Traceability as a mean to obtain worldwide useful reference intervals in clinical enzymology

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Summary

- Traceability in clinical enzymology
- Present situation of reference intervals in clinical enzymology
- The IFCC project for the definition of common reference intervals
  - Theory
  - Some preliminary data

Traceability of the catalytic concentration of enzymes

- For enzyme measurements, the definition of the derived coherent SI unit "mole per second cubic metre", also called "katal per cubic metre", is the top of the hierarchy followed by a primary reference measurement procedure to which lower level measurement procedures, calibrators, and control materials should be traced whenever possible.

Traceability of the catalytic concentration of enzymes

- Results of catalytic concentration measurements are only comparable if the enzyme activities are measured under the same conditions. Therefore, an enzyme measurand cannot be described only by kind of quantity, name of enzyme and of system, but requires also the specified measurement procedure and especially the indicator component of the measured reaction, e.g. 'creatine kinase measured by the conversion rate of NADH in the IFCC reference measurement procedure'.

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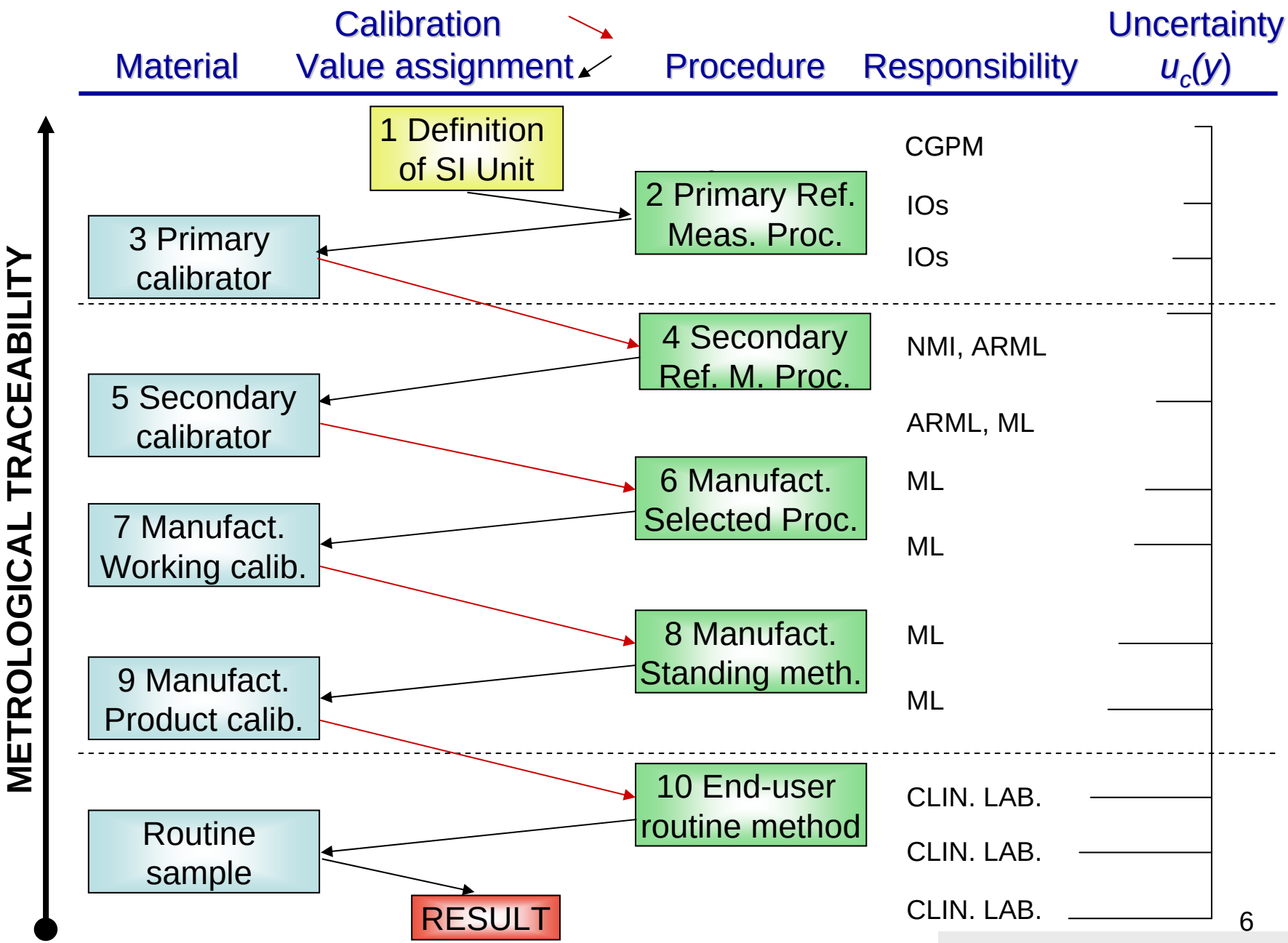
ISO 18153

***In vitro* diagnostic medical devices — Measurement of quantities in samples of biological origin — Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials**



EN ISO 18153: 2003, Fig 1

Elements of the Reference measurement system

- Reference methods
- Reference materials
- Network(s) of reference laboratories

Existing Reference Systems for enzymes

	Reference Method	Ref. Material
AST	Clin Chem Lab Med 2002;40:725-33	IRMM/IFCC 457
ALT	Clin Chem Lab Med 2002;40:718-24	IRMM/IFCC 454
γGT	Clin Chem Lab Med 2002;40:734-38	IRMM/IFCC 452
LDH	Clin Chem Lab Med 2002;40:643-48	IRMM/IFCC 453
CK	Clin Chem Lab Med 2002;40:635-42	IRMM/IFCC 455
AMY	Clin Chem Lab Med 2006;44:1146-55	IRMM/IFCC 456
ALP	In preparation	In preparation

Enzyme reference laboratories

C1RMS1	**CIRME** (Centro Interdipartimentale di Ricerca Sulla Riferibilita' Metrologica in Medicina di Laboratorio - Universita' di Milano), Italy Contact person: Prof. Mauro Panteghini mauro.panteghini@unimi.it
C1RMS146	**DGKL** (Reference Institut of the German Society of Clinical Chemistry and Laboratory Medicine), Germany Contact person: Prof. Dr. G. Schumann schumann.gerhard@mh-hannover.de
C1RMS107	**Instand** e.V., Germany Contact person: Prof. Dr. H. Reinauer Email: reinauer@instand-ev.de
C1RMS61	**KCHL** HagaZiekenhuis (Klinisch Chemisch en Hematologisch Laboratorium HagaZiekenhuis), The Netherlands Contact person: Dr. P.F.H. Franck p.franck@hagaziekenhuis.nl
C1RMS54	**Laboraf** (Diagnostica e Ricerca San Raffaele S.p.A.), Italy Contact person: Dr Ferruccio Ceriotti ceriotti.ferruccio@hsr.it

