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ERNDIMQA-LSD
EVALUATION SCHEME

External Quality Assessment
Enzyme Analysis for Lysosomal Storage Diseases



Pilots "large scale"

2006 (36)

2007 (46)

2008 (55)

2009 (59), DBS (27)

Otto van Diggelen

2010 (58)



Kees Schoonderwoerd(2011)



LYSOSOMAL DISORDERS

Sphingolipidosis

Gm1-gangliosidosis

Gm2-gangliosidosis

Fabry

Gaucher

Niemann-Pick A/B/C

MLD

Krabbe

CLN

Wolman

Oligosaccharidosis

Mucopolidosis

Fucosidosis

α -Mannosidosis

β -Mannosidosis

Aspartyl-glucosaminuria

Pompe

Schindler

Mucopolysaccharidosis

MPS-I

MPS-II

MPS-III A-D

MPS-IV A-B

MPS-VI

MPS-VII

What enzymes in the scheme?



DIAGNOSIS LYSOSOMAL DISORDERS

Diagnosis Sphingolipidosis

Clinical Suspicion+ Enzyme diagnostics+DNA

Diagnosis Oligosaccharidosis

Clinical Suspicion+Abnormal oligo-saccharides Urine+Enzyme diagnostics+DNA

Diagnosis MPS

Clinical Suspicion+Deviant MPS Urine + Enzyme diagnostics + DNA

Enzyme diagnostics

Leucocytes, Fibroblasts, DBS



ENZYME DIAGNOSTICS

Specificity Only one enzyme responsible for product formation (Matrix)

Sensitivity Enzyme measurement without prior purification

Stability Reproducibility (CV duplicate sample)

Accuracy Distinguish patient and control activities (Patient cell lines)

Challenge enzyme diagnostics

Distinguish patient enzyme activity from control and lowered enzyme activities



EQA for LSD's 2010

- **10 enzymes: 8 “conventional” samples**
 - EBV lympho's from LSD patients, 6 samples
 - At least one duplicate (EBV)
 - Preparation of 8 homogenates from EBV pellets
 - Freeze-drying, Shipping & Data Base managing
- Otto van Diggelen Scientific Advisor 2010
- Kees Schoonderwoerd Scientific Advisor for ERNDIM 2011



- **Problem with ECACC EBV-lympho's?**
 - low enzyme activities compared to leucocytes
 - 2011 some pellets not properly washed



