ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Lysosomal Enzymes in fibroblasts

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Annual Report 2018
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1. Scheme Design

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

Dr. Kees Schoonderwoerd has now retired as Scientific Advisor.

Ms Marie Jackson (Scientific Advisor from November 2018 onwards) therefore performed the scoring for the 2018 scheme and has organised and designed the scheme for 2019.

1.1. Sub-contracted activities:

The fibroblasts used as the EQA materials were cultured by AMC, Rotterdam.

The fibroblasts were prepared and aliquoted by SKML, Netherlands, which also hosts and manages the results submission website (www.erndimqa.nl) on behalf of ERNDIM.

2. Samples

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

Table 1: EQA samples included in the 2018 scheme

Sample	Disorder	Enzyme defect	Reporting deadline
LEFB2018.01	MPS I	alpha-iduronidase	30th March 2018
LEFB2018.02	Control	Normal activities	
LEFB2018.03	Control	Normal activities	25th May 2018
LEFB2018.04	Krabbe leucodystrophy	galactosylceramidase	
LEFB2018.05	Gaucher disease	beta-glucosidase	28th September 2018
LEFB2018.06	Fabry disease	alpha-galactosidase	

3. Shipment

One shipment of 6 samples was dispatched on the 13th February 2018, to the 73 laboratories, from 27 countries, which registered for the scheme.

4. Receipt of results

There were three submission deadlines for the 2018 scheme: 30th March (LEFB2018.01 &.02), 25th May (LEFB2018.03 &.04) and 28th September 2018 (LEFB2018.05 & 06). Laboratories were asked to submit results for each EQA sample by the relevant submission deadline using the results website www.erndimqa.nl. All submitted results are treated as confidential information and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting.

Laboratories were asked to report the total protein and the activities for 10 enzymes in absolute units and as a percentage of their own laboratory's control, see Table 2 for details. Laboratories

Version Number (& Date)	Amendments
[1] version 2 (25th June 2019)	• Page 8: Preview of 2019 scheme updated (item 11.1).

could submit results for as many, or as few, of these 10 enzymes as they wished and were 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	-
α -Galactosidase	nmol/h/mg	% mean control
Galactose-6-sulphate sulphatase	nmol/17h/mg	% mean control
β -Galactosidase	nmol/h/mg	% mean control
α -Glucosidase	nmol/h/mg	% mean control
β -Glucosidase	nmol/h/mg	% mean control
β -Hexosaminidase A	nmol/h/mg	% mean control
β -Hexosaminidase A+B	nmol/h/mg	% mean control
α -Iduronidase	nmol/h/mg	% mean control
Galactosylceramidase	nmol/17h/mg	% mean control
Sphingomyelinase	nmol/h/mg	% mean control

5. Reports

All data-transfer, the submission of data as well as request and viewing of reports is via the interactive website www.erndimqa.nl which can also be reached through the ERNDIM website (www.erndim.org). The results of each laboratory are confidential and only accessible by password protected laboratory accounts. The anonymised mean results of all labs are accessible to all participants. Statistics of the respective reports are explained in the general information section of the website.

Short-term reports on the six individual specimens are available two weeks after the submission deadline and provide up-to-date information on analytical performance. Although it is technically possible to produce reports immediately there is a delay of 14 days to enable the scientific advisor to inspect the results and add comments to the report when appropriate.

A second important characteristic of the website is the different levels of detail of results which allows individual laboratories the choice of fully detailed and/or summarised reports. The "Analyte in Detail" is the most detailed report and shows results of a specific analyte in a specific sample. Thus for the 10 enzymes in the year 2018 cycle, 6 x 10 = 60 such Analyte-in-Detail-reports can be requested. A more condensed report is the "Cycle Review" which summarises the performance for all enzymes in a specific sample (6 such Cycle Reviews can be requested in 2018).

6. Scoring scheme and Poor performance policy

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 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.

A letter pointing out failure to achieve these levels will be issued to those laboratories which do not achieve satisfactory performance. The letter is intended to instigate dialogue between the EQA Scientific Advisor and the participating laboratory in order to solve any particular analytical problems in order to improve quality of performance of labs in the pursuit of our overall aim to improve quality of diagnostic services in this field.

6.1. 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.

Diagnosis incorrect indicates incorrect interpretation and incorrect enzyme activity level.

6.2. Coefficient of variation

Results submitted for samples LF2 and LF3 were used to calculate the coefficient of variation (CV) according to the following formula.

