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 2016 scheme

Sample	Disorder	Enzyme defect
LF1	GM1	beta-Galactosidase
LF2	Fabry	Alpha-Galactosidase
LF3	Sandhoff	Beta-Hexosaminidase A+B
LF4	Hurler	alpha-iduronidase
LF5	Gaucher	beta-Glucosidase
LF6	Sandhoff	Beta-Hexosaminidase A+B

3. Shipment
One shipment of 6 samples was sent out on the 9th February 2016, to the 75 laboratories, from 30 countries, which registered for the scheme.

4. Receipt of results
There were three submission deadlines from April to October 2016, two before the summer holiday and one after the summer holiday. 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 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
Galactosamine-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 must 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 LF3 and LF 6 were used to calculate the coefficient of variation (CV) according to the following formula.

CV = Activity LF6-activity LF3/mean

With only two samples (LF3 and LF6) it was not possible to calculate the standard deviation.

6. Results

Seventy-one laboratories (94.6% of registered laboratories) submitted sufficient results for their performance to be assessed. One laboratory (1.3%) did not submit any results and three laboratories (4%) did not submit enough results for their performance to be assessed.

Full details of each participant's results are given in Appendix 1 but summaries are presented here:

- Over 69% 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.
- 63 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 (excluding non- and partial submitters)

Number of Enzymes for which results were submitted	Number of laboratories
0	0
1	3
2	2
3	0
4	5
5	4
6	6
7	4
8	8
9	11
10	28
Total number of labs	**71**

Table 5: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	74	n.a.	87	87
β-Hexosaminidase A+B	63	94	71	82
α-Galactosidase	68	83	80	82
β-Galactosidase	69	94	81	87
α-Glucosidase	52	80	72	76
β-Glucosidase	67	92	81	86
β-Hexosaminidase A	59	90	83	86
α-Iduronidase	57	96	81	88
Galactosamine-6-sulphate sulphatase	42	99	66	82
Galactocerebrosidase	48	96	70	83
Sphingomyelinase	45	99	69	84

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

Table 6: Percentage of maximum possible scores for laboratories that submitted results (excluding partial submitters)

%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%	0	0%
40% – 49%	2	2.8%
50% –59%	6	8.5%
60% –69%	0	0%
70% –79%	11	15.5%
80% –89%	20	28.2%
90% –99%	21	29.6%
100%	11	15.5%
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	10	10
2	8	8
3	10	9
4	9	0 (partial submitter)
5	10	9
6	9	9
7	7	7
8	10	10
9	10	6
10	4	3
11	10	5
12	9	7
13	4	0 (partial submitter)
14	10	7
15	10	8
16	8	8
17	10	10
18	10	10
19	10	5
20	8	8
21	6	6
22	7	4
23	5	1
24	9	6
25	9	8
26	10	10
27	5	5
28	10	9
29	8	8
30	10	9
31	7	7
32	10	8
33	8	7
34	6	6
35	10	10
36	10	9
37	1	1

Lab No	No of enzymes for which:	
	results were submitted by lab	lab had satisfactory performance
38	6	1
39	1	1
40	10	9
41	7	7
42	2	1
43	4	3
44	5	4
45	6	6
46	8	3
47	8	5
48	9	9
49	6	2
50	10	9
51	9	7
52	10	9
53	10	8
54	0	0 (non-submitter)
55	9	5
56	9	9
57	10	8
58	4	4
59	4	3
60	10	7
61	10	9
62	10	7
63	8	7
64	2	2
65	9	1
66	10	8
67	8	8
68	4	3
69	6	1
70	1	1
71	4	0 (partial submitter)
72	10	7
73	10	8
74	5	5
75	9	3

7. Comments here on overall scheme performance

Overall all samples were correctly interpreted, only for the enzymes α-Galactosidase(Fabry) and α-Glucosidase the % of maximal possible score was below 90%. For the sample of the Fabry patient(LF2) about 9% of the participants both the interpretation and the amount of enzyme activity measured was not correct. In sample LF4(Hurler patient) about 25% of the participants measured an enzyme activity of α-Glucosidase on the level of Pompe patient.

8. Comparison to previous years

In 2016 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 2014, 2015 and 2016 however there was a remarkable increase in the number of participants with CV>35 for the analytes α-Galactosidase α-Glucosidase and galactocerebrosidase in 2016.