ENZYMES 2010 PROFICIENCY TESTING EBV CELL LINES

Hurler/Scheie (MPS I) α -L-iduronidase EBV 2 + 7

Krabbe Galactocerebrosidase

Niemann Pick A/B Sphingomyelinase

Sanfilippo B (MPS IIIB) α -N-acetylglucosaminidase

Sandhoff (GM₂, β -Hexosaminidase A + B) EBV 3

Tay-Sachs (GM₂) β -Hexosaminidase A

MPS IVA, Galactose-6S sulphatase EBV 6

GM₁ (Morquio B) β -Galactosidase EBV 5

Mannosidosis β -mannosidase

Sly (MPS VII) β -Glucuronidase

Mucopolipidosis EBV 8

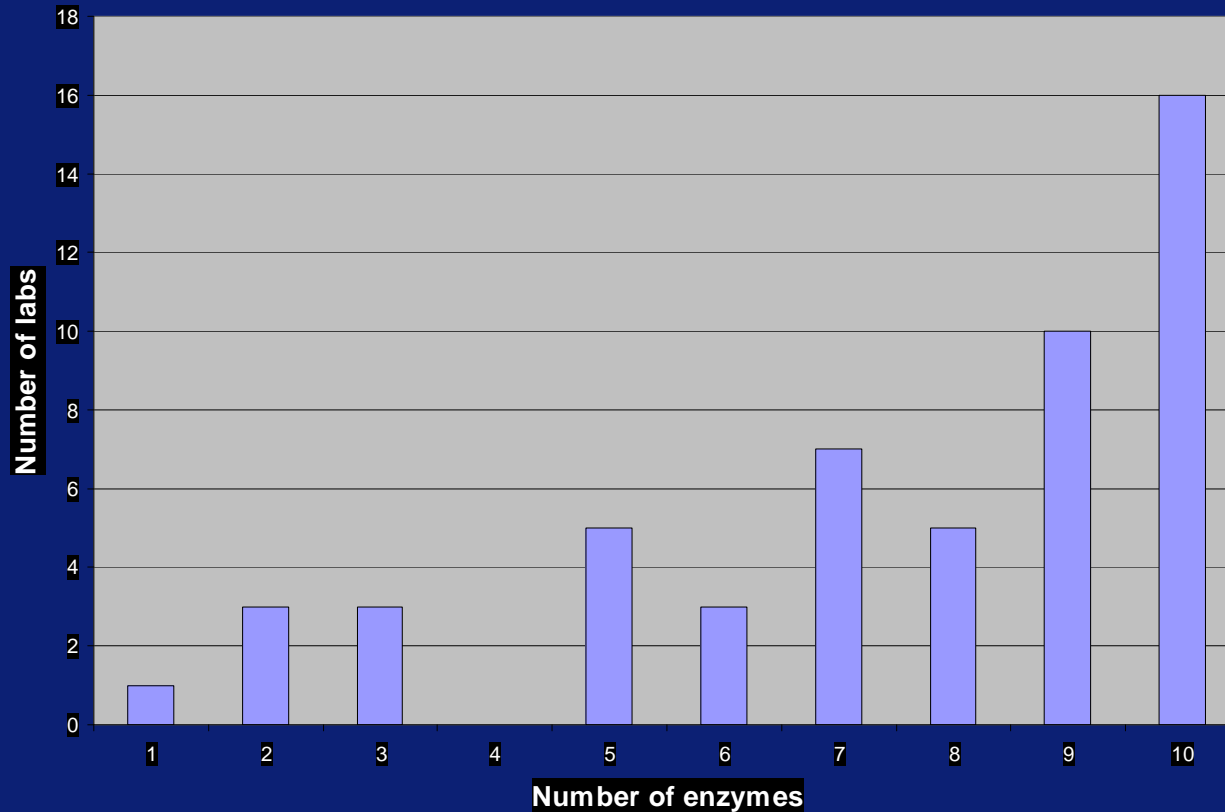
EBV nr 1 + 4 control EBV used for determination CV

Enzymes/Disorders: 5 Sphingolipidosis, 3 MPS, 2 Oligo



RESULTS 2010

NUMBER OF ENZYMES MEASURED



Reproducibility of protein/vial 2010 duplicate samples EBV

ERN042YIUF	?
ERN148DWP	?
ERN008KJIH	16
ERN398ZFN	3
ERN416BLXI	4
ERN405TDPA	11
ERN388QXE	11
ERN401HQ	0
ERN088PZ	2
ERN041WUSQ	2
ERN240UIY	73
ERN301RCP	?
ERN290BNO	9
ERN111HBX	9
ERN046BZXV	13
ERN108DYT	4
ERN043YWUS	2
ERN410TC	28
ERN005CMY	6
ERN403QAMB	0

ERN034RBNC	11
ERN141WPI	39
ERN036GRCQ	1
ERN119UOI	4
ERN342XFP	2
ERN407ITES	6
ERN414NYJX	8
ERN078EUQB	1
ERN210EUK	7
ERN408XHTE	4
ERN369RA	75
ERN243DRF	23
ERN066BYVS	28
ERN357NVD	7
ERN179IR	6
ERN140VOH	24
ERN413LWHV	11
ERN017GQA	14
ERN054NXH	0
ERN411EPAO	21

