ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Lysosomal Enzymes in fibroblasts

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Annual Report 2017

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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 2017 scheme

Sample	Disorder	Enzyme defect
LF1	Tay Sachs	beta- Hexosaminidase A
LF2	Pompe	Alpha-Glucosidase
LF3	Krabbe	Galactosylceramidase
LF4	Niemann-Pick A/B	Sfingomyelinase
LF5	MPS IV A	Galactosamine-6-sulphate sulphatase
LF6	Tay Sachs	Beta-Hexosaminidase A

3. Shipment

One shipment of 6 samples was sent out on the 14th February 2017, to the 72 laboratories, from 29 countries, which registered for the scheme.

4. Receipt of results

There were three submission deadlines from March to September 2017, 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.

Version Number (& Date)	Amendments
[1] version 2 (02 May 2018)	• Page 3, section 6: text updated to reflect change in performance for lab 27 (see below). • Page 3, table 4: number of labs which submitted results for 1 enzyme changed from 2 to 3 and total number of submitting labs changed from 66 to 67. • Page 4, table 6: numbers updated to reflect change in performance for lab 27 (see below). • Page 5, table 7: results for lab 27 changed to: – No of enzymes for which results were submitted by lab = 1 – No of enzymes for which lab had satisfactory performance = 1

Table 2: Analytes to be measured

Analyte	Parameter 1	Parameter 2
Protein	mg/vial	
beta-Hexosaminidase A+B	37 degr; nmol/h/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 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

Sixty-seven laboratories (93% of registered laboratories) submitted sufficient results for their performance to be assessed. Five laboratories (7%) did not submit enough results for their performance to be assessed.

Full details of each participants results are given in Appendix 1 but summaries are 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.
- All 67 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	0
3	0
4	5
5	3
6	5
7	1
8	11
9	11
10	28
Total number of labs	**67**

Table 5: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	71	n.a.	92	92
β-Hexosaminidase A+B	63	96	83	90
α-Galactosidase	66	97	80	88
β-Galactosidase	67	98	82	90
α-Glucosidase	54	87	82	85
β-Glucosidase	67	95	74	84
β-Hexosaminidase A	58	91	72	82
α-Iduronidase	57	94	83	89
Galactosamine-6-sulphate sulphatase	44	94	78	86
Galactocerebrosidase	44	81	75	78
Sphingomyelinase	44	88	74	81

[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%	0	0%
50% –59%	1	1.5%
60% –69%	6	8.9%
70% –79%	10	14.9%
80% –89%	18	26.9%
90% –99%	27	40.3%
100%	6	9.0%
Totals	**67**	**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	6
3	10	10
4	8	7
5	10	9
6	9	8
7	8	8
8	10	10
9	10	9
10	4	4
11	10	9
12	9	8
13	3	0 (partial submitter)
14	10	7
15	10	10
16	8	8
17	10	10
18	10	10
19	10	8
20	6	3
21	6	6
22	8	7
23	5	4
24	9	8
25	9	8
26	9	4
27	1	1
28	9	9
29	8	8
30	10	9
31	10	9
32	8	7
33	7	5
34	10	10
35	10	9
36	1	1
37	6	6
38	3	1 (partial submitter)
39	10	10

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

7. Comments on overall scheme performance

Overall all samples were correctly interpreted, only for the enzymes α-Iduronidase (Hurler), α-Glucosidase and ß-hexosaminidase the % of maximal possible score was just below 90%. For the sample of the Tay-Sachs patient (LF1 and LF6) the CV was low because of the patient value.

8. Comparison to previous years

In 2017 and 2016 arylsulfatase and iduronate-2-sulphate sulphatase activity measurements were not in the scheme, therefore no comparison can be made. For a lot of enzymes and the protein measurements there was an improvement in the reproducibility. Only for ß-Glucosidase, Sphingomyelinase and ß-Hexosaminidase A there was a decrease in the CV in 2017, especially for ß-Hexosaminidase, probably because of the rather low activity in the duplicate samples. The measurement of galactocerebrosidase activity remains rather difficult, seen the rather high CV.

