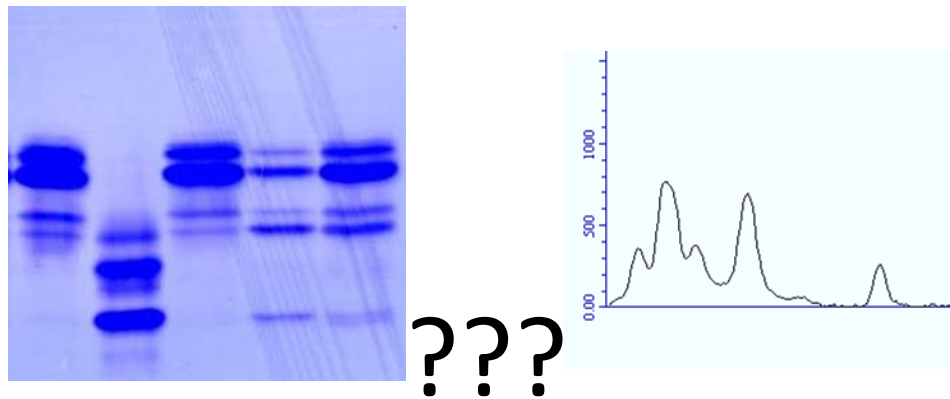


## Towards a QC scheme for the screening of Congenital Disorders of Glycosylation

From Euroglycanet to ERNDIM



**Dirk Lefeber**, Karin Huyben, Irma Versleijen, Cas Weycamp, Gert Matthijs, Ron Wevers

# Euroglycanet: European Glycosylation Disorders Network



FP6 EU program

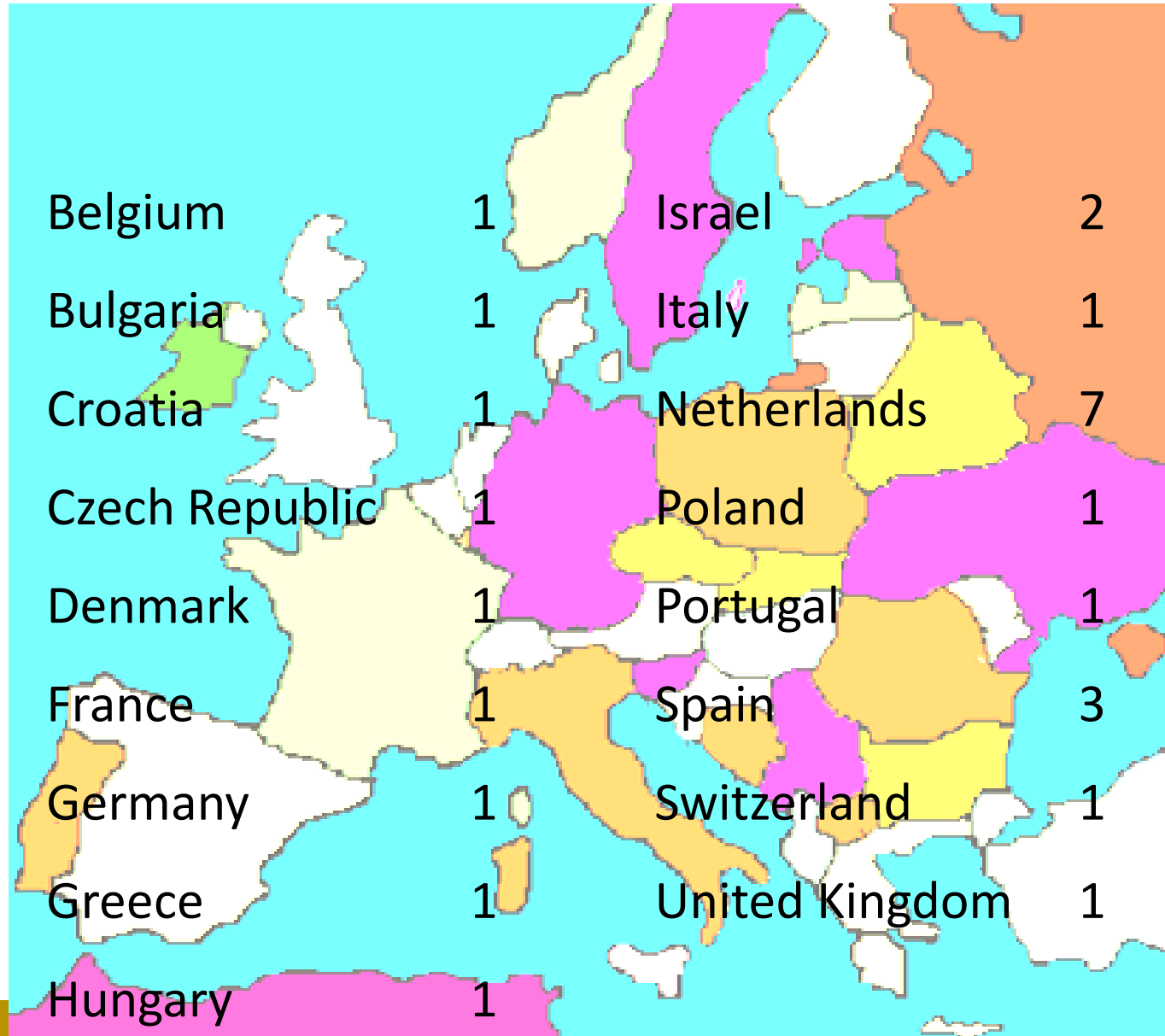
~28 metabolic centres in Europe and Israel