ERN417KVGU	30
ERN003FEDC	2
ERN158QIA	21
ERN418OZKY	30
ERN415GP	13
ERN395AKWH	
ERN077VFRC	5
ERN010WGS D	50
ERN316AJU	64
ERN284XJX	
ERN406SCOD	48
ERN194IZQ	19
ERN104ZUP	14
ERN218MWG	0,4

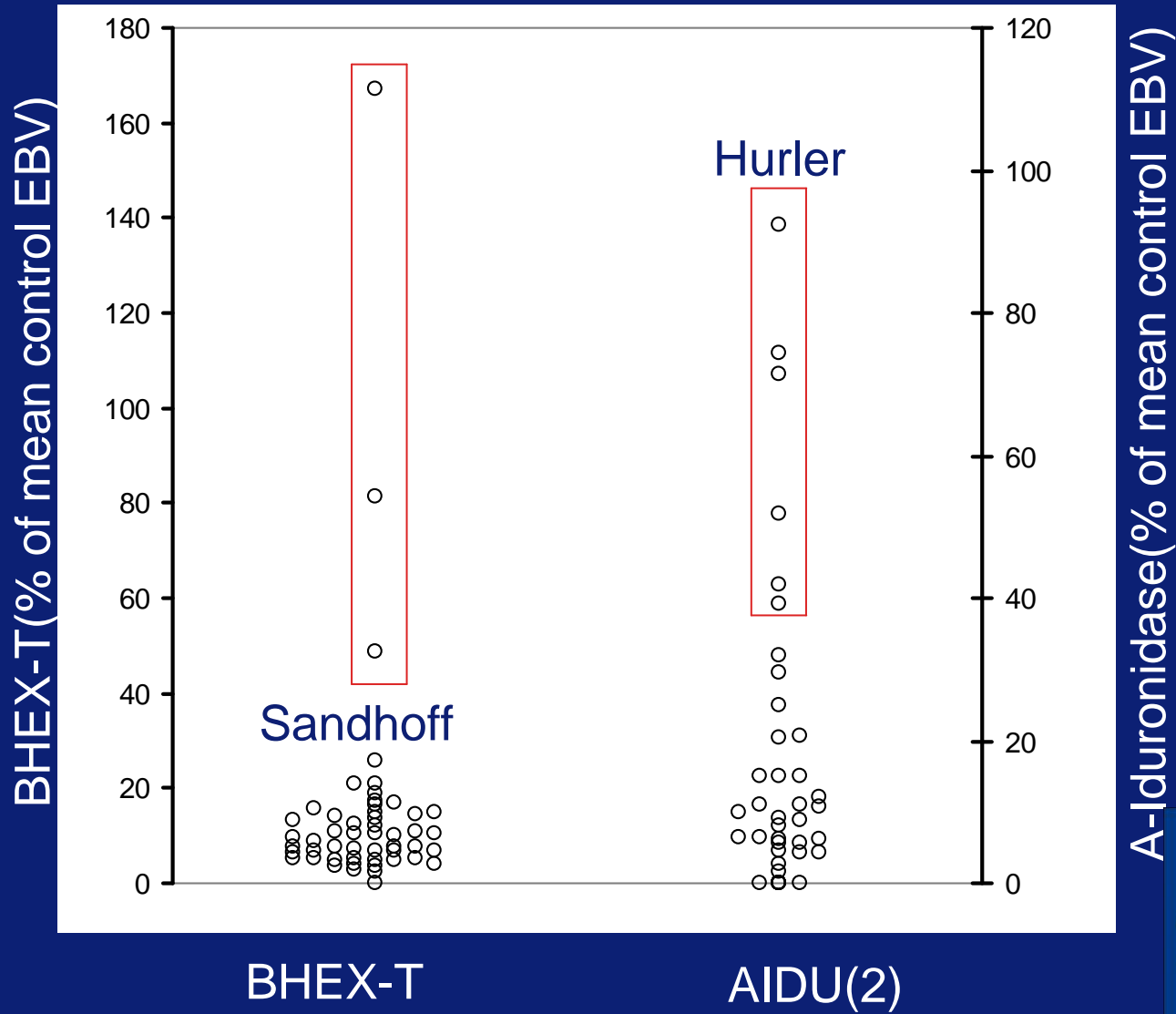
CV=
difference duplicate
samples/
mean value
duplicate samples
*100%

