Experience with an international EQAS for a rare disease: a clinical and analytical scheme for porphyrias

EQALM, Szeged- 2011

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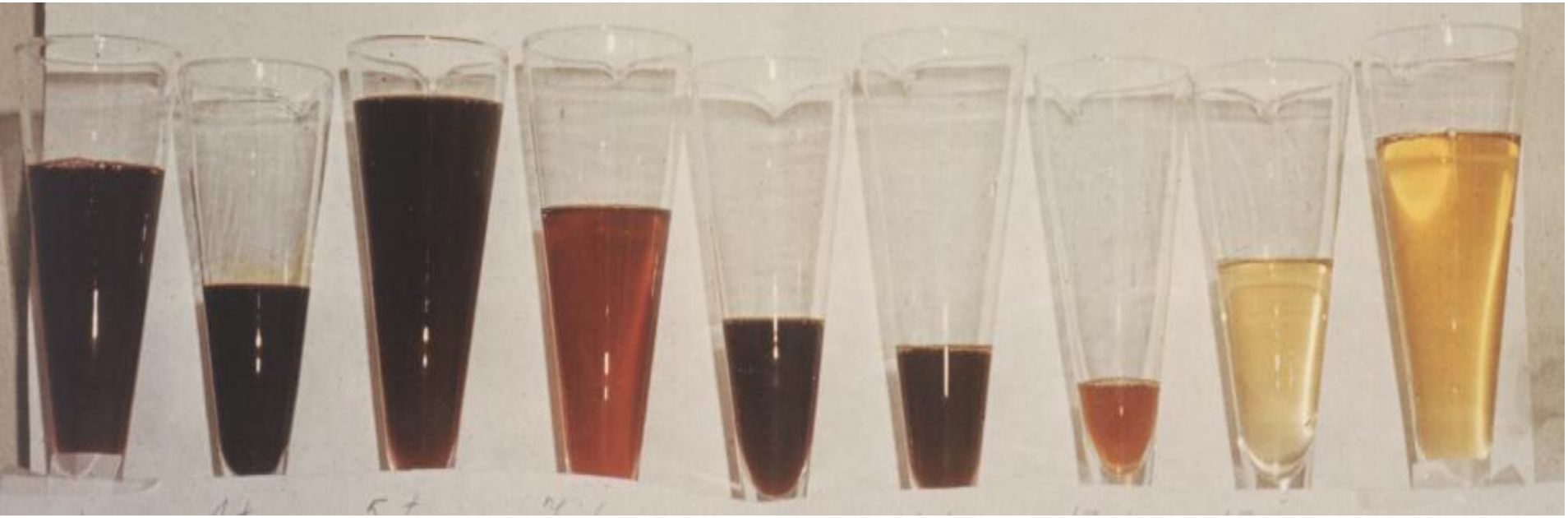
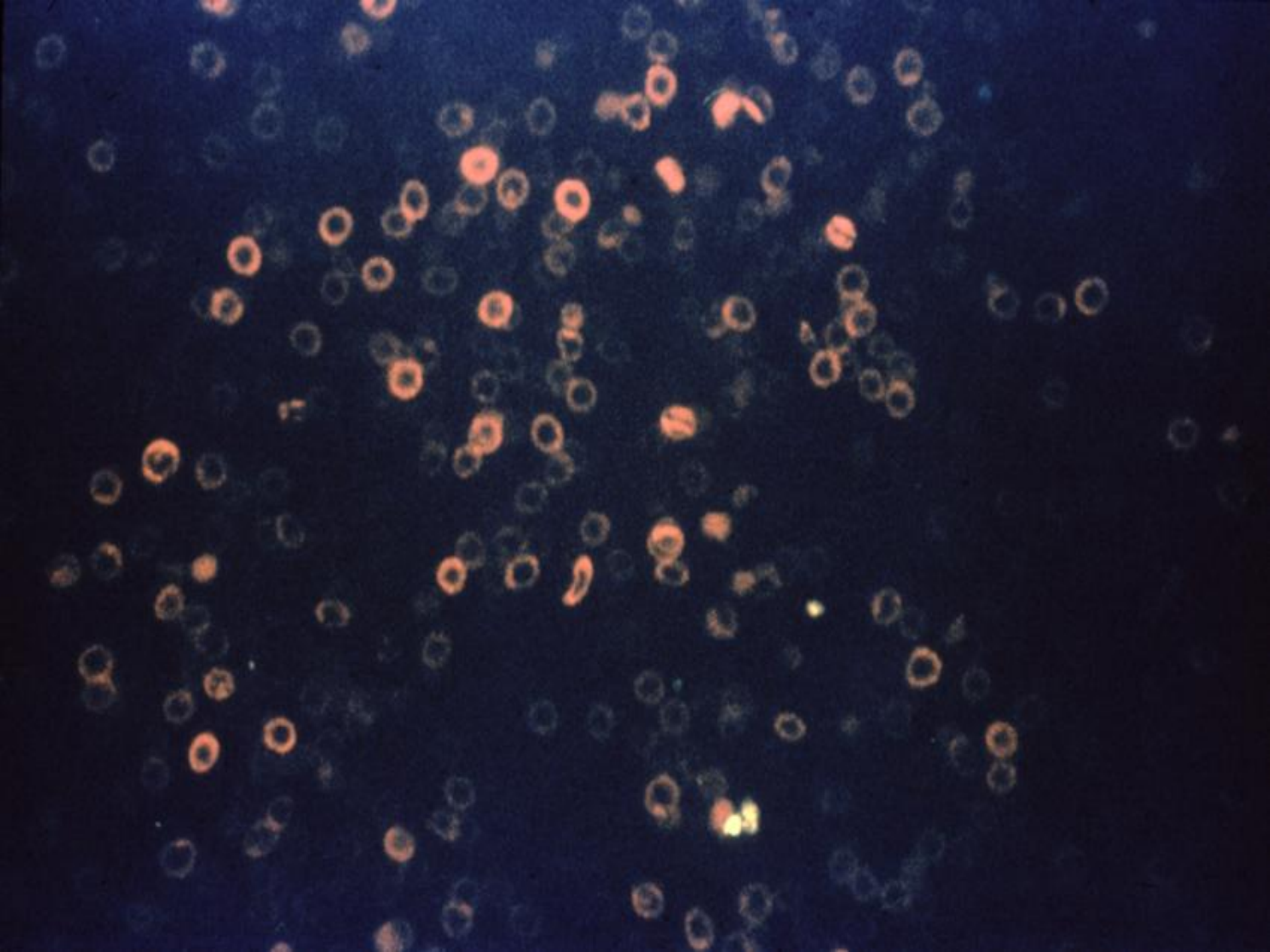
Porphyria

