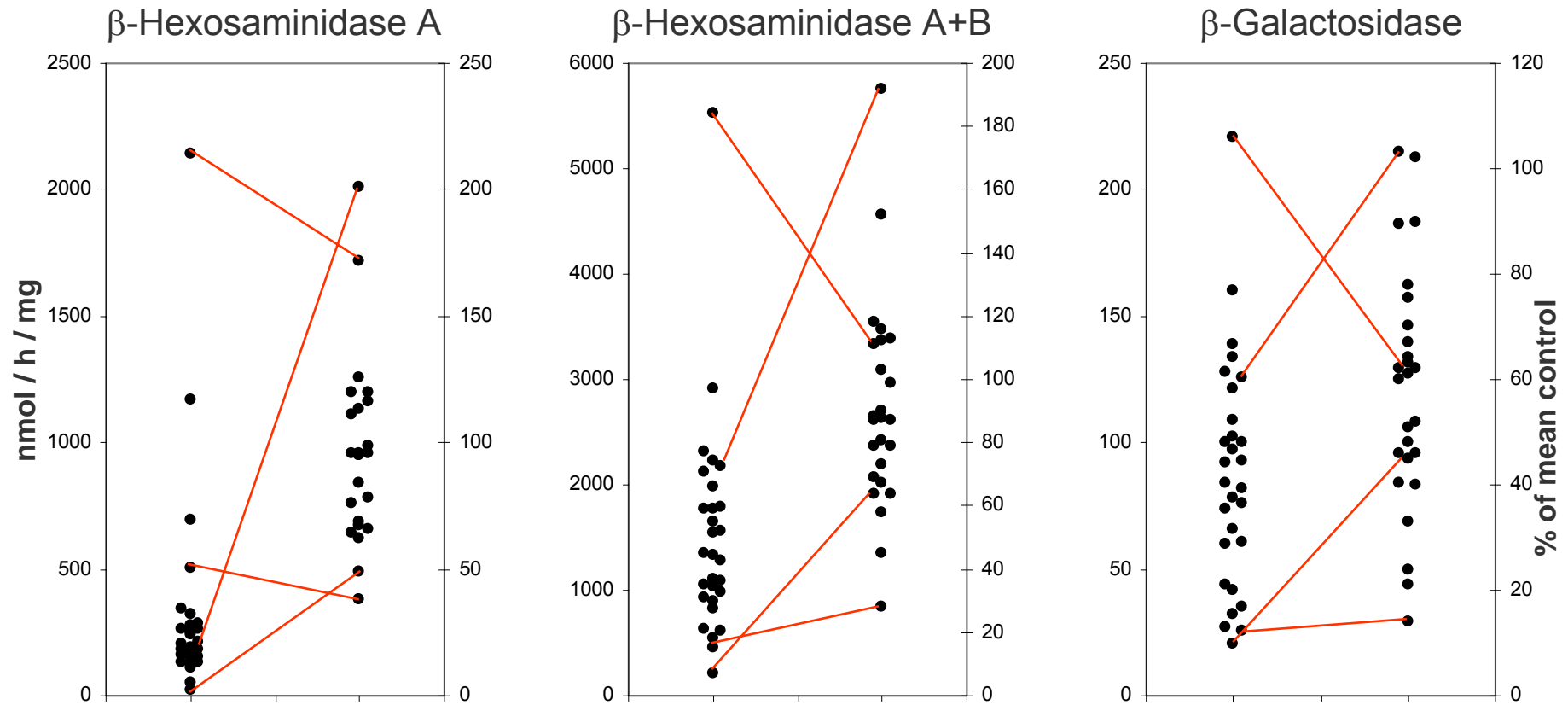
Control leukocytes

Specific enzyme activity (*left*) versus % of mean control (*right*)