	2009	2010
No data	27%	9%
CV<25	59%	72%
CV>25	14%	19%



PROFICIENCY TESTING HURLER AND GM₂-GANGLIOSIDOSIS IN EBV LYMPHOCYTES 2010

Activity patient/
Activity ctrEBV
*100%



Reproducibility of α -L-iduronidase 2010 duplicate samples EBV

ERN042YIUF	127;d-
ERN148DWP	d-
ERN008KJIH	5
ERN398ZFN	16
ERN416BLXI	1;d-
ERN405TDPA	5
ERN388QXE	
ERN401HQ	0
ERN088PZ	22
ERN041WUSQ	
ERN240UIY	
ERN301RCP	
ERN290BNO	36;2d-
ERN111HBX	
ERN046BZXV	
ERN108DYT	5
ERN043YWUS	21;d-
ERN410TC	60
ERN005CMY	1
ERN403QAMB	0

ERN034RBNC	22
ERN141WPI	43
ERN036GRCQ	80
ERN119UOI	56;d-
ERN342XFP	57;d-
ERN407ITES	67
ERN414NYJX	3
ERN078EUQB	9
ERN210EUK	6
ERN408XHTE	50
ERN369RA	
ERN243DRF	12;d-
ERN066BYVS	
ERN357NVD	24
ERN179IR	0
ERN140VOH	56
ERN413LWHV	
ERN017GQA	39
ERN054NXH	200;d
ERN411EPAO	158

ERN417KVGU	17
ERN003FEDC	0
ERN158QIA	
ERN418OZKY	43;d
ERN415GP	54
ERN395AKWH	d-
ERN077VFRC	21
ERN010WGSD	77;d
ERN316AJU	67
ERN284XJX	d-
ERN406SCOD	40
ERN194IZQ	50
ERN104ZUP	200
ERN218MWG	13

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	24%
CV<25	39%
CV>25	37%



Reproducibility of β -Hexosaminidase A+B 2010 duplicate samples EBV

ERN042YIUF	34
ERN148DWP	
ERN008KJIH	9
ERN398ZFN	32
ERN416BLXI	0,3
ERN405TDPA	8
ERN388QXE	3
ERN401HQ	21
ERN088PZ	15
ERN041WUSQ	39
ERN240UIY	8
ERN301RCP	
ERN290BNO	28
ERN111HBX	26
ERN046BZXV	d-
ERN108DYT	18
ERN043YWUS	76
ERN410TC	24
ERN005CMY	0,4
ERN403QAMB	13

ERN034RBNC	24
ERN141WPI	
ERN036GRCQ	12
ERN119UOI	1
ERN342XFP	4
ERN407ITES	2
ERN414NYJX	30
ERN078EUQB	5
ERN210EUK	4
ERN408XHTE	62
ERN369RA	119;d-
ERN243DRF	4
ERN066BYVS	41
ERN357NVD	19
ERN179IR	15
ERN140VOH	16
ERN413LWHV	2
ERN017GQA	100
ERN054NXH	9
ERN411EPAO	40

ERN417KVGU	16
ERN003FEDC	1
ERN158QIA	41
ERN418OZKY	7
ERN415GP	123
ERN395AKWH	
ERN077VFRC	1
ERN010WGSD	133;d-
ERN316AJU	45
ERN284XJX	
ERN406SCOD	55
ERN194IZQ	28
ERN104ZUP	
ERN218MWG	4

