Lysosomal Enzymes in fibroblasts

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1. Scheme Design

The scheme has been designed, planned and coordinated by Dr Kees Schoonderwoerd as Scientific Advisor and Dr Cas Weykamp as Scheme Organiser (sub-contractor on behalf of SKML); both appointed by and according to procedures laid down by the ERNDIM Board.

2. Samples

All EQA materials are lyophilised samples of human fibroblasts. All samples were obtained following local ethical and consent guidelines.

Table 1: Samples for the 2014 scheme

Sample	Disorder	Enzyme defect
LF1	MLD	Arylsulphatase A
LF2	Niemann Pick A/B	Sphingomyelinase
LF3	Hunter	Iduronate-2-sulphatase
LF4	Niemann Pick A/B	Sphingomyelinase
LF5	GM$_1$ (Morquio B)	beta-Galactosidase
LF6	Fabry	alpha-Galactosidase

3. Shipment

One shipment of 6 samples was sent out to the 76 laboratories, from 29 countries, which registered for the scheme.

4. Receipt of results

There were six submission deadlines from March to October 2014 at approximately 6 week intervals. Laboratories were asked to submit results for each EQA sample by the relevant submission deadline using the results website www.erndimqa.nl.

Laboratories were asked to report the total protein and the activities for 10 enzymes in absolute units and also as a percentage of their own laboratories control, see Table 2 for details. Laboratories could submit results for as many, or as few, of these 10 enzymes as they wished. Laboratories were also asked to select an 'interpretation' of the results from a dropdown list on the results website.

Table 2: Analytes to be measured

Analyte	Parameter 1	Parameter 2
Protein	mg/vial	
Arylsulphatase A	37 degr; nmol/17h/mg	37 degr; % mean control
alpha-Galactosidase	nmol/h/mg	% mean control
beta-Galactosidase	nmol/h/mg	% mean control
alpha-Glucosidase	nmol/h/mg	% mean control
beta-Glucosidase	nmol/h/mg	% mean control
beta-Hexosaminidase A	nmol/h/mg	% mean control
alpha-Iduronidase	nmol/h/mg	% mean control
Iduronate sulphatase	nmol/4h/mg	% mean control
Galactosylceramidase	nmol/17h/mg	% mean control
Sphingomyelinase	nmol/h/mg	% mean control

5. Scoring scheme

For each enzyme 2 criteria were scored: 1) diagnosis and 2) coefficient of variation (CV). A maximum of 2 points was awarded for each criterion. For the protein value a maximum of 2 points could be scored.

Table 3: Scoring criteria

	Criteria		Score
Protein	**CV**	CV<35%	2
		35%<CV<60%	1
		CV>60%	0
Enzymes	**Diagnosis**	Diagnosis correct	2
		Diagnosis partially correct	1
		Diagnosis incorrect	0
	CV	CV<35%	2
		35%<CV<60%	1
		CV>60%	0

The maximum possible score for the scheme was 42 points (10 enzymes plus the protein value). Laboratories that participated fully in the scheme (i.e. submitted enough results for their performance to be assessed) but scored less than 60% of their maximum possible score were considered to be unsatisfactory performers in the scheme. For example if a laboratory submitted results for 8 analytes (protein & 7 enzymes) their maximum possible score would be 30 points so they would need to score 18 or more points to be a satisfactory performer. If 60% of a laboratory's maximum possible score was not a full integer the number of points for satisfactory performance was rounded down to the next full integer.

5.1. Diagnosis

The participants had to select an interpretation from the dropdown list on the results website.

Diagnosis correct indicated correct interpretation and correct measurement of enzyme activity level. In cases of control enzyme activity, the activity should be >15% of the mean control while in case of a patient enzyme activity, the activity should be <30% of the mean control.

Diagnosis partially correct indicated incorrect interpretation and correct enzyme activity level or correct interpretation and incorrect enzyme activity level.

Diagnosis incorrect indicated incorrect interpretation and incorrect enzyme activity level.

5.2. Coefficient of variation

Results submitted for samples LF2 and LF 4 were used to calculate the coefficient of variation (CV) according to the following formula.

$$CV = \text{Activity LF4-activity LF2/mean}$$

With only two samples (LF2 and LF4) it was not possible to calculate the standard deviation.

6. Results

Sixty-eight laboratories (89.5% of registered laboratories) submitted sufficient results for their performance to be assessed and a further 3 laboratories (3.9%) did not submit enough results for their performance to be assessed (partial submitters). One laboratory (1.3%) withdrew from the scheme and 4 laboratories (5.2%) did not submit any results.

