ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Congenital Disorders of Glycosylation
Final Report 2015

[This final report was ratified by the Scientific Advisory Board in March 2016]

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Amended report issued: April 25 2016[1] and May 11 2016[2]

1. Scheme Design

The scheme has been designed and planned by the Scientific Advisor (SA) and Scheme Organiser (SO, subcontractor on behalf of SKML), both appointed by and according to procedures laid down by the ERNDIM Board.

2. Samples

All EQA materials are lyophilised plasma or serum samples (25 µl). Laboratories that need a larger sample volume due to their analysis method (e.g. HPLC) were offered a 50 µl sample volume for a reduced scheme price. All samples are obtained following local ethical and consent guidelines.

3. Shipment

The six samples were sent out to the 63 registered laboratories in one parcel on 11th February 2015. Twenty laboratories requested and were sent the larger sample volume.

4. Receipt of results

Returns were submitted by email to the SA. The returns for the first round (samples CDG 2015.01 - CDG 2015.03) and second round (samples CDG 2015.04 - CDG 2015.06) were received by the due date from 48 (76%) and 46 (73%) laboratories respectively.

There were seven laboratories who failed to make a return on either submission round.

5. Scoring scheme

In agreement with ERNDIM rules, we applied a scoring system of 2+2:

Item C: technical aspects: 1 point for identification of an abnormal profile and 1 point for correct identification of the profile as type I or II. For sample 005, assigning as either type I or type II is correct as well.

Item D: diagnostic suggestions: This section should be filled for scoring. Just referring to a specialised lab is insufficient. If required, advice can be obtained from a reference laboratory or in collaboration with a clinical colleague. For normal profiles 2 points are scored. For abnormal profiles, comments should be made on the possibility of secondary causes in view of the clinical indication. In addition, the right suggestions should be made for the next step in the diagnostic process that eventually will lead to the genetic defect. Scoring for this part is not so straightforward, but we tried to keep it as consistent as possible. For sample 02., exclusion of secondary causes and standard work-up for CDG-I defects have to be mentioned. This includes PMM2 enzyme analysis for this particular case. For sample 03., the possibility of a protein polymorphism should be

Version Date	Amendments
[1] April 25 2016	• Pages 4 & 5: The transposition of the text for samples 2015.05 & 2015.06 was corrected.
[2] May 11 2016	• Page 1, item 4: the text was changed from “There were eight laboratories who failed ...” to “There were seven laboratories who failed ...”. • Page 5, table 2: the number of returns per sample & diagnostic suggestions (%) were both updated to include results from 1 additional lab. • Page 5, table 3: the score for lab 6 sample 06, item D was changed from 1 to 2 • Page 6, table 3: the results for lab 57 were added to the table.

mentioned (if using IEF or CE). For sample 05., the possibility of PGM1-CDG should be mentioned for full scoring.

CDG 2015.04 is classed as an educational sample, so the scoring does not count for satisfactory performance.

The maximum score achievable with full submission for all five remaining samples is 20, while a maximum of 12 and 8 points are available for labs that only submitted results for the first or second round respectively. The level for satisfactory performance is 12 points. Laboratories that participate only in one circulation are treated as partial-submitters and can achieve satisfactory performance with 8 points if results were submitted for the first round only or 5 points if results were submitted for the second round only. For the 2014 scheme onwards, another criterion for satisfactory performance will be the absence of any "critical error", which is defined as an error resulting from seriously misleading analytical findings and /or interpretations with serious clinical consequences for the patient. For the 2015 CDG scheme, no critical errors were identified. This has been agreed at the meeting of the Scientific Advisory Board on 17th March 2016.

6. Results of samples and evaluation of reporting

All submitted results are treated as confidential information and are only shared with ERNDIM approved persons for the purposes of evaluation and reporting. For the purposes of evaluation, the Scientific Advisor's centre is not included in the following results.