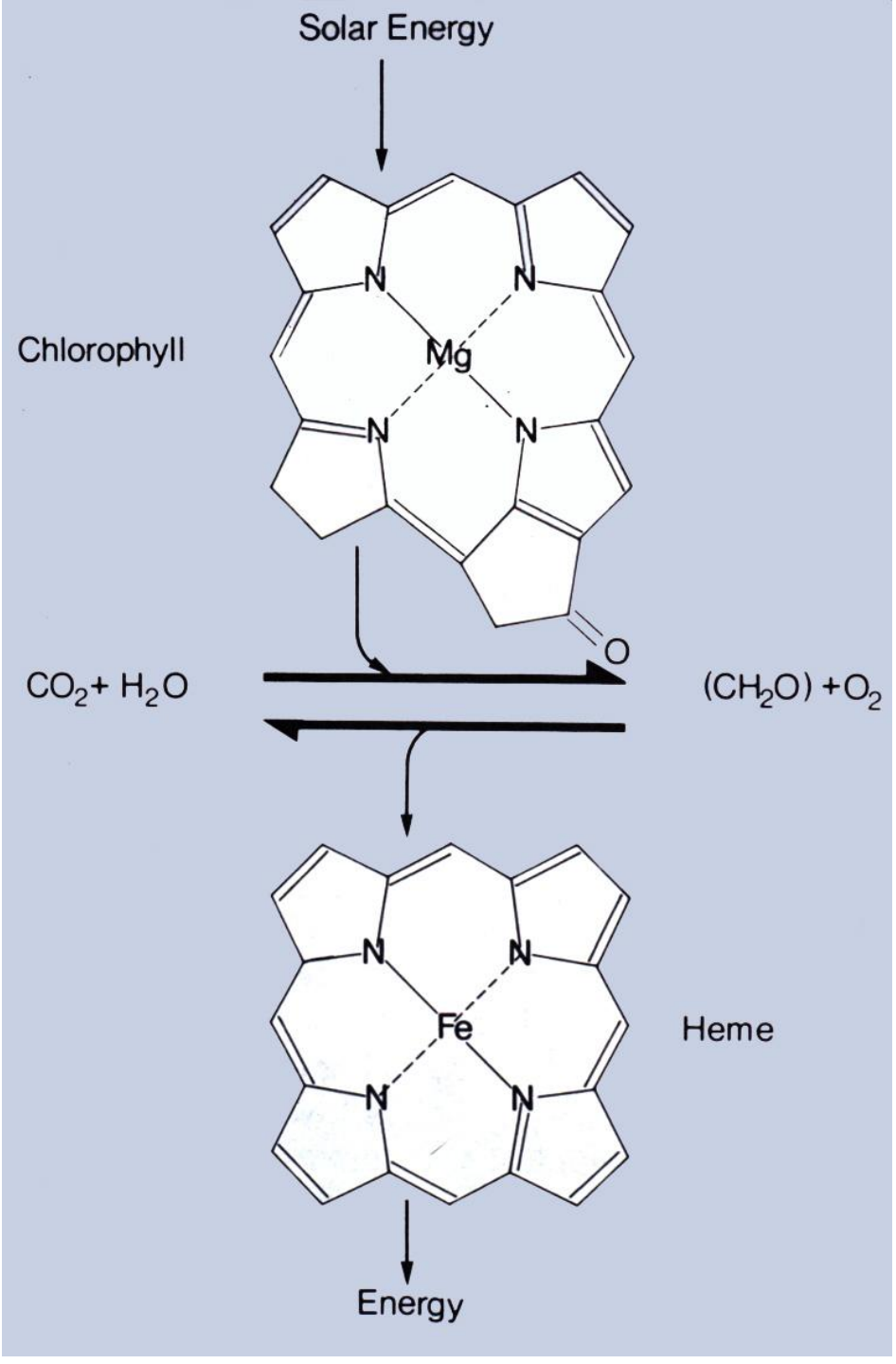


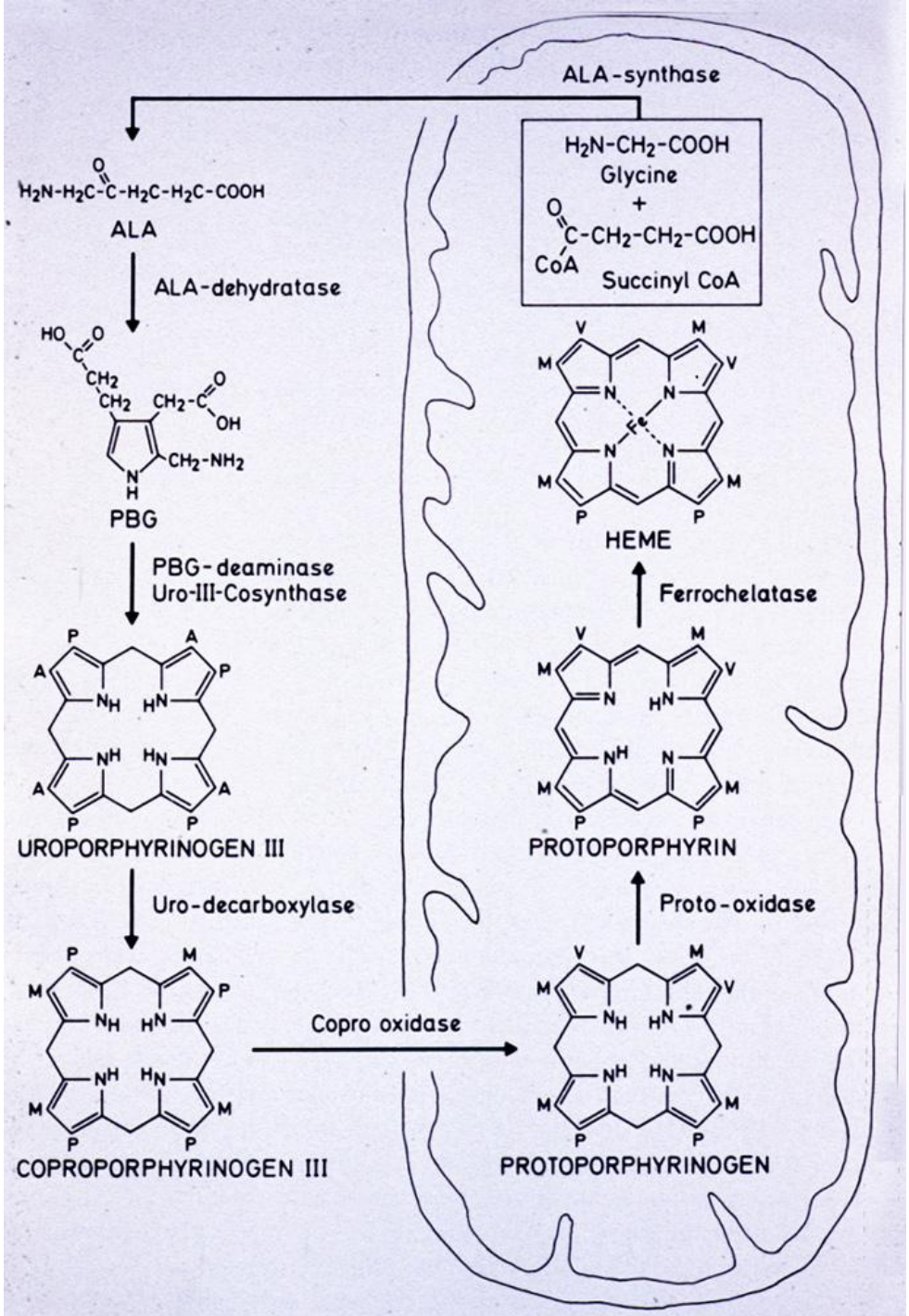
The greek name of the musling (murex brandaris) was porphyria. In ancient time a strong red or dark violet color was extracted from this. In latin the name of the color was purpurus