CV = Activity LF3-activity LF2/mean

7. Results

Sixty-two laboratories (85% of registered laboratories) submitted sufficient results for their performance to be assessed. Eight laboratories (11% of registered laboratories) did not submit enough results for their performance to be assessed; 2 laboratories (3% of registered laboratories) did not submit any results; and 1 (1%) laboratory withdrew from the scheme.

Table 4: Results returns for the 2018 scheme

	Submission Deadline					
	30th March 2018		**25th May 2018**		**28th Sept. 2018**	
Sample Numbers:	2018.01	2018.02	2018.03	2018.04	2018.05	2018.06
No. of labs that submitted results:						
By the submission deadline	68 *(93.2%)*	66 *(90.4%)*	67 *(91.8%)*	67 *(91.8%)*	63 *(86.3%)*	63 *(86.3%)*
Within 7 days of the submission deadline	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*	3 *(4.1%)*
Within 2 weeks of the submission deadline	0	0	0	0	0	0
Did not submit	4 *(5.5%)*	6 *(8.2%)*	5 *(6.8%)*	5 *(6.8%)*	9 *(12.3%)*	7 *(9.6%)*

The results for each sample were published on the results website 14 days after the relevant submission deadline

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

- Over 70% of participating laboratories submitted results for 7 or more enzymes, see Table 5.
- The proficiency per analyte is given in Table 6.
- Table 7 shows the percentage of the maximum possible score for the laboratories that submitted results.
- Of the 62 laboratories that submitted results 60 scored 60% or more of their maximum possible score and were classed as satisfactory performers in this current scheme.

Table 5: Number of enzymes for which laboratories submitted results (excluding non/partial submitters)

Number of Enzymes for which results were submitted	Number of laboratories
0	0
1	1
2	1
3	4
4	7
5	2
6	1
7	4
8	14
9	11
10	17
Total number of labs	**62**

Table 6: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	70	n.a.	90	90
α -Galactosidase	66	77	85	81
Galactose-6-sulphate sulphatase	44	98	82	90
β -Galactosidase	66	99	79	89
α -Glucosidase	53	100	79	90
β -Glucosidase	66	85	80	82
β -Hexosaminidase A	60	99	79	89
β -Hexosaminidase A+B	64	98	74	86
α -Iduronidase	56	98	86	92
Galactosylceramidase	45	87	74	81
Sphingomyelinase	43	100	79	90

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

Table 7: 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%	1	1.6%
50% –59%	1	1.6%
60% –69%	4	6.5%
70% –79%	5	8.1%
80% –89%	16	25.8%
90% –99%	21	33.9%
100%	14	22.6%
Totals	**62**	**100%**

Table 8: 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	9
5	10	10
6	9	9
7	8	8
8	10	10
9	10	10
10	3	0 (partial submitter)
11	10	10
12	9	9
13	3	0 (partial submitter)
14	10	10
15	10	10
16	8	8
17	10	10
18	10	10
19	10	8
20	7	7
21	6	5
22	7	0 (partial submitter)
23	5	3
24	9	7
25	9	9
26	10	0 (partial submitter)
27	8	8
28	10	9
29	10	10
30	6	4
31	10	10
32	10	0 (partial submitter)
33	10	8
34	1	1
35	6	5
36	3	3
37	10	0 (partial submitter)
38	6	4
39	5	3
40	5	4
41	5	4
42	0	0 (non-submitter)
43	8	0 (partial submitter)
44	8	8
45	9	9
46	6	3
47	10	8
48	9	8
49	10	9
50	10	10
51	10	10
52	10	10
53	9	9
54	10	9
55	4	4
56	4	4
57	10	10
58	10	8
59	10	7
60	8	8
61	2	2
62	10	8
63	8	7
64	10	10
65	8	8
66	4	4
67	10	10
68	10	9
69	6	0 (partial submitter)
70	0	0 (non-submitter)
71	6	6
72	8	8

8. Certificates of Participation

As for other schemes, the performance for this scheme is summarised in the annual Certificate of participation. The certificate lists the total number of enzymes in the scheme, the number for which results have been submitted and the number for which satisfactory performance has been achieved. It is important to bear in mind that the certificate has to be backed up by the laboratory's individual on-line reports in the case of internal or external auditing.

9. Comments on overall scheme performance

The majority of participants made the correct interpretation: that is, the correct enzyme deficiency was observed in the samples from affected patients and normal activity was observed in the unaffected samples.