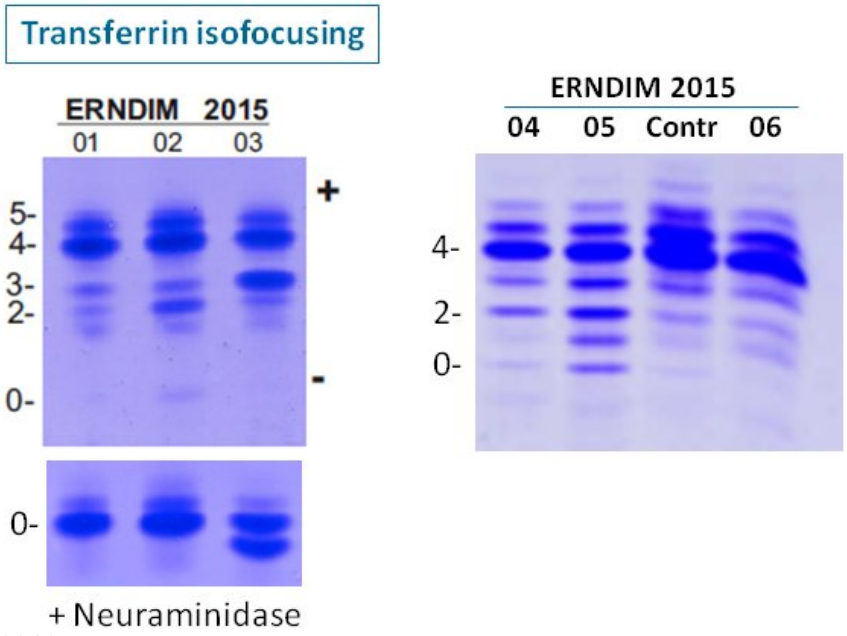
For the reporting laboratories, isofocusing was the method employed most often (30), followed by HPLC (10) and CE (10), mass spectrometry (2) and western blot (1).

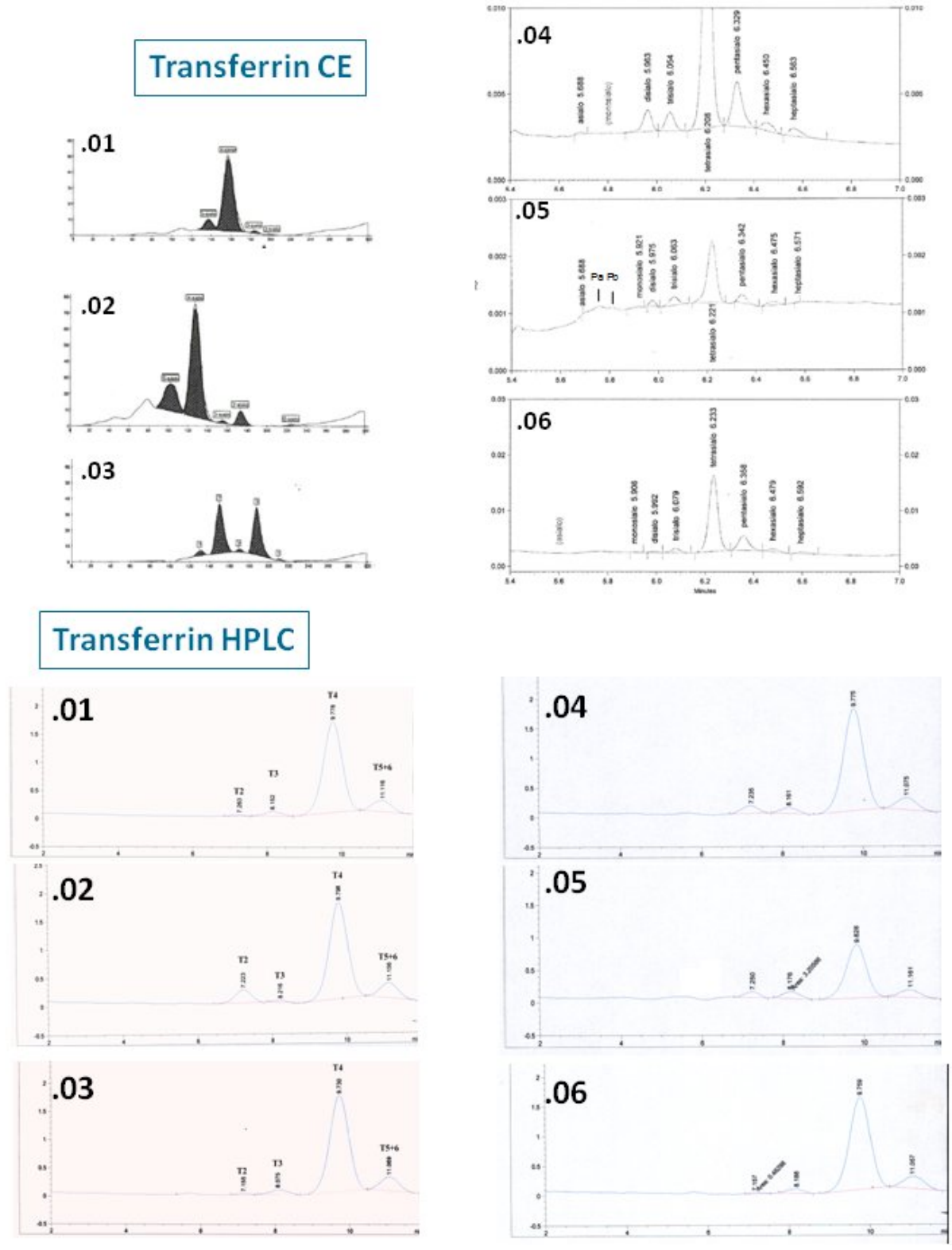
The shipped samples were from CDG patients, from controls and of patients with established excessive intake of alcohol. The final results of the six samples with respect to CDG are summarized in Table 1 below.