Full details of each participating results are given in Appendix 1 but a brief summary is presented here:

- Over 70% of all laboratories submitted results for 7 or more enzymes, see Table 4.
- The proficiency per analyte is given in Table 5.
- Table 6 shows the percentage of the maximum possible score for the laboratories that submitted results.
- 64 laboratories that submitted results scored 60% or more of their maximum possible score and were classed as satisfactory performers.

Table 4: Number of enzymes for which laboratories submitted results

Number of Enzymes for which results were submitted	Number of laboratories
0	4
1	2
2	1
3	3
4	5
5	2
6	4
7	11
8	9
9	10
10	24
Total number of labs	**75**

Table 5: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	71	n.a.	91	91
Arylsulfatase A	57	43	71	57
α-Galactosidase	62	86	81	83
β-Galactosidase	68	93	78	85
α-Glucosidase	48	90	76	83
β-Glucosidase	63	95	77	86
β-Hexosaminidase A	62	96	80	88
α-Iduronidase	53	92	79	85
Iduronate-2-sulphate sulphatase	41	85	70	77
Galactocerebrosidase	46	80	69	74
Sphingomyelinase	46	39	77	58

[1] = percentage of maximum possible score (for all laboratories that submitted results)

Table 6: Percentage of maximum possible scores for laboratories that submitted results

%age of maximum possible score	No of submitting labs	%age of submitting labs
0% – 9%	0	0%
10% – 19%	0	0%
20% – 29%	1	1.4%
30% –39%	0	0
40% – 49%	0	0%
50% –59%	7	9.9%
60% –69%	9	12.7%
70% –79%	16	22.5%
80% –89%	20	28.2%
90% –99%	14	19.7%
100%	4	5.60%
Totals	**71**	**100%**

Sample LF1 can be regarded as an educational sample, derived from a MLD patient with a high residual arylsulphatase A activity. Samples LF2 and LF4 will also be regarded as educational samples. They were derived from a Niemann-Pick A/B patient with a mutation in the substrate binding site. Therefore they were missed by the participants that used the artificial substrate without sphingomyelin and many participants did not interpret this as a patient.

Table 7: Number of enzymes for which submitting laboratories had satisfactory performance

Lab No	No of enzymes for which:		Lab No	No of enzymes for which:	
	results were submitted by lab	lab had satisfactory performance		results were submitted by lab	lab had satisfactory performance
1	8	8	39	10	10
2	10	10	40	5	1
3	9	8	41	9	9
4	10	5	42	10	10
5	9	8	43	10	10
6	1	1	44	7	7
7	10	8	45	9	8
8	8	5	46	10	8
9	7	1	47	7	4
10	5	5	48	9	7
11	9	7	49	6	6
12	4	4	50	10	0
13	8	8	51	7	7
14	10	6	52	7	6
15	7	5	53	6	6
16	3	3	54	7	4
17	9	9	55	10	9
18	10	10	56	9	9
19	10	10	57	7	6
20	2	2	58	10	10
21	10	8	59	1	1
22	10	10	60	6	5
23	10	8	61	4	4
24	7	6	62	8	5
25	10	6	63	4	0
26	8	8	64	9	6
27	7	4	65	10	5
28	8	8	66	4	4
29	8	7	67	8	7
30	10	9	68	10	9
31	10	9	69	4	3
32	9	9	70	10	10
33	6	1	71	10	5
34	3	3			
35	3	2			
36	7	5			
37	10	10			
38	7	6			

7. Comments here on overall scheme performance
Overall all samples were correctly interpreted besides the educational samples.

8. Comparison to previous years
In 2013 arylsulfatase and iduronate-2-sulphate sulphatase activity measurements were not in the scheme, therefore no comparison can be made. For most enzymes there was no difference in the CV between 2013 and 2014 however there was a remarkable improvement in the number of participants with CV<35 for the analyte galactocerebrosidase.