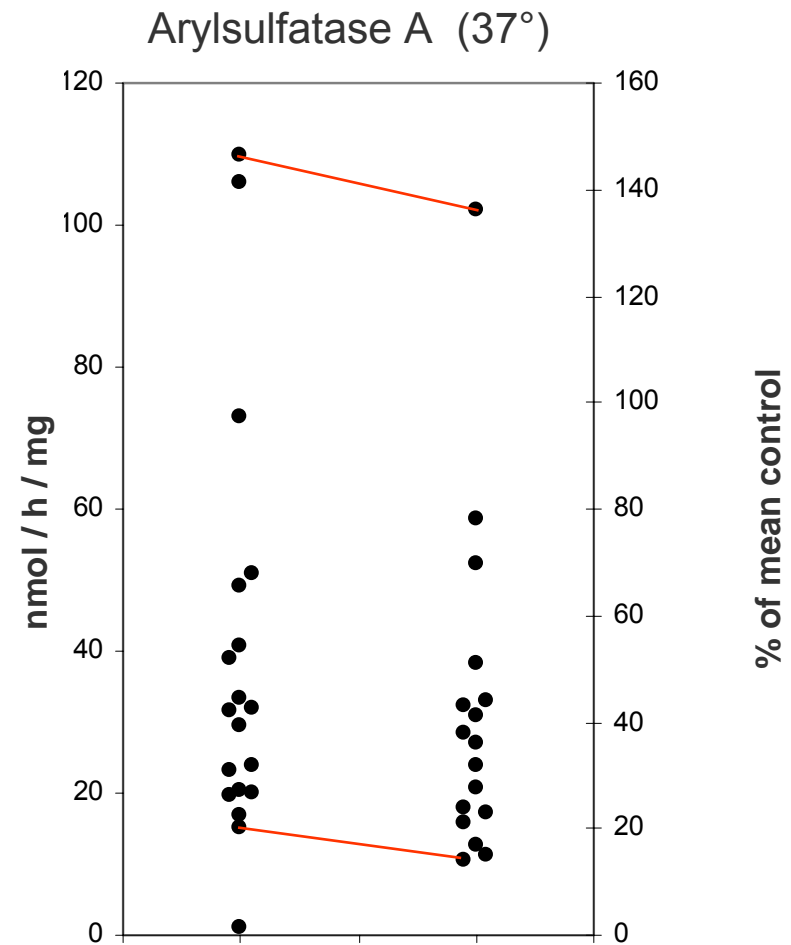
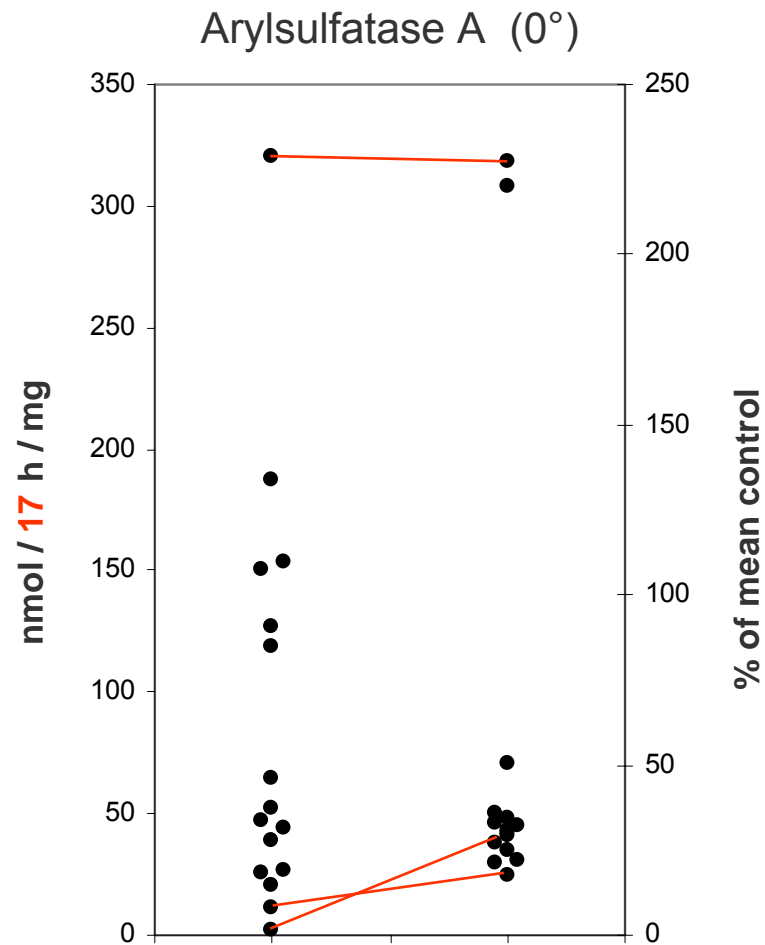
Control EBV lymphoblasts

Specific enzyme activity (*left*) versus % of mean control (*right*)



