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

Scientific Advisor
Dr Dirk Lefeber
830- Laboratory of Genetic, Endocrine and
Metabolic Disease
Radboud University Nijmegen Medical
Centre
Geert Grooteplein 10
6525 GA Nijmegen, The Netherlands
Tel: +31 24 3614567 / 3953
Fax: +31 24 3668754
Email: Dirk.Lefeber@Radboudumc.nl

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

Congenital Disorders of Glycosylation
Final Report 2017

[This final report was ratified by the Scientific Advisory Board in November 2017]
Date of issue: 13 April 2018
Amended report issued: 20 April 2018[1]

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 64 registered laboratories in one parcel on 14th February 2017. Twenty-one laboratories requested a total of 26 extra sample sets 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 2017.01 - CDG 2017.03) and second round (samples CDG 2017.04 - CDG 2017.06) were received by the due date from 59 (91%) and 57 (88%) laboratories respectively. An additional 1 (2%) lab submitted their results for the first round within 2 weeks of the submission deadline. Two labs only submitted results for the first round and 5 labs only submitted results for the second round. There were three 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: 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 the presence of a secondary cause in light 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 .01, PGM1-CDG should be mentioned for full scores. For sample .04, PMM2-CDG should be mentioned as a possible diagnosis. For sample .06, exclusion of liver disease as secondary cause can be mentioned.

   The maximum score achievable with full submission for all samples is 24, while a maximum of 12 points are available for labs that only submitted results for the first or second round. The level for satisfactory performance is 15 points. Laboratories that participate only in one circulation are treated as partial-submitters and can achieve satisfactory performance with 8 points. 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

Version Number (& Date)	Amendments
[1] version 2 (20 April 2018)	• Page 2: Table 2 updated to reflect changes to scores in table 3 (see below). • Page 5: In table 3 scores for samples 2017.01-03 have been inserted for lab 28; and scores for samples 2017.04-06 have been inserted for labs 13 and 20.

misleading analytical findings and/or interpretations with serious clinical consequences for the patient. For the 2017 CDG scheme, one critical error was identified. This has been agreed at the meeting of the Scientific Advisory Board on 23rd November 2017.

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 (34), followed by HPLC (11) and CE (14), mass spectrometry (2) and western blot (1).

The shipped samples were from (CDG) patients and from controls. The final results of the six samples with respect to CDG are summarized in Table 1 below.

Table 1: Samples in the 2017 scheme

| Sample	Clinical information (age, sex, phenotype)	Diagnosis
2017.01	F, 12 years, short stature, increased transaminases, cleft palate	PGM1-CDG
2017.02	M, 4 years, cataract, hepatomegaly	Control
2017.03	M, 19 years, hypercholesterolemia, increased alkaline phosphatase and transaminases	Control
2017.04	M, 32 years, intellectual disability, hypotonia and balance problems	PMM2-CDG
2017.05	F, 2 years, encephalopathic epilepsy	Control
2017.06	M, 55 years, increased transaminases, gGT, and CRP	Secondary cause, liver cancer

Table 2: Scoring of samples in the 2017 scheme

| Sample	No of returns	Technical Aspects (%)	Diagnostic Suggestions (%)	Total (%)
CDG2017.01	59	80%	73%	76%
CDG2017.02	60	99%	99%	99%
CDG2017.03	55	98%	98%	98%
CDG2017.04	51	96%	78%	87%
CDG2017.05	59	99%	99%	99%
CDG2017.06	50	85%	89%	87%

The full anonymised results for all labs that submitted results are given in Table 3 on page 5-6 at the end of this report.