WP 3: Carousel testing

WP 8: Quality Management of the diagnostic tests

WP 9: Training of new centres

## Participating Centres



## Outline of the QC scheme

	Samples	Centers	Date of shipment
Round 1	EUCDG001- EUCDG004	20/26	26-06-2006
Round 2	EUCDG005- EUCDG008	27/28	20-11-2006
Round 3	EUCDG009- EUCDG012	25/27	22-02-2007
Round 4	EUCDG013- EUCDG018	24/26	22-04-2008

### Rounds 1-3:

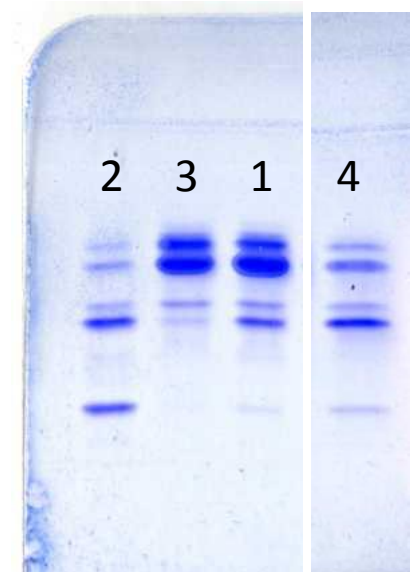
- 4 samples (20 µl) of patients with (ab)normal TIEF profiles
- Brief clinical information, age and sex
- Scoring on basis of:
  - abnormal profile (3pts)
  - type I/II (1pt) and follow-up suggestions (1pt)

### Round 4:

- Reproducibility and shipment conditions
- 2 samples (control and CDG-I) in triplo