Table 8: Comparison between CV data from 2014, 2015 and 2016

	2014				2015				2016			
	%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	7%	83%	10%	71	10%	80%	10%	71	5%	84%	11%	74
Arylsulfatase A	14%	63%	23%	56	-	-	-	-	-	-	-	-
α-Galactosidase	8%	73%	19%	64	11%	66%	23%	62	10%	57%	32%	68
β-Galactosidase	12%	63%	25%	69	11%	64%	26%	66	6%	73%	22%	69
α-Glucosidase	8%	68%	24%	49	10%	63%	27%	48	10%	46%	44%	52
β-Glucosidase	11%	70%	19%	64	14%	67%	19%	63	9%	70%	21%	67
β-Hexosaminidase A	13%	66%	21%	62	15%	72%	13%	60	7%	70%	24%	59
β-Hexosaminidase A+B	-	-	-	-	12%	60%	28%	60	6%	59%	35%	63
α-Iduronidase	4%	76%	20%	54	13%	69%	19%	54	7%	70%	23%	57
Iduronate-2-sulphate sulphatase	10%	61%	29%	43	-	-	-	-	-	-	-	-
Galactosamine-6-sulphate sulphatase	-	-	-	-	15%	59%	27%	41	12%	60%	29%	42
Galactocerebrosidase	7%	65%	28%	46	13%	63%	24%	46	10%	52%	38%	48
Sphingomyelinase	9%	59%	17%	46	16%	71%	14%	44	9%	64%	27%	45

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Scheme Organiser

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	0	2	12	2	2	3	2	2	3	2	2
2	25	2	22	2	2	3	2	2	10	2	2
3	23	2	15	2	2	10;d-	2	1	66	0	2
4	R0	0	R0	0	2	R0	0	2	R0	0	2
5	17	2	200	0	2	7	2	2	13	2	2
6	8	2	33	2	2	9	2	2	1	2	2
7	1	2	8	2	2	10	2	2	5	2	2
8	6	2	0	2	2	16	2	2	31	2	2
9	10	2	128	0	2	R0	0	2	78	0	2
10	22	2	16	2	2				30	2	2
11	6	2	R0;D0	0	2	21	2	2	5	2	2
12	6	2	22	2	2	12	2	2	14	2	2
13	R0	0	R0;D0	0	0	200;D+	0	1			
14	10	2	59	1	2	10	2	2	56	1	2
15	1	2	160	0	2	8	2	2	30	2	2
16	4	2	13	2	2	2	2	2	4	2	2
17	4	2	17	2	2	11	2	2	2	2	2
18	9	2	2	2	2	4	2	2	0	2	2
19	5	2	77	0	2	4;d-D-	2	0	90	0	2
20	0	2	36	1	2				8	2	2
21	8	2	10	2	2	12	2	2	0	2	2
22	11	2	18	2	2	3;D-d-	2	0	5;D-d-	2	0
23	0	2	147	0	2	35;D-	1	1	73;D-	0	1
24	4	2	139	0	2	9;d-D-	2	0	7	2	2
25	3	2	9	2	2	19	2	2	7	2	2
26	39	1	19	2	2	2	2	2	8	2	2
27	9	2							29	2	2
28	34	2	7	2	2	0	2	2	1	2	2
29	2	2	3	2	2	0	2	2	6	2	2
30	8	2	17	2	2	15	2	2	8	2	2
31	1	2	2	2	2	2	2	2	1	2	2
32	42	1	65	0	2	46	1	2	34	2	2
33	3	2	46	1	2	54	1	2	45	1	2
34	38	1	1	2	2	3	2	2	6	2	2
35	12	2	17	2	2	15	2	2	3	2	2
36	11	2	26	2	2	15	2	2	13	2	2
37	67	0				15D+D0	2	1			
38	62	0				R0	0	2	R0	0	2
39	6	2									
40	12	2	1	2	2	1	2	2	13	2	2
41	16	2	39	1	2	20;D-	2	1	16	2	2
42	4	2				19;d-D-	2	0	20	2	2
43	6	2	1;2D-	2	0				0	2	2
44	20	2	27	2	2	32;D-d-	2	0	24;D-	2	1
45	40	1				28	2	2	34	2	2
46	192	0	4	2	2	R0	0	2	8	2	2