Figure 1. Example profiles of the six 2017 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.
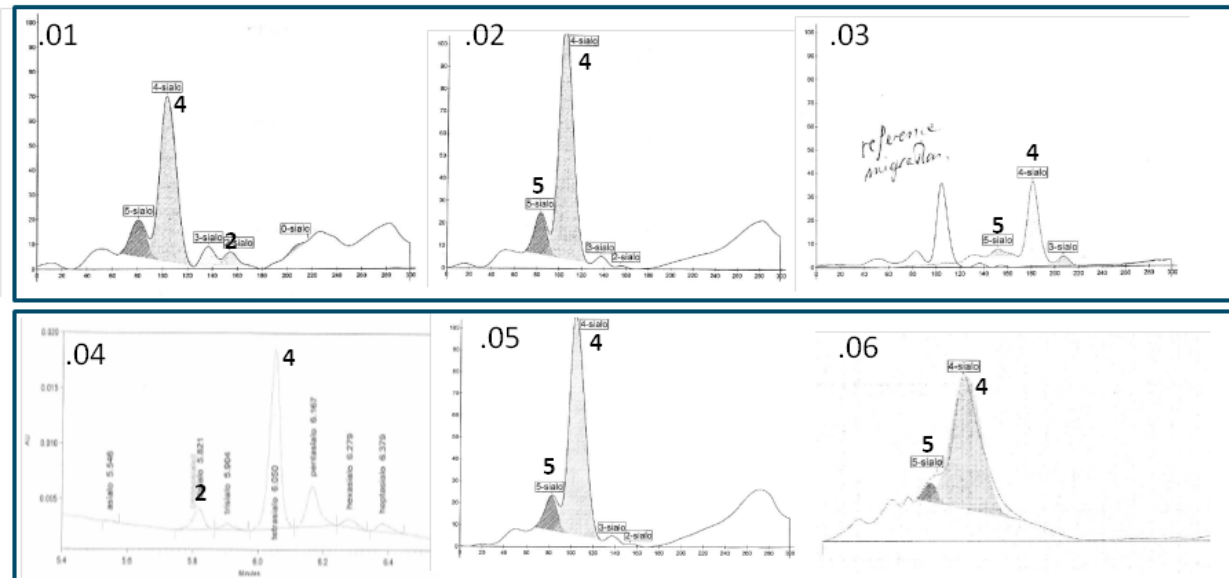

Transferrin isofocusing

.01 .02 .03   .04 .05 .06   .06   .06   .06
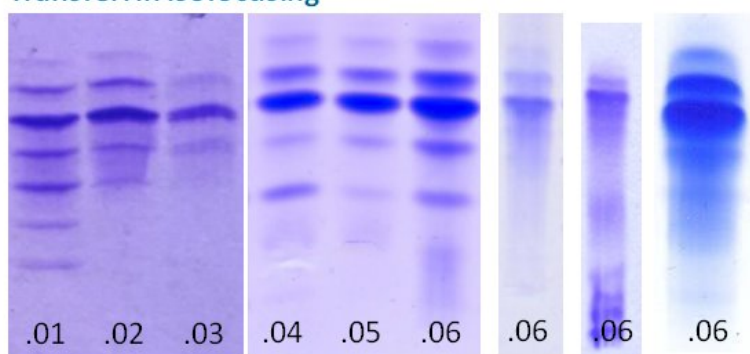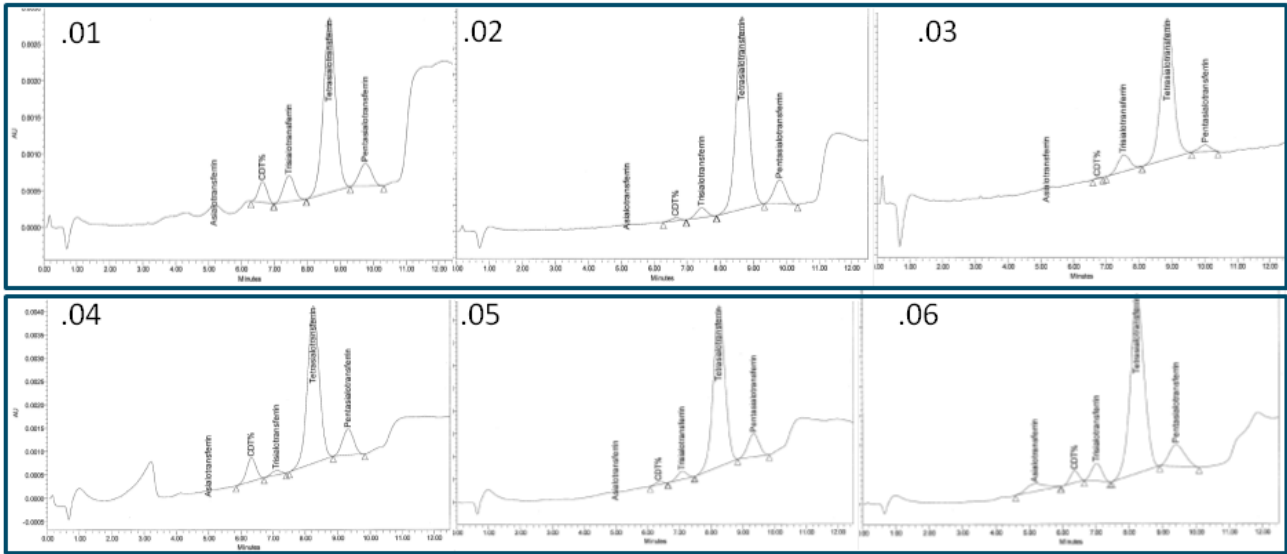
Transferrin HPLC