The diseases are called porphyria since the urine get a

red colour.

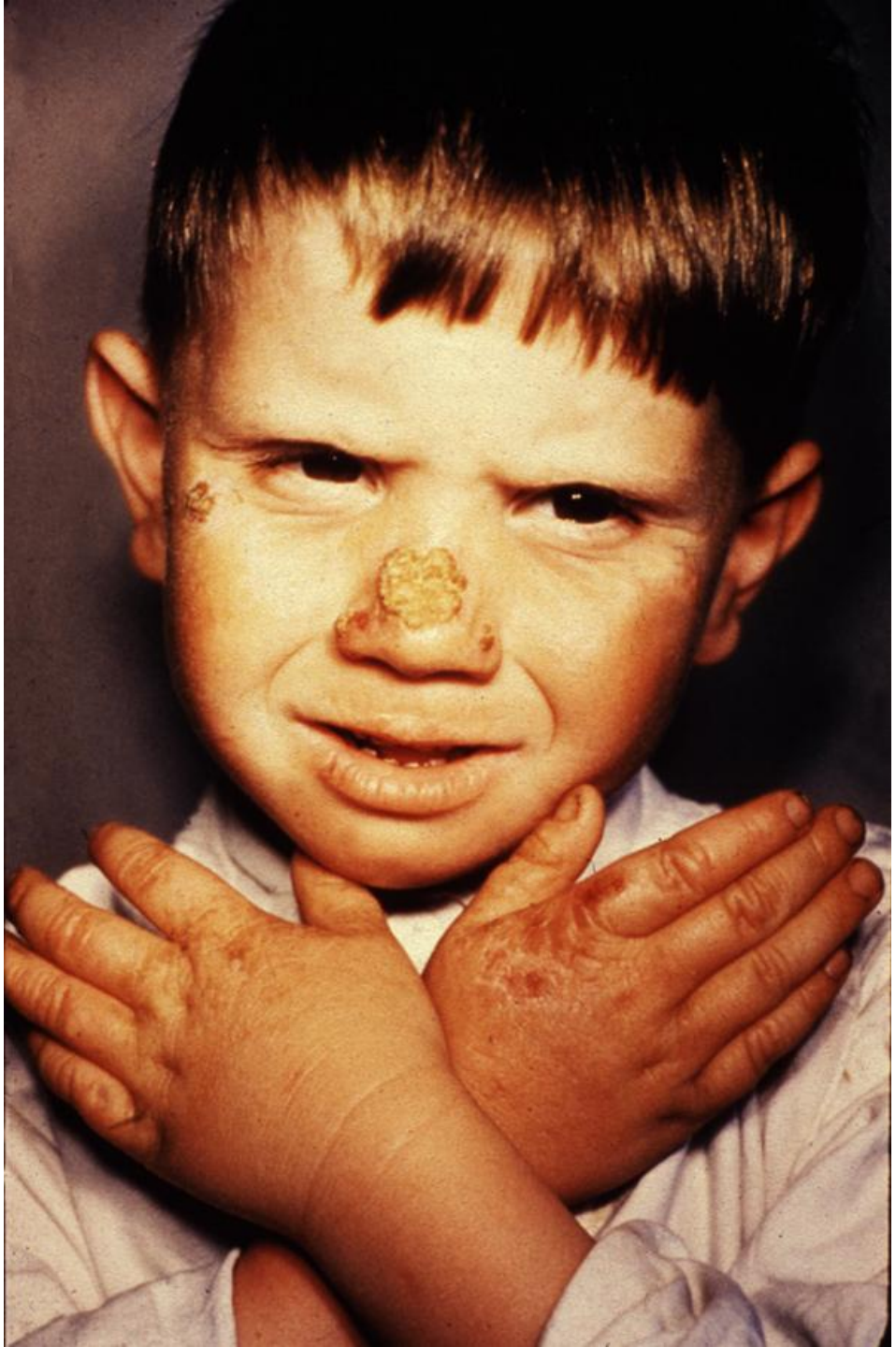



Solar Energy
Chlorophyll
N
N
Mg
N
N
O
$CO_2 + H_2O$
$(CH_2O) + O_2$
N
N
Fe
N
N
Heme
Energy


ALA-synthase
H2N-CH2-COOH
Glycine
+
C-CH2-CH2-COOH
CoA
Succinyl CoA
ALA
ALA-dehydratase
PBG
PBG-deaminase
Uro-III-Cosynthase
UROPORPHYRINOGEN III
Uro-decarboxylase
COPROPORPHYRINOGEN III
Copro oxidase
PROTOPORPHYRINOGEN
Proto-oxidase
PROTOPORPHYRIN
Ferrochelatase
HEME

The different porphyrias - seven different forms -

- Acute symptoms
  - Abdominal pain
  - Paresis
  - Psychiatric
- Photosensititivity
  - Burnt skin
  - Vesicles and bullae on light exposed areas



Porphyrias are rare diseases

- Prevalence of about 100-200 per million
- Difficult diagnosis
- Specialist centres in Europe. A specialist centre should be able to make all the different porphyria diagnosis and to give clinical advice

European EQAS for Porphyrias - for specialist laboratories

In the same scheme:

- Pre-analytical
- Analytical
- Post-analytical

NOKLUS

EQAS for porphyrias
28 specialist laboratories

- Samples from one patient are circulated within 48 hours.
- Case history
- What analysis would have been performed in your laboratory?
- Analytical results
- How was the results reported?
- What diagnosis?