Table 1: Samples in the 2015 scheme

Sample	Clinical information (age, sex, phenotype)	Diagnosis
2015.01	3y, M, intellectual disability, low coagulation parameters, increased transaminases	Control
2015.02	30y, F, cerebellar ataxia	PMM2-CDG (CDG-Ia) OMIM: 212065
2015.03	11y, F, cataract, dysmorphic features, epilepsy	Control, transferrin polymorphism
2015.04	Educational sample, adult with increased alcohol intake	Increased CDT
2015.05	52y, M, rhabdomyolysis, dilated cardiomyopathy	PGM1-CDG OMIM: 171900
2015.06	1y, F, epileptic encephalopathy	Control

Figure 1. Example profiles of the six 2015 samples are shown below, as analysed by the most commonly employed methods: isofocusing, HPLC or CE. Examples were randomly selected from the submissions and shown anonimized.





ERNDIM CDG 2015.01
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 99%.

ERNDIM CDG 2015.02
All labs reported this sample as abnormal and nearly all centers correctly assigned this profile as type I profile. The profile was rather mild with elevation of disialotransferrin and a minor number of participants reported a slight elevation of asialotransferrin as well. The age and clinical presentation of cerebellar ataxia could hint in the direction of PMM2-CDG. The advice for further diagnostics should include the option of PMM2-CDG as most frequent CDG-I subtype and known to be associated with this clinical presentation. Of

course, if not PMM2-CDG other CDG-I subtypes are possible. As a secondary cause, in view of age, alcohol abuse should be considered.

ERNDIM CDG 2015.03

All labs using IEF or CE reported an abnormal profile of transferrin, either directly suggesting a protein polymorphism or an abnormal type II profile. It is important to note that the polymorphism was only visible by IEF or CE and not by HPLC, WB, and mass spectrometry. Several laboratories performed neuraminidase incubation to confirm a polymorphism (shown in Figure 1).

The presence of a polymorphism is clinically without any complication, but in this case could complicate interpretation of the profile type. Quite a number of labs performed neuraminidase incubation and confirmed the presence of a transferrin polymorphism. For IEF, this additional band migrates exactly at the position of trisialotransferrin, while for CE, the polymorphism is found at the position of pentasialotransferrin or disialotransferrin.

For IEF, the presence of an additional band at trisialotransferrin could also be indicative for MAN1B1-CDG, and care should be taken to really exclude or confirm this polymorphism, in order not to miss a diagnosis of MAN1B1-CDG. Below, a Figure is shown of the transferrin polymorphism and MAN1B1-CDG, showing the high level of similarity on IEF. Only a single lab suggested the possibility of MAN1B1-CDG