Performance overall was very good for the following enzymes: galactose-6-sulphatase, beta-galactosidase, total hexosamindase, hexosaminidase A, alpha-iduronidase and sphingomyelinase, A correct diagnosis was achieved by 98 – 100% of participants.

LEFB 06 was a patient with an alpha galactosidase deficiency (Fabry disease): The correct diagnosis of Fabry disease was made by 77% of participants. The lower proficiency is most likely to be a reflection of the difficulties of measuring alpha galactosidase in cultured fibroblasts. The assay is rarely performed in cultured cells; the majority of participating laboratories will now be offering this assay in either plasma, leucocytes and/or dried blood spots.

LEFB 02 & 03 were duplicates of a sample included as an unaffected control, and to calculate the % CV data. However, this sample was from an individual who was a heterozygote for Pompe disease and alpha-glucosidase activity in this sample was near the lower end of the normal reference range. Subsequently, some laboratories reported LEFB 02 and/or LEFB 03 as having an alpha-glucosidase deficiency. The Scientific Advisory Board has decided to class ***LEFB 02 & 03 as educational samples for alpha-glucosidase. Laboratories interpreting either or both these samples as having an alpha-glucosidase deficiency have therefore not been penalised for this result and no marks have been deducted.***

Note: The level of alpha glucosidase activity is very low in infantile patients affected with Pompe disease, but can be as high as 40% of normal levels in later onset cases of this disorder in cultured fibroblasts.

LEFB 05 was a patient with a beta-glucosidase deficiency (Gaucher disease). Most laboratories (83%) had no problems making the correct diagnosis. However, some laboratories did note this sample to have a slightly higher residual activity.

LEFB 04 was a patient with a galactosylceramidase deficiency (Krabbe Leucodystrophy): 86% of participants made the correct diagnosis in this scheme. The diagnosis of Krabbe leucodystrophy can be difficult and this is possibly related to different substrates/methods used to assay this enzyme amongst participants.

10. Comparison to previous years

Table 9: Comparison between CV data from 2016, 2017 and 2018

	2016				2017				2018			
	%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	5%	84%	11%	74	7%	86%	8%	71	6%	90%	4%	70
α-Galactosidase	10%	57%	32%	68	5%	74%	21%	66	6%	82%	12%	66
Galactose-6-sulphate sulphatase	12%	60%	29%	42	5%	70%	25%	44	7%	80%	14%	44
β-Galactosidase	6%	73%	22%	69	6%	76%	18%	67	5%	71%	24%	66
α-Glucosidase	10%	46%	44%	52	11%	78%	11%	54	2%	70%	28%	53
β-Glucosidase	9%	70%	21%	67	10%	67%	24%	67	5%	77%	18%	66
β-Hexosaminidase A	7%	70%	24%	59	0%	62%	38%	58	5%	72%	23%	60
β-Hexosaminidase A+B	6%	59%	35%	63	3%	76%	21%	63	6%	70%	23%	64
α-Iduronidase	7%	70%	23%	57	2%	79%	19%	57	4%	82%	14%	56
Galactosylceramidase	10%	52%	38%	48	0%	68%	32%	44	7%	67%	27%	45
Sphingomyelinase	9%	64%	27%	44	5%	68%	27%	44	5%	74%	21%	43

11. Preview of the scheme in 2019.

a) There will be two submission deadlines for the 2019 scheme:
- Samples 01, 02 & 03 to be submitted by 31/05/2019
- Samples 04, 05 & 06 to be submitted by 30/08/2019

b) In recent years there has been little variation in the enzymes offered in this scheme. Therefore some of the enzymes included in the scheme for 2019 have been changed, see table 10 below for details. For purposes of laboratory accreditation there is an increasing demand for the inclusion of further /different enzymes in the scheme. In order to address this requirement in future there will be regular rotation of the enzymes included each year.

Table 10: Analytes to be measured

Analyte	**2018**	**2019**
Protein	✓	✓
α -Galactosidase	✓	✓
Galactose-6-sulphate sulphatase	✓	×
β -Galactosidase	✓	✓
α -Glucosidase	✓	✓
β -Glucosidase	✓	✓
β -Hexosaminidase A	✓	×
β -Hexosaminidase A+B	✓	×
α -Iduronidase	✓	×
Galactosylceramidase	✓	✓
Sphingomyelinase	✓	×
Arylsulphatase A	×	✓
Iduronate-sulphatase	×	✓
Lysosomal acid lipase (LAL/acid/esterase)	×	✓
Palmitoyl protein thioesterase	×	✓
Tripeptidyl peptidase	×	✓

c) There are some issues which we hope to address by making some modifications to the website:

