ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

ERNDIM Administration Office
Manchester Centre for Genomic Medicine
6th Floor, St Mary's Hospital, Oxford Road,
Manchester M13 9WL, United Kingdom.
Tel: +44 161 276 6741
Fax: +44 161 850 1145
Email: admin@erndim.org

Lysosomal Enzymes in fibroblasts

Scientific Advisor
Ms Marie Jackson
Biochemical Genetics Laboratory
Viapath Genetics Laboratories,
5th Floor Guys Tower, Guys Hospital,
London SE1 9RT
United Kingdom
Email: Marie.Jackson@viapath.co.uk

Scheme Organiser
Dr. C.W. Weykamp
Queen Beatrix Hospital
MCA Laboratory
P.O. Box 9005
NL – 7100 GG Winterswijk
Netherlands
Email: c.w.weykamp@skbwinterswijk.nl

Annual Report 2019

Date of issue: 06 May 2020

Amended report issued: 02 June 2020[1]

1. Scheme Design

The scheme has been designed, planned and coordinated by Ms Marie Jackson (as 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.

1.1. Sub-contracted activities:

The fibroblasts used as the EQA materials were cultured by Centre de Biotechnologie Cellulaire, CHU de Lyon. 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: Samples included in EQA scheme

Sample	Disorder	Enzyme defect	Reporting deadline
LEFB2019.01	Normal control	All enzyme activities normal	31st May 2019
LEFB2019.02	MLD	Arylsulphatase A deficiency	
LEFB2019.03	NCL2	Tripeptidyl peptidase I deficiency	
LEFB2019.04	Normal control	All enzyme activities normal	30th August 2019
LEFB2019.05	GM1 gangliosidosis	beta-galactosidase deficiency	
LEFB2019.06	Pompe (GSD II)	alpha-glucosidase deficiency	

3. Shipment

One shipment of six samples was dispatched on 12th February 2019, to the 73 laboratories, from 30 countries, which registered for the scheme.

4. Receipt of results

There were two submission deadlines for the 2019 scheme: (LEFB2018.01, 02 & 03 on 31st May) and (LEFB2018.04, 05 & 06 on 30th August). 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 in mg/vial and the activities for 10 enzymes in:

Version Number (& Date)	Amendments
version 1 (06 May 2020)	• 2019 Annual Report published
[1] version 2 (02 June 2020)	• Page 4: tables 5, 6 & 7 updated to reflect the changes made to Appendix 1. • Page 5: table 8 updated to reflect the changes made to Appendix 1. • Page 8: table 11 updated to reflect changes made to Appendix 1. • Pages 10-11, Appendix 1 (part 1): LAL – score corrected for lab 15; β-Galactosidase – score corrected for lab 15 score corrected & score added for lab 35 score. • Pages 12-13, Appendix 1 (part 2): α- Glucosidase – scores added for labs 16, 25 & 67; β-Glucosidase – score added for lab 6 score & score corrected for lab 47; PPT – score added for lab 3. • Pages 14-15, Appendix 1 (part 3): Iduronate sulphatase – scores added for labs 2, 6, 30, 37, 48 & 59; Arylsulphatase A – score added for lab 21 & score removed for lab 47.

- Absolute units
- As the percentage of activity in sample LEFB 01.

See Table 2 for details. Laboratories 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 protein	% of sample LEFB 01
Iduronate sulphatase	nmol/4h/mg protein	% of sample LEFB 01
β -Galactosidase	nmol/h/mg protein	% of sample LEFB 01
α -Glucosidase	nmol/h/mg protein	% of sample LEFB 01
β -Glucosidase	nmol/h/mg protein	% of sample LEFB 01
Arylsulphatase A	nmol/h/mg protein	% of sample LEFB 01
Lysosomal acid lipase (LAL)	nmol/h/mg protein	% of sample LEFB 01
Tripeptidyl peptidase (TPP)	nmol/h/mg protein	% of sample LEFB 01
Palmitoyl protein thioesterase (PPT)	nmol/h/mg protein	% of sample LEFB 01
Galactosylceramidase	nmol/17h/mg protein	% of sample LEFB 01

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 the results of a specific analyte in a specific sample. Thus for the 10 enzymes in the year 2019 cycle, 6 x 10 (60) such Analyte-in-Detail-reports can be requested.

