ERNDIM
QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

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Congenital Disorders of Glycosylation

Final Report 2014

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[This final report was ratified by the Scientific Advisory Board in March 2015]

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 59 registered laboratories in one parcel on 11th February 2014. Seven 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 CDG032, CDG033, and CDG034) and second round (samples CDG035, CDG036 and CDG037) were received from 49 laboratories (83%) by the due date.

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.

Item D: diagnostic suggestions: For normal profiles in general 2 points. 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.

CDG 037 is classed as an educational sample, so full points have been scored for all centers.

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 7 points if results were submitted for the first round only or 5 points if results were submitted for the second round only. This has been agreed at the meeting of the Scientific Advisory Board on 19th March 2015.

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 CDG scheme, identification of the mild CDG-I sample 033 as normal has been advised by the SAB as critical error.

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 (9), CE (7), 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 2014 scheme

Sample	Clinical information (age, sex, phenotype)	Diagnosis
CDG 032	2y, M, developmental delay, epilepsy, deafness	Control (no CDG)
CDG 033	8y, M, myasthenic syndrome, mental retardation	DPAGT1-CDG (CDG-Ij)
CDG 034	6y, F, cataract, mental retardation, skeletal abnormalities	Control (no CDG)
CDG 035	3 y, F, coagulopathy, epilepsy, psychomotor retardation, liver disease	PMM2-CDG (CDG-Ia) and protein polymorphism
CDG 036	16y, M, hepatopathy, increased transaminases, liver fibrosis	Control (no CDG)
CDG 037	**Educational sample**, adult with increased alcohol intake	Increased CDT

ERNDIM CDG032/CDG034/CDG036

A normal profile was identified and interpreted by nearly all centers.

ERNDIM CDG033

The vast majority of labs reported an abnormal type I profile of transferrin. The profile was rather mild with some elevation of disialotransferrin and in quite a number of analyses a similar level of trisialotransferrin. However, the relative level of trisialotransferrin in the majority of cases was not increased above reference ranges, which results in an isolated increase of disialotransferrin and thus a CDG-I profile. For some of the laboratories, this resulted in complications in the interpretation of the profile type (for example annotated as type II). In Figure 1, an example is shown of an HPLC profile of samples CDG032 and CDG033. In CDG033, a relative increase in disialotransferrin is shown (T2) as in a mild CDG-I profile.

The clinical information includes a description of myasthenic syndrome. This clinical entity has recently been related to CDG defects with mutations in GFPT1, DPAGT1, ALG2 and ALG14. Thus far, abnormal CDG screening has been shown in DPAGT1-CDG, and ALG2-CDG patients with a clear multisystem phenotype. ALG14-CDG and GFPT1-CDG patients have thus far not been reported with abnormal glycosylation of serum transferrin. Nevertheless, in view of the limited number of patients reported, neither one of these gene defects can be excluded on basis of normal/abnormal CDG screening in blood. For Advice, one point was scored for mentioning enzyme assays or other follow-up for CDG-I defect, and one additional point for mentioning the occurrence of myasthenic syndrome in the gene defects as discussed above.