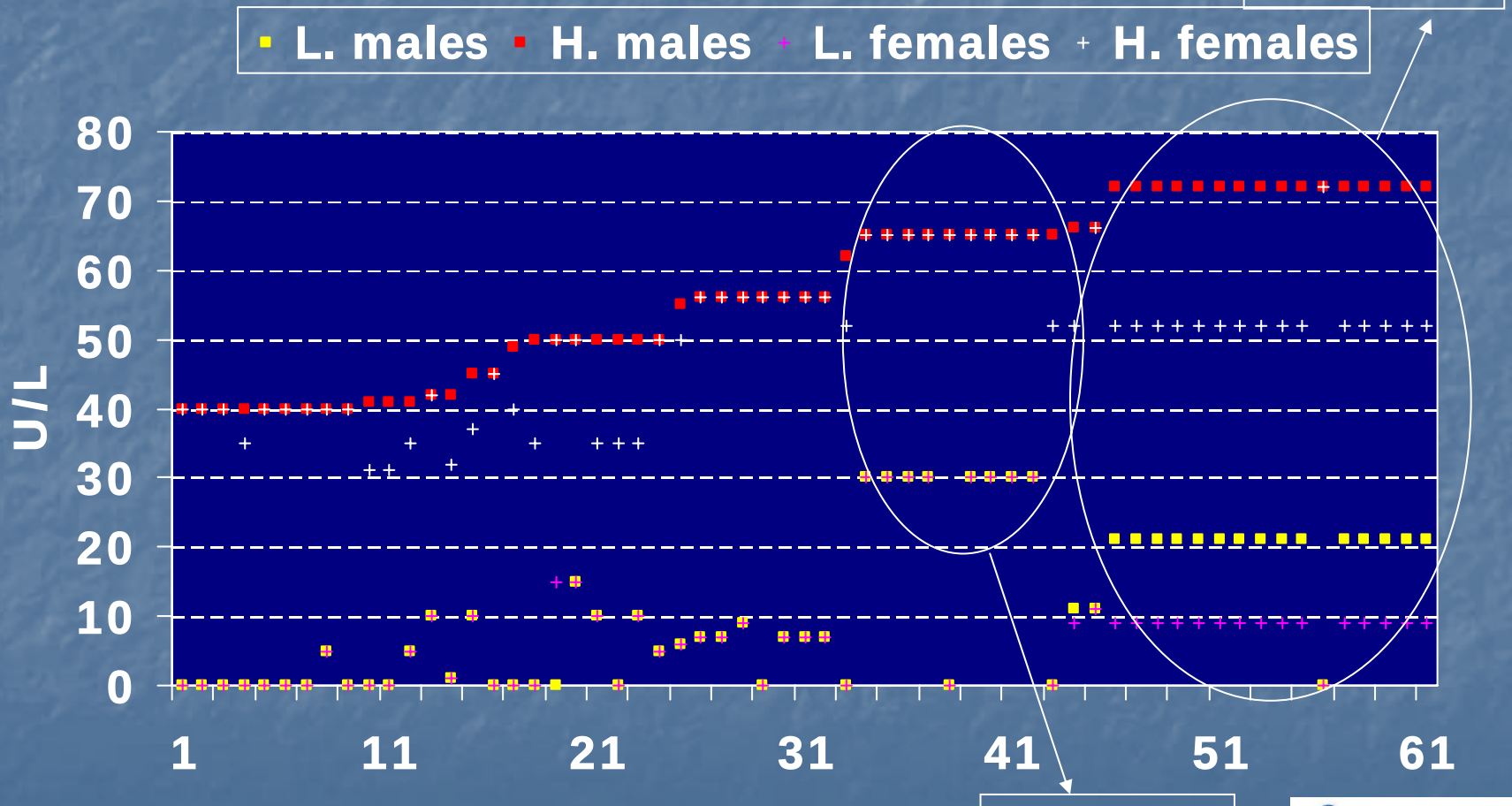
Key for traceability in clinical enzymology

- Specific routine methods
- Commutable calibrators

Improvement in comparability among laboratories

ALT (IFCC method)

Reference intervals (61 labs)



Consequences of R.I. not in line with the adopted method

- Wrong patients' classification

Ferard et al. CCLM 2005;43:549

Table 2 Inter-laboratory variation of patient results and of results expressed in multiples of the upper reference limit for routine procedures.

Laboratory	Mean, U/L (range) **AST**	URL[a]		Multiples of URL[a]
		Female	Male	
Laboratory 1[b]	40.7 ± 27.9 (12–119)	31	31	0.94
Laboratory 2	51.6 ± 36.8 (15–159)	31	31	1.18
Laboratory 3[b]	42.6 ± 28.6 (15–122)	35	40	0.64
Laboratory 4	43.2 ± 34.8 (18–143)	35	40	0.75
Laboratory 5	47.0 ± 33.5 (12–143)	31	31	0.97
Laboratory 6	49.4 ± 33.8 (15–146)	31	31	1.02
Laboratory 7	50.3 ± 35.7 (20–149)	30	40	1.60
Laboratory 8	46.0 ± 34.9 (12–143)	31	35	1.08
Laboratory 9	50.2 ± 33.5 (16–147)	32	35	1.03
Laboratory 10	50.3 ± 35.7 (16–148)	35	45	0.94
Laboratory 11	52.0 ± 35.6 (19–158)	32	35	0.89
Laboratory 12	55.8 ± 37.2 (18–166)	27	32	1.26
Laboratory 13	54.6 ± 36.9 (17–162)	27	32	1.27
Mean, U/L	46.7			0.99
Number of procedures	13			13
SD, U/L	4.60			0.18
CV, %	9.85			18.5

[a] URL: upper limit of reference values used by the laboratory. [b] Performed with reagents without pyridoxal-5′-phosphate. Results were significantly lower ($p < 0.0001$) when compared to those obtained in laboratories 2 and 4.

Reasons for different R.I.

- The requirement that each laboratory defines its own R.I.: method-dependent results
- Changes in methodology not accompanied by R.I. modification
- Adoption of R.I. proposed by the manufactures (sources often not declared)
- Adoption of literature data (without any critical appraisal)

The definition of

Common Reference Intervals

could be the solution to overcome these problems

Rationale for common reference intervals

- With a good level of standardization only population differences justify different R.I.
- The use of the "old" reference intervals with the "new" methods can impair the clinical interpretation of the results
- The absence of reliable reference intervals for the newly standardized methods hampers their adoption

Multicenter studies, possible approaches

1. Each center, properly standardized, analyzes its own fresh samples
2. The samples are collected in the different centers, frozen and shipped to a central laboratory where all the analyses are performed

Organization of Multicenter Reference Intervals Studies

Approach 1:

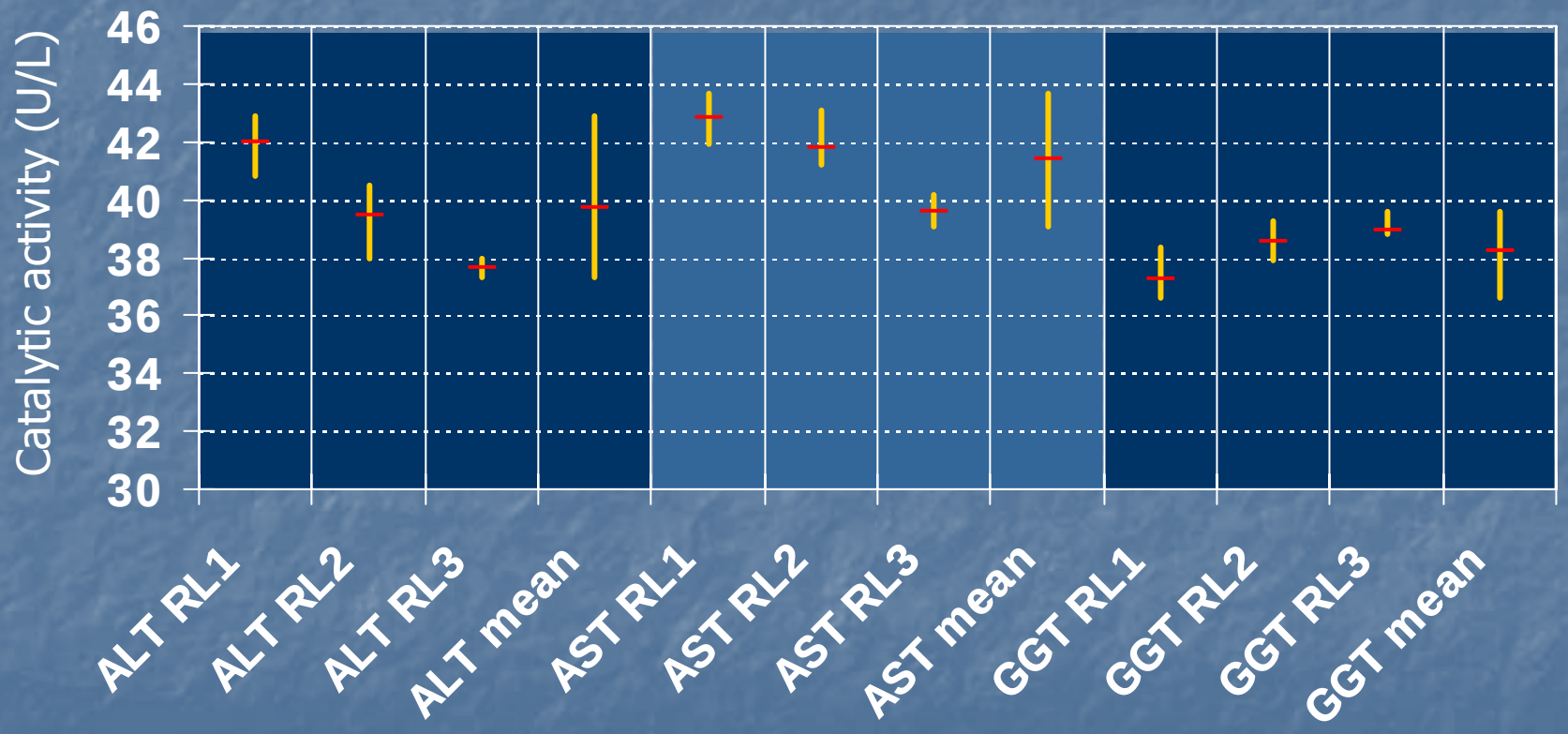
- only clinical laboratories using methods for which the traceability to primary reference methods and / or material is clearly stated by the manufactures ;
- goals for maximal allowable interlaboratory variability i.e. maximum allowable bias must be stated a priori and verified in a preliminary experiment;
- the analytical quality during the collection of reference values must be monitored carefully by an ad hoc quality control program.