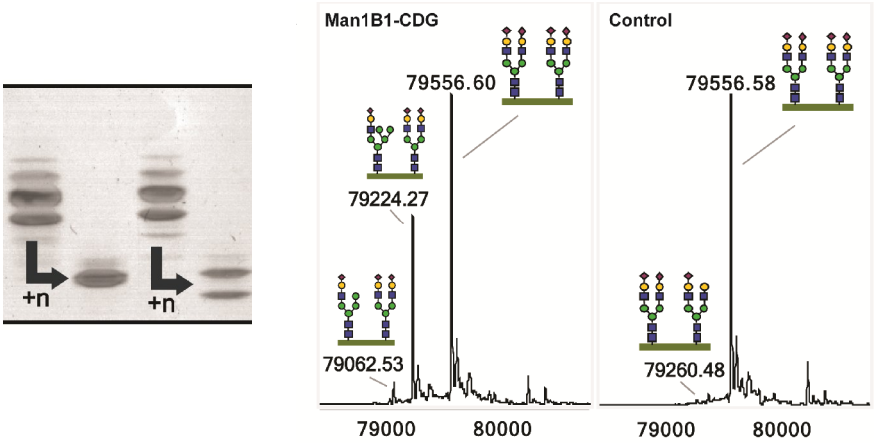


Figure 2. MAN1B1-CDG as compared to the trisialotransferrin polymorphism by IEF on the left (adapted from Van Scherpenzeel et al, Brain 2014), and compared with mass spectrometry of transferrin. +n indicates treatment with neuraminidase. It is unknown how MAN1B1-CDG presents on CE or HPLC analysis.

ERNDIM CDG 2015.04 [EDUCATIONAL SAMPLE]

This sample was derived from an adult patient with excessive alcohol intake. In general, this results in CDG-I abnormal profiles, although 4 labs reported as normal. As this concerns an educational sample, it is sufficient to mention that the identified profile is in agreement with alcohol abuse. The sample was not scored for individual performance, a proficiency score was calculated (see Table 2).

ERNDIM CDG 2015.05

An abnormal profile was mentioned by the vast majority of labs. Assigning the profile type was more variable, some centres indicating type I, others type II. PGM1-CDG in most patients presents with a mixed type of CDG-I and CDG-II (lack of galactose-sialic acid disaccharides) abnormalities. As far as reported in literature, PGM1-CDG is the only subtype that presents with a mixed type I/II profile of transferrin, at least on IEF. As far as we know, CE and HPLC profiles of PGM1-CDG have not yet been reported in literature. In this sample, the type I abnormalities are a bit more prominent as compared to the type II abnormalities.

By IEF, several laboratories recognized the mixed profile type and, in combination with the clinical symptoms, suggested direct testing for PGM1-CDG. This can be done by enzyme analysis or direct genetic testing. By CE and HPLC, it looks like additional very minor peaks are visible in the chromatograms. This could indicate that the various glycoforms of transferrin in PGM1-CDG migrate at different positions. Because of a lack of experience with PGM1-CDG, these mild abnormalities could not directly be linked to a diagnosis of PGM1-CDG. However, the combination of an abnormal profile and a clinical phenotype of myopathy and dilated cardiomyopathy should be sufficient to indicate the possibility of PGM1-CDG as diagnosis.

Especially in view of ongoing therapeutic trials with galactose, the recognition of clinical symptoms and profile types suggestive of PGM1-CDG is very important. Below, a series of different PGM1-CDG profiles by IEF is provided.

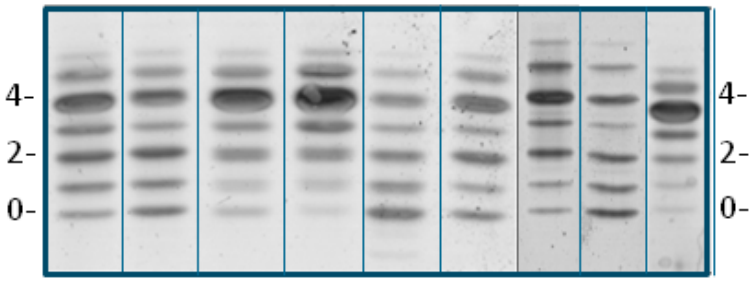


Figure 3. Transferrin isofocusing profiles of 9 PGM1-CDG. As can be seen, some profiles show a dominant type I profile, others a mild type II profile or a clear mixture of type I and II.

ERNDIM CDG 2015.06
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 99%.