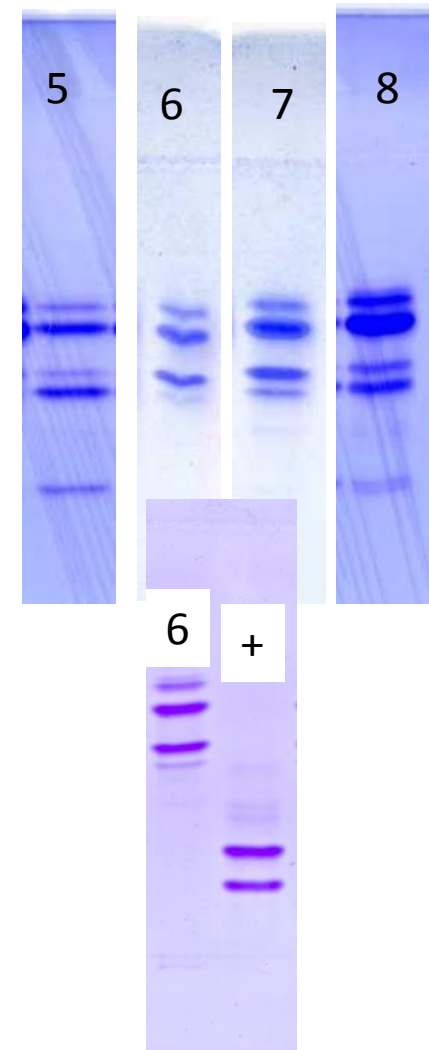
## Results 1st round

Sample Code	Clinical information	Patient data	Diagnosis
EUCDG001	Ataxia, cardiac anomalies,	F, 30 years	Mild CDG-Ia
EUCDG002	Hypoglycaemia, feeding difficulties, hypothyroidism	F, 2 weeks	Severe CDG-Ia
EUCDG003	Strabismus, mental retardation	M, 3 years	Control
EUCDG004	Hypotonia, knik in ontw.	M, 7 years	CDG-Ic



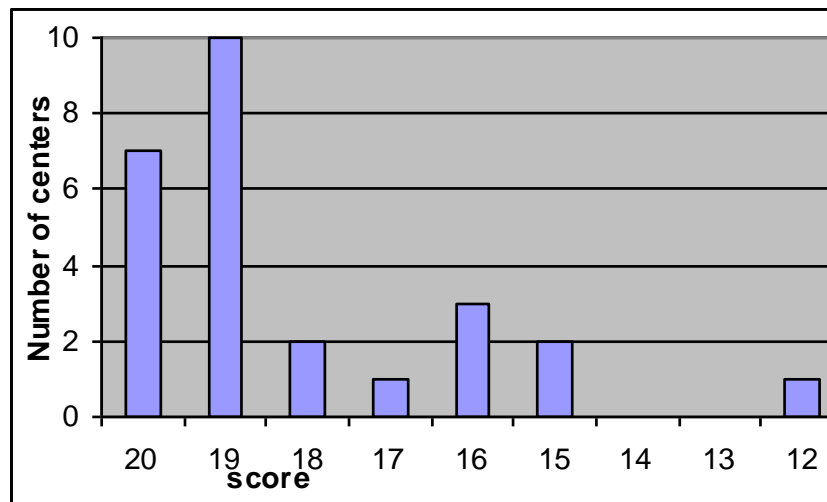
## Results 2nd round

Sample Code	Clinical information	Patient data	Diagnosis
EUCDG005	Abnormal fat distribution, vomiting, behavioural abnormalities	F, 6 years	CDG-Ia
EUCDG006	Diarrhea, hypertonia	M, 38 years	Polymorphism
EUCDG007	Microcephaly, hypotonia, skeletal abnormalities, feeding problems	F, 4 years	CDG-II
EUCDG008	neuropathy	M, 50 years	Alcohol abuse