Multicenter reference intervals studies

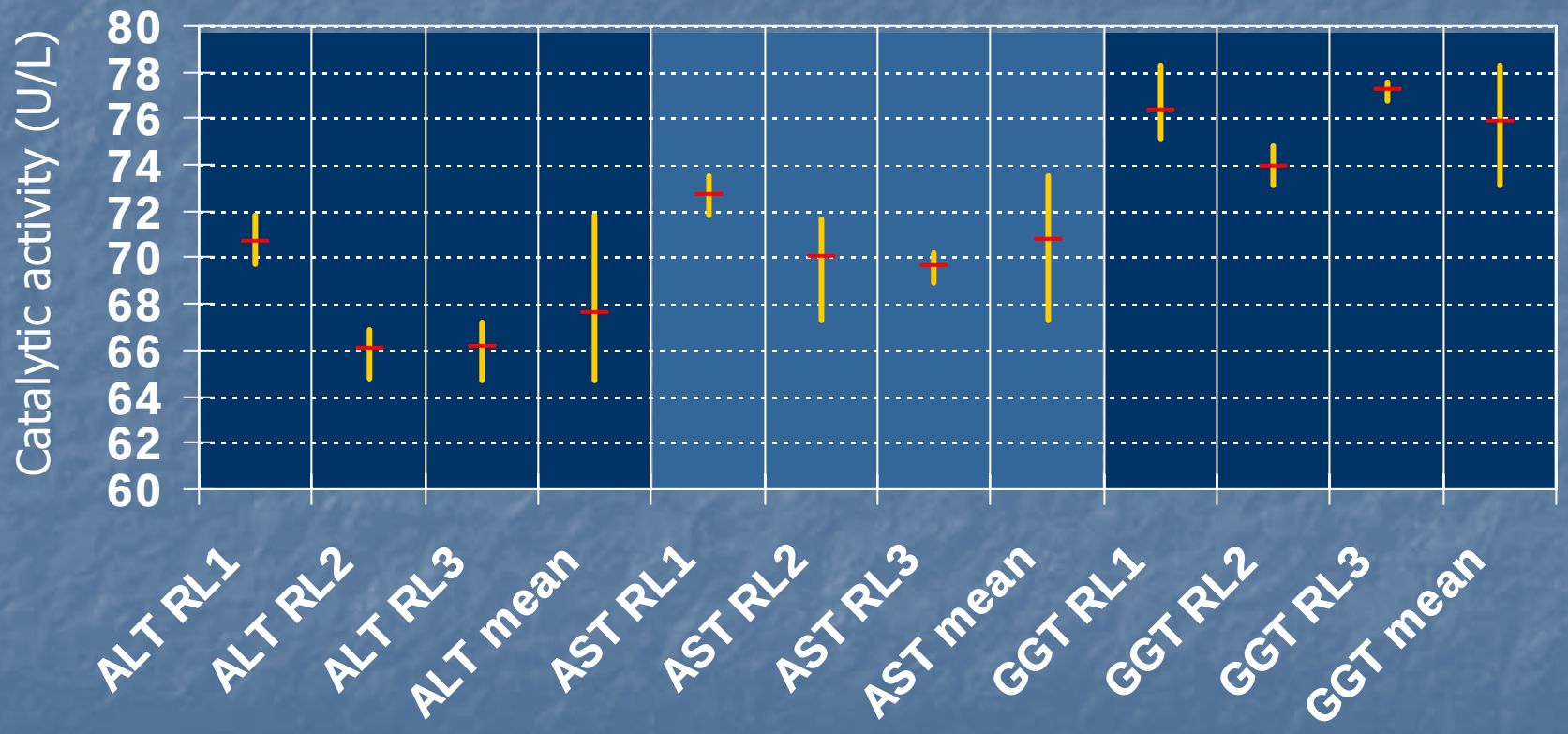
1. Preparation of the material for trueness – traceability control
   - serum pools (2 or 3 levels) at borderline concentration, minimally processed (filtering, freezing at -80°C);
   - value assignment by a reference method possibly by a network of at least two reference laboratories according to a defined protocol (three batches, two or three replicate measurements per batch);

IFCC experiment for AST, ALT and GGT reference values

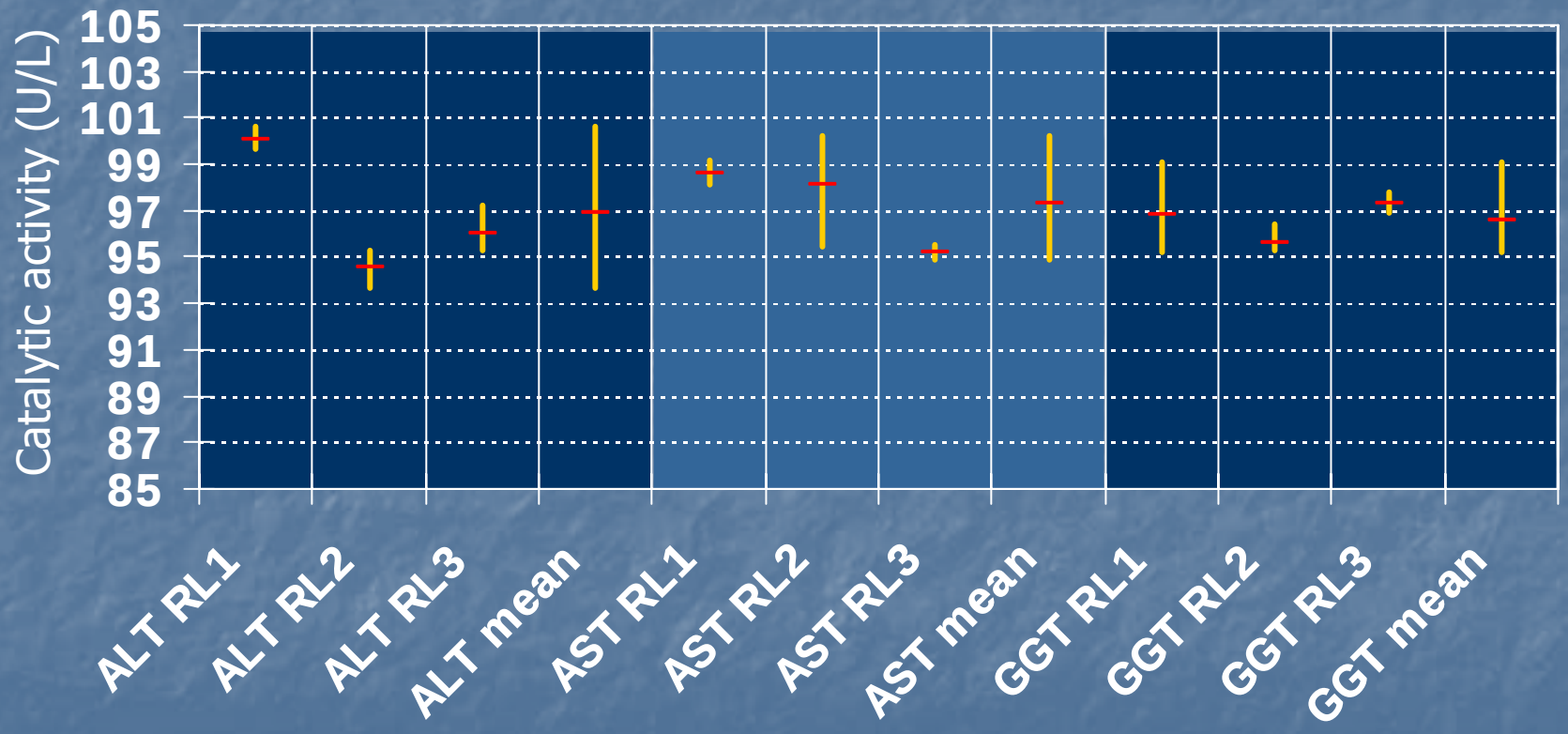
Trueness material Level 1: value assignment