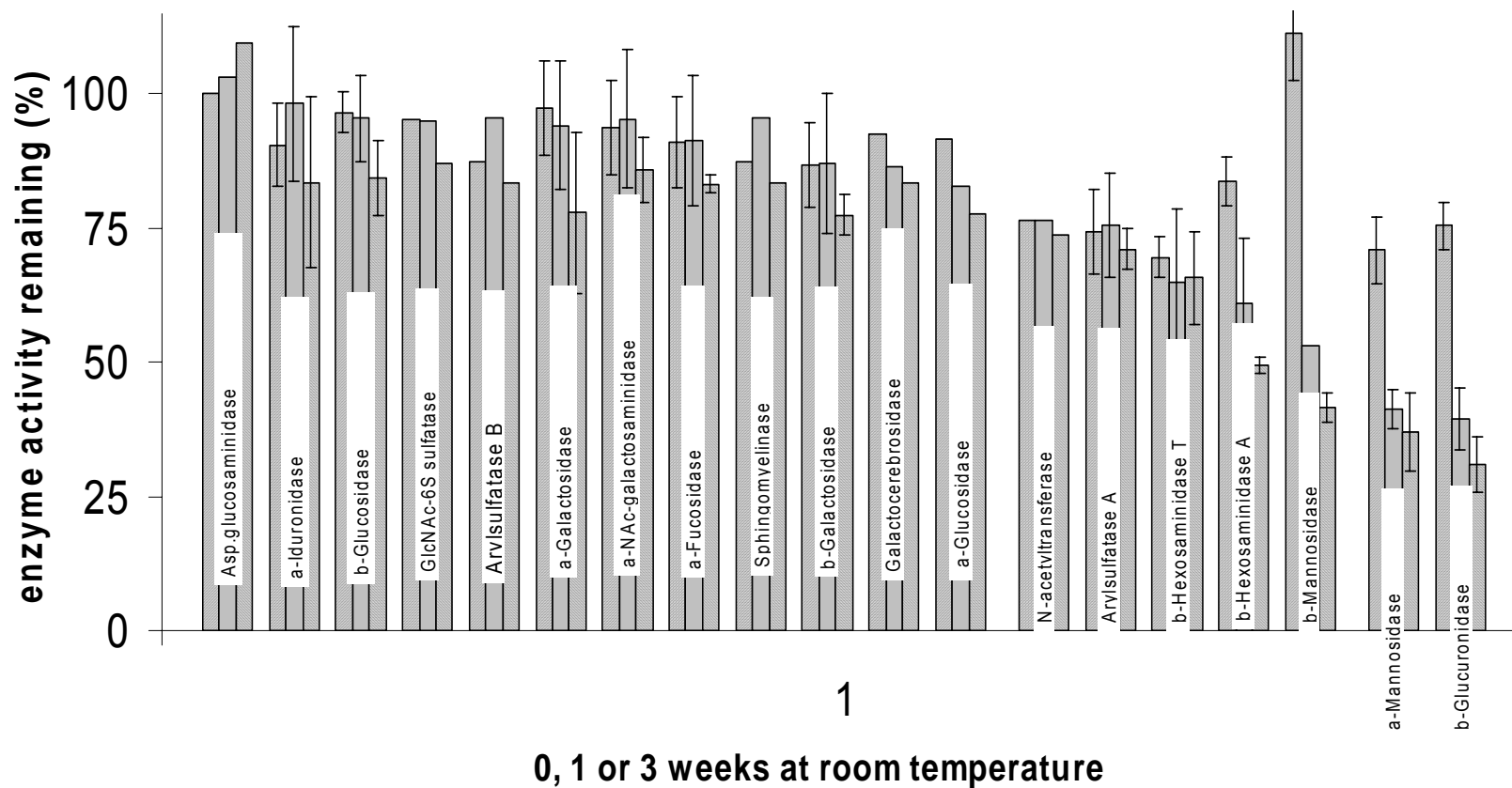
Control EBV lymphoblasts

Specific enzyme activity (*left*) versus % of mean control (*right*)



EBV lymphoblasts are suitable for
QA for enzyme analysis

Stability of 20 lysosomal enzymes in lyophilised leukocyte pellets (n=4)