Table 8: Comparison between CV data from 2013 and 2014

Analyte	2013				2014			
	%age of labs with:			No of labs	%age of labs with:			No of labs
	No data	CV<35	CV>35		No data	CV<35	CV>35	
Protein/vial	8%	84%	8%	71	7%	83%	10%	71
Arylsulfatase A	-	-	-	-	14%	63%	23%	56
α-Galactosidase	7%	80%	13%	60	8%	73%	19%	64
β-Galactosidase	10%	62%	28%	69	12%	63%	25%	69
α-Glucosidase	9%	64%	27%	44	8%	68%	24%	49
β-Glucosidase	11%	64%	25%	64	11%	70%	19%	64
β-Hexosaminidase A	8%	67%	25%	61	13%	66%	21%	62
α-Iduronidase	11%	59%	30%	53	4%	76%	20%	54
Iduronate-2-sulphate sulphatase	-	-	-	-	10%	61%	29%	43
Galactocerebrosidase	10%	46%	44%	39	7%	65%	28%	46
Sphingomyelinase	15%	58%	27%	41	9%	59%	17% (32%)	46

Kees Schoonderwoerd   **Cas Weykamp**
Scientific advisor   **Scheme Organiser**

Appendix 1 (part 1): Results per laboratory

(see page 8 for key)

Lab No	Protein/vial CV	Score CV	Arylsulfatase A CV	Score CV	Score Diagnosis	α-Galactosidase CV	Score CV	Score Diagnosis	β-Galactosidase CV	Score CV	Score Diagnosis
1	3	2				3	2	1	7	2	2
2	5	2	6;D-	2	0	10	2	2	12	2	2
3	7	2	18	2	2	20	2	2	14	2	2
4	9	2	181	0	0	63	0	2	112	0	2
5	0	2	2	2	2	13	2	2	1	2	2
6	-	1									
7	47	1	6	2	2	6	2	2	1	2	2
8	8	2	165;D-D+	0	0	4	2	2	84	0	2
9	64	0	D0;R0	1	2	80	0	1	39	1	2
10	5	2	8	2	0	44	1	2	19	2	2
11	17	2	26	2	1	5	2	2	119	0	2
12	16	2	19	2	1				4	2	2
13	19	2	7	2	2	1	2	2	23	2	2
14	3	2	69	0	2	78	0	2	120	0	2
15	4	2	10;D-	2	0	8	2	1	5	2	2
16	-	1.5				-	1.5	2	R0	1.5	2
17	-	1	R0;D+	1	2	-	1	2	R0	1	2
18	9	2	9	2	0	3	2	2	23	2	2
19	8	2	3	2	1	9	2	2	12	2	2
20	14	2				20	2	2			
21	6	2	47	1	0	63	0	2	51	1	2
22	60	0	1	2	0	2	2	2	2	2	2
23	11	2	4;D-	2	1	31	2	2	33	2	2
24	12	2	6;D-	2	0	26	2	2	14	2	2
25	38	1	32	1	1	45	1	2	49;D+	1	0
26	14	2	23	2	0	28	2	2	41	1	2
27	15	2	R0;D-	0	0	64	0	1	12	2	2
28	2	2				3	2	2	1	2	2
29	45	1	58	1	1	11	2	2	45	1	2
30	3	2	D0;R0	1	1	12	2	2	16	2	2
31	15	2	33	2	2	14	2	2	36	1	2
32	2	2	38;D-	1	0	14	2	2	7	2	2
33	13	2	68:D-	0	0	-	1	2	136	0	2
34	10	2							12	2	2
35	15	2	106;D+	0	0				55	1	2
36	109	0	195:D-	0	0	9	2	0	21	2	2
37	8	2	0	2	2	5	2	2	7	2	2
38	13	2	12	2	2				33	2	2
39	10	2	12;D-	2	1	12	2	2	3	2	2
40	46	1	R0	1	1	2	2	2	R0	1	2
41	13	2	2:D-	2	0	28	2	2	25	2	2
42	5	2	9	2	1	14	2	2	1	2	2
43	0	2	4	2	2	2	2	2	5	2	2
44	5	2	135	0	1	4	2	2	10	2	2
45	0	2	32	2	2	28;D-	2	1	2	2	2
46	27	2	24	2	0	28:D-	2	1	15	2	2