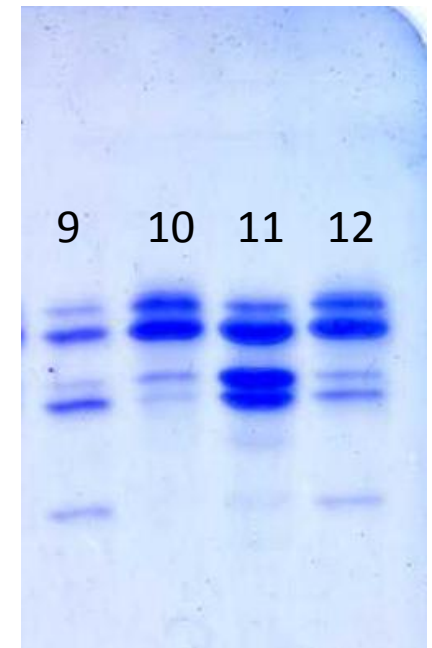
## Performance 2nd round

- Degradation in 4 (of 112) samples
- Polymorphism indicated as CDG-II, suggestions for further CDG work-up
- Alcohol abuse missed



## Results 3rd round

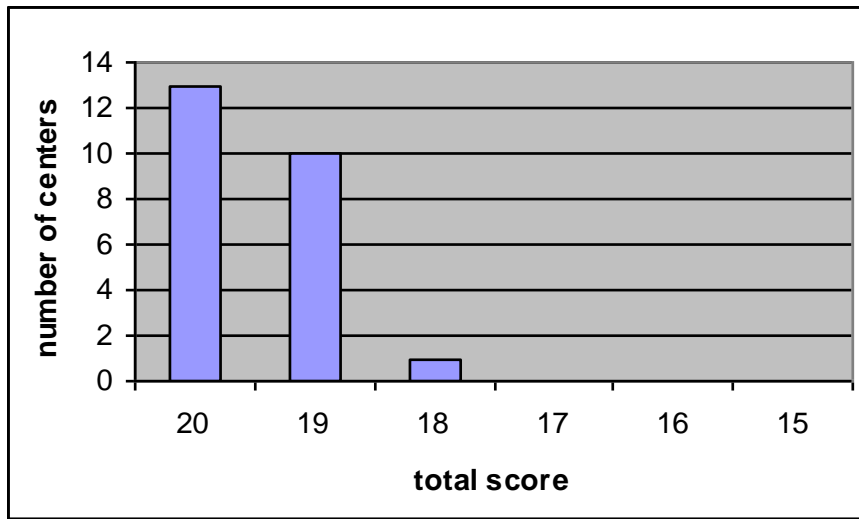
Sample Code	Clinical information	Patient data	Diagnosis
EUCDG009	vomiting, behavioural abnormalities, mental retardation	Female, 5 years	CDG-Ia
EUCDG010	epilepsy	Male, 3 years	control
EUCDG011	Epilepsy, severe mental retardation	Female, 20 years	CDG-II
EUCDG012	Failure-to-thrive, gastro-enteritis	Female, 1.5 years	fructosemia





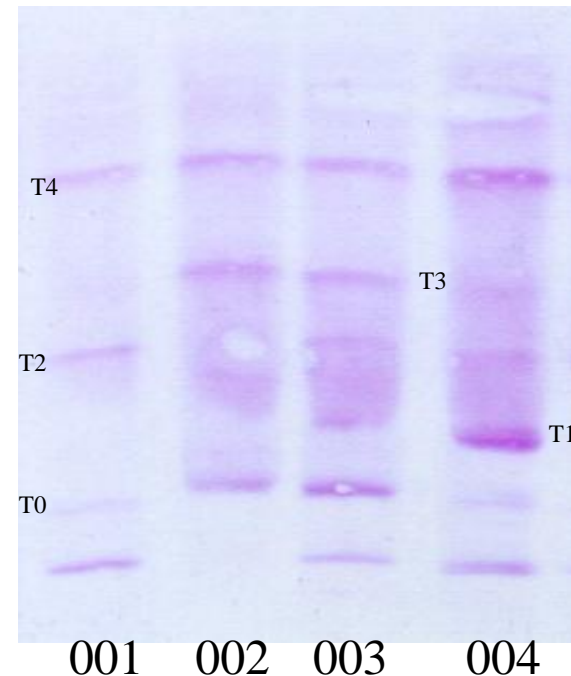
## Performance 3rd round

- Quantification does not improve diagnostics
- Degradation in 4 (of 108) samples
- fructosemia