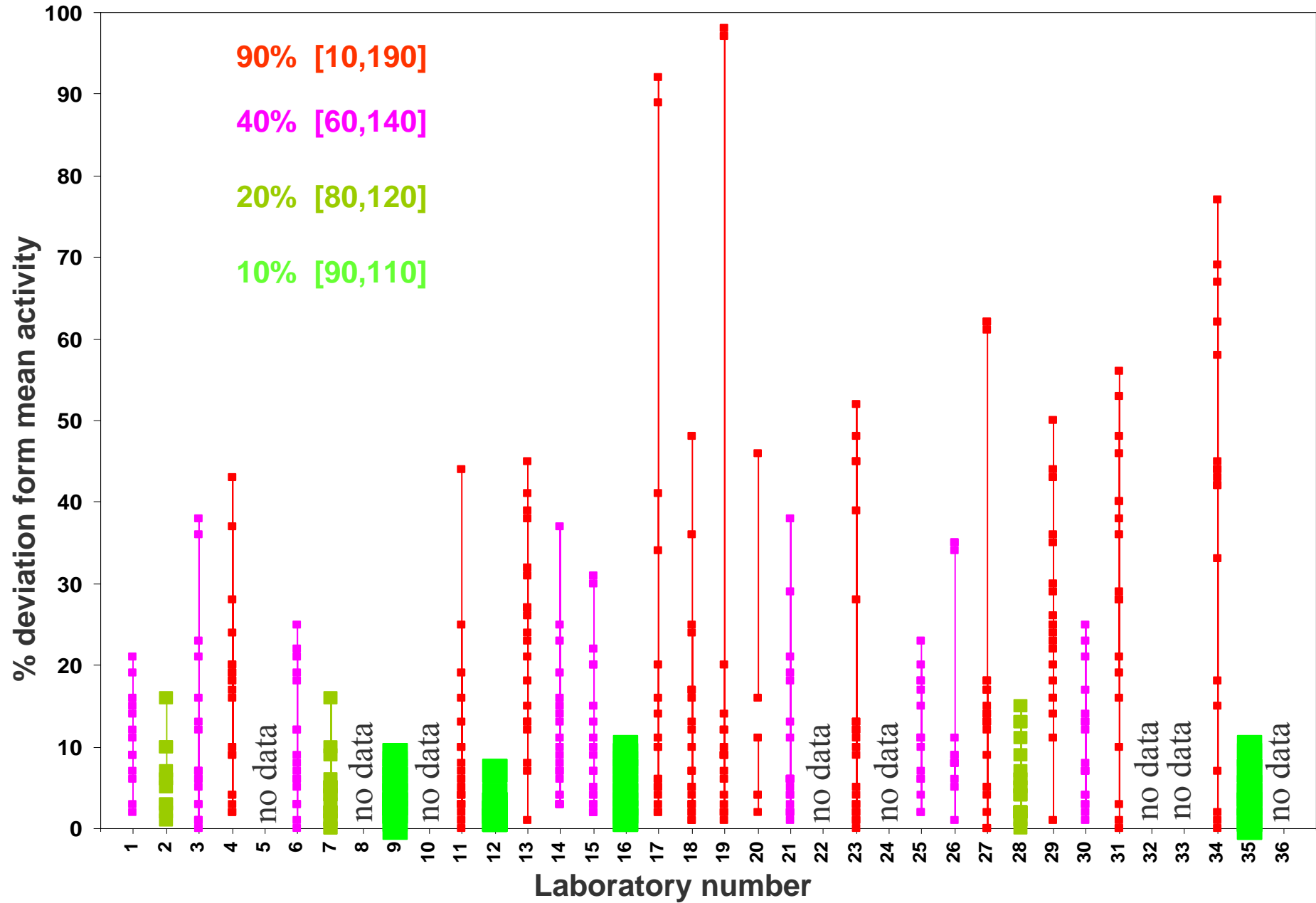
Reproducibility of enzyme analysis in duplicate leukocyte samples

Blind duplicate samples were analysed in 2 experiments