Lab No	Protein/vial CV	Protein/vial Score CV	Arylsulfatase A CV	Arylsulfatase A Score CV	Arylsulfatase A Score Diagnosis	α-Galactosidase CV	α-Galactosidase Score CV	α-Galactosidase Score Diagnosis	β-Galactosidase CV	β-Galactosidase Score CV	β-Galactosidase Score Diagnosis
47	18	2	31;D-;D+	2	0	28	2	2	13	2	2
48	30	2	37	1	0	43	1	1	13	2	2
49	-	1.5	R0;	1.5	2	-	1.5	2	R0	1.5	2
50	-	1	R0	1	1	-	1	1	R0;D0	1	2
51	0	2	1	2	1	4	2	2	5	2	2
52	5	2		0	0	24	2	2	27	2	2
53	5	2				12	2	2	7	2	2
54	10	2	65	0	0				71	0	2
55	14	2	10	1.5	1.5	38	1	1	2	1.5	1.5
56	5	2				6	2	1	21	2	2
57	16	2				21	2	1.5	19	2	1.5
58	19	2	33:D-	2	1	29	2	2	31	2	2
59	8	2				14	2	1			
60	29	2	15:D-	2	0				19	2	2
61	27	2							76;D-	0	0
62	0	2							1	2	2
63	17	2	15:D0	2	0	-	0	0	-	0	0
64	13	2				42	1	1	42	1	2
65	3	2	194;D-	0	1	63	0	2	52	1	2
66	19	2	19	2	1	13	2	2	5	2	2
67	27	2	8	2	2	0	2	2	2	2	2
68	6	2	10	2	1	27	2	2	33	2	2
69	6	2				8	2	1	19;D-	2	0
70	7	2	14	2	1	45	1	2	11	2	2
71	6	2	2:D-	2	1	31	2	2	57	1	2
Mean CV (±SD)	15.5 ±18.1%		39 ±55%			23 ±20%			28 ±32%		
% Diagnoses incorrect					2%			0%			4%

Key

green cells = correct CV (<35), correct interpretation and correct enzyme level

red cells =Incorrect measurement, CV (>35) or incorrect interpretation or enzyme level

blue cells =not all samples measured

D- = enzyme activity patient sample > 30% control Fibroblast

D+ = enzyme activity other samples < 20 % control Fibroblasts

D0 = patient sample not measured

R0 = CV calculation not possible as one or both of LF2 and LF4 (duplicate samples) were not measured

Appendix 1 (part 2): Results per laboratory

(see page 8 for key)

Lab No	α-Glucosidase			β-Glucosidase			β-Hexosaminidase A			α-Iduronidase		
			Score			Score			Score			Score
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
1	-	0	0	8	2	2	32	2	2	-	0	0
2	41	1	2	3	2	2	4	2	2	11	2	2
3	2	2	2	67	0	2	5	2	2	3	2	2
4	1	2	2	88	0	2	9	2	2	28	2	2
5	2	2	2	3	2	2	1	2	2	2	2	2
6						-	-	1	2			
7	13	2	1	47	1	2	38	1	2	13	2	2
8	26	2	2	21	2	2	19	2	2			
9							-	2	2	86	0	2
10				22	2	2	53	1	2			
11	14	2	2	61	0	2	9	2	2			
12				3	2	2				24	2	2
13	12	2	2	21	2	2	7	2	2	28	2	2
14	33	2	2	3	2	2	6	2	2	45	1	2
15	59	1	2	6	2	2	15	2	2	5	2	2
16							-	1.5	2			
17	-	1	2	-	1	2	-	1	2	-	1	2
18	4	2	2	8	2	2	6	2	2	5	2	2
19	6	2	2	3	2	2	0	2	2	4	2	2
20												
21	8	2	2	21	2	2	16	2	2	85	0	2
22	2	2	2	24	2	2	-	2	2	8	2	2
23	39	1	2	94	0	2	22	2	2	102;D+	0	1
24	35	1	0	-	0	1	16	2	2	9	2	2
25	52	1	2	31	2	2	45;D+	1	1	36;D+	1	1
26				36	1	2	57	1	2	23	2	2
27				18	2	2	17	2	1	10	2	2
28				20	2	2	24	2	2	4	2	2
29	40	1	2	16	2	2	131	0	2	41	1	2
30	27	2	2	16	2	2	18	2	2	7	2	2
31	31	2	2	20	2	2	7	2	2	4	2	2
32	12	2	2	30	2	2	8	2	2	11	2	2
33	28	2	2	153	0	2						
34							9	2	2			
35				39	1	2						
36	-	1	2	32	2	2	12	2	2	61	0	2
37	24	2	2	18	2	2	6	2	2	7	2	2
38	30	2	2	8	2	2	2	2	2	4;D+	2	1
39	1	2	2	6	2	2	1	2	2	13	2	2
40				-	1	1	73	0	2			
41	12	2	2	43	1	2	36	1	2	7	2	2
42	0	2	2	0	2	2	10	2	2	2	2	2
43	1	2	2	6	2	2	14	2	2	4	2	2
44	73	0	2	-	2	2						
45	3	2	2	3	2	2	6	2	2	12	2	2
46	49	1	2	25;D+	2	1	7	2	2	20	2	2