EQAS 1/10

Female born 1962, has experienced three "attacks" of abdominal pain with additional complaints of muscle weakness and nausea, the first of which occurred during her first pregnancy. She has periodically suffered from depression. Her GP sent urine, blood and faecal samples for porphyrin analysis, obtained two months after she had recovered from the last attack.

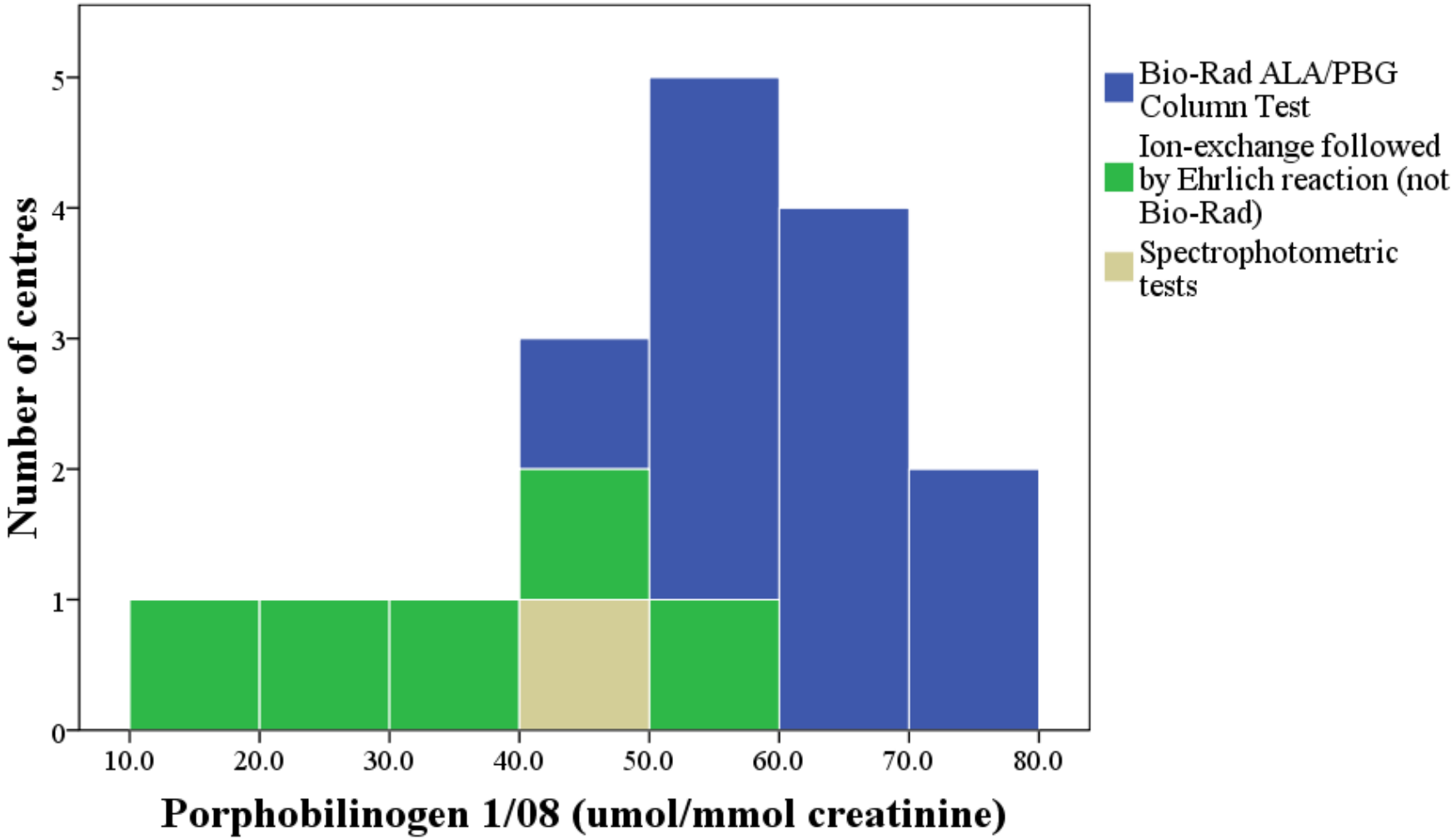
Pre-analytical: Given the case history, what would you analyse?

	Your data #	No of centres that selected this analyte
u-ALA	1	27
u-PBG	1	28
Total u-porphyrin	1	23
u-porphyrin fractionation	(1)	23
Total f-porphyrin	1	20
f-porphyrin fractionation	0	21
Total e-proto-porphyrin	1	5
Zinc proto-porphyrin	0	1
Metal free protoporphyrin	0	2
Total plasma porphyrin	0	5
Plasma scan	1	25
Enzymes: PBG deaminase*	1	15
DNA analysis: HMBS-gene**	1	14

Analytical

Material	Analyte	
Urine	δ-aminolevulinic acid (ALA)	
	Porphobilinogen (PBG)	
	Qualitative/semiquantitative PBG[a]	
	Total porphyrins	
	Porphyrin fractions	
	Uroporphyrin	Total, *isomers I and III*
	Heptaporphyrin	
	Hexaporphyrin	
	Pentaporphyrin	
	Coproporphyrin	Total, *isomers I and III*
Feces	*Percentage dry weight*	
	Total porphyrins	
	Porphyrin fractions	
	Uroporphyrin	*Total, isomers I and III*
	Heptaporphyrin	
	Hexaporphyrin	
	Pentaporphyrin	
	Coproporphyrin	Total, isomers I and III
	Isocoproporphyrin	
	Protoporphyrin	
	Other dicarboxylated porphyrins	*Deutero- and mesoporphyrins*
Whole blood	Erythrocyte protoporphyrin	
	Erythrocyte protoporphyrin fractions	
	Zinc protoporphyrin	
	Free protoporphyrin	
	Porphobilinogen deaminase[b]	
	Uroporphyrinogen decarboxylase[b]	
Plasma	*Total porphyrins*[c]	
	Plasma fluorescence scanning	Wavelengths for excitation and emission peaks

Analytical:
Example Porphobilinogen (PBG)



Can the variation in results be explained by different reference limits / upper cut offs?