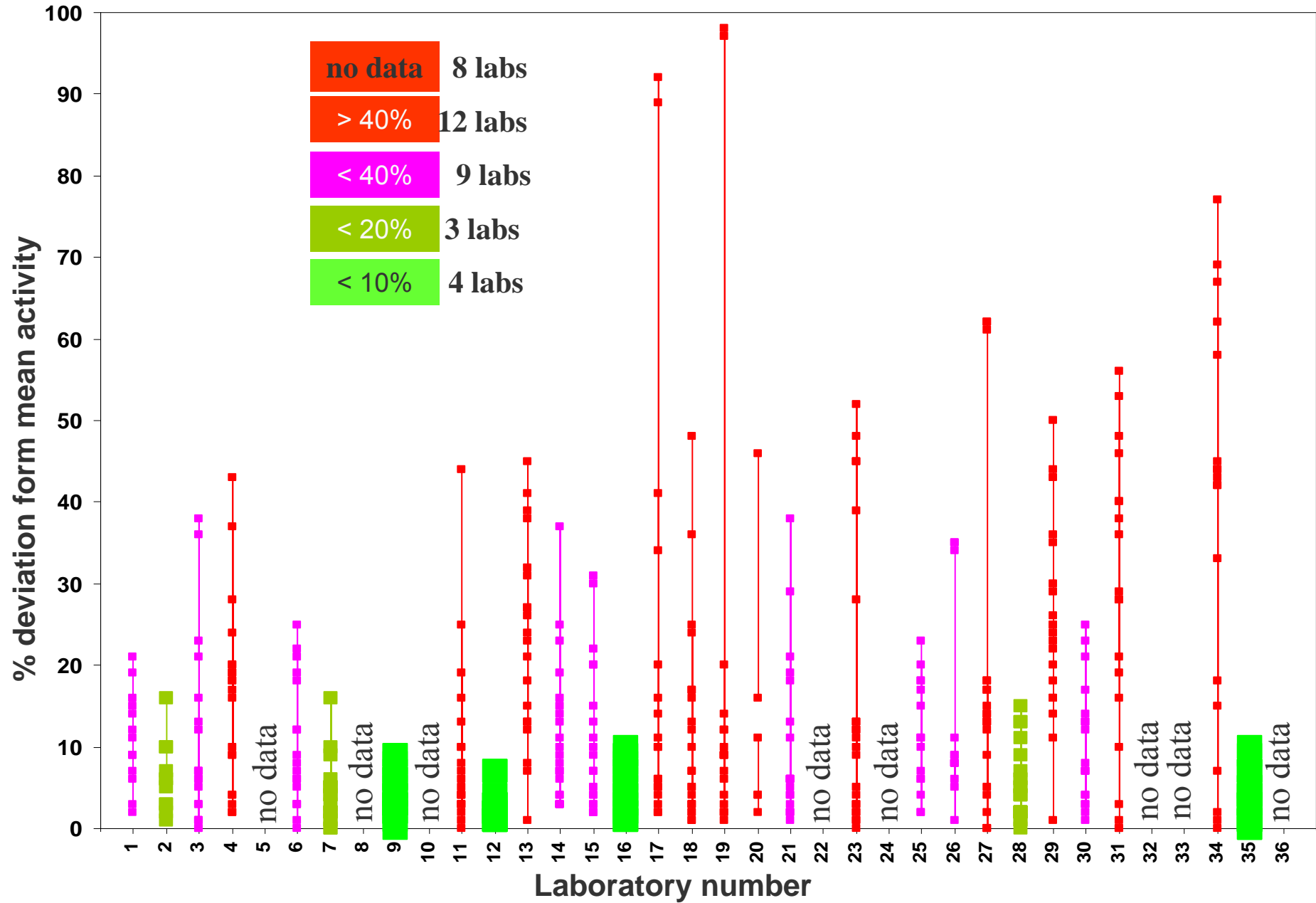
- 10 different enzymes / sample
- 2 couples of identical samples
- 20 matched pairs of enzyme activities / laboratory