Transferrin CE

ERNDIM CDG 2017.01: PGM1-CDG
The majority of labs reported this sample as abnormal. The major difficulty was in assigning this profile as a mixed type I/II profile, which is suggestive of PGM1-CDG. Most labs interpreted this correctly, while many others interpreted the profile as type I. The clinical presentation, including cleft palate and short stature, is highly suggestive for PGM1-CDG (within the context of CDG). Correct interpretation of the profile and advice for further diagnostics in the direction of PGM1-CDG should be included for full scoring. Proficiency score: 76%.

ERNDIM CDG 2017.02: control
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 99%.

ERNDIM CDG 2017.03: control
A normal profile was identified by all centers and interpreted as normal by nearly all centers with a proficiency score of 98%.

ERNDIM CDG 2017.04: PMM2-CDG
Nearly all laboratories reported this sample as abnormal and correctly assigned the profile as type I. Quite a number of laboratories, all except one performing CE analysis, had problems with interpretation of the profile, possibly due to interfering substances or maybe sample preparation (see further under 2017.06). Sample volume does not seem to explain this, since all labs using HPLC analysis received full scores for technical interpretation. The age and clinical presentation fits very well with a mild presentation of adult patients with PMM2-CDG, which is the final diagnosis. 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. In view of age and symptoms, secondary causes are unlikely. Proficiency score: 89%.

ERNDIM CDG 2017.05: control
A normal profile was identified by all centers and interpreted as normal by nearly all centers. Proficiency score: 99%.

ERNDIM CDG 2017.06: non-CDG liver pathology
Many laboratories reported this sample as abnormal and indicated a possible type II profile with mild increase of tri- and disialotransferrin. Many laboratories using isofocusing reported possible sample degradation in view of the smearing profiles (see examples in Figure 1). In addition, most CE using laboratories reported problems with interpretation of the profile with a possible explanation of interfering substances. The laboratories using HPLC scored very well in the technical interpretation of this sample. This sample was derived from an adult patient with a mild type II profile and later turned out to have liver cancer. Liver pathology is a known secondary cause for type II profiles. Proficiency score: 87%.

In view of the smearing profiles on isofocusing, we set out to investigate the sample preparation procedures of different laboratories. In the past, we had experienced similar isofocusing profiles in our own laboratory, in patients with low levels of serum iron levels. Increasing the level of Fe(III)citrate solved the problem.

Sample preparation for transferrin isofocusing:
- Add 200 µl 10 mM Fe(III)citrate and 100 µl 0.5 M NaHCO3-sol. together and mix.
- Use 3 µl of this solution and 10 µl plasma or serum .
- Mix and 1 hour incubation at roomtemperature.
- Dilute samples 6 times by adding 50 µl milliporewater.

For HPLC, most labs use the protocols from literature (Helander et al 2003, Clinical Chemistry 49(11):1881-1890 and Jeppsson et al 2007 Clinical Chemistry and Laboratory Medicine 45(4):558-562). For CE, the Sebia CE system is most often used. The protocol uses premade solutions of which we could not get information about the concentration of iron. The protocol for transferrin iron saturation as shown above for isofocusing was applied for CE sample preparation (courtesy Dr. Francois Boemer), showing improved peak shape (Figure 2).

Figure 2. Sample preparation using the standard Sebia protocol and after use of increased Fe3+ concentration.

Sample preparation for transferrin CE:
- Mix 200 µL of 10 mM Fe(III)-Citrate and 100 µL of 0.5 M NaHCO3 solutions.
- Add 15 µL of this solution to 50 µL of serum sample.
- Mix the solution and centrifuge.
- Dilute the supernatant as follow :
  - 40µL supernatant
  - 40µL sample diluent (provided by manufacturer)
  - 120µL distillated water
  Use 200 µL of this diluted solution on the Capillary Electrophoresis device.

Although this is an n=1 test, we strongly advice CE and IEF users to critically check their protocols for sample preparation and test if the profiles can be improved by using higher concentrations of iron in this preparation. Possibly, HPLC profiles could also be improved, but this remains to be tested.

