ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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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	Pompe	alpha-Glucosidase
LF2	Gaucher	beta-Glucosidase
LF3	Tay Sachs	Beta-Hexosaminidase A
LF4	Morquio A	Galactosamine-6-sulphate sulphatase
LF5	Hurler	alpha-iduronidase
LF6	Pompe	alpha-Glucosidase

3. Shipment
One shipment of 6 samples was sent out in February 2015, to the 74 laboratories, from 29 countries, which registered for the scheme.

4. Receipt of results
There were six submission deadlines from March to October 2015 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	
beta-Hexosaminidase A+B	37 degr; nmol/7h/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
Galactosamin-6-sulphate sulphatase	nmol/17h/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
		CV=35 or 35%<CV<60%	1
		CV>60%	0
Enzymes	**Diagnosis**	Diagnosis correct	2
		Diagnosis partially correct	1
		Diagnosis incorrect	0
	CV	CV<35%	2
		CV=35 or 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 have to select an interpretation from the dropdown list on the results website.

Diagnosis correct indicates 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 indicates incorrect interpretation and correct enzyme activity level or correct interpretation and incorrect enzyme activity level.

Diagnosis incorrect indicates incorrect interpretation and incorrect enzyme activity level.

5.2. Coefficient of variation

Results submitted for samples LF1 and LF 6 were used to calculate the coefficient of variation (CV) according to the following formula.

CV = Activity LF6-activity LF1/mean

With only two samples (LF1 and LF6) it was not possible to calculate the standard deviation.

6. Results

Seventy one laboratories (95.9% of registered laboratories) submitted sufficient results for their performance to be assessed; one laboratory (1.4%) withdrew from the scheme and 2 laboratories (2.7%) did not submit any results.

Full details of each participating results are given in Appendix 1 but summaries are presented here:

- Over 67% 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.
- 65 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	3
1	3
2	2
3	0
4	4
5	5
6	7
7	7
8	8
9	9
10	26
Total number of labs	**74**

Table 5: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	71	n.a.	89	89
β-Hexosaminidase A+B	60	98	75	86
α-Galactosidase	62	94	77	85
β-Galactosidase	66	95	77	86
α-Glucosidase	48	65	74	69
β-Glucosidase	63	85	78	81
β-Hexosaminidase A	60	88	82	85
α-Iduronidase	54	94	78	86
Galactosamine-6-sulphate sulphatase	41	91	73	82
Galactocerebrosidase	46	88	78	83
Sphingomyelinase	44	98	82	90

[1]= percentage of maximum possible score (for 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%	0	0%
30% –39%	2	2.8%
40% – 49%	1	1.4%
50% –59%	3	4.2%
60% –69%	8	11.3%
70% –79%	10	14.1%
80% –89%	15	21.1%
90% –99%	20	28.2%
100%	12	16.9%
Totals	**71**	**100%**

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

Lab No	No of enzymes for which:	
	results were submitted by lab	lab had satisfactory performance
1	2	0
2	6	6
3	9	9
4	9	5
5	10	7
6	6	5
7	1	1
8	8	8
9	5	5
10	4	4
11	7	5
12	10	10
13	7	5
14	5	3
15	8	7
16	5	3
17	6	5
18	9	6
19	10	10
20	9	9
21	10	1
22	9	5
23	7	Not enough for analysis
24	10	9
25	10	8
26	7	2
27	8	7
28	4	4
29	9	9
30	9	9
31	10	10
32	10	8
33	6	5
34	6	6
35	8	6
36	10	8
37	6	6

Lab No	No of enzymes for which:	
	results were submitted by lab	lab had satisfactory performance
38	10	10
39	8	8
40	8	8
41	10	9
42	10	9
43	9	6
44	10	8
45	6	5
46	10	10
47	4	3
48	10	10
49	2	2
50	1	1
51	10	9
52	9	6
53	8	8
54	10	7
55	10	10
56	7	7
57	10	10
58	10	9
59	10	6
60	10	10
61	4	1
62	1	0
63	8	6
64	5	5
65	10	8
66	7	7
67	10	7
68	5	2
69	7	6
70	10	9
71	10	8

7. Comments here on overall scheme performance

Overall all samples were correctly interpreted.

8. Comparison to previous years

In 2013 and 2015 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 in 2014 and 2015.