$$\text{Variation} = \frac{\text{difference between 2 activities}}{\text{sum of 2 activities}}$$

Reproducibility, duplicate leukocyte samples



Reproducibility, duplicate leukocyte samples



Urgently needed:

QA for enzyme analysis
of LSD's

Proficiency testing

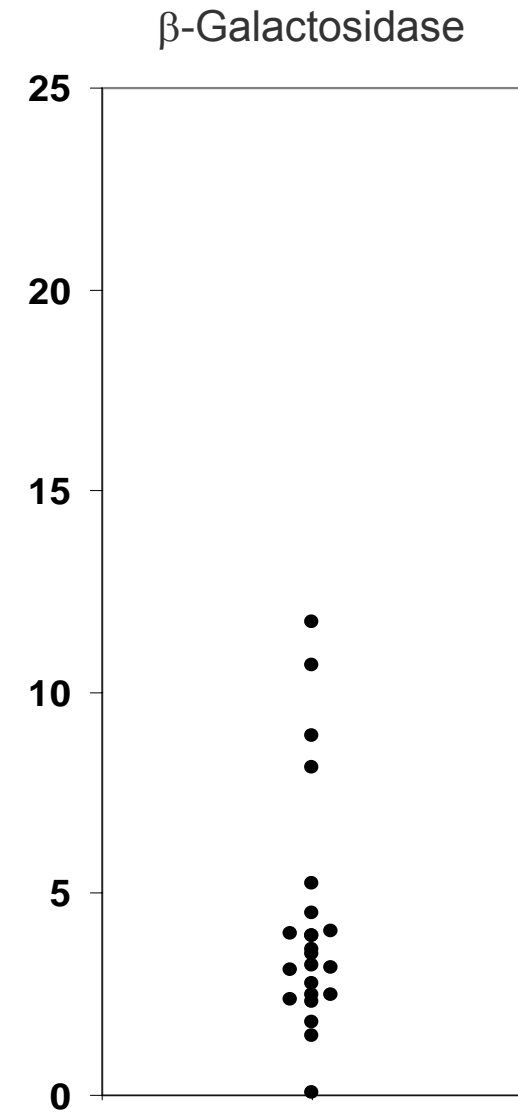
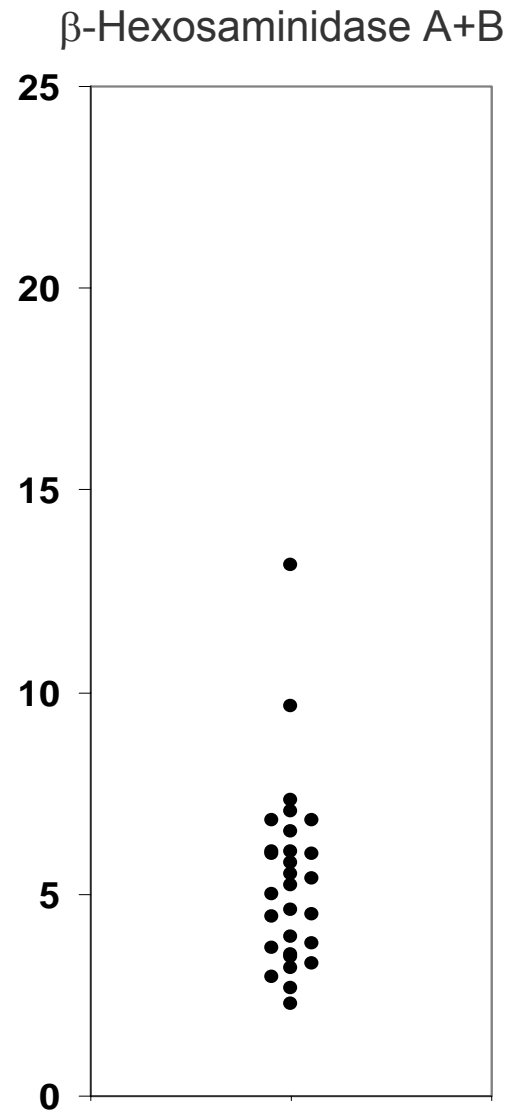
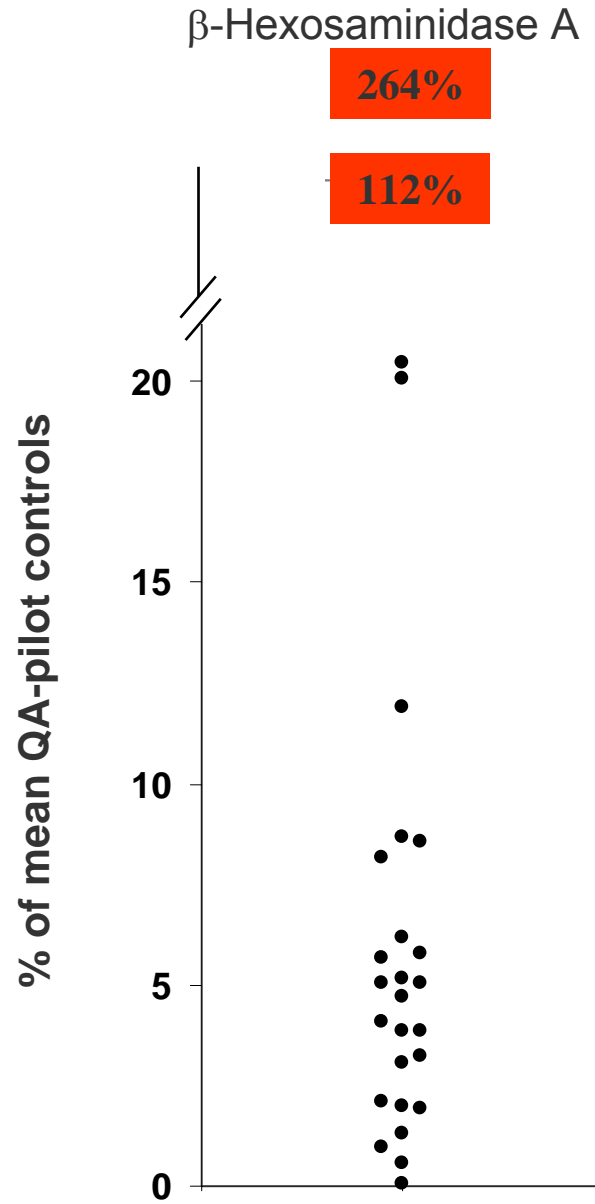
Lysosomal Storage Diseases