- *Variation in substrates used for assays*: When entering results on the website each laboratory will be required to enter the method used for each enzyme assay (i.e. fluorimetric, colorimetric, radio-labelled or 'other').
- *Several laboratories participating in the ERNDIM LEFB scheme do not assay lysosomal enzymes in cultured fibroblasts:* In order to address this issue, laboratories will be asked to report results as absolute units (as usual), and as a percentage of the enzyme activity obtained in sample 1 (LEFB-01) instead of entering the result as a percentage of their own laboratory control.
- *Comments box*: Participant comments may in future, be taken into account by the Scientific Advisor. Please use this box to note any issues.

11.1. Scoring for the 2019 Scheme:

The ERNDIM Scientific Advisory Board have agreed that the inclusion of scoring of interpretation in addition to scoring of quantitative results may improve the utility of this scheme for participants. Therefore during 2019 pilot scoring of interpretation will be performed by the Scientific Advisor based on the interpretations selected by participants when submitting their quantitative results. The planned pilot scoring will assign 1 point for a correct interpretation and 0 points for incorrect or missing interpretations. No negative scores will be assigned and where a laboratory does not perform the necessary testing required to identify an abnormality an interpretation of 'normal' will be assigned a score of 1.

As scoring of interpretation will be in the pilot phase for the 2019 scheme, it will not affect the performance assessment for participants and will not be included in the 2019 certificates of participation. Further information about these changes will be included in the ERNDIM annual newsletter later in 2019.

11.2. Participant workshop:

A Lysosomal Enzymes Workshop is planned at the ERNDIM Participants meeting prior to SSIEM 2019 in Rotterdam, where changes and improvements to the scheme can be discussed. Full details will be sent to all scheme participants nearer the date.

12. Questions, Comments and Suggestions

If you have any questions, comments or suggestions in addition to specific user comments please address these to the either the ERNDIM Administration Office (admin@erndim.org), the scientific advisor of the scheme, Ms Marie Jackson, (Marie.Jackson@viapath.co.uk) or the scheme organiser Dr Cas Weykamp (c.w.weykamp@skbwinterswijk.nl).

13. Confidentiality Statement

This annual report is intended for participants of the ERNDIM Lysosomal Enzymes in fibroblasts scheme. The contents should not be used for any publication without the permission of the Scientific Advisor and Administration Office.

Marie Jackson
Scientific Advisor

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	2	2	0	2	2	0	2	2	0	2	2
2	12	2	16	2	2	25	2	2	74	0	2
3	33	2	96	0	2	54	1	0	55	1	2
4	5	2	21	2	2	24	2	2	2	2	2
5	6	2	6	2	2	22	2	2	23	2	2
6	7	2	16	2	2	15	2	2	16	2	2
7	14	2	12	2	2	14	2	2	22	2	2
8	43	1	23	2	2	32	2	2	63	0	2
9	2	2	4	2	2	2	2	2	6	2	2
10	4	2	30	2	2				42	1	2
11	9	2	13	2	2	3	2	2	9	2	2
12	3	2	47	1	2	9	2	2	50	1	2
13	R0	0	R0 D-	0	0	R0	0	0			
14	2	2	20	2	2	3	2	2	9	2	2
15	5	2	14	2	2	8	2	2	28	2	2
16	1	2	2	2	2	4	2	2	2	2	2
17	2	2	8	2	2	1	2	2	0	2	2
18	29	2	1	2	2	0	2	2	6	2	2
19	2	2	40	1	2	16	2	2	41	1	2
20	1	2	65	0	2				8	2	2
21	11	2	14	2	2	2	2	0	1	2	2
22	R0	0	R0	0	2	R0	0	2	R0	0	2
23	4	2	16	2	2	48	1	0	9	2	2
24	4	2	7	2	2	2	2	0	10	2	2
25	32	2	70	0	2	37	1	2	21	2	2
26	R0	0	R0	0	2	R0	0	2	R0	0	2
27	5	2	6	2	2	119	0	2	20	2	2
28	3	2	43	1	2	2	2	2	0	2	2
29	18	2	7	2	2	16	2	2	20	2	2
30	38	1	138 (D-)	0	2	20 (D-)	2	0	14 (D-)	2	2
31	8	2	0	2	2	14	2	2	47	1	2
32	R0	0	84	0	2	13	2	2	38	1	2
33	19	2	118	0	2	5	2	0	38	1	2
34	2	2				40	1	2			
35	1	2				10	2	2	13	2	2
36	0	2									
37	14	2	24	2	2	12	2	2	17	2	2
38	8	2	29	2	2	13	2	0	6	2	2
39	8	2	16	2	2	9	2	0	20	2	2
40	8	2	13	2	2	23	2	0	14	2	2
41	14	2				9	2	2	13	2	2
42											
43	0	2	18	2	2	0	2	0	15	2	2
44	2	2	25	2	2	76	0	2	81	0	2
45	2	2	ND	0	2	ND	0	2	ND	0	2
46	9	2	33	2	2	18	2	2	27	2	2
47	2	2	73	0	2	2	2	0	6	2	2
48	12	2	3	2	2	29	2	2	36	1	2
49	2	2	8	2	2	18	2	2	25	2	2
50	5	2	20	2	2	11	2	2	5	2	2
51	2	2	39	1	2	14	2	2	13	2	2
52	10	2	1	2	2	9	2	2	15	2	2
53	2	2	1	2	2	7	2	2	2	2	2