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	13%
CV<25	54%
CV>25	33%



Reproducibility of β -Galactosidase 2010 duplicate samples EBV

ERN042YIUF	11;d-
ERN148DWP	
ERN008KJIH	176
ERN398ZFN	75
ERN416BLXI	5
ERN405TDPA	14
ERN388QXE	24
ERN401HQ	0
ERN088PZ	32
ERN041WUSQ	29
ERN240UIY	30
ERN301RCP	?
ERN290BNO	58
ERN111HBX	17
ERN046BZXV	d-
ERN108DYT	8
ERN043YWUS	31
ERN410TC	33; d-
ERN005CMY	6
ERN403QAMB	17

ERN034RBNC	50
ERN141WPI	23
ERN036GRCQ	40
ERN119UOI	41;d-
ERN342XFP	4
ERN407ITES	48
ERN414NYJX	6
ERN078EUQB	1
ERN210EUK	7
ERN408XHTE	2
ERN369RA	88
ERN243DRF	8
ERN066BYVS	1
ERN357NVD	33
ERN179IR	15
ERN140VOH	12
ERN413LWHV	1;d-
ERN017GQA	
ERN054NXH	14
ERN411EPAO	27

ERN417KVGU	8;d+
ERN003FEDC	17
ERN158QIA	
ERN418OZKY	14
ERN415GP	36
ERN395AKWH	
ERN077VFRC	24
ERN010WGSD	0
ERN316AJU	91
ERN284XJX	
ERN406SCOD	130
ERN194IZQ	4
ERN104ZUP	20;d-
ERN218MWG	5

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	13%
CV<25	54%
CV>25	33%



Reproducibility of Galactose-6S-sulfatase 2010 duplicate samples EBV

ERN042YIUF	d-
ERN148DWP	
ERN008KJIH	18
ERN398ZFN	16
ERN416BLXI	
ERN405TDPA	24
ERN388QXE	
ERN401HQ	11
ERN088PZ	25
ERN041WUSQ	
ERN240UIY	
ERN301RCP	
ERN290BNO	172;2d+
ERN111HBX	
ERN046BZXV	
ERN108DYT	
ERN043YWUS	13; d-
ERN410TC	
ERN005CMY	2
ERN403QAMB	86

ERN034RBNC	
ERN141WPI	
ERN036GRCQ	
ERN119UOI	
ERN342XFP	
ERN407ITES	27
ERN414NYJX	13
ERN078EUQB	0
ERN210EUK	118
ERN408XHTE	10;d+
ERN369RA	191;2d+
ERN243DRF	67
ERN066BYVS	
ERN357NVD	0;d-
ERN179IR	0;d-
ERN140VOH	17;d-
ERN413LWHV	
ERN017GQA	8
ERN054NXH	93
ERN411EPAO	67

ERN417KVGU	133
ERN003FEDC	
ERN158QIA	
ERN418OZKY	11;d-
ERN415GP	82
ERN395AKWH	
ERN077VFRC	11
ERN010WGSD	
ERN316AJU	
ERN284XJX	
ERN406SCOD	
ERN194IZQ	68;d-
ERN104ZUP	
ERN218MWG	13

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	48%
CV<25	31%
CV>25	20%



Reproducibility of β -Glucuronidase 2010 duplicate samples EBV

ERN042YIUF	8
ERN148DWP	
ERN008KJIH	28
ERN398ZFN	64
ERN416BLXI	0
ERN405TDPA	22
ERN388QXE	
ERN401HQ	150
ERN088PZ	11
ERN041WUSQ	62
ERN240UIY	8
ERN301RCP	
ERN290BNO	23
ERN111HBX	0,5
ERN046BZXV	
ERN108DYT	19
ERN043YWUS	12
ERN410TC	22
ERN005CMY	9
ERN403QAMB	11

ERN034RBNC	9
ERN141WPI	5
ERN036GRCQ	4
ERN119UOI	
ERN342XFP	3
ERN407ITES	3
ERN414NYJX	27
ERN078EUQB	2
ERN210EUK	35
ERN408XHTE	3
ERN369RA	53
ERN243DRF	30
ERN066BYVS	46
ERN357NVD	14
ERN179IR	1
ERN140VOH	69
ERN413LWHV	
ERN017GQA	9
ERN054NXH	30
ERN411EPAO	30