Table 8: Comparison between CV data from 2015, 2016 and 2017

	2015				2016				2017			
	%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	10%	80%	10%	71	5%	84%	11%	74	7%	86%	8%	71
α-Galactosidase	11%	66%	23%	62	10%	57%	32%	68	5%	74%	21%	66
β-Galactosidase	11%	64%	26%	66	6%	73%	22%	69	6%	76%	18%	67
α-Glucosidase	10%	63%	27%	48	10%	46%	44%	52	11%	78%	11%	54
β-Glucosidase	14%	67%	19%	63	9%	70%	21%	67	10%	67%	24%	67
β-Hexosaminidase A	15%	72%	13%	60	7%	70%	24%	59	0%	62%	38%	58
β-Hexosaminidase A+B	12%	60%	28%	60	6%	59%	35%	63	3%	76%	21%	63
α-Iduronidase	13%	69%	19%	54	7%	70%	23%	57	2%	79%	19%	57
Galactosamine-6-sulphate sulphatase	15%	59%	27%	41	12%	60%	29%	42	5%	70%	25%	44
Galactocerebrosidase	13%	63%	24%	46	10%	52%	38%	48	0%	68%	32%	44
Sphingomyelinase	16%	71%	14%	44	9%	64%	27%	44	5%	68%	27%	44

Kees Schoonderwoerd
Scientific advisor

Cas Weykamp
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			Score	
Lab No	CV	CV	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
1	1	2	10	2	2	4	2	2	14	2	2
2	8	2	11	2	2	95	0	2	6	2	2
3	5	2	41	1	2	18	2	2	9	2	2
4	5	2	8	2	2	29	2	2	5	2	2
5	4	2	9	2	2	1	2	2	11	2	2
6	5	2	15	2	2	0	2	2	4	2	2
7	20	2	26	2	2	12	2	2	10	2	2
8	11	2	4	2	2	18	2	2	14	2	2
9	18	2	0	2	2	4	2	2	31	2	2
10	5	2	10	2	2				42	1	2
11	0	2	15	2	2	1	2	2	3	2	2
12	16	2	33	2	2	17	2	2	27	2	2
13	R0	0	R0	0	0	R0	0	2			
14	73	0	52	1	2	62	0	2	35	1	2
15	14	2	4d+	2	1	5	2	2	13	2	2
16	6	2	5	2	2	11	2	2	6	2	2
17	16	2	9	2	2	6	2	2	0	2	2
18	4	2	7	2	2	9	2	2	3	2	2
19	22	2	35	1	2	56	1	2	35	1	2
20	26	2	64	0	2				26	2	2
21	48	1	11	2	2	13	2	2	42	1	2
22	10	2	1	2	2	2	2	2	11	2	2
23	9	2	19	2	2	18	2	2	15	2	2
24	3	2	7	2	2	3	2	2	17	2	2
25	1	2	9	2	2	6	2	2	36	1	2
26	63	0	120	0	2	88	0	2	71	0	2
27	2	2							R0	0	2
28	2	2	11	2	2	3	2	2	15	2	2
29	0	2	6	2	2	8	2	2	47	1	2
30	14	2	34	2	2	53	1	2	41	1	2
31	0	2	24	2	2	5	2	2	54	1	2
32	0	2	6	2	2	3	2	2	11	2	2
33	R0	0	2	2	2	29	2	2	19	2	2
34	31	2	6	2	2	22	2	2	9	2	2
35	36	1	2	2	2	8	2	2	23	2	2
36	22	2				16;D+	2	1			
37	3	2				6	2	2	2	2	2
38	14	2									
39	4	2	4	2	2	19	2	2	34	2	2
40	4	2	3	2	2	23	2	2	15	2	2
41	1	2	10	2	2				16	2	2
42	5	2	3	2	2	12	2	2	6;D+	2	1
43	5	2				1	2	2	3	2	2
44	9	2	26	2	2	11	2	2	199	0	2
45	9	2	9	2	2	25	2	2	13	2	2