The "Cycle Review" summarises the performance for all enzymes in a specific sample (6 such Cycle Reviews can be requested in 2019).

6. Scoring scheme and Poor performance policy

For the 2019 scheme, for each enzyme two 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%<CV<60%	1
		CV>60%	0
Enzymes	**Diagnosis**	Diagnosis correct	2
		Diagnosis incorrect	0
	CV	CV<35%	2
		CV= 35%<CV<60%	1
		CV>60%	0

The maximum possible score for the scheme (10 enzymes plus the protein value) was 42 points. 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 is 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 LF1 and LF4 were used to calculate the coefficient of variation (CV) according to the following formula: *CV = Activity LF4-activity LF1/mean.*

7. Results

Seventy three laboratories were registered in the 2019 scheme. Seventy laboratories (96% of registered laboratories) submitted sufficient results for their performance to be assessed. Three laboratories (4% of registered laboratories) did not submit enough results for their performance to be assessed.

Table 4: Results returns for the 2019 scheme

	Submission Deadline					
	31st May 2019			**30th August 2019**		
Sample Numbers:	**2019.01**	**2019.02**	**2019.03**	**2019.04**	**2019.05**	**2019.06**
No. of labs that submitted results:						
By the submission deadline	68 *(93.2%)*	69 *(94.5%)*	70 *(95.9%)*	71 *(97.3%)*	71 *(97.3%)*	71 *(97.3%)*
Within 7 days of the submission deadline	2 *(2.7%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*
Within 2 weeks of the submission deadline	0	0	0	0	0	0
Did not submit	3 *(4.1%)*	3 *(4.1%)*	2 *(2.7%)*	1 *(1.4%)*	1 *(1.4%)*	1 *(1.4%)*

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:

- 80% of participating laboratories submitted results for 5 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 70 laboratories that submitted results, 67 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	3
3	5
4	5
5	5
6	8
7	7
8	10
9	8
10	18
Total number of labs	**70**

Table 6: Proficiency per analyte

Analyte	No of returns	Diagnosis (%[1])	CV (%[1])	Total Proficiency (%[1])
Protein	71	n.a.	88	88
α -Galactosidase	66	100	91	96
Iduronate sulphatase	43	100	79	90
β -Galactosidase	68	97	82	90
α -Glucosidase	57	93	83	88
β -Glucosidase	66	97	96	96
Arylsulphatase A	62	95	85	90
Lysosomal acid lipase (LAL)	35	94	81	88
Tripeptidyl peptidase (TPP)	32	100	84	92
Palmitoyl protein thioesterase (PPT)	31	100	87	94
Galactosylceramidase	42	100	73	86

[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%	1	1.4%
20% – 29%	0	0%
30% –39%	0	0%
40% – 49%	0	0%
50% –59%	2	2.9%
60% –69%	2	2.9%
70% –79%	4	5.7%
80% –89%	9	12.9%
90% –99%	26	37.1%
100%	26	37.1%
Totals	**70**	**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	8	8
4	7	7
5	10	10
6	10	10
7	6	6
8	9	9
9	8	8
10	2	2
11	10	10
12	9	9
13	3	0 (partial submitter)
14	8	8
15	10	9
16	9	9
17	10	10
18	9	9
19	10	10
20	4	4
21	4	4
22	4	4
23	3	3
24	9	9
25	10	10
26	10	10
27	7	7
28	6	6
29	10	10
30	6	6
31	10	10
32	7	7
33	10	10
34	10	8
35	7	7
36	1	1
37	6	6
38	2	2
39	8	8
40	5	5
41	6	6
42	4	4
43	3	3
44	5	5
45	5	5
46	7	7
47	7	7
48	8	8
49	6	6
50	9	9
51	9	9
52	10	10
53	8	8
54	10	10
55	7	7
56	3	3
57	3	3
58	8	6
59	10	10
60	8	8
61	6	6
62	2	2
63	10	10
64	8	7
65	5	3
66	3	0 (partial submitter)
67	3	0
68	0	0 (non-submitter)
69	10	10
70	9	9
71	4	4
72	5	5
73	6	6

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.