ERN417KVGU	67
ERN003FEDC	3
ERN158QIA	2
ERN418OZKY	43
ERN415GP	20
ERN395AKWH	
ERN077VFRC	16
ERN010WGSD	
ERN316AJU	25
ERN284XJX	
ERN406SCOD	125
ERN194IZQ	10
ERN104ZUP	
ERN218MWG	4

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	19%
CV<25	54%
CV>25	28%



Reproducibility of β -Hexosaminidase A 2010 duplicate samples EBV

ERN042YIUF	15
ERN148DWP	
ERN008KJIH	19
ERN398ZFN	34
ERN416BLXI	0,4
ERN405TDPA	18
ERN388QXE	32
ERN401HQ	8
ERN088PZ	18
ERN041WUSQ	48
ERN240UIY	20
ERN301RCP	
ERN290BNO	133;d+
ERN111HBX	
ERN046BZXV	?
ERN108DYT	161;3d+
ERN043YWUS	7
ERN410TC	16
ERN005CMY	11
ERN403QAMB	22

ERN034RBNC	22
ERN141WPI	10
ERN036GRCQ	8
ERN119UOI	31
ERN342XFP	6
ERN407ITES	33
ERN414NYJX	30
ERN078EUQB	9
ERN210EUK	35
ERN408XHTE	68
ERN369RA	
ERN243DRF	24
ERN066BYVS	13
ERN357NVD	17
ERN179IR	22
ERN140VOH	21
ERN413LWHV	3
ERN017GQA	16
ERN054NXH	49
ERN411EPAO	40

ERN417KVGU	9
ERN003FEDC	0
ERN158QIA	19
ERN418OZKY	19
ERN415GP	79
ERN395AKWH	3d+
ERN077VFRC	13
ERN010WGSD	129
ERN316AJU	48
ERN284XJX	
ERN406SCOD	44
ERN194IZQ	12
ERN104ZUP	8
ERN218MWG	3

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	13%
CV<25	59%
CV>25	28%



Reproducibility of Galactocerebrosidase 2010 duplicate samples EBV

ERN042YIUF	d+?
ERN148DWP	
ERN008KJIH	64
ERN398ZFN	31
ERN416BLXI	167;d+
ERN405TDPA	20
ERN388QXE	
ERN401HQ	58
ERN088PZ	200;3d+?
ERN041WUSQ	
ERN240UIY	
ERN301RCP	
ERN290BNO	47
ERN111HBX	
ERN046BZXV	?
ERN108DYT	
ERN043YWUS	
ERN410TC	143
ERN005CMY	5
ERN403QAMB	5

ERN034RBNC	
ERN141WPI	21
ERN036GRCQ	150
ERN119UOI	143
ERN342XFP	
ERN407ITES	
ERN414NYJX	19
ERN078EUQB	22
ERN210EUK	
ERN408XHTE	10;d+
ERN369RA	
ERN243DRF	37
ERN066BYVS	26
ERN357NVD	28
ERN179IR	200;2d+?
ERN140VOH	8
ERN413LWHV	
ERN017GQA	60
ERN054NXH	
ERN411EPAO	71

ERN417KVGU	49
ERN003FEDC	15
ERN158QIA	24
ERN418OZKY	33
ERN415GP	
ERN395AKWH	
ERN077VFRC	17
ERN010WGSD	
ERN316AJU	d+?
ERN284XJX	
ERN406SCOD	
ERN194IZQ	52
ERN104ZUP	
ERN218MWG	