Table 8: Comparison between CV data from 2013, 2014 and 2015

	2013				2014				2015			
	%age of labs with:				%age of labs with:				%age of labs with:			
Analyte	**No data**	**CV <35**	**CV >35**	**No of labs**	**No data**	**CV <35**	**CV >35**	**No of labs**	**No data**	**CV <35**	**CV >35**	**No of labs**
Protein/vial	8%	84%	8%	71	7%	83%	10%	71	10%	80%	10%	71
Arylsulfatase A	-	-	-	-	14%	63%	23%	56				
α-Galactosidase	7%	80%	13%	60	8%	73%	19%	64	11%	66%	23%	62
β-Galactosidase	10%	62%	28%	69	12%	63%	25%	69	11%	64%	26%	66
α-Glucosidase	9%	64%	27%	44	8%	68%	24%	49	10%	63%	27%	48
β-Glucosidase	11%	64%	25%	64	11%	70%	19%	64	14%	67%	19%	63
β-Hexosaminidase A	8%	67%	25%	61	13%	66%	21%	62	15%	72%	13%	60
β-Hexosaminidase A+B									12%	60%	28%	60
α-Iduronidase	11%	59%	30%	53	4%	76%	20%	54	13%	69%	19%	54
Iduronate-2-sulphate sulphatase	-	-	-	-	10%	61%	29%	43				
Galactosamine-6-sulphate sulphatase									15%	59%	27%	41
Galactocerebrosidase	10%	46%	44%	39	7%	65%	28%	46	13%	63%	24%	46
Sphingomyelinase	15%	58%	27%	41	9%	59%	17% (32%)	46	16%	71%	14%	44

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Appendix 1 (part 1): Results per laboratory

(see page 8 for key)

	Protein/vial		B-Hexosaminidase A+B			α-Galactosidase			β-Galactosidase		
		Score		Score			Score		CV	Score	
Lab No	CV	CV	CV	CV	Diagnosis	CV	CV	Diagnosis		CV	Diagnosis
1	35	1				158;d+	0	1			
2	4	2							12	2	2
3	4	2	20	2	2	8	2	2	7	2	2
4	20	2	50	1	2	4	2	2	38;d+	1	1
5	50	1	59	1	2	88	0	2	80	0	2
6	36	1	50	1	2	46	1	2	6	2	2
7	13	2									
8	0	2	2	2	2	1	2	2	2	2	2
9	26	2	30	2	2	29;d+	2	1	40	1	2
10	14	2	14	2	2	41	1	2	12	2	2
11	11	2	55	1	2	6	2	2	42	1	2
12	9	2	20	2	2	23	2	2	9	2	2
13	0	2	6	2	2	28	2	2	6	2	2
14	200	0	12	2	2				20	2	2
15	14	2	3	2	2	4	2	2	18	2	2
16	66	0				0	2	2	75	0	2
17	16	2				23	2	2	31	2	2
18	8	2	200	0	2	Ro;d+	1	1	2	2	2
19	1	2	6	2	2	2	2	2	2	2	2
20	4	2	20	2	2	0	2	2	13	2	2
21	57	1	94	0	2	104	0	2	100	0	2
22	2	2	46;D+	1	0	200;D+	0	0	2	2	2
23	R0	1	R0	1	2	R0	1	2	R0	1	2
24	22	2	68	0	2	27	2	2	5	2	2
25	12	2	35	2	2	27	2	2	38;D+	1	1
26	28	2	23	2	2	120	0	2	31	2	2
27	8	2	10	2	2	10	2	2	52	1	2
28	22	2				28	2	2			
29	2	2	2	2	2	2	2	2	2	2	2
30	8	2	16	2	2	29	2	2	20	2	2
31	18	2	13	2	2	13	2	2	14	2	2
32	R0	1	R0	1	2	R0	1	2	R0	1	2
33	0	2	8	2	2				20	2	2
34	4	2	39	1	2	13	2	2	3	2	2
35	R0	1	R0	1	2				R0	1	2
36	9	2	14	2	2	8	2	2	11	2	2
37	16	2				16	2	2	20	2	2
38	5	2	14	2	2	9	2	2	8	2	2
39	14	2	3	2	2				17	2	2
40	11	2	17	2	2	35	2	2	36	1	2
41	22	2	25	2	2	30	2	2	46	1	2
42	10	2	10	2	2	2	2	2	8	2	2
43	27	2	62	0	2	26	2	2	29	2	2
44	31	2	0	2	2	37	1	2	50	1	2
45	7	2				0	2	2	29	2	2
46	3	2	0	2	2	0	2	2	13	2	2