4 patient samples were included

- 3 EBV lymphoblasts
 - Tay-Sachs (β -hexosaminidase A)
 - Sandhoff (β -hexosaminidase A+B)
 - GM1-gangliosidosis (β -galactosidase)
- 1 fibroblast cell line
 - Gaucher type II (β -glucosidase)

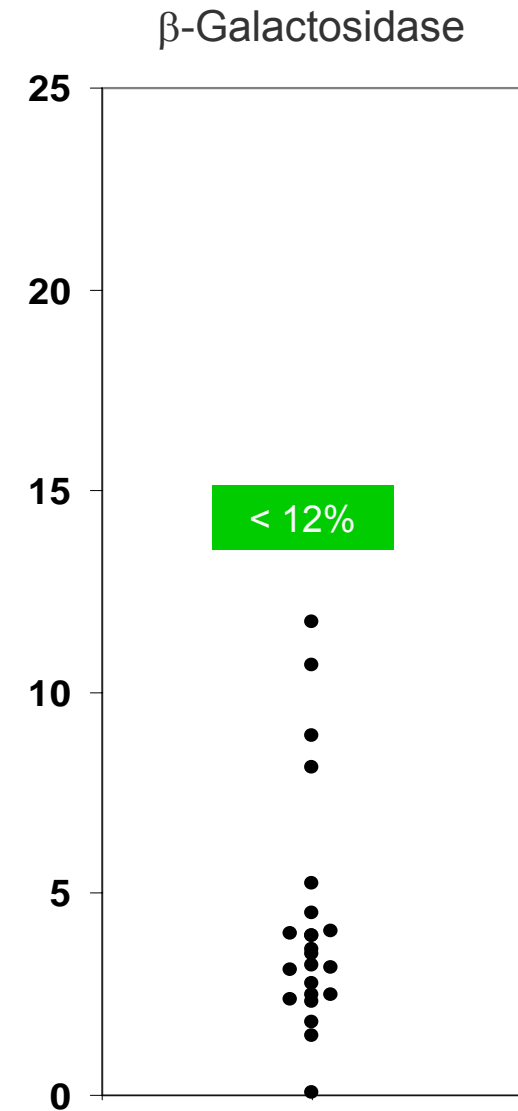
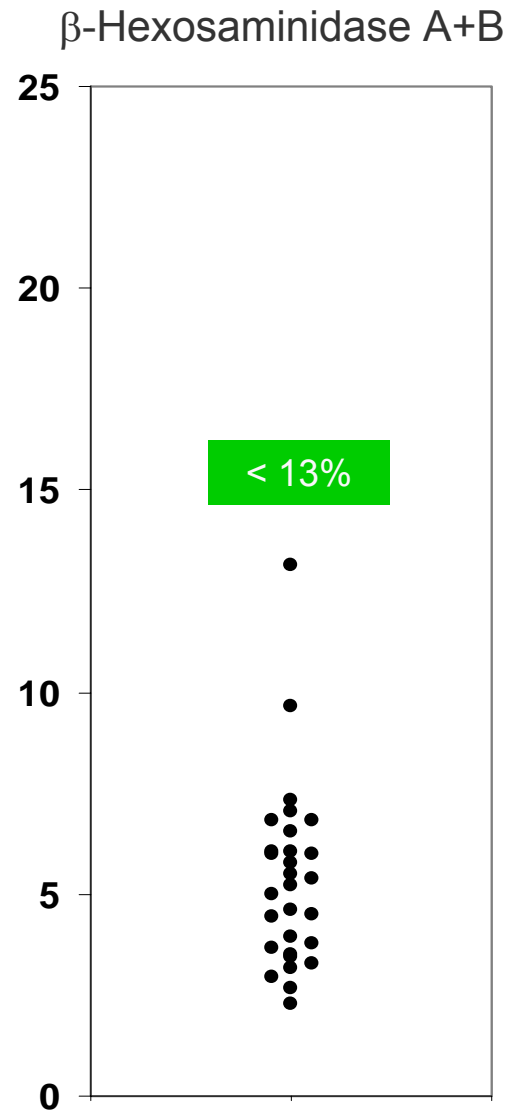
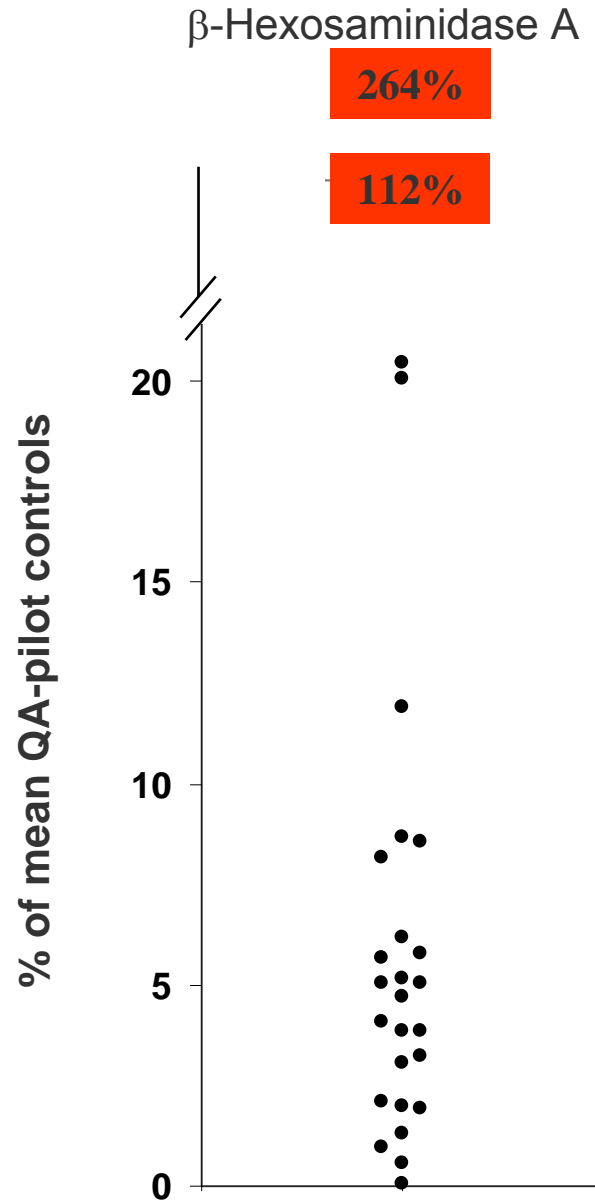
EBV lymphoblasts form patients