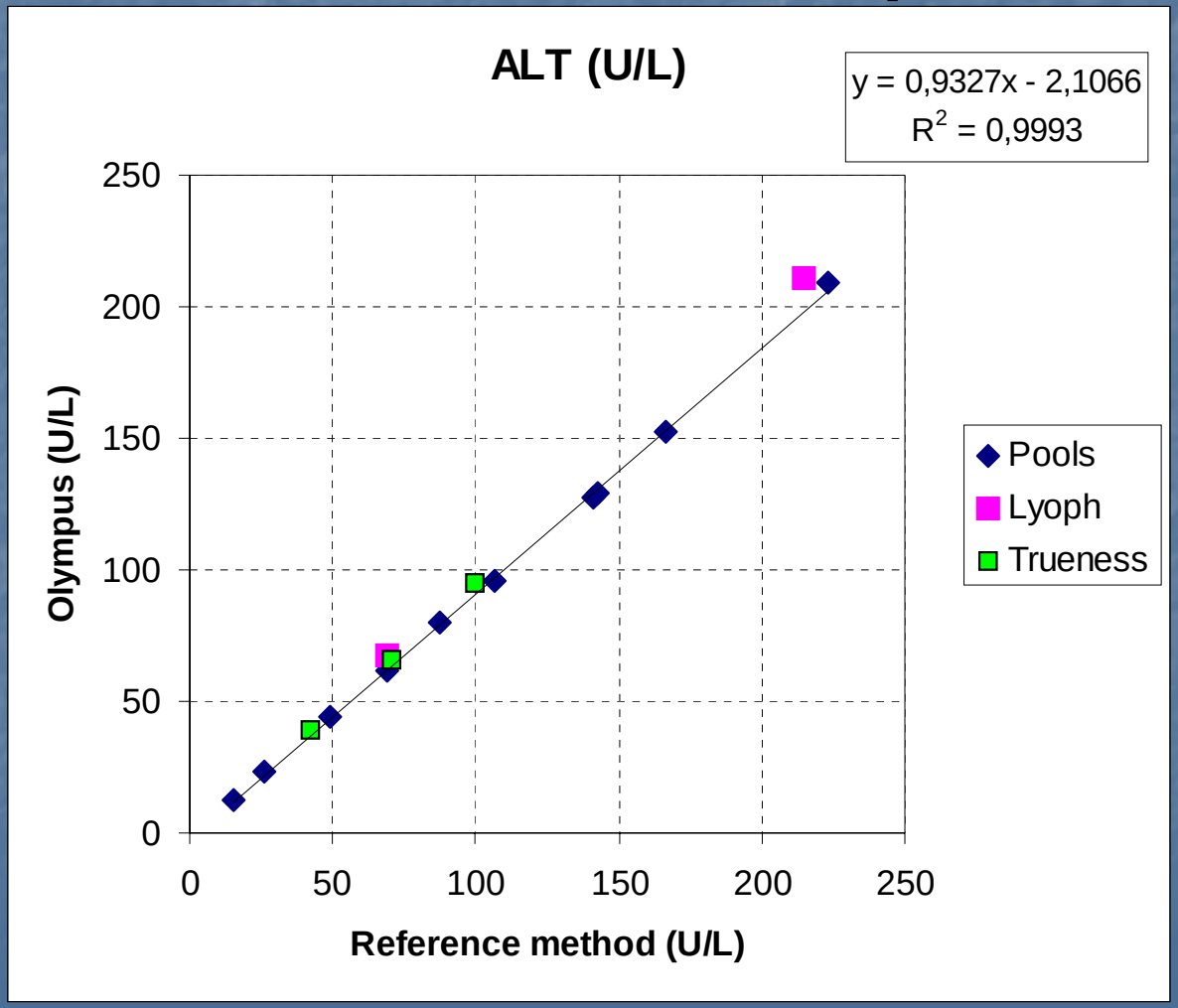
Trueness material Level 2: value assignment



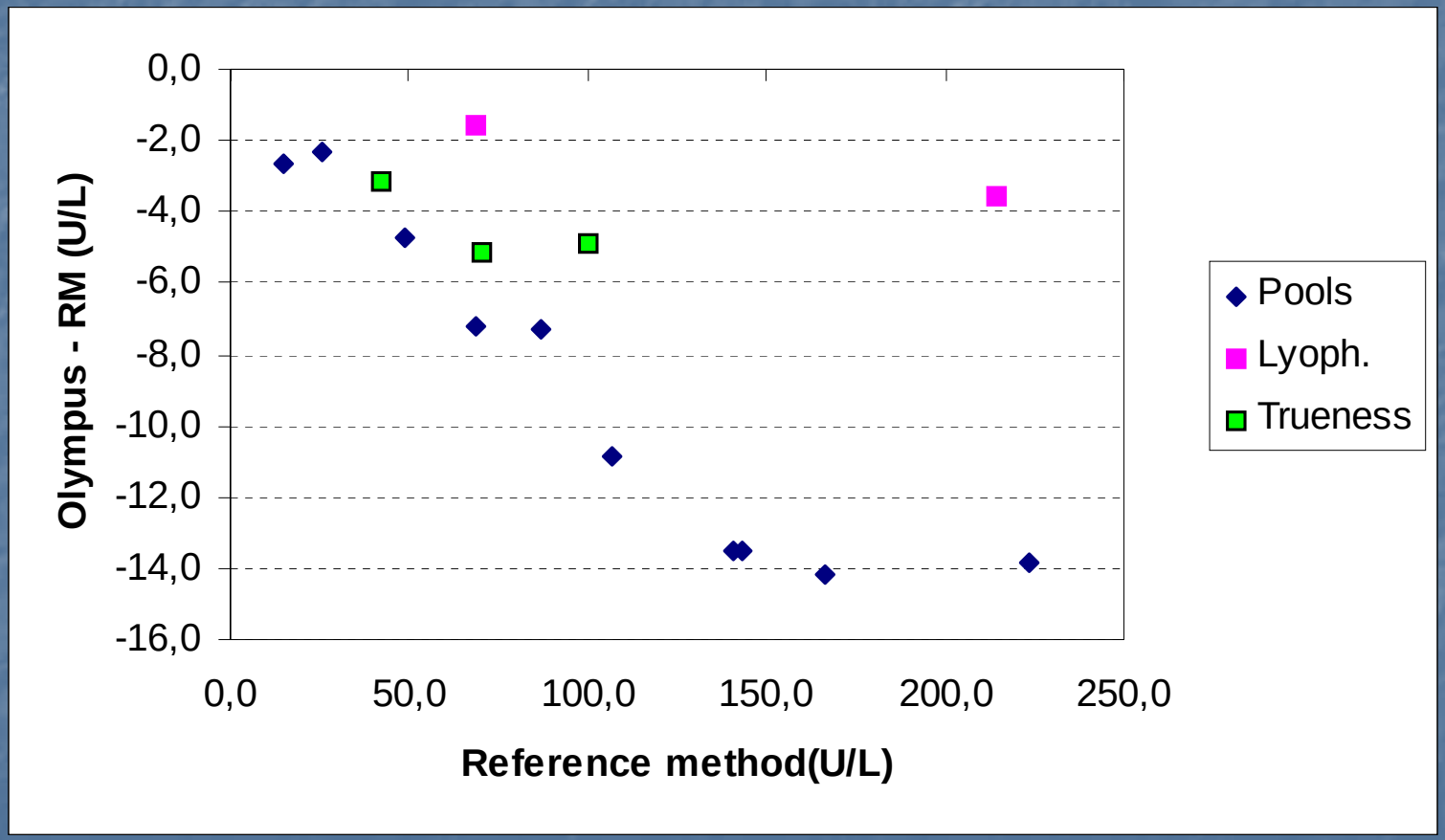
Trueness material Level 3: value assignment