## Conclusions after 3 rounds

- Methods used:  
PhastSystem/Minigel (11), Multiphor(11), other (4)
- Controls: variation in Transferrin-2 intensity
- Qualitative vs Quantitative (12/20): no difference
- Sample stability issue



# Stability of TIEF profiles on storage

Matrix	Storage Temperature	Short Term				Medium Term			Long Term		
		Day 0	1 wk	2 wk	4 wk	2 md	3 md	6 md	1 jr	2 jr	
Native	Frozen-80	101	102	103	104	105	106	107	108	109	
	Frozen-20		110	111	112	113	114	115	116	117	
	Fridge+4		118	119							
	RT+22		126	127							
	+37										
Lyo Basis	Frozen-80	201	202	203	204	205	206	207	208	209	
	Frozen-20		210	211	212	213	214	215	216	217	
	Fridge+4		218	219	220	221	222				
	RT+22		226	227							
	+ 37										
Lyo Cryo	Frozen-80	301	302	303	304	305	306	307	308	309	
	Frozen-20		310	311	312	313	314	315	316	317	
	Fridge+4		318	319	320	321	322	323	324	325	
	RT+22		326	327	328	329					
	+ 37		334	335							



mildly degraded

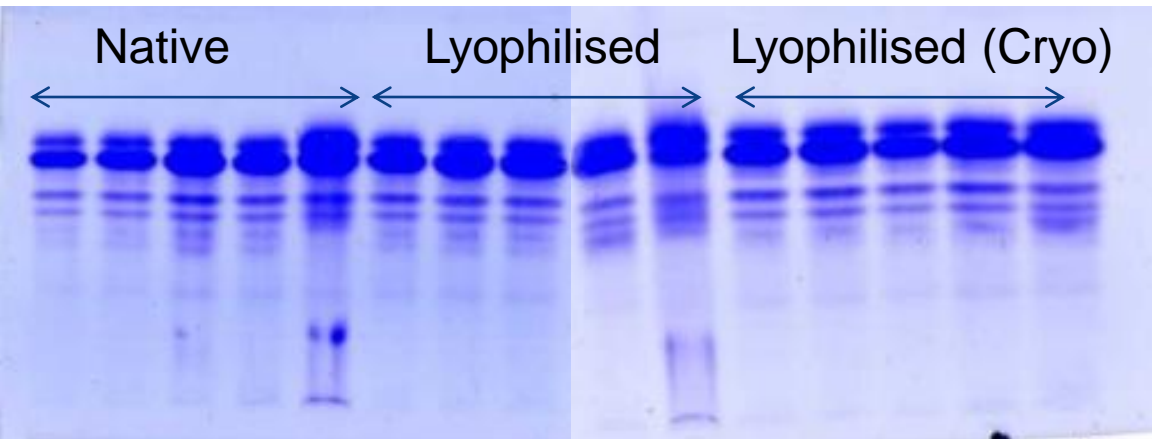


degraded

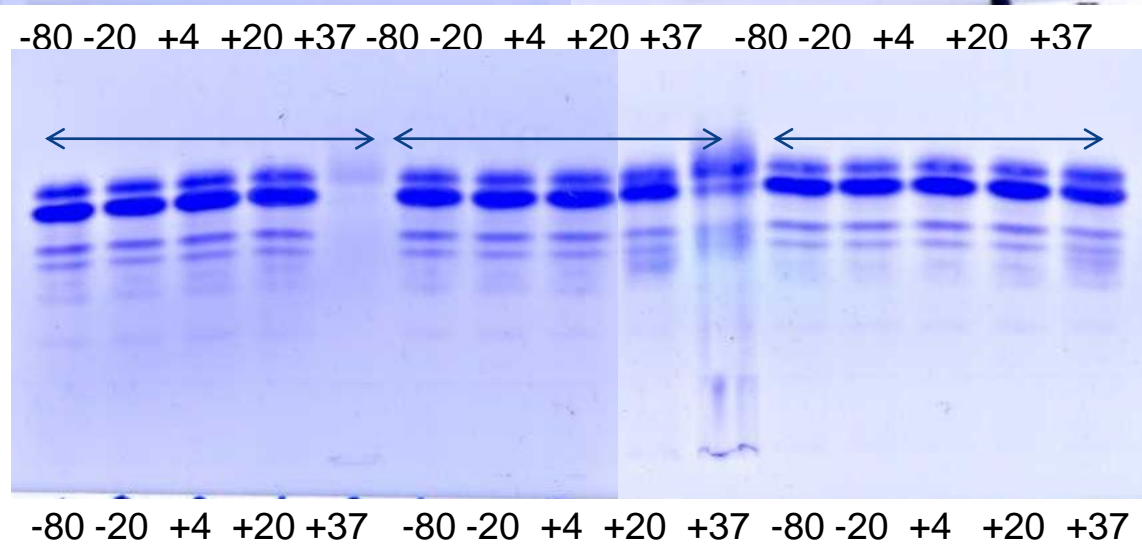


# Stability of TIEF profiles on storage

- Effect of lyophilisation



1 week storage

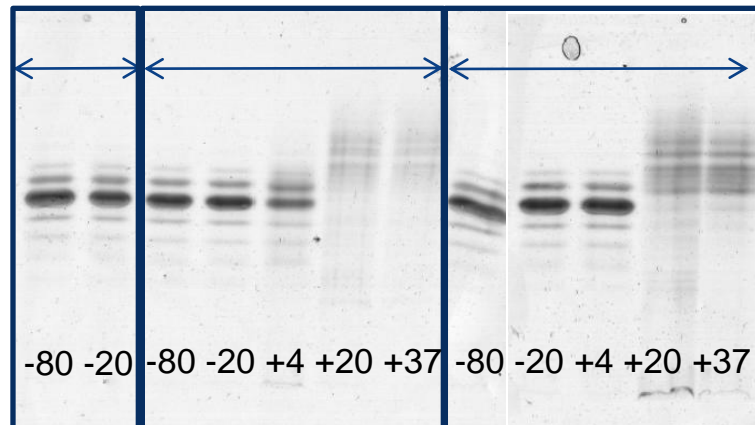


2 weeks storage



## After 2 years of storage

Native Lyophilised Cryoprotected

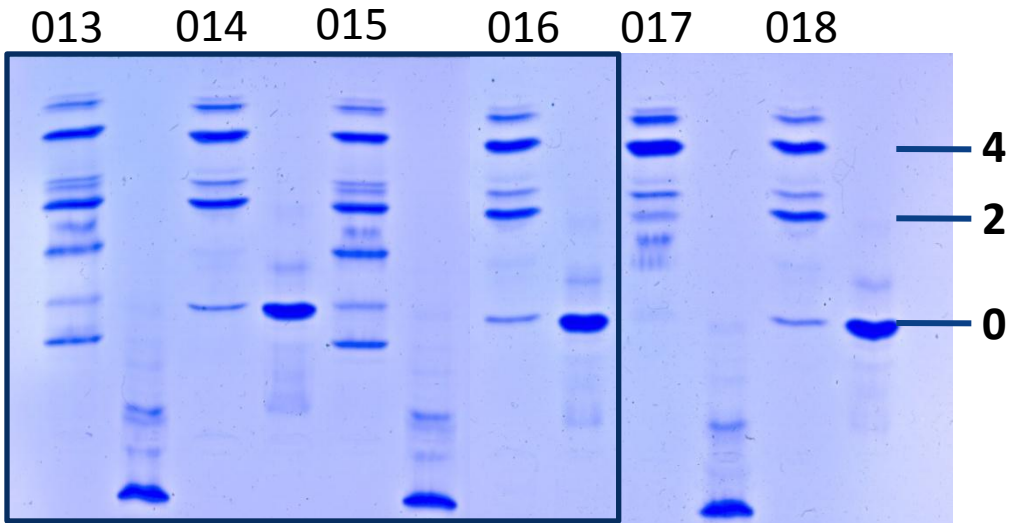
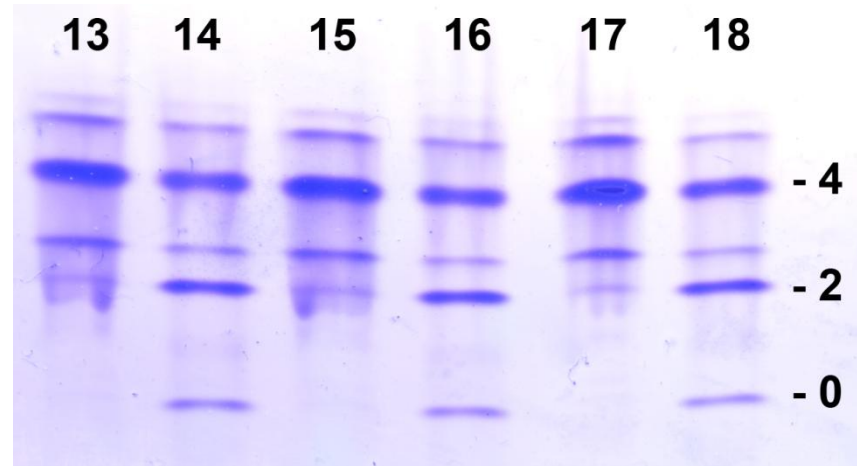


- Store Native samples frozen: 24 months
- Room temperature: Lyophilised (Cryo): 2 months
- Sterile storage!!!
- Other causes of degradation not included!!

# 4th round: room temperature vs lyophilisation (cryo)

## Reproducibility

EUCDG013/15: control  
 EUCDG014/16: CDG-Ia  
 EUCDG017 : control (Cryo)  
 EUCDG018 : CDG-Ia (Cryo)



## Stability



## Overall conclusions

- All centers find TIEF abnormalities
- Exclusion of secondary causes!