Lab No	Protein/vial		B-Hexosaminidase A+B			α-Galactosidase			β-Galactosidase		
		Score		Score			Score			Score	
	CV	CV	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
46	4	2	24	2	2	36	1	2	5	2	2
47	R0	0	R0	0	1	R0	0	2	R0	0	2
48	3	2	9	2	2	11	2	2	1	2	2
49	3	2	52	1	2	1	2	2	17	2	2
50	6	2	12	2	2	39	1	2	5	2	2
51	30	2	5	2	2	34	2	2	29	2	2
52	18	2	5	2	2	13	2	2	14	2	2
53	16	2	37	1	2	22	2	2	30	2	2
54	11	2	21	2	2	8	2	2	2	2	2
55	9	2	29	2	2	5	2	2	11	2	2
56	9	2				9	2	2			
57	32	2	28	2	2	31	2	2	34	2	2
58	1	2	42	1	2	37	1	2	200:d+	0	1
59	2	2	199	0	2	62	0	2	22	2	2
60	13	2	52	1	2	36	1	2	8	2	2
61	9	2	1	2	2	30	2	2	12	2	2
62		0							R0	0	2
63	25	2	0	2	2	11	2	2	5	2	2
64	5	2	91	0	2	17	2	2	7	2	2
65	5	2	38	1	2	176;D+d+	0	0	76	0	2
66	16	2	13	2	2	7	2	2	1	2	2
67	59	1				54	1	2			
68	R0	0				R0;D+	0	1	R0	0	2
69	11	2	49	1	2	113	0	2	17	2	2
70	5	2	3	2	2	90	0	2	1	2	1
71	4	2	23	2	1	8	2	2	2	2	2
72	66	0	27	2	2	11	2	2	2	2	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	1	2	2	1	2	2	11	2	2	5	2	2
2	34	2	2	82	0	2	30	2	2	12;d+	2	1
3	15	2	2	5	2	2	12	2	2	14	2	2
4	2	2	2	17	2	2	67	0	2	11	2	2
5	1	2	2	0	2	2	200	0	2	57	1	2
6	1	2	2	9	2	2	97	0	2	16	2	2
7	10	2	2	21	2	2	50	1	2	9	2	2
8	10	2	2	8	2	2	5	2	2	12	2	2
9	10	2	2	11	2	2	24	2	2	75	0	2
10				28	2	2	9	2	2			
11	6;d0	2	0	15	2	1	0	2	2	4	2	2
12	16	2	2	40	1	2	67	0	2	4	2	2
13				R0	0	2						
14	11	2	2	1	2	2	25	2	2	0	2	2
15	22	2	2	13	2	2	17;d+	2	1	20:d+	2	1
16	2	2	2	1	2	2	7	2	2			
17	8	2	2	11	2	2	17	2	2	5	2	2
18	3	2	2	2	2	2	20	2	2	31	2	2
19	68	0	2	42	1	2	0	2	2	20	2	2
20				77	0	2	18	2	2	24	2	2
21				10	2	2	58	1	2	18:D+	2	1
22				23	2	2	16	2	2	17	2	2
23				149	0	2	41	1	2			
24				6	2	2	40	1	2	75	0	2
25	7	2	2	7	2	2	36	1	2	76	0	2
26	105	0	2				197;D-D+	0	1	0	2	2
27				R0	0	2				R0;D+	0	1
28	5	2	2	4	2	2	6	2	2	5;d+	2	1
29	1	2	2	7	2	2				4;d+	2	1
30	63	0	2	26	2	2	18	2	2	34	2	2
31	11	2	2	18	2	2	22	2	2	8	2	2
32	1	2	2	1	2	2	51	1	2	13	2	2
33				177	0	2	46	1	2			
34	22	2	2	16	2	2	18	2	2	3	2	2
35	27	2	2	3	2	2	20	2	2	11	2	2
36												
37	56	1	2	19	2	2				9	2	2
38	Ro;D0	0	0	R0	0	0				26	2	2
39	21;D0	2	1	16	2	2	3	2	2	8	2	2
40	16	2	2	41	1	2				50	1	2
41				4	2	2	70	0	2			
42				9	2	2	14	2	2			
43	R0;D0	0	0	2	2	2	41:D--	1	1	17	2	2
44	21	2	2	96	0	2	76	0	2	22	2	2
45				3	2	2	78	0	2	19	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
46	41	1	2	12	2	2	5	2	2	6	2	2
