Lysosomal Enzymes in fibroblasts

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Note: 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 permission of the Scientific Advisor.

The fact that your laboratory participates in ERNDIM schemes is not confidential. However, the raw data and performance scores are confidential and will only be shared within ERNDIM for the purpose of evaluating performance of your laboratory, unless ERNDIM is required to disclose performance data by a relevant government agency. For details, please see the terms and conditions in the EQA Schemes Catalogue and Participant Guide and the ERNDIM Privacy Policy on www.erndim.org.

1. Scheme Design
The scheme has been designed, planned and coordinated by Ms Marie Jackson (as Scientific Advisor) and Dr Eline van der Hagen as Scheme Organiser (sub-contractor on behalf of MCA Laboratory); both 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 MCA Laboratory, 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 the EQA scheme

Sample	Disorder	Enzyme Defect	Reporting deadline
LEFB2022.01	Control	All normal	27 May 2022
LEFB2022.02	Pompe disease	Alpha-glucosidase	
LEFB2022.03	Sandhoff disease	Hexosaminidase A + B	
LEFB2022.04	Krabbe leucodystrophy	Galactosylceramidase	26 August 2022
LEFB2022.05	Niemann Pick (A/B)	Sphingomyelinase	
LEFB2022.06	Fabry disease	Alpha-galactosidase	

3. Shipment
One shipment of six samples was dispatched 8th February 2022, to the 69 laboratories, from 27 countries, which registered for the scheme.

4. Receipt of results
There were two submission deadlines for the 2022 scheme: (LEFB2022.01, 02 & 03 on 27th May) and (LEFB2022.04, 05 & 06 on 26thAugust).

[1] **If this Annual Report is not Version 1 for this scheme year, go to APPENDIX 2 (page12) for details of the changes made since the last version of this document.**

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:

- 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	-
Arylsulphatase A	nmol/h/mg protein	% of sample LEFB 01
Aspartylglucosaminidas	nmol/h/mg protein	% of sample LEFB 01
Galactosylceramidase	nmol/h/mg protein	% of sample LEFB 01
Sphingomyelinase	nmol/h/mg protein	% of sample LEFB 01
α -Galactosidase	nmol/h/mg protein	% of sample LEFB 01
α -Glucosidase	nmol/h/mg protein	% of sample LEFB 01
β -Galactosidase	nmol/h/mg protein	% of sample LEFB 01
β -Glucosidase	nmol/h/mg protein	% of sample LEFB 01
β -Hexosaminidase (A+B)	nmol/h/mg protein	% of sample LEFB 01
β -Hexosaminidase A	nmol/h/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 2022 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 2022).

6. Scoring scheme and Poor performance policy

It was approved by the Scientific Advisory Board at their meeting in November 2019 that scoring of interpretation would be formally introduced for the 2020 scheme onwards.

For the 2021 Scheme and subsequent years, the %CV for each enzyme will no longer be provided.

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, to solve any particular analytical problems and to help you improve performance.

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

The **diagnostic proficiency** was scored for each enzyme: i.e. is the interpretation correct or incorrect. One point was awarded for a correct diagnosis.

For the protein value a maximum of 2 points could be scored depending on the %CV.

Table 3: Scoring criteria

	Criteria		Score
Protein	**CV**	CV<35%	2
		CV= 35%<CV<60%	1
		CV>60%	0
Enzymes	**Diagnosis**	Diagnosis correct	1
		Diagnosis incorrect	0
	CV	Not scored from 2020 onwards	

Laboratories could participate in as many of the ten enzymes offered in the scheme plus the protein assay as required. Each enzyme is assessed individually, the emphasis being on the correct interpretation of the result. 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.

If a laboratory misinterprets a result, then a performance support letter is sent relating to **that specific enzyme only**. 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: correct interpretation and correct measurement of enzyme activity level.

Diagnosis incorrect: incorrect interpretation and incorrect enzyme activity level.

6.2. Coefficient of variation

CV results for enzymes included in the scheme are **not** determined and do not contribute to the scoring. *Only CV for proteins will contribute to scoring: this is calculated from median results for all labs.*

6.3. Appeals

If your laboratory has been sent a performance support letter for the 2022 scheme and you wish to appeal against this classification please complete the online appeal form (see below) within one month of the date of the relevant Performance Support Letter. Full details of the reason for the appeal should be included. Initial appeals will be considered by the relevant Scientific Advisor and a decision sent within 21 days of receipt of the appeal.

Appeal form: **https://www.formdesk.com/erndim/Poor_Performance_Appeals_Form** [please note this form will only be accessible for one month after the performance support letters have been sent].