% of QA-pilot controls



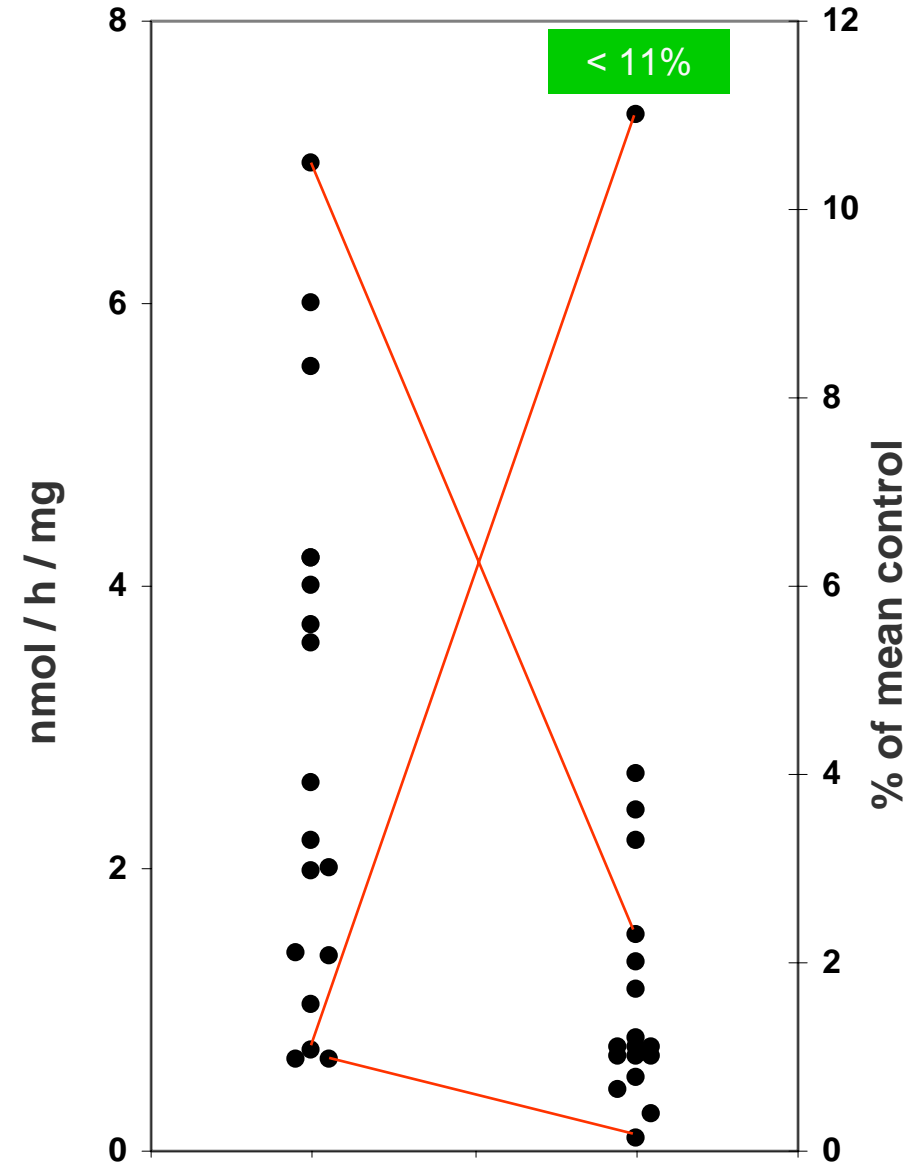
EBV lymphoblasts form patients

% of QA-pilot controls



Fibroblasts from Gaucher II patient

Normal β -glucosidase from QA-pilot:
4, 19, 113 – 460 nmol/h/mg (n=18)



QA for LSD's

What needs to be done

- Report results to participants
- Website adjustment for enzyme analysis
 - show all data, without cut-off (e.g. $> 3SD$)
 - show data on reproducibility of duplicate samples
 - show track records of *all* laboratories
- More information from participants
 - which enzyme assay? [substrate, pH]
 - which homogenisation procedure? [sonication, freeze-thaw]
 - enzyme assay in: [full-homogenate, supernatant]
- Increase compliance of participants
 - 10% of participants did not return any data
 - 21 - 41% of participants did not return all data

QA for LSD's

Plans for 2007 / 2008 and after

- **Towards a new ERNDIM activity: QA-for LSD**
 - Start regular ERNDIM programme in 2009
 - Prolong the pilot phase with 2 years (2007 and 2008).
 - How to obtain permanent support for making the samples?
 - Make samples for coming QA-pilots on larger scale
Leukocytes, EBV lymphoblasts, fibroblasts, DBS
 - Determine costs for participants for of regular ERNDIM QA-programme
no cost for participants in the pilot phase (estimated participants: 50)
 - Start with DBS enzyme analysis, collect blood from patients
 - Assist to improve poor performance.
- **QA pilots for 2007 / 2008**

Genzyme will financially support the coming 2 years.

 - February 2007
 - small pilot, leukocytes, EBV lymphoblasts (fibroblasts)
 - February 2008 :
 - big pilot, leukocytes, EBV lymphoblasts (fibroblasts)
one shipment, many samples, 4 independent experiment

Enduring QA-Programme for Enzyme Analysis for Lysosomal Storage Diseases