- Interpretation of profile and further suggestions!
- Shipment conditions sufficient for ERNDIM  
20  $\mu$ l lyophilised serum with cryoprotectant

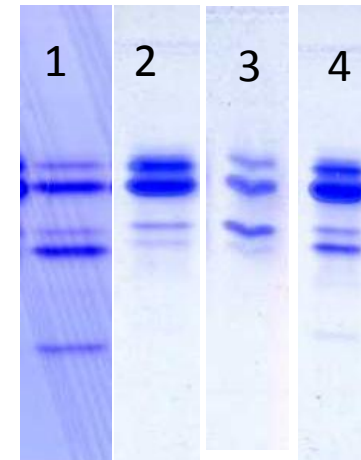
## Results 5th round; 1st ERNDIM pilot

- 46 participants, 10 non-EU
- Methods: Isofocusing (27), CE/HPLC (11), MS, WB
- No interference of the cryoprotectant
- 5 samples resend, no sample degradation
- Volume limited for some centres



# Final results

Sample Code	Clinical information	Patient data	Final diagnosis
ERNDIMCDG001	Protein-losing enteropathy, coagulation problems, epilepsy	F, 3 years	CDG-Ia
ERNDIMCDG002	Mental retardation, increased transaminases	F, 40 years	No known CDG
ERNDIMCDG003	Congenital myopathy, mental retardation	M, 20 years	Transferrin polymorphism
ERNDIMCDG004	Cerebellar ataxia	F, 22 years	CDG-Ia



- Profile: 2 points
- Type I/II: 2 points
- Further suggestions: 2 points