	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
47	11	2	9	2	2	2;D-d-	2	0	1	2	2
48	9	2	21	2	2	14	2	2	2	2	2
49	15	2	18;D-	2	1	46;D0	1	1	28	2	2
50	2	2	14	2	2	14	2	2	14	2	2
51	6	2	21	2	2	11	2	2	22	2	2
52	18	2	14	2	2	18;D-	2	1	5	2	2
53	11	1	5	2	2	14	2	2	21	2	2
54	nd		nd			nd			nd		
55	9	2	84	0	2	103	0	2	79	0	2
56	4	2	16	2	2	1	2	2	9	2	2
57	9	2	18	2	2	124	0	2	21	2	2
58	8	2				0	2	2			
59	3	2	4;2D-	2	0	15;D-	2	1	5;d-	2	1
60	26	2	200	0	2	29	2	2	81;2d+	0	0
61	16	2	3	2	2	24	2	2	10	2	2
62	12	2	1	2	2	59	1	2	24	2	2
63	8	2	54	1	2	18	2	2	13	2	2
64	2	2							1;D-	2	1
65	R0	0	R0;D0	0	2	R0	0	2	R0	0	2
66	16	2	64	0	2	19	2	2	27	2	2
67	6	2	21	2	2	2	2	2	1	2	2
68	24	2	47	1	2	9	2	2	28	2	2
69	27	2				R0;D0	0	1	33	2	2
70	10	2				1;D0	2	1	nd		
71	R0	0				R0;D0;D+	0	1	R0;D0	0	1
72	3	2	57	1	2	29	2	2	53	1	2
73	12	2	90	0	2	43	1	2	60	0	2
74	11	2	7;D-	2	1	2	2	2	16	2	2
75	44	1	11	2	2	54	1	2	36	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
D- = patient sample not indicated as patient in drop down list
D+ = sample falsely indicated as patient sample in drop down list
D0 = patient sample not measured
R0 = CV calculation not possible as one or both of LF3 and LF6 (duplicate samples) were not measured
nd = not detected (i.e. result not submitted)

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	4	2	2	10	2	2	6	2	2	6	2	2
2	19;2d+	2	1	5	2	2	20	2	2	7	2	2
3	7	2	2	47	1	2	5	2	2	35	1	2
4	R0	0	2	R0	0	2	R0	0	2	R0;D0	0	2
5	11;2d+	2	1	43	1	2	28	2	2	21	2	2
6	12	2	2	1	2	2	11;D+	2	1	3	2	2
7	8;2d+	**2**	**1**	8	2	2	18	2	2	3	2	2
8	5;d+	2	1	1	2	2	26	2	2	0	2	2
9	3	2	2	56	1	2	29	2	2	19	2	2
10				29	2	2	90:D+	0	1			
11	7;D+d+	2	0	12	2	2	R0	0	2	25;D0	2	0
12	80	0	2	4	2	2	62	0	2	8	2	2
13				R0;d-D-	0	0						
14	126	0	2	0	2	2	43	1	2	38	1	2
15	6;2d+	2	1	38	1	2	8	2	2	7	2	2
16	2;D+	2	1	7	2	2	4	2	2			
17	6	2	2	0	2	2	4	2	2	5	2	2
18	3	2	2	3	2	2	5	2	2	8	2	2
19	21;d+D+	2	0	9	2	2	0;D+d+	2	0	5	2	2
20				29	2	2	3	2	2	22	2	2
21				1	2	2	8	2	2	11	2	2
22				5	2	2	54;D+	1	1	44	1	2
23				113;D-	0	1	14	2	2			
24				8	2	2	7	2	2	135	0	2
25	55;d+	1	1	2	2	2	21	2	2	29	2	2
26	6	2	2	14;D-	2	1	0	2	2	13	2	2
27				1	2	2				10	2	2
28	41;d+	1	1	3	2	2	0	2	2	15	2	2
29	4	2	2	4	2	2				10	2	2
30	77;d+	0	1	19	2	2	37	1	2	6	2	2
31	4	2	2	2	2	2						
32	24	2	2	30	2	2	31	2	2	19	2	2
33	42;2d+	1	1	57	1	2	30	2	2	36	1	2
34				10	2	2	1	2	2			
35	3	2	2	5	2	2	14	2	2	9	2	2
36	5	2	2	5	2	2	12	2	2	6	2	2
37												
38	R0	0	2	R0;D0	0	2				R0	0	2
39										8	2	2
40	12;d+	2	1	3	2	2	4	2	2	25	2	2
41	24	2	2	24	2	2	4	2	2	43	1	2
42												
43							7	2	2			
44				9	2	2	25:D+	2	1			
45	36	1	2	17:D-	2	1	29	2	2	17;D-	2	1
46	R0;d+D+	0	0	88;D-d+	0	0	23	2	2	109	0	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
47				1	2	2	11	2	2	3	2	2
48	15	2	2	9;d-	2	1	11	2	2	15	2	2
49	92;D+	0	1	160;D-	0	1						
50	5	2	2	22	2	2	10	2	2	11	2	2
51	117	0	2	47	1	2	24	2	2	86	0	2
52	11	2	2	4	2	2	8	2	2	9	2	2
53	144	0	2	4	2	2	6	2	2	26	2	2
54	nd			nd			nd			nd		
55	4	2	2	12	2	2	37	1	2	55	1	2
56	4	2	2	6	2	2	2	2	2	9	2	2
57	36	1	2	32	2	2	22	2	2	32	2	2
58	11	2	2	5	2	2				0	2	2
59							30	2	2			
60	16;d+	2	1	0	2	2	10;D+	2	1	2	2	2
61	15	2	2	3	2	2	24	2	2	6	2	2
62	21;d+D+	2	0	33	2	2	39;D+	1	1	1	2	2
63	32	2	2	34	2	2	13	2	2	31	2	2
64				7	2	2						
65	R0	0	2	R0	0	2	R0	0	2	R0;D0	0	2
66	4	2	2	3	2	2	17	2	2	26	2	2
67	16	2	2	7	2	2				5	2	2
68							48;2D+	1	0			
69				R0	0	2				R0;D0	0	1
70												
71	R0	0	2	R0;D0D+	0	1						
72	62	0	2	2	2	2	60	0	2	14	2	2
73	8	2	2	9	2	2	1	2	2	15;D0	2	1
74				22;D-	2	1	0;D+	2	1			
75				60	0	2	R0	0	1	131	0	2