Normalization by dividing the result on the upper reference limit

- If your result is PBG=10mmol/creatinine
- Upper reference limit/cut off = 1.2mmol/creatinine
- “Normalized” result would be 10/1.2=8

RATIO BETWEEN MEASURED VALUE AND REPORTED UPPER REFERENCE LIMIT

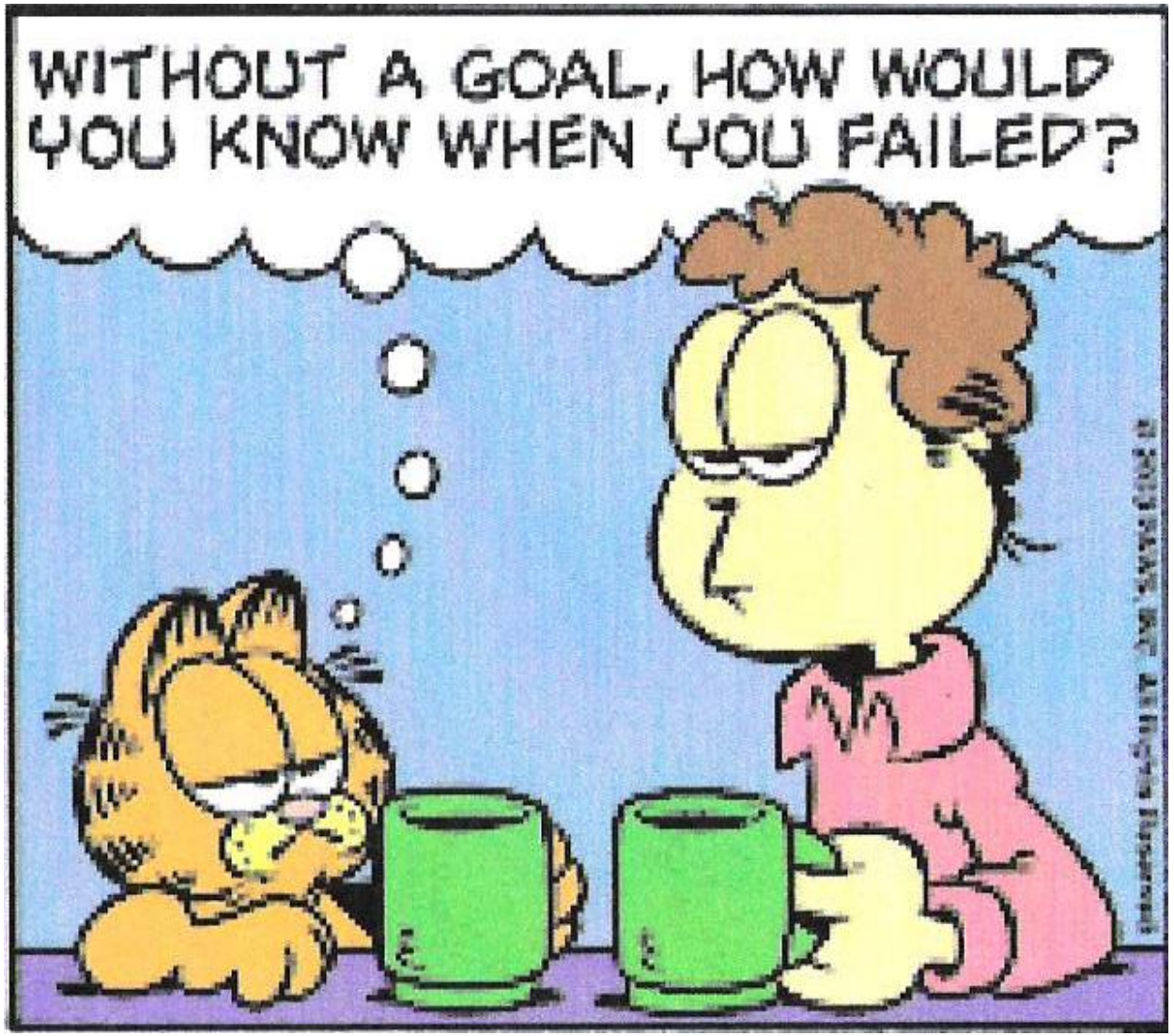
	Your ratio #	Median	Range	Mean
u-ALA	1.9	1.5	0.7 - 4.9	1.7
u-PBG	6.9	4.2	0.3 - 11.8	5.1
Total u-porphyrin	1.4	1.3	0.6 - 2.7	1.5
Total f-porphyrin	0.6	0.4	0.0 - 1.1	0.4
Total e-protoporphyrin	1.2	0.6	0.1 - 1.5	0.7
Total plasma porphyrin	0.6	0.5	0.0 - 1.1	0.4

“Normalization” did not decrease the inter-laboratory variation and can therefore not explain the variation seen