ALT, trueness materials commutability



ALT, trueness materials commutability

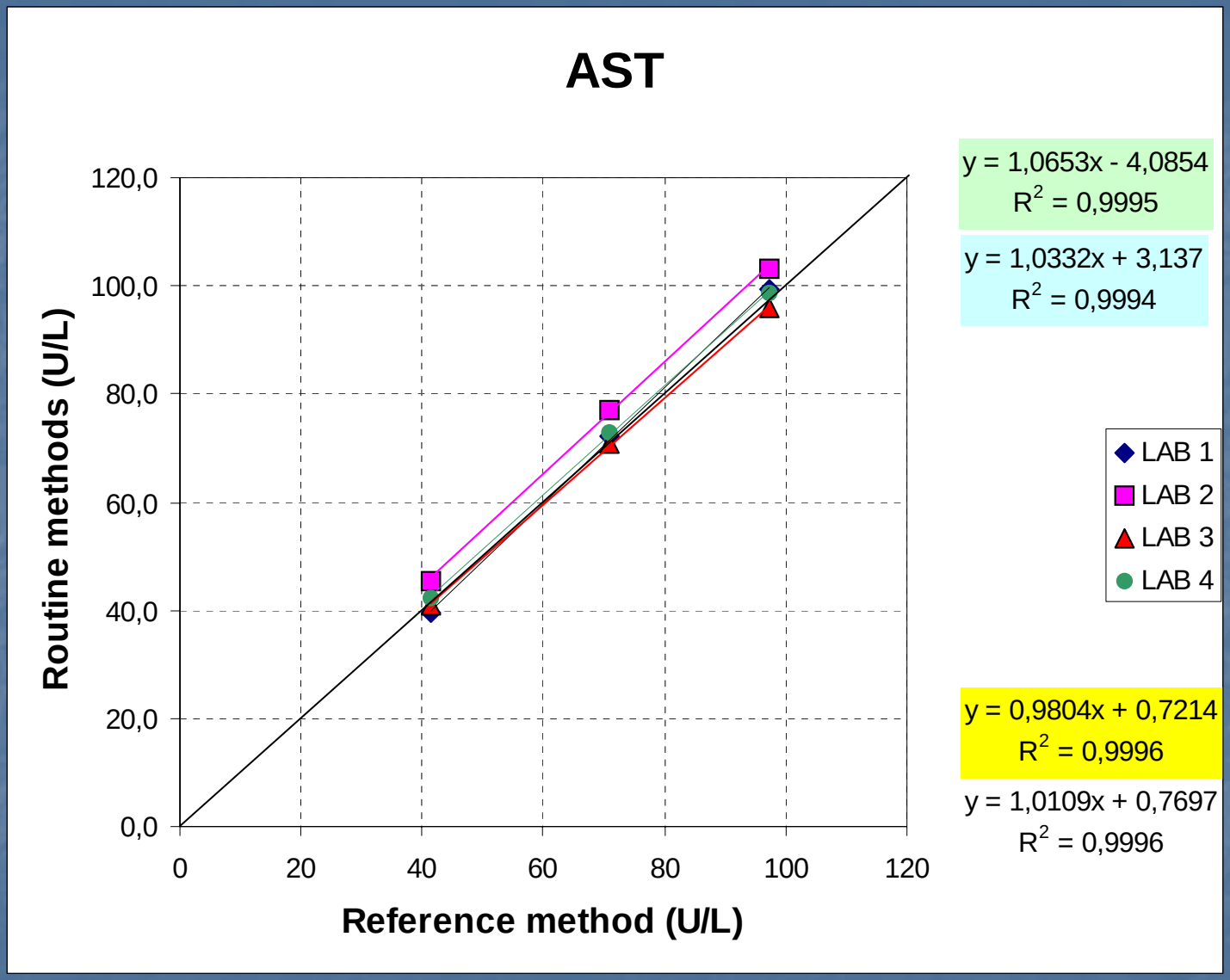


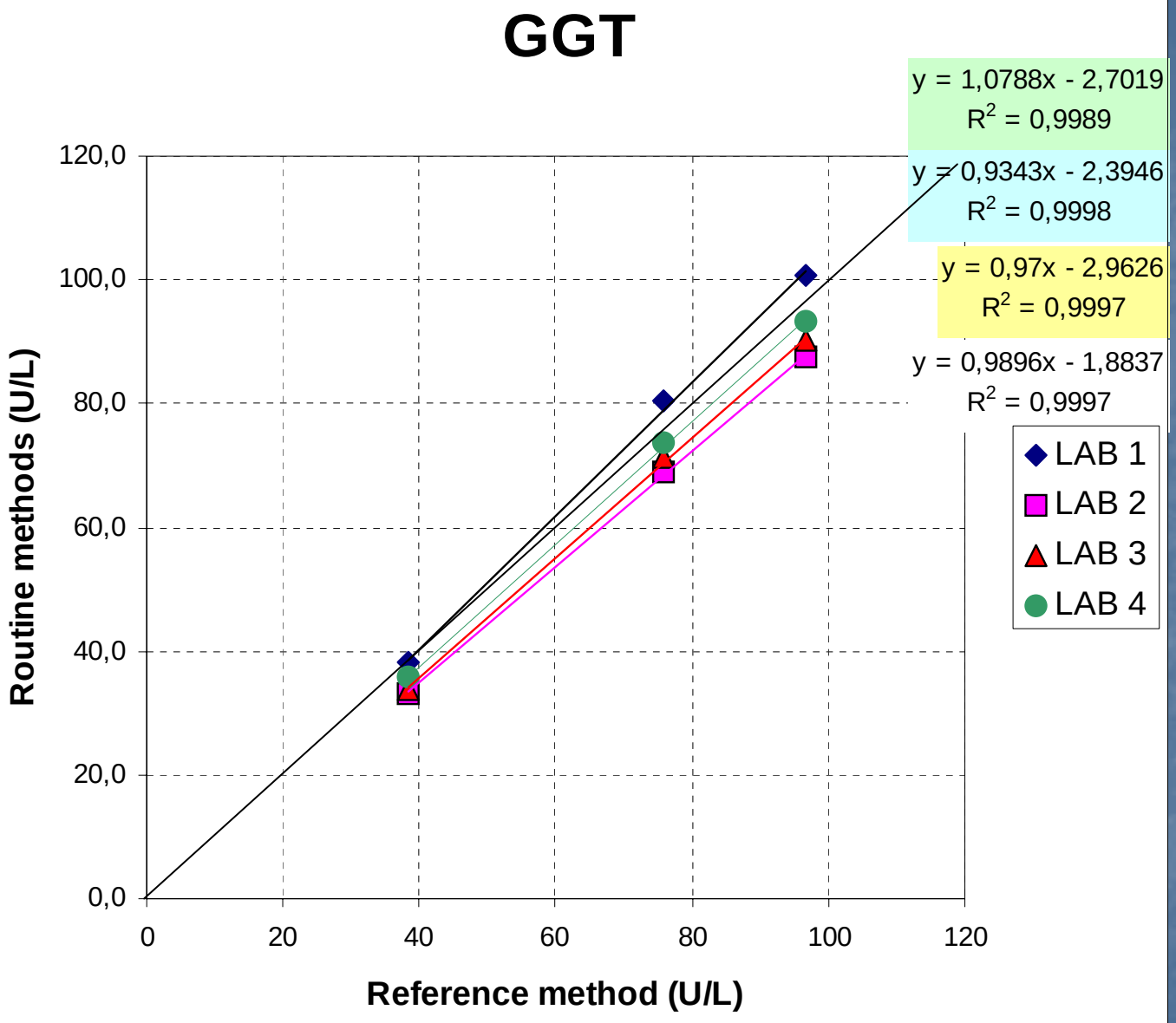
Multicenter reference intervals studies

2. Verification of trueness and precision of the participating laboratories
   - 3 replicate measurements in 5 different runs (if relevant with different calibrations) (total of 15 results per laboratory);

Analytical performances during the preliminary phase

	ALT			AST			GGT		
	Lev 1	Lev 2	Lev 3	Lev 1	Lev 2	Lev 3	Lev 1	Lev 2	Lev 3
Lab1	2,3%	2,1%	1,7%	2,0%	0,9%	0,7%	4,8%	1,7%	1,7%
Lab2	3,7%	2,0%	1,5%	2,5%	0,9%	0,9%	3,4%	3,2%	2,4%
Lab3	4,8%	3,7%	2,5%	1,8%	1,3%	1,5%	1,5%	0,7%	0,7%
Lab4	0,7%	0,6%	0,7%	0,7%	0,9%	0,8%	2,0%	0,8%	0,5%


AST
Routine methods (U/L)
Reference method (U/L)
120,0
100,0
80,0
60,0
40,0
20,0
0,0
0
20
40
60
80
100
120
y = 1,0653x - 4,0854
R2 = 0,9995
y = 1,0332x + 3,137
R2 = 0,9994
LAB 1
LAB 2
LAB 3
LAB 4
y = 0,9804x + 0,7214
R2 = 0,9996
y = 1,0109x + 0,7697
R2 = 0,9996


GGT
y = 1,0788x - 2,7019
R2 = 0,9989
y = 0,9343x - 2,3946
R2 = 0,9998
y = 0,97x - 2,9626
R2 = 0,9997
y = 0,9896x - 1,8837
R2 = 0,9997
LAB 1
LAB 2
LAB 3
LAB 4
Routine methods (U/L)
Reference method (U/L)
0,0
20,0
40,0
60,0
80,0
100,0
120,0
0
20
40
60
80
100
120

Multicenter reference intervals studies

3. Selection of reference individuals and measurement of the samples.
4. Calculation of the reference intervals

Multicenter R.I. study AST, ALT, GGT – reference population –

	Males			Females		
	African	Caucasian	Asian	African	Caucasian	Asian
<10 years	10	30	15	10	30	15
10 – 20 y	15	40	20	15	40	20
20 – 40 y	40	60	45	40	60	45
40 – 60 y	40	60	50	40	60	50
60 – 80 y	15	40	20	15	40	20

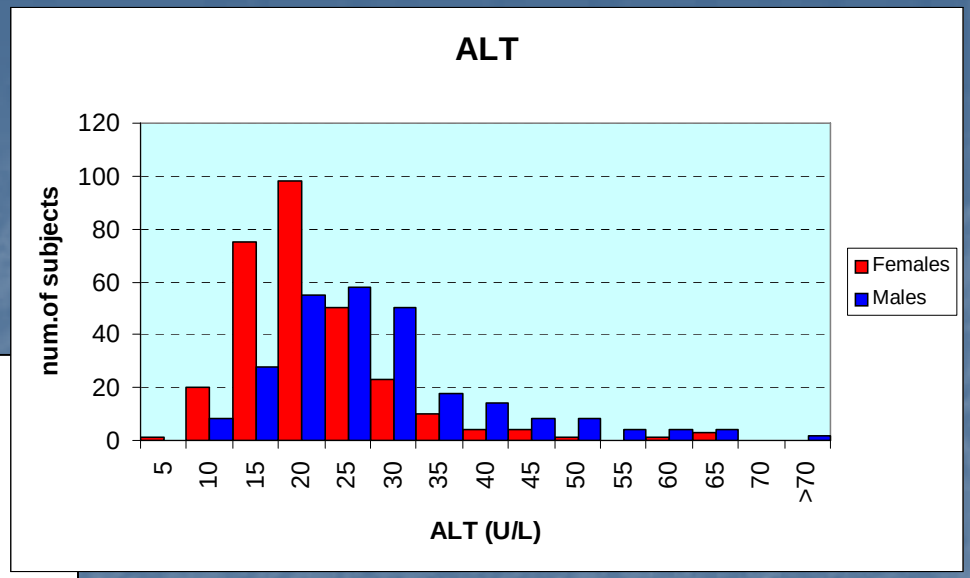
In total 1000 subjects: 240 African, 460 Caucasian and 300 Asian subjects. [Four laboratories in Europe, 3 in Asia and 3 in US collecting preferentially samples from African – American people].