Further information on the affected samples provided for testing can be found in Table 9 below.

Table 9: Cultured fibroblast samples included in the EQA scheme:

Sample	Diagnosis *[& enzyme deficiency]*	Age at diagnosis	Clinical information	Other information
LEFB2019.01	Normal control *[all enzyme activities normal]*			All 10 enzymes in 2019 scheme were assayed prior to distribution and confirmed to have normal levels of enzyme activity
LEFB2019.02	Metachromatic Leucodystrophy (MLD) *[Arylsulphatase A deficiency]*	2 years, 2months	Motor impairment from the age of 2 years (unstable sitting, limb spasticity) and feeding refusal. High proteins in CSF (0.6g/L). MRI: leucodystrophy	Iduronate sulphatase activity is normal excluding the diagnosis of a multiple sulphatase deficiency
LEFB2019.03	NCL2 (classic late infantile neuronal ceroid lipofuscinosis) *[Tripeptidyl peptidase I deficiency]*	7.5 years	Drug resistant epilepsy, psychomotor retardation	
LEFB2019.04	Normal control *[all enzyme activities normal]*			Duplicate of LEFB2019.**01**. This sample was used to calculate %CV scores
LEFB2019.05	GM1 gangliosidosis *[beta-galactosidase deficiency]*	10 months	Psychomotor retardation, hypotonia, nystagmus, hepatomegaly	
LEFB2019.06	Pompe disease (Glycogen storage disease type II- GSD II) *[alpha-glucosidase deficiency]*	35 years	Progressive muscle weakness, exercise dyspnea	

LEFB 01 & 04 were duplicates of a sample included as an unaffected control which was used to calculate the % CV data. **Participants were asked to express enzyme results as a percentage of sample LEFB 01.** LEFB 01 was included as a control to enable an improved comparison of overall results from all participants, and to include laboratories that do not use fibroblasts. However, not all laboratories followed this instruction when entering their results. On this occasion the Scientific Advisor has made the appropriate corrections. ***For the 2020 scheme all participants MUST enter this data correctly.***

LEFB 02 was a patient with a deficiency of arylsulphatase A (metachromatic leucodystrophy (MLD)): The correct diagnosis of MLD was made by 95% of participants.

Note: Individuals with pseudodeficiency of arylsulfatase A can have results in the affected range, but are otherwise unaffected with MLD. Abnormal results can be confirmed by looking for the presence of

sulphatides in the urine and/or DNA testing to check for the presence of the pseudodeficiency variant. ASA is also reduced, along with other sulphatase enzymes in cases of multiple sulphatase deficiency (MSD) and the possibility of this diagnosis should always be considered when low ASA is found. MSD can usually be confirmed or excluded by assaying one or more further sulphatases.

Assay temperature: Approximately 50% of participants use incubation temperature of 0°C and 50% incubate at 37°C.

Substrate: The majority of participants (87%) use the colorimetric substrate (para-nitrocatechol sulphate) for this assay. Since other arylsulphatases (ASB & ASC) will also hydrolyse this substrate, incubation conditions are chosen to maximise ASA activity but minimise activity of ASB and ASC. The most convenient method is to carry out the incubation at 0°C for a relatively long time. At 0°C ASA still has 25% of the activity measurable at 37°C, whereas ASB and ASC are practically inactive. Previous assays relied on ionic inhibition of ASB but the assay at 0°C has been shown to be more reliable diagnostically.

11% of participants are using a fluorimetric substrate and a single laboratory entered their methodology used as 'other'.

LEFB 03 was a patient affected with classic late infantile neuronal ceroid lipofuscinosis (NCL2). Fewer laboratories participated in this enzyme but 100% provided the correct diagnosis.

LEFB 05 was a patient with a beta-galactosidase deficiency (GM1 gangliosidosis). Most laboratories offer this enzyme test and had no problems achieving the correct diagnosis (97%).

Note: Deficiency of lysosomal β-galactosidase activity is the primary defect in GM1-gangliosidosis. However, β-gal is also deficient in Morquio disease type B (mucopolysaccharidosis IVB), which is allelic with GM1-gangliosidosis, and in galactosialidosis, which is non-allelic.