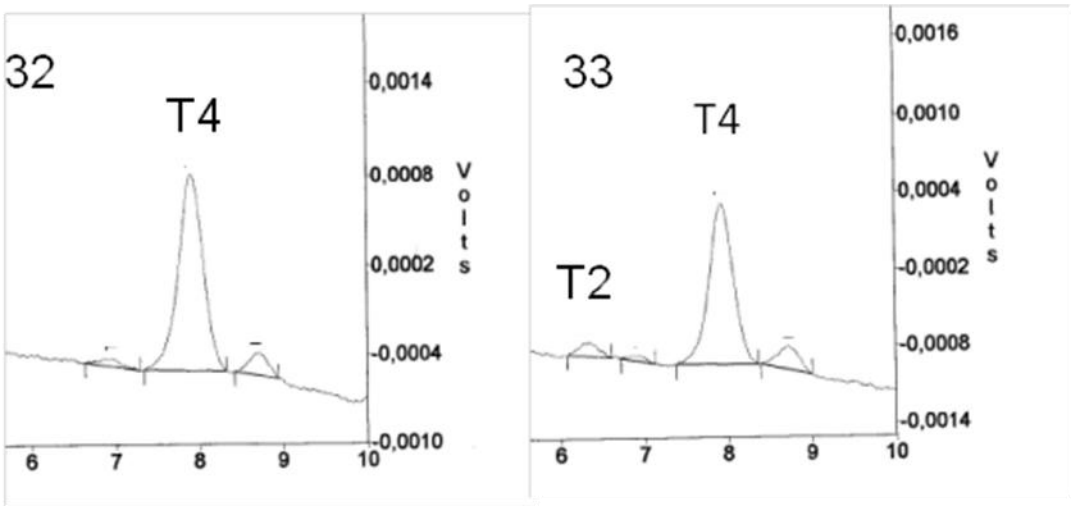

Figure 1: HPLC profile of samples CDG032 and CDG033

ERNDIM CDG035

The majority of labs reported an abnormal type I profile of transferrin and in addition a polymorphism of transferrin. The presence of a polymorphism is clinically without any complication, but in this case complicates interpretation of the profile type. Figure 2 (below) shows 3 examples. Migration of the polymorphism bands on isofocusing apparently depends quite a bit on the conditions used (such as for example the ampholites, pH range, etc). In some cases, this results in the appearance of the transferrin polymorphism band of disialotransferrin (T2) at exactly the same position as trisialotransferrin (2a, HPLC profile). This can be misinterpreted as a CDG-II profile. In other cases (2b, 2c, IEF profiles), the presence of polymorphic bands is more clear, as the polymorphic bands migrate in between the “normal” disialo- and trisialotransferrin bands.

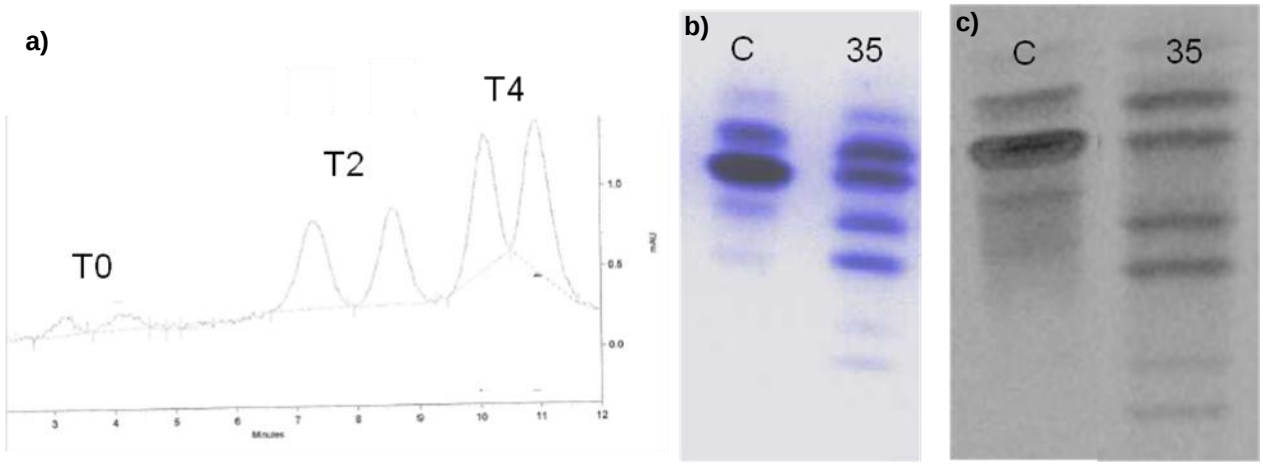

Figure 2: CDG035 **a)** HPLC profile**; b)** Isofocusing profile **c)** Isofocusing profile

The clinical indication in this patient was rather non-specific, at least not directly suggesting a certain CDG-I subtype. In this case, the next step would be enzyme analysis for the most frequent subtype PMM2-CDG (CDG-Ia), followed (if needed) by analysis of lipid-linked oligosaccharides, next-generation sequencing (gene panels/exome sequencing, etc), or else.

ERNDIM CDG037 [EDUCATIONAL SAMPLE]

This sample was derived from an adult patient with excessive alcohol intake. In general, this results in CDG-I abnormal profiles. In this case, disialotransferrin was increased, but also trisialotransferrin was slightly elevated. Quite a number of centers commented on this elevation of trisialotransferrin. It is not completely clear why trisialotransferrin was increased. It could be that secondary liver pathology results in a mild type II profile, as is known in CDG screening. Mass spectrometry of transferrin revealed a loss of a complete glycan (in agreement with CDG-I), but also a slightly elevated loss of a single sialic acid, likely responsible for the mild increase of trisialotransferrin.