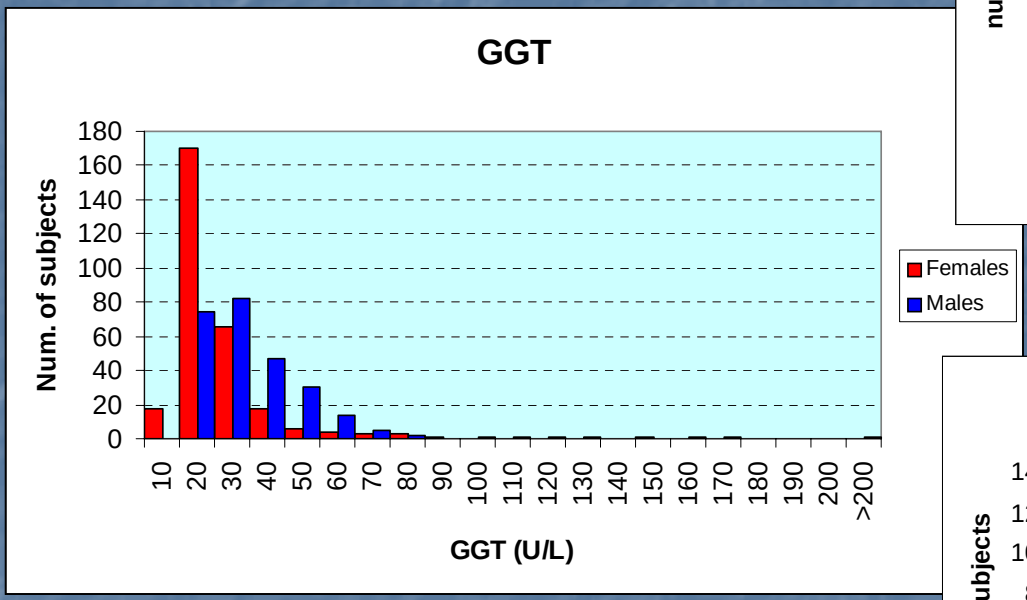
Exclusion criteria

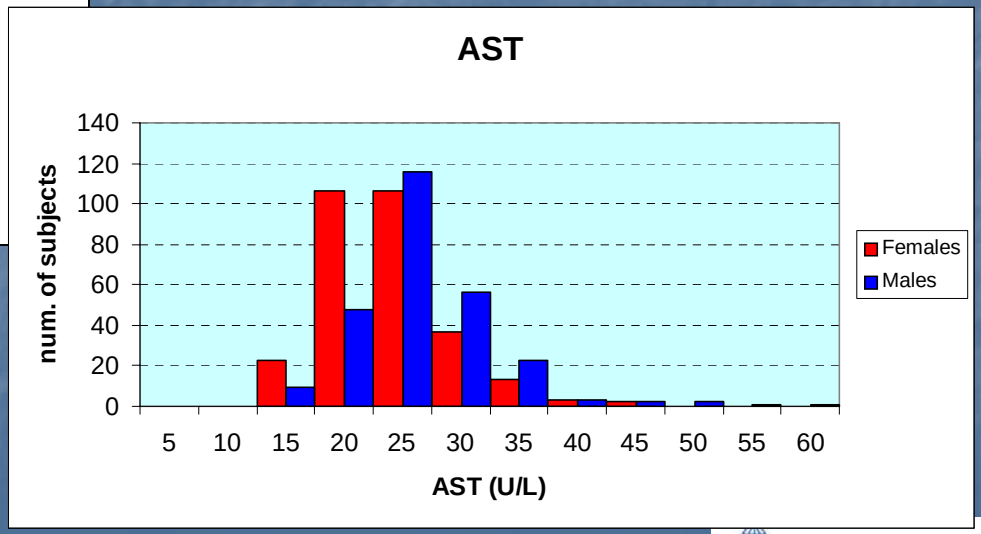
- Diabetes mellitus type 1 and type 2
- Myopathies
- Burns and muscle traumas
- Hypothyroidism
- Chronic nephropathies
- Acute and chronic infection
- Hepato-biliary diseases
- Therapeutic drugs with influence on serum and plasma enzyme concentration (e.g. warfarin, antiepileptics, diphenylhydantoin, aminopyrin, antidepressants, analgesics).
- Antibiotics
- Pregnancy
- BMI >30
- Heavy exercise in the previous days
- Alcohol > 30 grams per day