	2010
No data	48%
CV<25	19%
CV>25	35%

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV



Reproducibility of Sphingomyelinase 2010 duplicate samples EBV

ERN042YIUF	6
ERN148DWP	
ERN008KJIH	175;d+
ERN398ZFN	13
ERN416BLXI	174;d+
ERN405TDPA	0
ERN388QXE	
ERN401HQ	4
ERN088PZ	0
ERN041WUSQ	
ERN240UIY	39
ERN301RCP	
ERN290BNO	182;3d+
ERN111HBX	
ERN046BZXV	
ERN108DYT	
ERN043YWUS	10
ERN410TC	41
ERN005CMY	6
ERN403QAMB	

ERN034RBNC	14
ERN141WPI	
ERN036GRCQ	7
ERN119UOI	
ERN342XFP	13
ERN407ITES	
ERN414NYJX	35
ERN078EUQB	1
ERN210EUK	23
ERN408XHTE	32
ERN369RA	
ERN243DRF	
ERN066BYVS	27
ERN357NVD	
ERN179IR	
ERN140VOH	22
ERN413LWHV	
ERN017GQA	43
ERN054NXH	49
ERN411EPAO	29

ERN417KVGU	4
ERN003FEDC	3
ERN158QIA	187; 3d+
ERN418OZKY	23
ERN415GP	25
ERN395AKWH	
ERN077VFRC	7
ERN010WGSD	
ERN316AJU	
ERN284XJX	
ERN406SCOD	61
ERN194IZQ	13
ERN104ZUP	48
ERN218MWG	

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV

	2010
No data	39%
CV<25	35%
CV>25	26%



Reproducibility of β -mannosidase 2010 duplicate samples EBV

ERN042YIUF	3
ERN148DWP	
ERN008KJIH	0
ERN398ZFN	40
ERN416BLXI	
ERN405TDPA	42
ERN388QXE	
ERN401HQ	3
ERN088PZ	19
ERN041WUSQ	39
ERN240UIY	0
ERN301RCP	
ERN290BNO	70
ERN111HBX	?
ERN046BZXV	
ERN108DYT	17
ERN043YWUS	8
ERN410TC	
ERN005CMY	4
ERN403QAMB	3

ERN034RBNC	18
ERN141WPI	22
ERN036GRCQ	4
ERN119UOI	
ERN342XFP	
ERN407ITES	1
ERN414NYJX	12
ERN078EUQB	3
ERN210EUK	6
ERN408XHTE	4
ERN369RA	
ERN243DRF	12
ERN066BYVS	4
ERN357NVD	27
ERN179IR	15
ERN140VOH	33
ERN413LWHV	
ERN017GQA	27
ERN054NXH	3
ERN411EPAO	43

ERN417KVGU	109
ERN003FEDC	23
ERN158QIA	
ERN418OZKY	26
ERN415GP	63
ERN395AKWH	
ERN077VFRC	13
ERN010WGSD	46
ERN316AJU	32
ERN284XJX	
ERN406SCOD	111
ERN194IZQ	11
ERN104ZUP	
ERN218MWG	1

	2010
No data	26%
CV<25	46%
CV>25	28%

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV



Reproducibility of α -N-acetylglucosaminidase duplicate samples EBV

ERN042YIUF	
ERN148DWP	
ERN008KJIH	3d+
ERN398ZFN	44
ERN416BLXI	4
ERN405TDPA	10
ERN388QXE	
ERN401HQ	11
ERN088PZ	32
ERN041WUSQ	
ERN240UIY	
ERN301RCP	
ERN290BNO	30
ERN111HBX	
ERN046BZXV	
ERN108DYT	
ERN043YWUS	15
ERN410TC	
ERN005CMY	8
ERN403QAMB	40

ERN034RBNC	
ERN141WPI	4
ERN036GRCQ	8
ERN119UOI	
ERN342XFP	15
ERN407ITES	52
ERN414NYJX	14
ERN078EUQB	7
ERN210EUK	17
ERN408XHTE	
ERN369RA	79
ERN243DRF	24
ERN066BYVS	
ERN357NVD	
ERN179IR	
ERN140VOH	174; 2d+
ERN413LWHV	
ERN017GQA	177; 2d+
ERN054NXH	15
ERN411EPAO	44