LEFB 06 was a patient with an alpha-glucosidase deficiency (Pompe /Glycogen storage disease type II (GSDII): 93% of participants made the correct diagnosis in this scheme.

The facility to record substrates used was added to the website in 2019. Overall, the majority of participants use fluorimetric substrates in their laboratories (the exception is arylsulphatase A): see Table 10 below.

Table 10: Substrates used

Enzyme	Colorimetric	Fluorimetric	Radiolabelled	Other
Alpha-galactosidase A	1.5%	94.5%		4%
Alpha-glucosidase	2%	92%		6%
Arylsulphatase A	87%	11%		2%
Beta-galactosidase	3.5%	94.5%		2%
Beta-glucosidase		95%		3%
Galactosylceramidase	2.5%	85%	7.5%	5%
Iduronate sulphatase		97%		3%
Lysosomal acid lipase (LAL/ acid esterase)	18%	76%	6%	
Palmitoyl protein thioesterase (PPT)		100%		
Tripeptidyl peptidase I (TPPI)		100%		

10. Pilot for Scoring of Interpretation (to be implemented in 2020 Scheme – see 11.1)

During the 2019 scheme participants were asked to submit a suggested diagnosis for each sample. The aim was to investigate if it was possible to introduce formal evaluation of interpretation for this scheme. Table 11 shows a comparison between overall performance under the current scoring scheme and the performance under the pilot for scoring of interpretation. Only labs that would be poor performers under the pilot scoring system are included in Table 11. Under the current scoring system 3 labs (4.3% of participating labs) were classed as poor performers (see item 7) but under the pilot scoring system 6 labs (8.6% of participating labs) would be classed as poor performers, including the 3 labs classed a poor performers under the current scoring system.

The ERNDIM Scientific Advisory Board has agreed that scoring of interpretation will be formally evaluated for the 2020 scheme, see item 11.1).

Table 11: Pilot score excludes partial and non-submitters

Lab No.	No. of enzymes results submitted for	Current scheme		Pilot Scheme	
		Score	Overall performance	Good performance	Poor performance for enzymes:
15	10	84%	Good performer	9/10	LAL
34	10	71%	Good performer	8/10	Arylsulphatase A & α-Glucosidase
58	8	59%	Poor performer	6/8	LAL & α-Glucosidase
64	8	74%	Good performer	7/8	α-Glucosidase
65	5	59%	Poor performer	3/5	ß-Galactosidase & ß-Glucosidase
67	3	14%	Poor performer	0/3	ß-Galactosidase, ß-Glucosidase & Arylsulphatase A

11. Preview of the scheme in 2020.

a) There will be two submission deadlines for the 2020 scheme:
- Samples 01, 02 & 03 to be submitted by 26 June 2020
- Samples 04, 05 & 06 to be submitted by 28 August 2020

b) Some changes have been made to the enzymes included in the 2020 LEFB scheme; see Table 12 below for comparison. 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 it is intended to provide regular rotation of the enzymes included each year.

Table 12: Analytes to be measured in 2020

Analyte	2018	2019	2020
Protein	✓	✓	✓
α -Galactosidase	✓	✓	✓
Galactose-6-sulphate sulphatase	✓	×	×
β -Galactosidase	✓	✓	✓
α -Glucosidase	✓	✓	✓
β -Glucosidase	✓	✓	✓
β -Glucuronidase	×	×	✓
β -Hexosaminidase A	✓	×	×
β -Hexosaminidase A+B	✓	×	×
α -Iduronidase	✓	×	×
Galactosylceramidase	✓	✓	✓
Sphingomyelinase	✓	×	×
Arylsulphatase A	×	✓	✓
Iduronate-sulphatase	×	✓	×
α-N-Ac-glucosaminidase	×	×	✓
Lysosomal acid lipase (LAL/acid/esterase)	×	✓	✓
Palmitoyl protein thioesterase	×	✓	✓
Tripeptidyl peptidase	×	✓	×

11.1. Scoring for the 2020 Scheme:

The results of the pilot for interpretation scoring (see item 10) were discussed by the Scientific Advisory Board at their meeting in November 2019 and it was agreed that **scoring of interpretation will be formally evaluated for the 2020 scheme**.