7. Results

Sixty-nine laboratories were registered in the 2022 scheme. Sixty-three laboratories (91% of registered laboratories) submitted sufficient results for their performance to be assessed.

Five laboratories (7.2% of registered laboratories) did not submit enough results for their performance to be assessed.

One lab did not submit any results for the 2022 scheme and no labs registered as educational participants.

Table 4: Results returns for the 2022 scheme

	Submission Deadline					
	27th May 2022			26th August, 2022		
Sample Numbers:	**2022.01**	**2022.02**	**2022.03**	**2022.04**	**2022.05**	**2022.06**
No. of labs that submitted results:						
By the submission deadline	65	66	67	66	66	65
Within 7 days of the submission deadline	1	1	1	0	0	0
Within 2 weeks of the submission deadline	0	0	0	1	1	1
Did not submit	3	2	1	2	2	3

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

Full details of the results for each participant's results (for labs that submitted results) are given in Appendix 1 but summaries are presented here:

- 90% of participating laboratories submitted results for 5 or more enzymes, see Table 5.
- The proficiency per analyte is given in Table 6.
- The majority of participants made the correct interpretation.
- **73.0% of participating laboratories achieved >90% of their maximum possible score (i.e. of enzymes plus proteins). See Table 7 which shows the percentage of the maximum possible score for the laboratories that submitted results.**

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	0
2	2
3	2
4	2
5	2
6	7
7	7
8	8
9	11
10	22
Total number of labs	**63**

Table 6: Proficiency per analyte

Analyte	No of returns	Correct interpretation* (diagnostic proficiency)
Protein	68	77.9%
Arylsulphatase A (ASA)	54	100%
Aspartylglucosaminidase	25	100%
Galactosylceramidase	42	88.1%
Sphingomyelinase	41	97.6%
α -Galactosidase	59	89.8%
α -Glucosidase	50	94.0%
β -Galactosidase	59	98.3%
β -Glucosidase	61	100%
β -Hexosaminidase (A+B)	56	94.6%
β -Hexosaminidase A	55	100%

*** = 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	0.0%
10% – 19%	0	0.0%
20% – 29%	0	0.0%
30% –39%	0	0.0%
40% – 49%	0	0.0%
50% –59%	0	0.0%
60% –69%	0	0.0%
70% –79%	6	9.5%
80% –89%	11	17.5%
90% –99%	9	14.3%
100%	37	58.7%
Total	**63**	**100%**

Table 8: Number of enzymes for which laboratories had satisfactory performance

Anon Lab No.	No of enzymes for which:	
	results were submitted by lab	lab had satisfactory performance
1	8	8
2	5	5
3	10	10
4	3	3
5	10	10
6	7	6
7	10	10
8	3	3
9	10	10
10	5	4
11	0	0
12	8	7
13	10	10
14	7	7
15	2	2
16	10	10
17	9	9
18	7	6
19	4	4
20	6	5
21	0	0
22	10	10
23	10	10
24	0	0
25	9	8
26	9	8
27	0	0
28	0	0
29	10	10
30	10	10
31	8	7
32	8	8
33	7	7
34	10	10
35	10	10
36	9	9
37	9	8
38	10	9
39	0	0
40	4	4
41	8	7

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

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 must 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.

Cultured fibroblast samples included in the EQA scheme:

All ten enzymes included in the 2022 scheme were assayed in all six samples prior to distribution for validation.

- The five affected cell lines had clear enzyme deficiencies confirming the specific disorder in each case.
- The remaining enzymes in all six samples included in the scheme had confirmed normal levels of enzyme activity.

LEFB 01 was included as a control to enable an improved comparison of overall results from all participants, and to provide a control to laboratories that do not use fibroblasts.

Participants were asked to express enzyme results as a percentage of sample LEFB 01: all participants must enter this data correctly.

LEFB 02 was a patient affected with **Pompe disease.** The correct interpretation for this sample was: **deficiency of alpha-glucosidase activity.** Proficiency for this enzyme was 94%.

LEFB 03 was a patient affected with **Sandhoff disease (GM2 gangliosidosis- Sandhoff variant).** The correct interpretation expected for this sample was: **deficiency of hexosaminidase A+B.** Proficiency for this enzyme was 94.6%.

The laboratories who made the incorrect interpretation all submitted results showing a clear deficiency in both the total hexosaminidase and hexosaminidase A enzyme activities but made the incorrect interpretation of hexosaminidase A deficiency (Tay Sachs disease).

LEFB 04 was a patient affected with Krabbe leukodystrophy. The correct interpretation expected for this sample was: deficiency of galactosylceramidase activity. The proficiency for this enzyme was 88.1%.