Dirk Lefeber

Scientific Advisor

Table 3: Detailed scores for submitting laboratories

2017	Technical, item C							Advice, item D							Total score (max 24)
Sample ID	.01	.02	.03	.04	.05	.06		.01	.02	.03	.04	.05	.06		
Average score	1,59	1,98	1,96	1,96	1,98	1,69		1,45	1,98	1,96	1,61	1,98	1,77		
Lab ID							Total							Total	
1	1	2	2	2	2	1	10	1	2	2	1	2	2	10	**20**
2	2	2	2	2	2	1	11	1	2	2	1	2	2	10	**21**
3	2	2	2	2	2	2	12	2	2	2	1	2	2	11	**23**
4	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
5	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
6	1	2	2	2	2	1	10	1	2	2	2	2	2	11	**21**
7	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
8	2	2	2	-	2	-	8	1	2	2	-	2	-	7	**15**
9	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
10	2	2	2	-	2	-	8	2	2	2	-	2	-	8	**16**
11	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
12	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
13	2	2	2	-	2	2	10	2	2	2	-	2	2	10	**20**
14	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
15	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
16	2	2	2	2	2	2	12	2	2	2	1	2	2	11	**23**
17	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
18	-	2	2				4	-	2	2				4	**8**
19	2	2	2	2	2	-	10	2	2	2	2	2	-	10	**20**
20	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
21	1	2	2				5	1	2	2				5	**10**
22	2	2	2	2	2	1	11	2	2	2	2	2	2	12	**23**
23	1	2	2	2	2	2	11	2	2	2	2	2	2	12	**23**
24	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
25	1	2	2	2	2	2	11	1	2	2	2	2	1	10	**21**
26	1	2	2	2	2	1	10	1	2	2	2	2	2	11	**21**
27	1	2	-	-	2	-	5	1	2	-	-	2	-	5	**10**
28	2	2	2	2	2	1	11	2	2	2	2	2	1	11	**22**
29	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
30	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
31	2	2	2	2	2	2	12	2	2	2	2	2	1	11	**23**
32	1	2	2	2	2	2	11	1	2	2	1	2	2	10	**21**
33	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
34	2	2	2	2	2	2	12	2	2	2	0	2	1	9	**21**
35	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
36	1	2	-	-	2	-	5	2	2	-	-	2	-	6	**11**
37	0	2	1	2	2	1	8	0	2	1	1	2	1	7	**15**
38	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
39	1	2	2	2	2	2	11	1	2	2	2	2	2	11	**22**
40	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
41	2	2	2	2	2	2	12	2	2	2	2	2	2	12	**24**
42	1	2	-	2	2	1	8	0	2	-	1	2	2	7	**15**
43	1	2	2	2	2	0	9	0	2	2	0	2	0	6	**15**
44	1	1	1	-	2	-	5	1	1	1	-	2	-	5	**10**
45	2	2	2	2	2	2	12	2	2	2	2	2	1	11	**23**

2017	Technical, item C							Advice, item D							Total score (max 24)
Sample ID	.01	.02	.03	.04	.05	.06		.01	.02	.03	.04	.05	.06		
Average score	1,59	1,98	1,96	1,96	1,98	1,69		1,45	1,98	1,96	1,61	1,98	1,77		
Lab ID							Total							Total	
46	1	2	2				5	1	2	2				5	10
47	1	2	-	-	2	-	5	1	2	-	-	2	-	5	10
48	1	2	2	2	2	2	11	0	2	2	0	2	2	8	19
49	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
50	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
51	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24
52	2	2	2	2	2	2	12	1	2	2	2	2	2	11	23
53				2	2	1	5				2	2	2	6	11
54	1	2	2	2	2	2	11	1	2	2	1	2	2	10	21
55	1	2	2	2	2	1	10	0	2	2	1	2	2	9	19
56	1	2	2	2	2	1	10	2	2	2	2	2	2	12	22
57	-	-	2	2	2	1	7	-	-	2	1	2	2	7	14
58	1	2	-	-	1	-	4	0	2	-	-	1	-	3	7
59	1	2	-	-	2	-	5	1	2	-	-	2	-	5	10
60	2	2	2	1	2	2	11	0	2	2	0	2	1	7	18
61	1	2	2	1	2	1	9	0	2	2	0	2	0	6	15
62	2	2	2	2	2	2	12	2	2	2	2	2	2	12	24

Note: This annual report is intended for the participants of the CDG 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.