Each enzyme will be assessed individually, the emphasis being on the correct interpretation of the result (i.e. qualitative aspect). Making the correct interpretation /diagnosis for each enzyme/ sample is

the priority: i.e. identifying a deficiency in an affected patient and reporting normal activity in unaffected samples.

Table 13: Scoring criteria 2020

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

For the 2020 Scheme the %CV for each enzyme will continue to be provided as participants indicated that they find this information useful. **Note: the %CV for each enzyme will not contribute to scores from 2020.**

If the interpretation of a result is incorrect for a specific enzyme a performance support letter may be issued, but only for that particular enzyme assay. This is to initiate a dialogue between us, the EQA scheme advisor/organiser and you, the participating laboratory, in order to solve any particular analytical problems and to help you improve performance.

***Comments box*:** Participant comments for this scheme may now be taken into account by the Scientific Advisor. Please use this box to note any issues noted regarding the sample or assay.

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 of this report or data derived from the use or analysis of ERNDIM EQA materials must not be used in written publications or oral presentations unless the explicit prior consent of ERNDIM has been granted.

Marie Jackson
Scientific Advisor

Appendix 1 (part 1): Results per laboratory
(see page 11 for key)

	Protein/vial		LAL			α-Galactosidase			β-Galactosidase		
		Score		Score			Score			Score	
Lab No	CV	CV	CV	Diagnosis	CV	%CV	Diagnosis	CV	%CV	Diagnosis	CV
1	9	2	1	2	2	3	2	2	12	2	2
2	14	2				5	2	2	2	2	2
3	69	0				90	2	0	43	2	1
4	5	2	5	2	2	2	2	2	1	2	2
5	13	2	33	2	2	16	2	2	14	2	2
6	14	2	6	2	2	19	2	2	11	2	2
7	0	2				11	2	2	24	2	2
8	16	2				13	2	2	28	2	2
9	11	2	10	2	2				3	2	2
10	37	1							56	2	1
11	4	2	16	2	2	3	2	2	7	2	2
12	10	2	11	2	2	22	2	2	8	2	2
13	10	2				20	2	2			
14	14	2	2	2	2	10	2	2	20	2	2
15	1	2	95	0	0	2	2	2	68	2	0
16	11	2	22	2	2	1	2	2	7	2	2
17	124	0	5	2	2	5	2	2	8	2	2
18	0	2				6	2	2	20	2	2
19	11	2	13	2	2	0	2	2	45	2	1
20	42	1							38	2	1
21	17	2				7	2	2	1	2	2
22	25	2				16	2	2	15	2	2
23	ND	0				9	2	2	9	2	2
24	13	2	20	2	2	1	2	2	5	2	2
25	36	1	163	2	0	110	2	0	100	2	0
26	13	2	2	2	2	10	2	2	3	2	2
27	0	2				2	2	2	1	2	2
28	6	2	10	2	2	6	2	2	11	2	2
29	35	1	2	2	2	8	2	2	40	2	1
30	0	2	3	2	2	11	2	2	0	2	2
31	9	2	3	2	2	11	2	2	36	2	1
32	19	2				0	2	2	6	2	2
33	3	2	1	2	2	1	2	2	1	2	2
34	10	2	208	2	0	13	2	2	37	2	1
35	25	2				7	2	2	16	2	2
36	22	2				8	2	2			
37	14	2				6	2	2	20	2	2
38	23	2									
39	8	2	11	2	2	24	2	2	22	2	2
40	7	2				0	2	2	10	2	2
41	11	2				6	2	2	3	2	2
42	4	2				17	2	2	13	2	2
43	0	2				10	2	2	13	2	2
44	5	2				8	2	2	31	2	2
45	11	2				35	2	1	29	2	2
46	10	2				3	2	2	18	2	2
47	36	1				60	2	1	70	2	0
48	3	2	319	2	0	29	2	2	8	2	2
49	7	2	28	2	2	17	2	2	23	2	2
50	5	2				14	2	2	11	2	2