	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
54	13	2	12	2	2	12	2	2	21	2	2
55	7	2				6	2	2			
56	111	0	115	0	2	76	0	2	89	0	2
57	31	2	19	2	2	34	2	2	62	0	2
58	10	2	39	1	2	31	2	0	58	1	2
59	24	2	6	2	2	12	2	2	6	2	0
60	7	2	7	2	2	15	2	2	9	2	2
61	17	2							11	2	2
62	17	2	29	2	2	6	2	2	26	2	2
63	10	0	99	0	2	53	1	0	45	1	2
64	17	2	18	2	2	35	1	2	17	2	2
65	8	2	70	0	2	5	2	2	25	2	2
66	12	2	0.5	2	2	15	2	2	18	2	2
67	22	2	20	2	2	24	2	2	16	2	2
68	21	2	20	2	2	20	2	0	15	2	2
69	6	2	15	2	2	8	2	2	12	2	2
70											
71	8	2	10	2	2	9	2	2	60	0	2
72	12	2	20	2	2	19	2	2	18	2	2

Key

green cells = correct interpretation
red cells = incorrect interpretation
blue cells =not all samples measured
D- = patient sample not indicated as patient in drop down list
R0 = CV calculation not possible as one or both of LF2 and LF3 (duplicate samples) were not measured
ND = not done (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	0	2	2	2	2	2	1	2	2	1	2	2
2	44	1	2	35	1	2	34	2	2	5	2	2
3	20	2	2	11	2	2	50	1	2	17	2	2
4	4	2	2	11	2	2	15	2	2	12	2	2
5	9	2	2	4	2	2	21	2	2	7	2	2
6	10	2	2	16	2	2	11	2	2	17	2	2
7	0	2	2	14	2	2	16	2	2	4	2	2
8	43	1	2	23	2	2	62	0	2	34	2	2
9	33	2	2	13	2	2	36	1	2	17	2	2
10							45	1	2			
11	12	2	2	14	2	2	10	2	2	10	2	2
12	27	2	2	43	1	2	51	1	2	17	2	2
13				R0 D-	0	0						
14	0	2	2	11	2	2	11	2	2	11	2	2
15	8	2	2	2	2	2	13	2	2	7	2	2
16	17	2	2	15	2	2	14	2	2			
17	8	2	2	6	2	2	1	2	2	2	2	2
18	21	2	2	5	2	2	6	2	2	16	2	2
19	64	0	2	16	2	2	54	1	2	23	2	2
20				2	2	2	2	2	2	1	2	2
21				7	2	2	12	2	2	19	2	2
22				R0	0	2	R0	0	2	R0	0	2
23				124	0	0	0.5	2	2			
24				77	0	2	40	1	2	70	0	2
25	44	1	2	30	2	2	31	2	2	62	0	2
26	R0	0	2	R0	0	2	R0	0	1	R0	0	2
27	17	2	2	13	2	2				8	2	2
28	3	2	2	7	2	0	40	1	2	33	2	2
29	16	2	2	20	2	2	3	2	2	21	2	2
30				178	0	2	140	0	2			
31	2	2	2	5	2	2	9	2	2	10	2	2
32	45	1	2	34	2	2	0	2	2	50	1	2
33	16	2	2	52	1	2	65	0	2	7	2	2
34												
35	36	1	2	24	2	0				18	2	2
36	10	2	2	85	0	2				36	1	2
37	5	2	2	16	2	2	24	2	2	20	2	2
38	8	2	2	8	2	0				12	2	2
39				7	2	0	4	2	2			
40				23	2	2	14	2	2			
41				34	2	0	8	2	2	33	2	2
42												
43	7	2	2	76	0	2	11	2	2	10	2	2
44				14	2	2	14	2	2	63	0	2
45	51	1	2	28	2	2	ND	0	2	116	0	2
46	2	2	2	154	0	2						
47	39	1	2	12	2	2	0	2	2	13	2	2
48	5	2	2	32	2	0	1	2	2	1	2	2
49	12	2	2	15	2	0	41	1	2	11	2	2