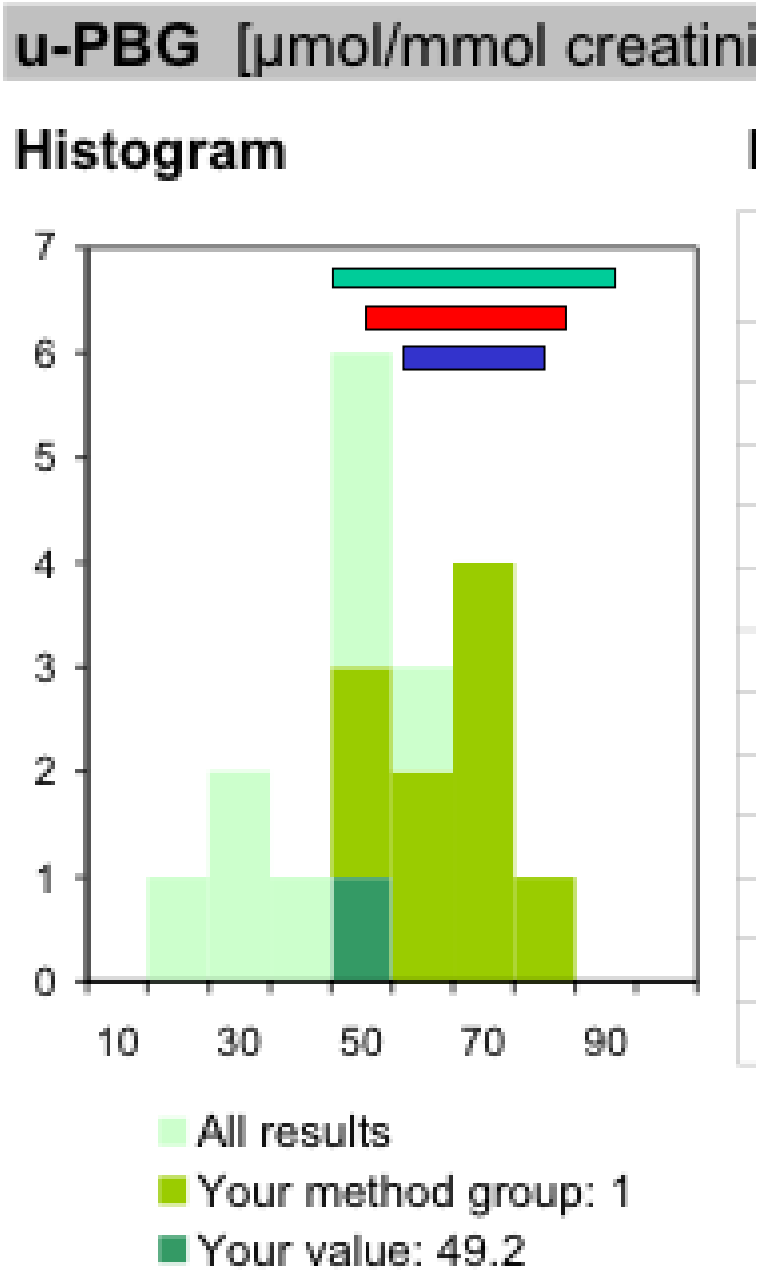
Analytical quality specifications

Courtesy: Per Hyltoft Petersen

Per Hyltoft Petersen



Per Hyltoft Petersen



QS for PBG

Minimum = ± 46%

Desirable = ± 31%

Optimum = ± 15%

Aarand et al, Clin Chem 2006;2:650-6

Fractions of participants (n=23) within *desirable* quality specifications

	QS	AIP	EPP	PCT	VP	AIP
U-ALA	20 %	83	50	70	90	86
U-PBG	30 %	76	37	56	75	45
U-tot-porf	50 %	100	68	86	95	100
F-tot-porf	50 %	47	67	70	71	62
E-protoporphyrin	30 %	64	47	25	74	53
P-tot-porf	50 %		25	63	43	42

Units

- Mg, mmol, ug, umol
- Per litre, per 24 hours, per mmol/creatinine

Can everyone report in same units

Results given in grams were transformed to moles using the factors given in the table below. Total porphyrin values were cal converting each porphyrin from grams to moles and then added up to a total value.

Component	Molcular weight [g/mol]	Factor of conversion		
		multiply by factor to convert	from	to
Creatinine	113.12	8.840		mmol
ALA	131.13	7.626		µmol
PBG	226.23	4.420	mg	µmol
Uroporphyrin I/III	830.76	1	µg	nmol
Heptacarboxylporphyrin I	786.75		µg	nmol
Hexacarboxylporphyrin I	742.74		µg	nmol
Pentacarboxylporphyrin I	698.73		µg	nmol
Coproporphyrin I/III	654.72	1.527	µg	nmol
Protoporphyrin IX	562.66	1.777	µg	nmol
Zinc protoporphyrin	626	1.597	µg	nmol
Isocoproporphyrin		1.527	µg	nmol
Deuteroporfyrin IX		1.959	µg	nmol
Mesoporfyrin IX		1.765	µg	nmol
If only total porphyrins ... following factors of conversion from gram to moles are used:				
Total u-porphyrins		1.356	µg	nmol
Total f-porphyrins		1.540	µg	nmol
Total e-protoporphyrins		1.687	µg	nmol
Total plasma porphyrins		1.652	µg	nmol

This has improved considerably

Post-analytical

Gathering all the written reports on this case.

- Was the diagnosis correct?
- Was the correct information/interpretation given to the physician?

Diagnostic Concluson

- 19 out of 28 participating laboratories would have made the correct diagnosis of Acute intermittent porphyria.
- Five laboratories stated that some form of acute porphyria was a possible diagnosis, but would have asked for a new sample.
- Four laboratories ruled out porphyria or gave no suggestion of a diagnosis

Reporting
- what should it include -

1) Laboratory name
2) Laboratory contact details
3) Date of report
4) Name of referring person
5) Patient name/date of birth
6) Date of sampling
7) Date of arrival
8) Material tested
9) Analysis performed
10) Results given
11) Units and reference intervals
12) Interpretation
13) Advice on further testing if approp
14) Signature of lab

Reporting results

80% of labs scored 13 or more (out of 14)

85% of labs provided an interpretation of the results

50% included clinical advice

Quality of interpretation (except for diagnosis) and clinical advice have not been studied