The majority of laboratories use the synthetic fluorescent substrate to measure this enzyme. Making the diagnosis of Krabbe disease can be difficult and there are known issues surrounding the use of this substrate. The lower proficiency for this enzyme may be a reflection of this. Laboratories using this substrate should be aware of these issues as a diagnosis of Krabbe disease may be missed.

Only one of the laboratories making an incorrect interpretation highlighted an awareness of these issues in the comments section.

LEFB 05 was a patient with a sphingomyelinase deficiency (Niemann Pick disease type A/B. Proficiency for this enzyme was 97.6%.

LEFB 06 was a patient affected with Fabry disease. The expected interpretation for this sample was: deficiency of alpha-galactosidase activity. The proficiency for this enzyme was 89.8%.

The lower proficiency for this enzyme is most likely to be a reflection of the difficulties of measuring alpha-galactosidase in cultured fibroblasts. This assay is rarely performed in cultured fibroblasts by the majority of participants as testing is more commonly offered in plasma, leucocytes and /or dried blood spots.

Proficiency for alpha-galactosidase 2018 - 2022

Year:	2018	2019	2020	2021	2022
Sample included:	Affected Fabry	Normal	Affected Fabry	Affected Fabry	Affected Fabry
Proficiency:	77%	93%	90.3%	86.5%	89.8%

10. Preview of the scheme in 2023.

a) There will be two submission deadlines for the 2023 scheme:
- Samples 01, 02 & 03 to be submitted by 26th May 2023
- Samples 04, 05 & 06 to be submitted by 25th August 2023

b) Some changes have been made to the enzymes included in the 2023 LEFB scheme: see

c) Table 9 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 that ERNDIM continue to provide regular rotation of the enzymes included each year.

Table 9: Analytes to be measured in 2023

Analyte	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**
Protein	✓	✓	✓	✓	✓	✓
Arylsulphatase A	×	✓	✓	×	✓	✓
Arylsulphatase B	×	×	×	✓	×	×
Aspartylglucosaminidase	×	×	×	×	✓	×
Galactose-6-sulphate sulphatase	✓	×	×	×	×	✓
Galactosylceramidase	✓	✓	✓	×	✓	×
Iduronate-sulphatase	×	✓	×	×	×	✓
Lysosomal acid lipase (LAL/acid/esterase)	×	✓	✓	×	×	×
Palmitoyl protein thioesterase	×	✓	✓	×	×	×
Sphingomyelinase	✓	×	×	✓	✓	×
Tripeptidyl peptidase	×	✓	×	×	×	×
α-Galactosidase	✓	✓	✓	✓	✓	✓
α-Glucosidase	✓	✓	✓	✓	✓	✓
α-Iduronidase	✓	×	×	×	×	✓
β-Galactosidase	✓	✓	✓	✓	✓	✓
β-Glucosidase	✓	✓	✓	✓	✓	✓
β-Glucuronidase	×	×	✓	×	×	×
β-Hexosaminidase A	✓	×	×	✓	✓	×
β-Hexosaminidase A+B	✓	×	×	✓	✓	✓
α-Fucosidase	×	×	×	✓	×	×
α-Mannosidase	×	×	×	✓	×	×
α-N-Ac-glucosaminidase	×	×	✓	×	×	×
Heparan-N-sulphatase	×	×	×	×	×	✓

11. 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, (admin@erndim.org) or the scheme organiser Dr Eline van der Hagen (mca.office@skbwinterswijk.nl).

12. 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. Results per laboratory (part 1)

(see page 12 for key)