Overview of scoring

Table 2: Proficiency per sample

Sample	No of returns	Technical Aspects (%)	Diagnostic Suggestions (%)	Total (%)
CDG2015.01	51	100%	98%	99%
CDG2015.02	52	97%	85%	91%
CDG2015.03	52	99%	91%	95%
CDG2015.04	51	90%	96%	93%
CDG2015.05	51	93%	71%	83%
CDG2015.06	51	99%	99%	99%

Table 3: Detailed scores for submitting laboratories

2015	**Technical, item C**							**Advice, item D**							
Sample ID	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**	**Total**	**.01**	**.02**	**.03**	**.04**	**.05**	**.06**	**Total**	**Total score**
Average score	2,00	1,96	1,98		1,89	1,98		1,96	1,77	1,85		1,49	1,98		**max 20**
1	2	1	1		1	2	7	0	0	0				0	7
2	2	2	2		2	2	10	2	2	2		2	2	10	20
3	2	2	2		2	2	10	2	2	2		2	2	10	20
4	2	2	2		2	2	10	2	2	2		2	2	10	20
5	2	2	2		2	2	10	2	2	2		2	2	10	20
6	2	2	2		2	2	10	2	1	2		1	2	8	18
7	2	2	2		2	2	10	2	1	2		0	2	7	17
8	2	2	2		2	2	10	2	0	0		0	2	4	14
9	2	2	2		2	2	10	2	2	2		2	2	10	20
10	2	2	2		2	2	10	2	2	2		2	2	10	20
11	2	2	2		2	2	10	2	2	2		2	2	10	20
12	2	2	2		1	2	9	2	2	2		0	2	8	17
13	2	2	2		2	2	10	2	2	2		2	2	10	20
14	2	2	2		2	2	10	2	2	2		1	2	9	19
15	2	2	2		2	2	10	2	2	2		2	2	10	20
16	2	2	2		2	2	10	2	2	2		2	2	10	20
17	2	2	2		2	2	10	2	2	2		2	2	10	20

2015	Technical, item C							Advice, item D							
Sample ID	.01	.02	.03	.04	.05	.06	Total	.01	.02	.03	.04	.05	.06	Total	Total score
Average score	2,00	1,96	1,98		1,89	1,98		1,96	1,77	1,85		1,49	1,98		max 20
18	2	2	2		2	1	9	2	2	2		1	2	9	18
19	2	2	2		2	2	10	2	2	2		0	2	8	18
20	2	2	2		2	2	10	2	2	2		2	2	10	20
21	2	2	2				6	2	2	2				6	12
22	2	2	2		2	2	10	2	2	2		2	2	10	20
23					2	2	4					2	2	4	8
24	2	2	2		2	2	10	2	2	2		2	2	10	20
25	2	2	2		2	2	10	2	2	2		2	2	10	20
26	2	2	2		2	2	10	2	2	2			2	8	18
27	2	2	2		2	2	10	2	2	2		2	2	10	20
28					2	2	4					2	2	4	8
29	2	2	2		2	2	10	2	2	2		2	2	10	20
30	2	2	2		1	2	9	2	2	2		1	2	9	18
31	2	2	2				6	2	2	0				4	10
32	2	2	2		2	2	10	2	2	2		2	2	10	20
33	2	2	2		2	2	10	2	2	2		1	2	9	19
34	2	2	2		2	2	10	2	2	2		2	2	10	20
35	2	1	2		2	2	9	2	0	2		0	2	6	15
36	2	2	2		2	2	10	2	2	2		2	2	10	20
37	2	2	2		2	2	10	2	2	2		0	2	8	18
38	2	2	2		2	2	10	2	2	2		2	2	10	20
39					2	2	4					0	2	2	6
40	2	2	2		2	2	10	2	2	2		2	2	10	20
41	2	2	2		2	2	10	2	2	2		2	2	10	20
42	2	2	2		2	2	10	2	2	2		2	2	10	20
43	2	2	2		1	2	9	2	2	2		0	2	8	17
44	2	2	2		2	2	10	2	1	2		1	2	8	18
45	2	2	2		1	2	9	2	1	2		1	2	8	17
46	2	2	2				6	2	2	2				6	12
47	2	2	2		2	2	10	2	2	1		2	2	9	19
48	2	2	2		2	2	10	2	2	2		2	2	10	20
49	2	2	2		2	2	10	2	1	2		2	2	9	19
50	2	2	2				6	2	2	2				6	12
51					2	2	4					2	2	4	8
52	2	2	2		1	2	9	2	1	2		0	2	7	16
53	2	2	2		1	2	9	2	0	0		0	2	4	13
54		2	2		2	2	8		2	2		2	2	8	16
55	2	1	2				5	2	0	2				4	9
56	2	2	2	2	2	2	12	2	2	2	2	1	2	11	23