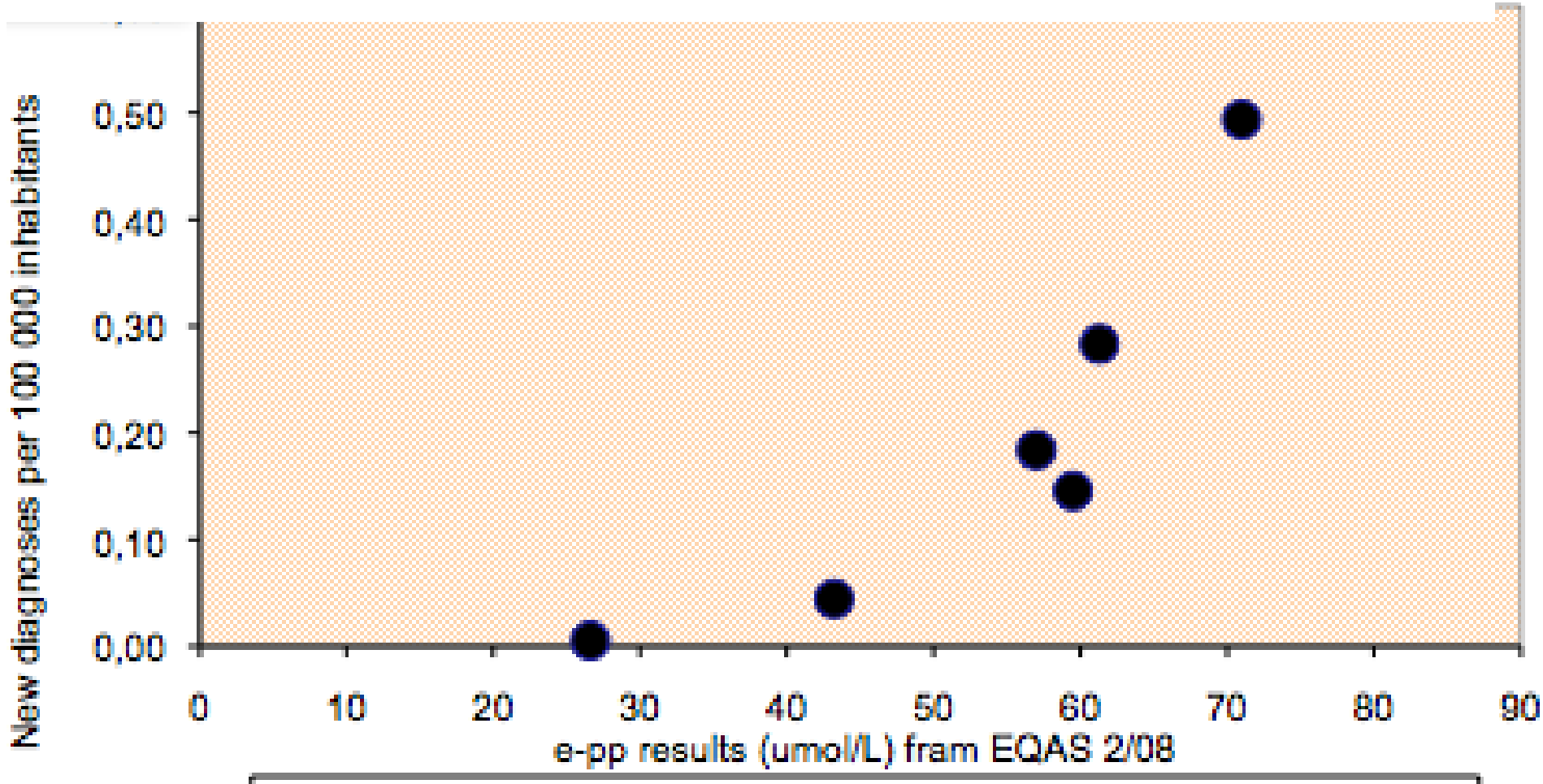
Post - post

- How are the results and comments interpreted by the person(s) who gets the report and what actions are taken?

We don’t know

(but be careful to say in your report something like “porphyria can not be completely excluded”)

E-pp vs diagnosis of erythropoietic protoporphyria in centres covering a whole country



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000–000 (2011)

European Specialist Porphyria Laboratories: Diagnostic Strategies, Analytical Quality, Clinical Interpretation, and Reporting as Assessed by an External Quality Assurance Program

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thank you
epi
Epnet
napos

Evidence-Based Medicine and Test Utilization

EUROPEAN SPECIALIST PORPHYRIA LABORATORIES: DIAGNOSTIC STRATEGIES, ANALYTICAL QUALITY, CLINICAL INTERPRETATION AND REPORTING AS ASSESSED BY AN EXTERNAL QUALITY ASSURANCE PROGRAMME

AASNE K. AARSAND[a,1], JØRILD H. VILLANGER[1], EGIL STØLE[1], JEAN-CHARLES DEYBACH[2], JOANNE MARSDEN[3], JORDI TO-FIGUERAS[4], MIKE BADMINTON[5], GEORGE H. ELDER[5], AND SVERRE SANDBERG[1,6]

Clin Chem 2011, in press

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NORWAY

Participant: 18
Survey: 2/10
Sent: 15.11.2010

Bergen, 26.02.2011

Report: Porphyria External Quality Assessment Scheme 2/10 (sent 15.11.2010)

In this EQAS distribution, a patient with porphyria cutanea tarda donated quality control material. The material was sent to 28 laboratories, all of which responded.

Materials

The laboratories received 6 ml urine, 5 g faeces, 3 x 1 ml plasma and 3 ml whole blood sent on ice and were instructed to freeze all samples upon arrival. The samples were sent by express service and delivered within the next day to all the laboratories except two, in which they arrived one and two days later.

Pre-analytical –analytical – post-analytical

The time glass model of laboratory errors

60% Pre pre

pre

10% Analytical

30% Post

Post post

Case history

- A 5 years old boy was referred to a paediatric clinic after having presented to the family physician on several occasions, after crying spells of unknown cause. According to the boys' parents the crying spells always occurred when outdoors. The family doctor referred the patient to a paediatric clinic. After extensive investigations over a 6 months period, blood, urine and faeces samples were sent for porphyrin analysis.

Anaytical performance
-interlaboratory CV - Total and by Method

Const	**CV_T**	**CV_M**
U-ALA	17-28	08-28
U-PBG	30-64	16-60
U-porf	26-60	16-38
F-porf	47-60	40-70
E-pp	30-66	33-66

The Scandinavian Journal of
Clinical & Laboratory Investigation

CONSENSUS STATEMENT*

The main outcome of the Conference was agreement that the following hierarchy of models should be applied to set analytical quality specifications.

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings
2. Evaluation of the effect of analytical performance on clinical decisions in general:
   a. Data based on components of biological variation
   b. Data based on analysis of clinicians' opinions

SJCLAY 59 (7) 475–586 (1999) NO ISSN 0036-5513

Consensus agreement

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*Department of Clinical Biochemistry, Our Lady's Hospital for Sick Children, Dublin, Ireland; †Directorate of Biochemical Medicine, Ninewells Hospital and Medical School, Dundee, Scotland; ‡Department of Clinical Chemistry, Odense University Hospital, Odense, Denmark; and §Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden

The Editors of this special issue of the *Scandinavian Journal of Clinical and Laboratory Investigation* and the Organising Committee of the Conference: *Strategies to set Global Quality Specifications in Laboratory Medicine*, Stockholm, 24–26 April 1999, are pleased to report that this recent Conference was most successful. Over 100 participants from 27 countries actively contributed to the discussions on the 22 formal presentations. Our primary aim in organizing the Conference was to provide a vehicle for reaching consensus on the setting of global quality specifications in laboratory medicine. This objective was achieved and lively constructive debate after the presentations were complete led to agreement on the principles laid down in the following Consensus Statement.

CONSENSUS STATEMENT*

The main outcome of the Conference was agreement that the following hierarchy of models should be applied to set analytical quality specifications.