Anon	Protein/vial		Score				
Lab No.	CV	Score	ASA	α-Galactosidase	β-Galactosidase	α-Glucosidase	β-Glucosidase
1	3	2	1	1	1	1	1
2	18	2	1	1	1	1	1
3	2034	0	1	1	1	1	1
4	7	2		1		1	1
5	8	2	1	1	1	1	1
6	10	2		1	1	1	1
7	30	2	1	1	1	1	1
8	1	2	1				1
9	11	2	1	1	1	1	1
10	55	1	1	0	1		
11	R0	0	(PS)	(PS)	(PS)	(PS)	(PS)
12	7	2	1	1	1		1
13	20	2	1	1	1	1	1
14	52	1	1	1	1	1	1
15	35	1		1			1
16	10	2	1	1	1	1	1
17	27	2	1	1	1	1	1
18	13	2	1	0	1	1	1
19	12	2		1	1		1
20	8	2	1	1	1	0	1
21	0	(NS)	(NS)	(NS)	(NS)	(NS)	(NS)
22	13	2	1	1	1	1	1
23	20	2	1	1	1	1	1
24	R0	0	(PS)	(PS)	(PS)	(PS)	(PS)
25	R0	0		0	1	1	1
26	34	2	1	1	1	1	1
27	R0	0	(PS)	(PS)	(PS)	(PS)	(PS)
28	R0	0	(PS)	(PS)	(PS)	(PS)	(PS)
29	R0	0	1	1	1	1	1
30	19	2	1	1	1	1	1
31	25	2	1	1	1	1	1
32	14	2	1	1	1	1	1
33	15	2	1		1		1
34	23	2	1	1	1	1	1
35	1	2	1	1	1	1	1
36	4	2	1	1	1	1	1
37	70	0	1	1	1	0	1
38	27	2	1	1	1	1	1
39	R0	0	(PS)	(PS)	(PS)	(PS)	(PS)
40	14	2		1	1		
41	22	2	1	0	1		1
42	35	1	1	1	1	1	1
43	8	2	1	1	1	1	1
44	12	2	1	1	1	1	1
45	86	0	1	1	1	1	1
46	25	2	1	1	1	1	1
47	3	2	1	1	1	1	1
48	30	2		1	1	1	1
49	61	0	1	1	1	1	1
50	3	2	1	1	1	1	1
51	6	2	1	1	1	1	1
52	13	2	1	1	1	1	1
53	45	1	1	0	1	1	1
54	14	2	1	1	1	0	1
55	7	2	1	1	1		1
56	8	2	1	1	1	1	1
57	39	1	1	1	1	1	1
58	5	2	1	1	1		1
59	16	2	1	1	1	1	1

Anon Lab No.	Protein/vial		Score				
	CV	Score	ASA	α-Galactosidase	β-Galactosidase	α-Glucosidase	β-Glucosidase
60	17	2		1	1	1	1
61	48	1	1	1	1		1
62	2	2				1	1
63	16	2	1	1	1	1	1
64	13	2	1		1		1
65	11	2	1	1	1	1	1
66	29	2	1	1	1	1	1
67	41	1	1	1	0		1
68	22	2	1	1	1	1	1
69	12	2	1	0	1	1	1

APPENDIX 1. Results per laboratory (part 2)

(see page 12 for key)

Anon Lab No.	Score				
	Galactosylceramidase	Hexosaminidase	Hexosaminidase A	Aspartylglucosaminidase	Sphingomyelinase
1	1	1	1		
2					
3	1	1	1	1	1
4					
5	1	1	1	1	1
6	0	1			1
7	1	1	1	1	1
8	1				
9	1	1	1	1	1
10		1	1		
11	(PS)	(PS)	(PS)	(PS)	(PS)
12	0	1	1		1
13	1	1	1	1	1
14		1	1		
15					
16	1	1	1	1	1
17	1	1	1		1
18		1	1		
19			1		
20			1		
21	(NS)	(NS)	(NS)	(NS)	(NS)
22	1	1	1	1	1
23	1	1	1	1	1
24	(PS)	(PS)	(PS)	(PS)	(PS)
25	1	1	1	1	1
26	1	1	1		0
27		(PS)			
28	(PS)	(PS)	(PS)		(PS)
29	1	1	1	1	1
30	1	1	1	1	1
31	0	1	1		
32	1	1	1		
33	1	1	1		1
34	1	1	1	1	1
35	1	1	1	1	1
36	1	1	1		1
37	1	1	1		1
38	1	0	1	1	1
39	(PS)	(PS)	(PS)		(PS)
40		1	1		
41	1	1	1		1
42	1	1	1		
43	1	1	1	1	1
44	1	1	1	1	1
45	1	1	1		1
46	1	1	1		1
47		1	1	1	1
48		1	1		1
49	1	1	1		1
50	1	1	1	1	1
51		1	1		
52		0	1		1
53	1	1	1	1	1
54		1			
55		1	1		
56	1	1	1	1	1
57	1	1	1	1	1

Anon Lab No.	Score				
	Galactosylceramidase	Hexosaminidase	Hexosaminidase A	Aspartylglucosaminidase	Sphingomyelinase
58		1	1		
59	1	1	1		1
60	1	1	1		1
61		0	1		
62					
63		1			1
64		1	1	1	
65	0	1	1	1	1
66	1	1	1	1	1
67		1	1		
68	1	1	1	1	1
69	0	1	1		1

Key

green cells = correct interpretation
orange cells = incorrect interpretation
R0 = CV calculation not possible as insufficient data.
NS = nonsubmitter
PS = partial submitter

APPENDIX 2. Change log (changes since the last version)

Version Number	Published	Amendments
1	17 October 2023	• 2022 annual report published

END OF REPORT