Lab No	α-Glucosidase			β-Glucosidase			β-Hexosaminidase A			α-Iduronidase		
		Score			Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
47	-	0	2	21	2	2				35;D+	2	0
48				91	0	2	7	2	2	12	2	2
49				-	1	2	-	1	2	-	1	2
50	D+	1	0	-	1	2	-	1	2	-	1	2
51	8	2	2	1	2	2	2	2	2	7	2	2
52				2	2	2	10	2	2	35	1	2
53	14	2	2	6	2	2						
54							100	0	2	4	2	1.5
55	9	1.5	1.5	8	1.5	1.5	15	1.5	1.5	20	1.5	1.5
56	3	2	2	6	2	2	55	1	2	57	1	2
57	36	1	1.5	32	2	1.5	45	1	1.5	29	2	1.5
58	13	2	2	22	2	2	38	1	2	57	1	2
59												
60				-	1	2	2	2	2	19	2	2
61				29	2	2	68	0	2			
62	5	2	2	3	2	2	-	0	0	2	2	2
63				-	0	0						
64	44	0	2	46	1	2	45	1	2	9	2	2
65	39	1	2	22	2	2	5	2	2	108	0	2
66							12	2	2			
67				14	2	2	57	1	2			
68	11	2	2	5	2	2	16	2	2	2	2	2
69				8	2	2	2	2	2			
70	48	1	2	35	1	2	4	2	2	43	1	2
71	70	0	0	84	0	2	26	2	2	32	2	2
Mean CV (±SD) % Diagnoses incorrect	24 ±20%		2%	26 ±29%		1.5%	23 ±26%		2%	24 ±27%		7%

Appendix 1 (part 3): Results per laboratory
(see page 8 for key)

Lab No	Iduronate-2-sulphate sulphatase CV	Score CV	Score Diagnosis	Galactocerebrosidase CV	Score CV	Score Diagnosis	Sphingomyelinase CV	Score CV	Score Diagnosis
1				-	0	0	-	1	1
2	2	2	2	18	2	2	1;D-	2	0
3				15	2	2	0	2	2
4	70	0	2	53	1	2	32;D-	2	0
5				166	0	2	2;D-	2	0
6									
7	4	2	2	12	2	0	0;D-	2	0
8				128	0	2	38;	1	2
9				177	0	2	200	0	2
10									
11	39	1	2	9	2	1	44	1	2
12									
13	19	2	2						
14	9:D+	2	0	2	2	2	121	2	2
15									
16									
17	-	1	2				R0	1	2
18	30	2	2	17	2	2	1	2	2
19	19	2	2	3	2	2	20	2	2
20	38	1	2						
21	35	1	2	10	2	2	4;D-	2	1
22	18	2	2	6	2	2	40;D-	1	0
23	15	2	2	16	2	2	14;D-	2	0
24									
25	36	1	2	87	0	2	19;D-	2	0
26	57	1	2	17	2	2			
27				6	2	2	30	2	0
28	19	2	2	30	2	2	2;D-	2	0
29	33	2	2						
30	11	2	2	38	1	1	-	0	2
31	36	1	0	61	0	2	16;D-	2	0
32	6	2	2	21	2	2			
33	158	0	2						
34				8	2	2			
35									
36									
37	10	2	2	3	2	2	23;D-	2	0
38							16;D-	2	0
39	4	2	2	9	2	2	80	2	2
40									
41	29	2	2	34	2	2			
42	5	2	2	5	2	2	4;D-	2	0
43	28	2	2	5	2	2	6;-	2	2
44				24	2	2	44;D-	1	1
45				16	2	2	9;D-	2	0
46	1	2	2	21	2	2	33;D-	2	0

Lab No	Iduronate-2-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
47							100	1	2
48	9	2	2	31	2	1	5;D-	2	0
49									
50	-	1	2	-	1	0	-	1	0
51									
52							200	2	2
53	2	2	2				113	2	2
54	-	0	2	51	1	2	19	2	1.5
55	29	2	0	10	1.5	1.5	96;D-	0	1
56	118	0	2	2	2	2	1;D-	2	0
57							-	0	0
58	17	2	2	31	2	2	55;D-	1	0
59									
60				86	0	0			
61				-	1	2			
62	-	0	0	-	0	0	21	2	2
63									
64	29	2	2	94	0	0	7;D-	2	0
65	67	0	2	81	0	2	53;D-	1	0
66									
67	-	0	0	18	2	2	16;D-	2	0
68	37	1	2	6	2	1	21;D-	2	0
69									
70	28	2	2	42	1	0	45;D-	1	0
71	82	0	0	71	0	2	-	0	0
Mean CV (±SD) **% Diagnoses incorrect**	31 ±33%		5%	37 ±43%		17%	21 ±20%		61%