1. Evaluation of the effect of analytical performance on clinical outcomes in specific clinical settings
2. Evaluation of the effect of analytical performance on clinical decisions in general:
   a. Data based on components of biological variation
   b. Data based on analysis of clinicians' opinions
3. Published professional recommendations
   a. From national and international expert bodies
   b. From expert local groups or individuals
4. Performance goals set by
   a. Regulatory bodies
   b. Organizers of External Quality Assessment (EQA) schemes
5. Goals based on the current state of the art
   a. As demonstrated by data from EQA or Proficiency Testing scheme
   b. As found in current publications on methodology.

Where available, and when appropriate for the intended purpose, models higher in the hierarchy are to be preferred to those at lower levels. The concept of such a hierarchy is described in a recent Editorial in Clinical Chemistry in which the relative merits of the above models are discussed (Clin Chem 1999; 45: 321–3). This hierarchy has also been proposed by the ISO/TC 212/WG 3 subgroup on "Analytical Performance Goals Based on Medical Needs" as the basis for the ongoing revision of ISO/CD 15196. The following matters were also discussed and agreed.

- The above hierarchy includes currently available models; however, new useful concepts will undoubtedly evolve. Implementation of any of the models should use well-defined and described procedures.
- To facilitate the future debate on the setting of analytical quality specifications, there is a need for agreement on concepts, definitions and terms.
- There is a need for continuous improvement in the exchange of information on quality issues between clinical laboratory professionals and the diagnostics industry, and between clinical laboratory professionals and the users of the laboratory service.

IFCC, IUPAC and WHO kindly sponsored the Conference but it must be noted that the Consensus Statement reflects the views of the presenters and registrants who participated in the Conference and does not necessarily represent those of the sponsoring bodies.

Kenny et al. SJCLI 1999; 59:585

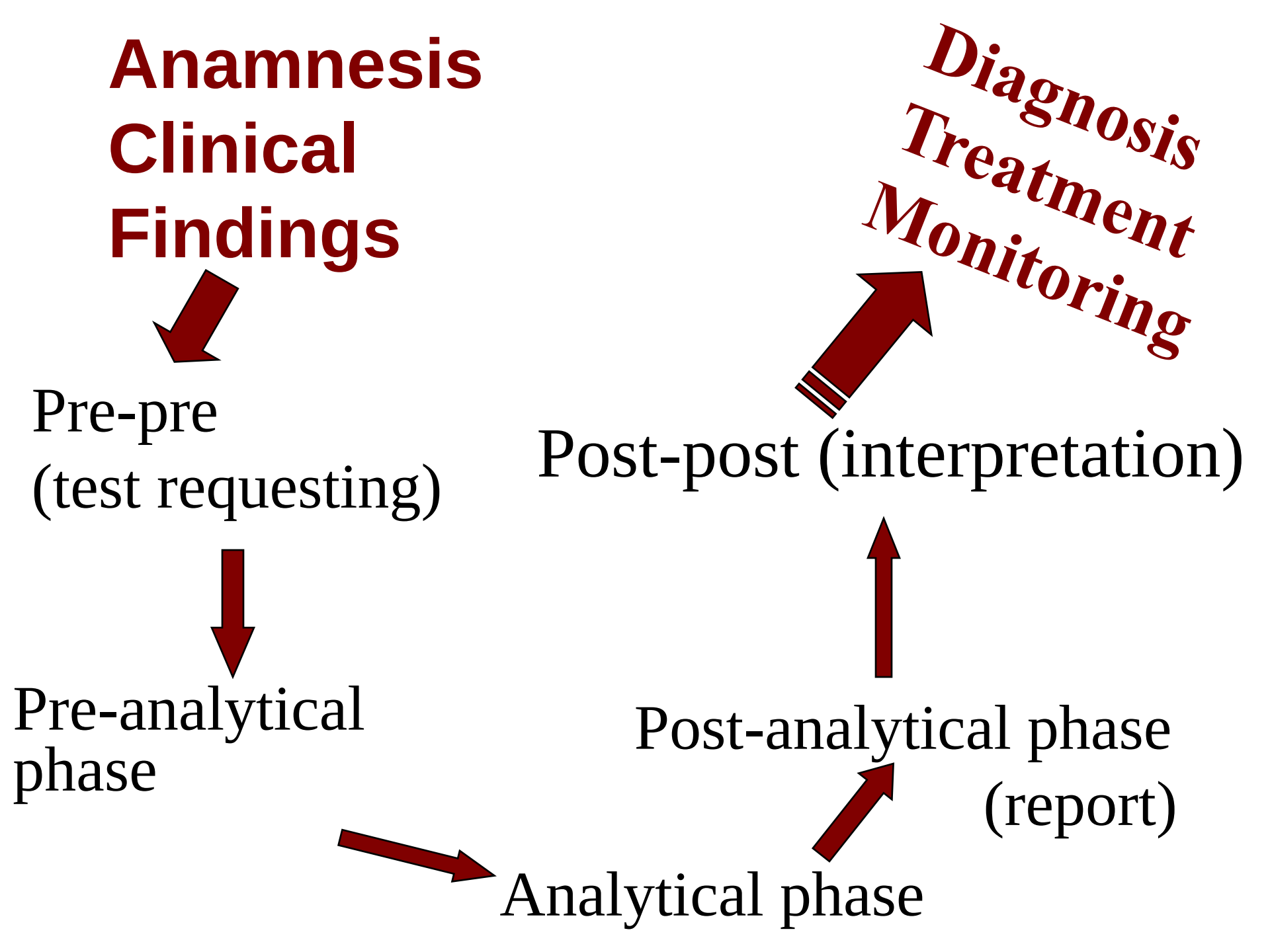
Total allowable error

$$TE_a = 0.375 * \sqrt{CV_{ws}^2 + CV_{bs}^2} + 1.65 * 0.75 * CV_{ws}$$

0.125 = optimal
0.250 = desirable
0.375 = minimum

0.25 = optimal
0.50 = desirable
0.75 = minimum

Libeer et al. Eur J Clin Chem Clin Biochem. 1996;34:665-78.


Anamnesis
Clinical
Findings
Pre-pre
(test requesting)
Pre-analytical
phase
Analytical phase
Post-analytical phase
(report)
Post-post (interpretation)
Diagnosis
Treatment
Monitoring

Medical laboratories – Particular requirements for quality and competence (ISO 15189:2003):

5.6.4.
External quality assessment programmes should, as far as possible, provide clinically relevant challenges that mimic patient samples and have the effect of checking the entire examination process, including pre- and post-examination procedures