Lab No	Protein/vial		B-Hexosaminidase A+B			α-Galactosidase			β-Galactosidase		
		Score		Score			Score		CV	Score	
	CV	CV	CV	CV	Diagnosis	CV	CV	Diagnosis		CV	Diagnosis
47	8	2	10	2	2				8	2	2
48	18	2	17	2	2	13	2	2	9	2	2
49	0	2							19	2	2
50	15	2				28	2	2			
51	R0	1	R0	1	2	r0	1	2	R0	1	2
52	6	2	10	2	2	120	0	2	29	2	2
53	R0	1	R0	1	2	R0	1	2	R0	1	2
54	19	2	102	0	2	9	2	2	3	2	2
55	15	2	15	2	2	12	2	2	11	2	2
56	8	2	11	2	2	18	2	2	18	2	2
57	29	2	29	2	2	24	2	2	36	1	2
58	17	2	39	1	2	23	2	2	50	1	2
59	17	2	110	0	2	1	2	2	149;d+	0	1
60	21	2	36	1	2	35	1	2	11	2	2
61	47	1	47	1	2	72	0	2	64	0	2
62	23	2				83;d+	0	1			
63	2	2	1	2	2	14	2	2	5;D+	2	0
64	17	2	4	2	2	12	2	2	12	2	2
65	9	2	33	2	2	28	2	2	24	2	2
66	14	2	8	2	2	4	2	2	4	2	2
67	R0	1	R0;d+	1	1	R0	1	2	R0	1	2
68	R0	1	R0	1	2	R0;d+	1	1	R0;d+	1	1
69	14	2	17	2	2				14	2	2
70	17	2	4	2	2	24	2	2	134	0	2
71	5	2	65	0	2	74	0	2	38	1	2

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)

	α-Glucosidase			β-Glucosidase			β-Hexosaminidase A			α-Iduronidase		
		Score			Score			Score			Score	
Lab No	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
1				R0;D-	1	0						
2				15	2	2				15	2	2
3	20;d-	2	1	15;d-	2	1	12	2	2	4	2	2
4	20;d-	2	1	79;d-	0	1	53;d-	1	1	42	1	2
5	119;D-	0	0	8	2	2	39	1	2	16	2	2
6	95	0	2	43	1	2						
7										16	2	2
8	25	2	2	20	2	2	3	2	2			
9				31	2	2	3	2	2			
10							23;d-	2	1			
11	15;D-	2	0	16	2	2	55	1	2			
12	11	2	2	23	2	2	18	2	2	22	2	2
13	171;d-	0	1	15;d-	2	1	R0	0	0	15	2	2
14				53	1	1	31;d0	2	1			
15	18	2	2	20	2	2	32	2	2	68	0	2
16										63	0	2
17	55;D-	1	0	28;d-	2	1				29	2	2
18	R0	1	2	R0	1	2	3	2	2	R0	0	0
19	2	2	2	8	2	2	1	2	2	1	2	2
20	0	2	2	31	2	2	10	2	2	29	2	2
21	Ro;d-	1	1	102	0	2	189;D-	0	0	93	0	2
22	18;D-	2	0	83;d-	0	1	3	2	2	14	2	2
23				R0;d0	0	0	R0;D0	0	0	R0;d0	0	0
24	49	1	2	15	2	2	4	2	2	11	2	2
25	33;d-	2	1	32;d+;d-	2	0	32	2	2	36	1	2
26				R0;d0	1	0	35	1	1	107	0	2
27				26	2	2	34	2	2	10	2	2
28	5	2	2	5	2	2				16	2	2
29	2	2	2	6	2	2	10	2	2	5	2	2
30	35	1	2	20	2	2	21	2	1	31	2	2
31	10;d+	2	1	29	2	2	3	2	2	40	1	2
32	R0;d-	1	1	R0	1	2	R0	1	2	R0;D0	1	1
33				1	2	2	1	2	2	45	1	2
34				2	2	2	12	2	2	14	2	2
35				R0	1	2	R0	1	2	R0	1	2
36	1;d-	2	1	87	0	2	12	2	2	8	2	2
37	13;d-	2	1	2;d-	2	1	24;d-	2	1	1	2	2
38	0;d-	2	1	2	2	2	17	2	2	6	2	2
39	26	2	2	14	2	2	12	2	2	7	2	2
40	6;d+	2	1	19	2	2	25	2	2	25	2	2
41	13;d+;d-	2	0	29	2	2	2	2	2	10	2	2
42	147;D-	0	0	11	2	2	7	2	2	11	2	2
43	39;D-	1	0	31;d-	2	1	79	0	2	29	2	2
44	48;d-	1	1	21	2	2	21	2	2	23	2	2
45	73	0	2	57	1	2						
46	22	2	2	5	2	2	12	2	2	6	2	2
47							3	2	2			