47	R0;D0	0	1	R0	0	0						
48	15	2	2	2	2	2	0	2	2	7	2	2
49	30	2	2	83	0	2	12	2	2	39	1	2
50	4	2	2	6	2	2	7	2	2	14	2	2
51	21;D-	2	1	33	2	2	35	1	2	13	2	2
52	7;D-	2	1	45	1	2	3	2	2	10;d+	2	1
53	20;D-d-	2	0	18	2	2	44	1	2	27	2	2
54	2	2	2	3	2	2	10	2	2	8	2	2
55	8	2	2	6	2	2	10	2	2	13	2	2
56	15	2	2	7	2	2				6	2	2
57							5;D-	2	1			
58	1	2	2	10	2	2	19;D-	2	1	11	2	2
59	34	2	2	100	0	2	34	2	2	1	2	2
60	17	2	2	4	2	2	76	0	2	41	1	2
61	13	2	2	64	0	2	48	1	2	15	2	2
62	R0	0	2	R0	0	2						
63	9;D+	2	1	0	2	2	6;D—d--	2	0	6	2	2
64	33	2	2	52	1	2	34	2	2	5	2	2
65	6	2	2	12	2	2				39	1	2
66							15	2	2			
67												
68	R0;D-	0	1	R0;D+	0	1						
69	26	2	2	56	1	2	12	2	2	61	0	2
70	38	1	2	46	1	2	135;D-	0	1	107	0	2
71				7;d0	2	1						
72				36	1	2	200;D-d-	0	0	133	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	1	2	2	3;d+	2	1	10	2	2
2	24	2	2						
3	12	2	2	0	2	2	25	2	2
4							16	2	2
5	12	2	2	23	2	2	23	2	2
6				21	2	2	1	2	2
7	24	2	2						
8	6	2	2	2	2	2	1	2	2
9	5	2	2	32	2	2	32	2	2
10									
11	26	2	2	43	1	2	8	2	1
12	57	1	2				14	2	2
13									
14	35;D0	2	1	83	0	2	76	0	2
15	2	2	2	4	2	2	20	2	2
16				47	1	2	1	2	2
17	9	2	2	7	2	2	3	2	2
18	2	2	2	30	2	2	5	2	2
19	36	1	2	23;d-d+	2	1	74	0	1
20	119;D-d-	0	0						
21									
22	R0;D0	0	1	25;D-	2	1			
23									
24	46	1	2	8	2	2	5	2	2
25	1	2	2	7	2	2			
26	10	2	2	0;D-d+	2	1	27;D0	2	1
27	26	2	2						
28				9;D-	2	1	10	2	2
29	3	2	2				15	2	2
30	59	1	2	28	2	2	9	2	2
31	5	2	2	13;D-	2	1	R0;D0	0	0
32	72	0	2						
33				15;d0	2	1	R0	0	0
34	21	2	2	19	2	2	7	2	2
35	15	2	2	68	0	2	4	2	2
36									
37	42	1	2						
38									
39	22	2	2	16	2	1	31	2	2
40									
41				2	2	2			
42									
43									
44	27	2	2						
45				7	2	2	66	0	2
46	17	2	2	41;d+	1	1			
47	R0	0	2						

Lab No	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
48	24	2	2	41	1	2	11	2	2
49				108;D-	0	1	46	1	2
50	11	2	2	134	0	2	5	2	2
51	29	2	2	31	2	2	26	2	2
52	119	0	2	16;D+d+	2	0	75	0	2
53	2	2	2	58	1	2	75	0	2
54				18	2	2	42	1	2
55	4	2	2	135;D-d-	0	0	23	2	2
56									
57									
58	12;D+	2	1	10;d+	2	1	103	0	2
59	60	1	2	120	0	2	49	1	2
60	6	2	2	56	1	2	10	2	2
61							19	2	2
62									
63				22	2	2	200;D+	0	1
64	18	2	2	15	2	2	5;d-	2	1
65				17;D-	2	1	39	1	2
66									
67									
68									
69	46	1	2	25	2	2	48;D-d-	1	0
70	9	2	2	60	0	2	34	2	2
71									
72	78	0	2	86;D+	0	1	10	2	2