ERN417KVGU	13
ERN003FEDC	0
ERN158QIA	200; 5d+?
ERN418OZKY	2
ERN415GP	10
ERN395AKWH	
ERN077VFRC	8
ERN010WGSD	
ERN316AJU	
ERN284XJX	3d+
ERN406SCOD	187; 5d+
ERN194IZQ	10
ERN104ZUP	14
ERN218MWG	

	2010
No data	43%
CV<25	37%
CV>25	20%

D- enzyme activity patient sample > 30% control EBV

D+ enzyme activity other EBV < 20 % control EBV



ACTIVITY ENZYMES CTRL EBV CELL LINE

ALL PARTICIPANTS 2010 SCHEME

	Act.EBV (% ctrl leuco)	Range participants
α -L-iduronidase	12 \pm 15	1.6-69%(0.08-12 nmol/h)
Galactocerebrosidase	16 \pm 16	2-70%(0.4-15 nmol/17h)
Sphingomyelinase	206 \pm 137	1.3-795%(2-127 nmol/17h)
α -N-acetylglucosaminidase	79 \pm 32	26-284%(0.5-44 nmol/h)
β -Hexosaminidase A + B	41 \pm 29	14-204%(49-2212 nmol/h)
β -Hexosaminidase A	42 \pm 25	12-152%(5-1737 nmol/h)
Galactose-6S sulfatase	6 \pm 5	0.6-19%(0.12-19 nmol/17h)
β -Galactosidase	25 \pm 17	9-126%(0.06-136 nmol/h)
β -mannosidase	26 \pm 12	3-56%(3-118 nmol/h)
β -Glucuronidase	36 \pm 26	9.8-131%(6.6-369 nmol/h)



ACTIVITY ENZYMES CTRL EBV CELL LINE 2010

	Act.EBV (% ctrl leuco)	Patient value(% ctrl leuco)
α -L-iduronidase	12 \pm 15	<3%
Galactocerebrosidase	16 \pm 16	<3%
Sphingomyelinase	206 \pm 137	<4%
α -N-acetylglucosaminidase	79 \pm 32	<1%
β -Hexosaminidase A + B	41 \pm 29	<10%
β -Hexosaminidase A	42 \pm 25	<2%
Galactose-6S sulfatase	6 \pm 5	<1%
β -Galactosidase	25 \pm 17	<4%
β -mannosidase	26 \pm 12	<1%
β -Glucuronidase	36 \pm 26	<3%



ACTIVITY ENZYMES CTRL EBV CELL LINE

2011 SCHEME (% and FLU)

	Act.EBV (% ctrl leuco)	FLU(1)	FLU(2)	FLU(bl)
α -L-iduronidase	4.4 \pm 2.4	145	54	29
Galactocerebrosidase	8.8 \pm 8	160	73	4
Sphingomyelinase	163 \pm 61	3060	1400	10
α -N-acetylglucosaminidase	72 \pm 30	415	249	0.5
A-mannosidase	12 \pm 6	196	72	12
Arylsulfatase B	14 \pm 10	200	150	90
Iduronate sulfatase	48 \pm 21	800	150	15
β -Galactosidase	26 \pm 13	320	12	1.3
β -mannosidase	36 \pm 35	145	21	2.5
β -Glucuronidase	20 \pm 11	285	165	5.5

Enzyme activities ebv 2-4 low, because of pink color, due to insufficient washing cell pellet by ECACC



QA for LSD's, a new ERNDIM scheme

What needs to be done

- Other layout of results presentation
- Information about the enzyme assay
- — Inclusion leucocyte sample
-
- **Certificate of Participation:**
 - score for detecting LSD patients (+)
 - score for reproducibility (+)
 - score for detecting enzyme deficiencies in “normal” samples (—)



QA for LSD's

What needs to be done

- SUGGESTIONS PARTICIPANTS