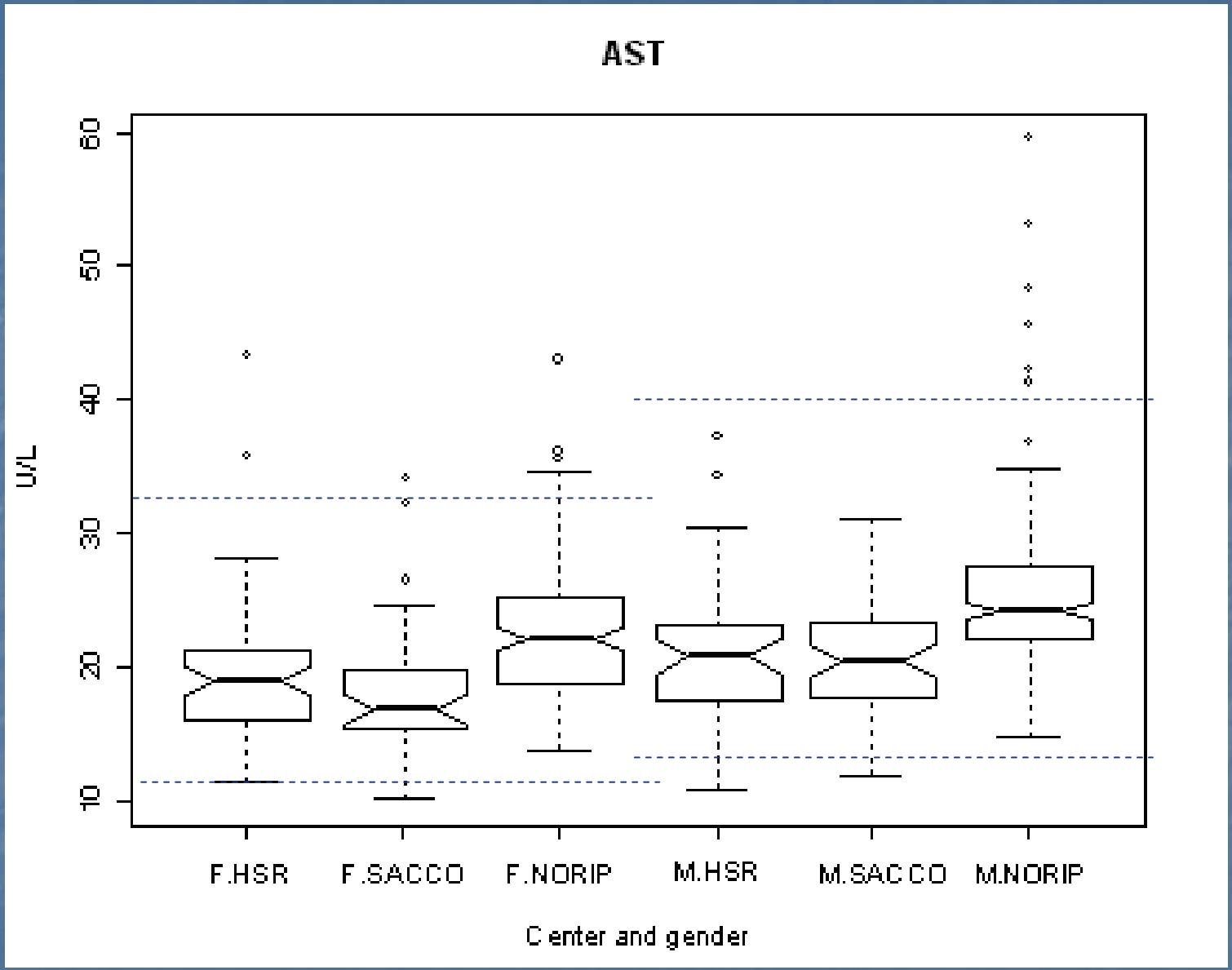
Exclusion criteria – laboratory tests

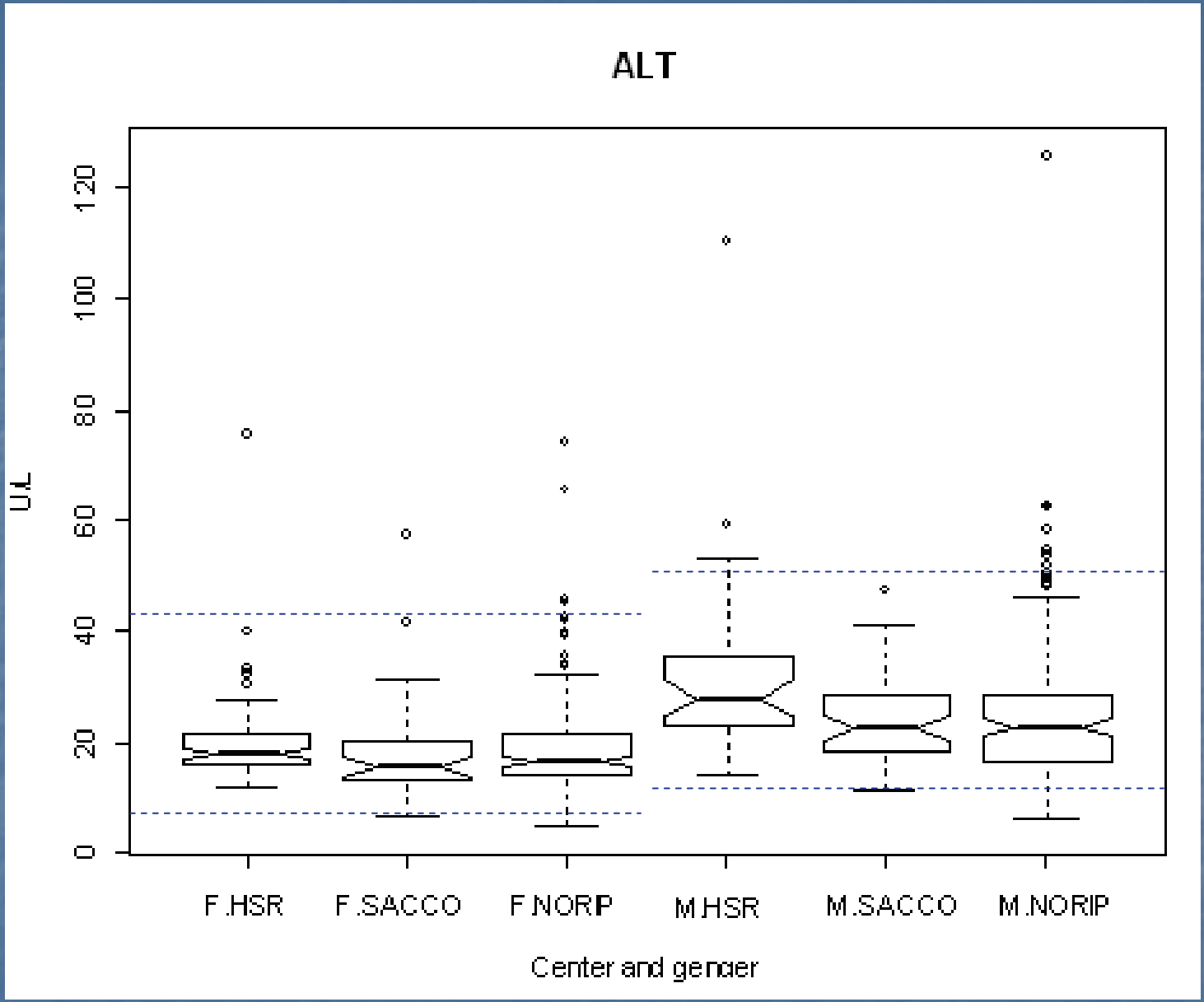
- Fasting Glucose > 126 mg/dl (7.0 mmol/L)
- Creatinine > 0.2 mg/dl above URL
- CK > 300 U/L
- CRP > 12 mg/L
- UA > 8.0 mg/dl (475 µmol/L)
- TG > 200 mg/dl (2.26 mmol/L)
- Chol > 260 mg/dl (6.7 mmol/L)
- Albumin < 32 g/L
- Erys (RBC) < 4.0 + > 5.5 mil/µL (males), < 3.4 + > 5.2 mil/µL (females)
- Hb < 13 g/dl (130 g/L)(males), < 11 g/dl (110 g/L) (females)
- WBC < 3000/µL + > 12000 /µL
- PLT < 100 /nl
- Hematocrit (HCT) < 42 + > 52% (males), < 37 + > 47% (females)
- MCV < 80 + > 96 fL
- HB surface antigen Positive
- IgG antibodies anti HB core antigen Positive
- Anti HCV antibodies Positive

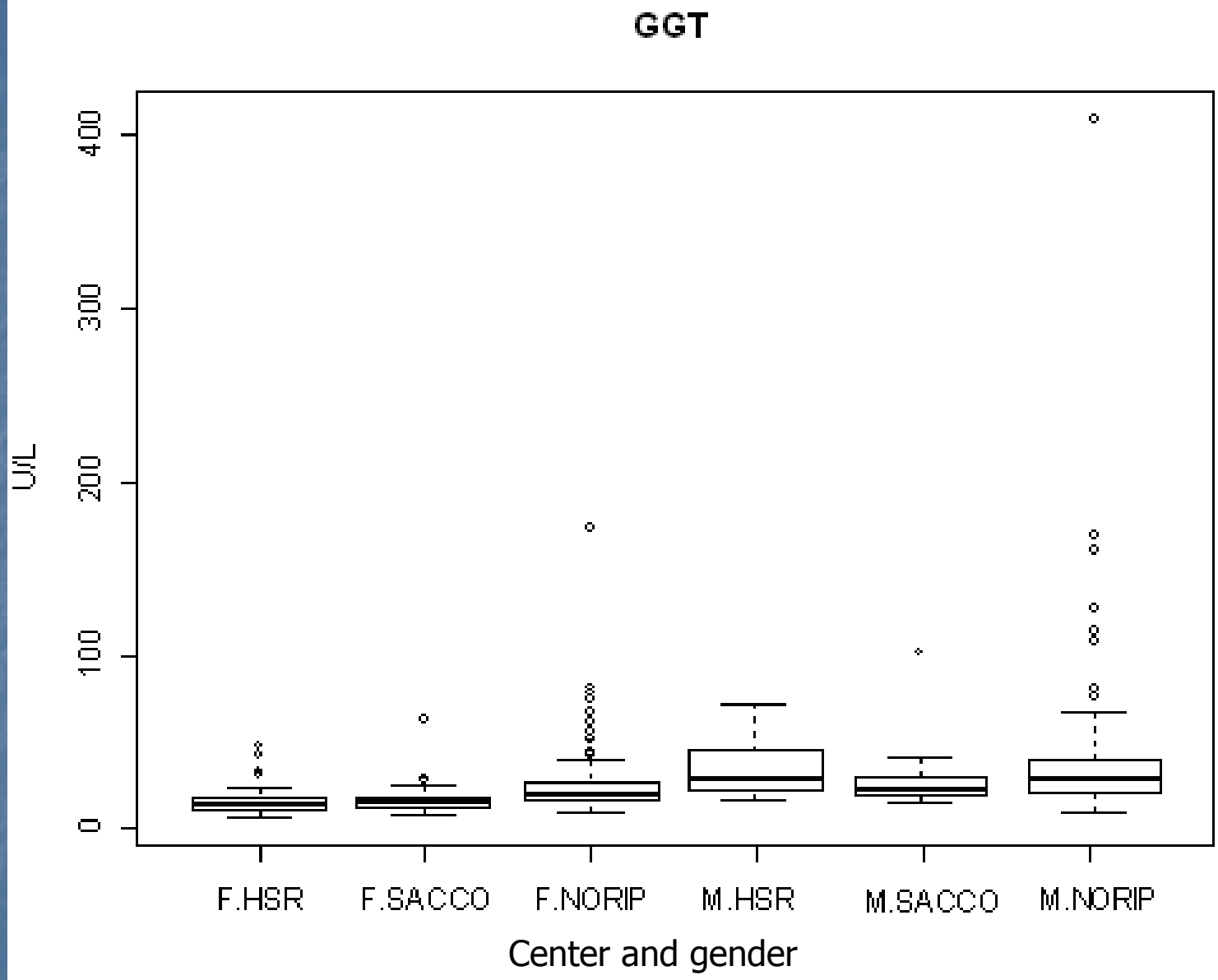

ALT
num.of subjects
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
>70
ALT (U/L)