50	27	2	2	1	2	2	9	2	2	21	2	2
51	75	0	2	10	2	2	1	2	2	8	2	2
52	35	1	2	3	2	2	30	2	2	5	2	2
53	9	2	2	1	2	2	11	2	2	3	2	2
54	59	1	2	6	2	2	9	2	2	15	2	2
55	0	2	2	1	2	2				1	2	2
56							110	0	2			
57	35	1	2	51	1	2	24	2	2	11	2	2
58	4	2	2	15	2	2	12	2	2	23	2	2
59	91	0	2	3	2	2	20	2	2	200	0	0
60	11	2	2	2	2	2	22	2	2	5	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**
61				11	2	2						
62	10	2	2	77	0	0	4	2	2	13	2	2
63	51	2	2	30	2	2	106	0	2	20	2	2
64	4	2	2	1	2	2	11	2	2	5	2	2
65	6	2	2	18	2	2				67	2	2
66							14	2	2			
67	108	0	2	45	1	2	32	2	2	9	2	2
68	28	2	2	27	2	2	18	2	2	17	2	2
69				6	2	2	0	2	2			
70												
71	55	1	2	62	0	2	38	1	2			
72	43	1	2	13	2	2	2	2	2	17	2	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	0	2	2	1	2	2
2	14	2	2						
3	81	0	2	24	2	2	56	1	2
4				85	0	2	5	2	2
5	13	2	2	3	2	2	23	2	2
6				13	2	2	14	2	2
7	15	2	2						
8	27	2	2	87	0	2	15	2	2
9	20	2	2	15	2	2	12	2	2
10									
11	5	2	2	7	2	2	11	2	2
12	12	2	2				16	2	2
13									
14	56	1	2	6	2	2	0	2	2
15	17	2	2	63	0	2	0	2	2
16				18	2	2	1	2	2
17	4	2	2	1	2	2	3	2	2
18	30	2	2	5	2	2	29	2	2
19	2	2	2	50	1	2	5	2	2
20	4	2	2	ND	0	2			
21									
22				ND	0	0			
23									
24	ND	0	0	41	1	2	87	0	2
25	68	0	2	15	2	2	ND	0	2
26	R0	0	2	R0	0	2	R0	0	2
27	11	2	2				15	2	2
28	18	2	2	23	2	2	21	2	2
29	63	0	2	29	2	2	47	1	2
30				36 D-	1	0			
31	9	2	2	0	2	2	3	2	2
32	71	0	2	112	0	2	4	2	2
33	25	2	2	53	1	0	74	0	2
34									
35	15	2	2						
36									
37	ND	0	2	16	2	2	3	2	2
38									
39									
40									
41									
42									
43	3	2	2						
44				25	2	2	18	2	2
45	26	2	2	13	2	2			
46	52	1	2						
47	1	2	2	32	2	0	54	1	2
48				8	2	2	12	2	2
49	18	2	2	35	1	2	18	2	2
50	6	2	2	11	2	2	7	2	2
51	4	2	2	50	1	2	2	2	2
52	11	2	2	40	1	2	86	0	2
53				18	2	2	2	2	2
54	5	2	2	3	2	0	28	2	2
55									

Lab No	Galactosamine-6-sulphate sulphatase			Galactocerebrosidase			Sphingomyelinase		
		Score			Score			Score	
	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis
56									
57	21	2	2	16	2	2	2	2	2
58	33	2	2	42	1	0	0	2	2
59	7	2	2	32	2	2	42	1	2
60							26	2	2
61									
62	28	2	2	11	2	2	123	0	2
63				36	1	2			
64	4	2	2	10	2	2	1	2	2
65				0	2	2	12	2	2
66									
67	15	2	2	31	2	2	114	0	2
68	27	2	2	15	2	2	17	2	2
69	12	2	2						
70									
71									
72	10	2	2						

END OF REPORT