	α-Glucosidase			β-Glucosidase			β-Hexosaminidase A			α-Iduronidase		
		Score			Score			Score			Score	
Lab No	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
48	14	2	2	8	2	2	19	2	2	6	2	2
49				5	2	2						
50												
51	R0;d+8	1	1	R0	1	2	R0	1	2	R0	1	2
52	4	2	2	30	2	2	R0;D0	0	0	2	2	2
53				R0	1	2	R0	1	2			
54	163;d+	0	1	70	0	2	17	2	2	3	2	2
55	9	2	2	12	2	2	12	2	2	7	2	2
56	17;d+	2	1	7	2	2	10	2	2	R0	1	2
57	22	2	2	48	1	2	35	2	2	18	2	2
58	13;D-	2	0	18	2	2	5	2	2	40	1	2
59	6;d-	2	1	55	1	2	7	2	2	41;d-	1	1
60	16	2	2	44	1	2	25	2	2	16	2	2
61							71	0	2			
62												
63	108;d-	0	1	7	2	2	6	2	2	5	2	2
64				18	2	2	27	2	2			
65	33	2	2	18;D-	2	0	24	2	2	4	2	2
66				7	2	2	8	2	2			
67	R0;d-	1	1	R0	1	2	R0;d+	1	1	R0	1	2
68				R0;d-	1	1	R0	1	2			
69							8	2	2	24;d-	2	1
70	7	2	2	44	1	2	3	2	2	16	2	2
71	74	1	2	4	2	2	29	2	2	28	2	2

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

Lab No	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
1									
2	33	2	2	28	2	2	26	2	2
3				17	2	2	18	2	2
4				10	2	2	17	2	2
5	51	1	2	36	1	2	24	2	2
6	14	2	2						
7									
8				7	2	2	16	2	2
9									
10									
11	180	0	2						
12	12	2	2	24	2	2	5	2	2
13									
14				41	1	1			
15	0	2	2						
16				11;d+	2	1	39	1	2
17	49	1	2						
18	2	2	2	5;d+	2	1			
19	6	2	2	11;d+	2	1	0	2	2
20	8	2	2				53	1	2
21	185	0	2	99;d+	0	1	22	2	2
22				28	2	2	34	2	2
23	R0;d0	0	1						
24	43	1	2	29	2	2	20	2	2
25	31;d+	2	1	3	2	2	34	2	2
26				R0	0	1			
27				48;d+	1	1	44	1	2
28									
29				10	2	1	8	2	2
30				22	2	2	8	2	2
31	24	2	2	21;d+	2	1	4	2	2
32	R0	1	2	R0	1	2	R0	1	2
33							119	0	2
34									
35	R0;D-	1	0	R0;d+	1	1	R0	1	2
36	4	2	2	114	0	2	22	2	2
37									
38	18	2	2	3	2	2	14	2	2
39	25	2	2	22	2	2			
40							9	2	2
41	30	2	2	33	2	2	16	2	2
42	4	2	2	8	2	2	5	2	2
43	55	1	2	38	1	2			
44	200;d+	0	1	6	2	2	9	2	2
45				40	1	2	27	2	2
46	30	2	2	4	2	2	16	2	2
47				51;d+	1	1			

Lab No	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
48	9	2	2	8	2	2	4	2	2
49									
50									
51	R0	1	2	R0	1	2	R0	1	2
52	15	2	2				R0;d0	0	0
53	R0	1	2	R0	1	2	R0	1	2
54	22	2	2	46	1	2	16	2	2
55	29	2	2	41	1	2	16	2	2
56									
57	51	1	2	31	2	2	14	2	2
58	40	1	2	14	2	2	10	2	2
59	10	2	2	0	2	2	83	0	2
60	52	1	2	22	2	2	20	2	2
61									
62									
63	30	2	2						
64									
65	67	0	2	38	1	2	48	1	2
66				0	2	2	14	2	2
67	R0	1	2	R0	1	2	R0	1	2
68									
69	11;D+	2	0	32	2	2	11	2	2
70	28	2	2	0	2	2	4	2	2
71	7	2	2	31	2	2	R0	1	2