	Protein/vial		LAL			α-Galactosidase			β-Galactosidase		
		Score		Score			Score			Score	
Lab No	CV	CV	CV	Diagnosis	CV	%CV	Diagnosis	CV	%CV	Diagnosis	CV
51	55	1	22	2	2	26	2	2	24	2	2
52	0	2	86	2	0	15	2	2	6	2	2
53	29	2	59	2	1	6	2	2	36	2	1
54	10	2	7	2	2	9	2	2	4	2	2
55	5	2				19	2	2	2	2	2
56	0	2				0	2	2			
57	2	2				12	2	2	9	2	2
58	17	2	76	0	0	82	2	0	R0	2	0
59	17	2	2	2	2	7	2	2	47	2	1
60	31	2	16	2	2	15	2	2	5	2	2
61	2	2	23	2	2	7	2	2	16	2	2
62	10	0							12	2	2
63	6	2	21	2	2	12	2	2	22	2	2
64	10	2	4	2	2	30	2	1	135	2	0
65	12	2				30	2	1	46	0	1
66	ND					R0	2	0	R0	2	0
67	66	0							44	0	1
68											
69	40	1	27	2	2	37	2	2	51	2	1
70	9	2				2	2	2	4	2	2
71	3	2				20	2	2	19	2	2
72	6	2				9.5	2	2	19	2	2
73	3	2				4	2	2	1	2	2

Key

green cells = correct interpretation
red cells = incorrect interpretation
blue cells =not all samples measured
R0 = CV calculation not possible as one or both of LF1 and LF4 (duplicate samples) were not measured
nd = not done (i.e. result not submitted)

Appendix 1 (part 2): Results per laboratory

(see page 11 for key)

	α-Glucosidase			β-Glucosidase			TPP			PPT		
		Score			Score			Score			Score	
Lab No	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV
1	4	2	2	0	2	2	1	2	2	1	2	2
2	4	2	2	4	2	2	5	2	2	2	2	2
3	20	2	2	16	2	2				90	2	0
4	21	2	2	6	2	2						
5	2	2	2	18	2	2	27	2	2	44	2	1
6	8	2	2	9	2	2	18	2	2	3	2	2
7	13	2	2	12	2	2						
8	16	2	2	6	2	2	8	2	2	15	2	2
9	56	2	1	6	2	2	24	2	2	1	2	2
10												
11	3	2	2	14	2	2	4	2	2	10	2	2
12	22	2	2	21	2	2	56	2	1	17	2	2
13				95	0	0						
14	22	2	2	9	2	2						
15	3	2	2	12	2	2	25	2	2	43	2	1
16	1	2	2	7	2	2	7	2	2	5	2	2
17	8	2	2	2	2	2	4	2	2	2	2	2
18	7	2	2	5	2	2	28	2	2	7	2	2
19	10	2	2	23	2	2	24	2	1	7	2	2
20				28	2	2						
21				2	2	2						
22	1	2	2	20	2	2						
23												
24				23	2	2	13	2	2	32	2	2
25	37	2	1	32	2	2	4	2	2	125	2	1
26	14	2	2	9	2	2	50	2	1	15	2	2
27	18	2	2	11	2	2	85	2	0			
28	18	2	2	65	2	0						
29	12	2	2	28	2	2	0	2	2	57	2	1
30	R0	2	0	4	2	2						
31	55	2	1	9	2	2	52	2	1	15	2	2
32				17	2	2	1	2	2	11	2	2
33	1	2	2	1	2	2	1	2	2	1	2	2
34	548	0	0	1	2	2	21	2	2	8	2	2
35	143	2	0	9	2	2						
36												
37	10	2	2	9	2	2						
38	10	2	2	38	2	1						
39	3	2	2	16	2	2						
40	20	2	2	14	2	2						
41	14	2	2	5	2	2						
42				18	2	2						
43				19	2	2						
44	13	2	2	21	2	2						
45				0	2	2						
46	63	2	0	5	2	2						
47	43	2	1	28	2	2	72	2	0	55	2	1
48	24	2	2	12	2	2						
49	8	2	2	0	2	2						
50	13	2	2	4	2	2	30	2	2	31	2	2