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

	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
Lab No	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
1	3	2	2	0	2	2	1	2	2
2	19	2	2						
3	15	2	2	0	2	2	52	1	2
4				R0	0	2	R0	0	2
5	16	2	2	42	1	2	5	2	2
6				15	2	2	22	2	2
7									
8	10	2	2	0	2	2	23	2	2
9	51	1	2	10	2	2	71	0	2
10									
11	R0	0	2	9	2	2	1	2	2
12	40	1	2				8	2	2
13				R0	0	2			
14	R0	0	2	49	1	2	R0;d+	0	1
15	83	0	2	0	2	2	40	1	2
16				1	2	2	1	2	2
17	1	2	2	11	2	2	4	2	2
18	2	2	2	0	2	2	1	2	2
19	22	2	2	55	1	2	42	1	2
20	50	1	2	9;d+	2	1	12	2	2
21									
22				45	1	2			
23									
24	31	2	2	5	2	2	6	2	2
25	5	2	2	22	2	2			
26	4	2	2	5	2	2	28	2	2
27	2	2	2	10;d+	2	1			
28	0	2	2	12	2	2	7	2	2
29	9	2	2				14	2	2
30	40	1	2	14;d+	2	1	6	2	2
31	1	2	2				8	2	2
32	9	2	2	0	2	2	74	0	2
33	21	2	2						
34				0	2	2			
35	3	2	2	1	2	2	5	2	2
36	113	0	2	42	1	2	27	2	2
37									
38	R0	0	2						
39									
40	66	0	2	3	2	2	1	2	2
41									
42									
43				45	1	2			
44									
45									
46	R0;d+	0	1						

Lab No	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
47				66	0	2	62	0	2
48	19	2	2	15	2	2			
49	140	0	2						
50	12	2	2	72	0	2	22	2	2
51				3	2	2	46	1	2
52	1	2	2	82	0	2	22	2	2
53	9	2	2	91	0	2	14	2	2
54	nd			nd			nd		
55				57	1	2	94	0	2
56				4	2	2	5	2	2
57	158	0	2	5	2	2	33	2	2
58									
59									
60	41	1	2	4;d+	2	1	63	0	2
61	8	2	2	61	0	2	13	2	2
62	13	2	2	32	2	2	105	0	2
63							63	0	2
64									
65				R0	0	2	R0	0	2
66	80	0	2	28	2	2	14	2	2
67				18	2	2	3	2	2
68									
69				R0	0	2	R0	0	2
70									
71									
72	95	0	2	48	1	2	8	2	2
73	2	2	2	47	1	2	4	2	2
74									
75	R0	0	2	R0	0	2	70	0	2