Table 2: Proficiency per sample

Sample	No of returns	Technical Aspects (%)	Diagnostic Suggestions (%)	Total (%)
CDG032	49	100%	98%	99%
CDG033	49	90%	65%	78%
CDG034	49	97%	96%	96%
CDG035	49	91%	78%	84%
CDG036	49	100%	98%	99%
CDG037	49	100%	100%	100%

Table 3: Detailed scores for submitting laboratories

2014	Technical, item C							Advice, item D							
Sample ID	**032**	**033**	**034**	**035**	**036**	**037**	**Total**	**032**	**033**	**034**	**035**	**036**	**037**	**Total**	**Total score**
Average score	2	1,80	1,94	1,82	2,00			1,96	1,31	1,92	1,55	1,96			**max 20**
1	2	0	2	1	2		7	0	0	0	0	0		0	**7**
2	2	2	2	2	2		10	2	1	2	1	2		8	**18**
3				2	2		4				2	2		4	**8**
4	2	2	2	2	2		10	2	2	2	2	2		10	**20**
5				1	2		3				2	2		4	**7**
6	2	2	2	2	2		10	2	2	2	2	2		10	**20**
7	2	1	1	2	2		8	2	2	1	1	2		8	**16**
8	2	2	2	2	2		10	2	0	2	0	2		6	**16**
9	2	2	2	2	2		10	2	1	2	2	2		9	**19**
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	0	2	2	2		8	2	0	2	2	2		8	**16**
13	2	2	2	2	2		10	2	2	2	1	2		9	**19**
14	2	2	2	2	2		10	2	0	2	2	2		8	**18**
15	2	2	2	2	2		10	2	2	2	2	2		10	**20**
16	2	2	2	2	2		10	2	1	2	2	2		9	**19**
17	2	2	2	1	2		9	2	1	2	0	2		7	**16**
18	2	2	2	2	2		10	2	2	2	2	2		10	**20**
19	2	2	2	2	2		10	2	2	2	2	2		10	**20**
20	2	2	2	1	2		9	2	2	2	1	2		9	**18**
21	2	2	2	2	2		10	2	2	2	1	2		9	**19**
22	2	2	2	2	2		10	2	1	2	2	2		9	**19**
23	2	2	2				6	2	1	2				5	**11**
24	2	2	2	1	2		9	2	1	2	1	2		8	**17**
25	2	2	2	2	2		10	2	2	2	2	2		10	**20**
26	2	2	0	2	2		8	2	1	1	2	2		8	**16**
27	2	2	2	2	2		10	2	2	2	2	2		10	**20**
28	2	2	2	2	2		10	2	1	2	2	2		9	**19**
29	2	0	2				4	2	0	2				4	**8**
30	2	2	2	2	2		10	2	2	2	2	2		10	**20**
31	2	2	2	2	2		10	2	2	2	2	2		10	**20**
32	2	2	2	2	2		10	2	1	2	2	2		9	**19**
33	2	2	2	2	2		10	2	2	2	2	2		10	**20**
34	2	2	2	2	2		10	2	1	2	1	2		8	**18**
35	2	2	2	2	2		10	2	1	2	2	2		9	**19**

Table 3: Detailed scores for submitting laboratories (continued)

2014	Technical, item C							Advice, item D							
Sample ID	**032**	**033**	**034**	**035**	**036**	**037**	**Total**	**032**	**033**	**034**	**035**	**036**	**037**	**Total**	**Total score**
Average score	2	1,80	1,94	1,82	2,00			1,96	1,31	1,92	1,55	1,96			**max 24**
36	2	2	2	2	2		10	2	2	2	2	2		10	**20**
37	2	1	2	1	2		8	2	0	2	1	2		7	**15**
38	2	2	2	2	2		10	2	2	2	2	2		10	**20**
39	2	2	2	2	2		10	2	1	2	2	2		9	**19**
40	2	2	2	2	2		10	2	2	2	2	2		10	**20**
41	2	1	2	2	2		9	2	0	2	1	2		7	**16**
42	2	2	2	2	2		10	2	2	2	2	2		10	**20**
43	2	2	2	2	2		10	2	1	2	2	2		9	**19**
44	2	1	2	2	2		9	2	1	2	1	2		8	**17**
45	2	2	2	2	2		10	2	0	2	1	2		7	**17**
46	2	2	2	1	2		9	2	1	2	1	2		8	**17**
47	2	2	2	2	2		10	2	2	2	2	2		10	**20**
48	2	2	2	2	2		10	2	1	2	1	2		8	**18**
49	2	2	2	2	2		10	2	2	2	2	2		10	**20**
50	2	2	2	1	2		9	2	2	2	0	2		8	**17**
51	2	2	2	1	2		9	2	1	2	1	2		8	**17**