	α-Glucosidase			β-Glucosidase			TPP			PPT		
		Score			Score			Score			Score	
Lab No	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV
51	3	2	2	22	2	2	12	2	2			
52	13	2	2	53	2	2	27	2	2	9	2	2
53	63	2	0	18	2	2						
54	12	2	2	5	2	2	1	2	2	3	2	2
55	12	2	2	14	2	2						
56	8	2	2	9	2	2						
57												
58	59	0	1	22	2	2						
59	4	2	2	7	2	2	3	2	2	16	2	2
60	0	2	2	1	2	2						
61	1	2	2	14	2	2						
62				4	2	2						
63	15	2	2	2	2	2	40	2	1	23	2	2
64	186	0	0	11	2	2	29	2	2	49	2	1
65	50	2	1	14	0	2						
66												
67	39	0	1									
68												
69	14	2	2	22	2	2	27	2	1	8	2	2
70	2	2	2	6	2	2	2	2	2	7	2	2
71				21	2	2						
72	33	2	2	10	2	2						
73	28	2	2	40	2	1						

Appendix 1 (part 3): Results per laboratory
(see page 11 for key)

	Iduronate sulphatase			Galactocerebrosidase			Arylsulphatase A		
		Score			Score			Score	
Lab No	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV
1	5	2	2	1	2	2	8	2	2
2	25	2	2				3	2	2
3	60	2	0	84	2	0	110	2	0
4				53	2	1	7	2	2
5	1	2	2	18	2	2	10	2	2
6	78	2	0	4	2	2	6	2	2
7	9	2	2				15	2	2
8	7	2	2	76	2	0	12	2	2
9	R0	2	0				15	2	2
10							52	2	1
11	7	2	2	1	2	2	2	2	2
12	6	2	2				6	2	2
13							94	0	0
14	6	2	2	0	2	2	38	2	1
15	2	2	2	14	2	2	2	2	2
16				1	2	2	2	2	2
17	5	2	2	9	2	2	39	2	1
18	3	2	2	10	2	2	8	2	2
19	39	2	1	49	2	1	14	2	2
20				59	2	1	21	2	2
21							3	2	2
22									
23							20	2	2
24	34	2	2	52	2	1	21	2	2
25	29	2	2	35	2	1	20	2	2
26	23	2	2	95	2	0	5	2	2
27				84	2	0	8	2	2
28							3	2	2
29	27	2	2	49	2	1	18	2	2
30	5	2	2						
31	5	2	2	24	2	2	17	2	2
32				16	2	2	7	2	2
33	0.5	2	2	1	2	2	1	2	2
34	43	2	1	33	2	2	60	0	0
35	45	2	1	192	2	0	28	2	2
36									
37	11	2	2				14	2	2
38									
39	42	2	1	14	2	2	20	2	2
40							16	2	2
41	0	2	2				1	2	2
42							4	2	2
43									
44							91	2	0
45				49	2	1	26	2	0
46	13	2	2	43	2	1	2	2	2
47	246	2	0						
48	12	2	2	10	2	2	14	2	2
49				16	2	2			
50	46	2	1	13	2	2	34	2	2
51	30	2	2	21	2	2	22	2	2

	Iduronate sulphatase			Galactocerebrosidase			Arylsulphatase A		
		Score			Score			Score	
Lab No	CV	Diagnosis	CV	CV	Diagnosis	CV	CV	Diagnosis	CV
52	35	2	1	48	2	1	42	2	1
53	170	2	0	13	2	2	24	2	2
54	29	2	2	13	2	2	6	2	2
55	16	2	2	22	2	2	21	2	2
56									
57							10	2	2
58	65	2	0	52	2	1	10	2	2
59	24	2	2	2	2	2	27	2	2
60	0	2	2	1	2	2	15	2	2
61							1	2	2
62									
63	1	2	2	4	2	2	7	2	2
64							38	2	1
65				73	2	0			
66							R0	2	0
67							645	0	0
68									
69	27	2	2	35	2	1	26	2	2
70	10	2	2	0	2	2	1	2	2
71							21	2	2
72							25	2	2
73	30	2	2				22	2	2

END OF REPORT