Females
Males


GGT
Num. of subjects
180
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
>200
GGT (U/L)
Females
Males


AST
num. of subjects
140
120
100
80
60
40
20
0
5
10
15
20
25
30
35
40
45
50
55
60
AST (U/L)
Females
Males


AST
U/L
10
20
30
40
50
60
F.HSR
F.SACCO
F.NORIP
M.HSR
M.SACCO
M.NORIP
Center and gender


ALT
U/L
0
20
40
60
80
100
120
F.HSR
F.SACCO
F.NORIP
M.HSR
M.SACCO
M.NORIP
Center and gender


GGT
U/L
0
100
200
300
400
F.HSR
F.SACCO
F.NORIP
M.HSR
M.SACCO
M.NORIP
Center and gender

Preliminary reference intervals:
Caucasians (Italy + Nordic, 550 subjects)

	Present study	Rustad (NORIP)	Schumann (Clin Chim Acta)
AST (U/L)	**F 13 – 33** **M 15 - 38**	F 15 – 35 M 15 - 45	F < 31 M < 35
ALT (U/L)	**F 8 – 42** **M 10 - 52**	F 8 - 46 M 10 - 68	F < 34 M < 45
GGT (U/L)	**F 8 – 37*** **M 14 - 57***	F 10 – 45 M 10 – 80	F < 38 M < 55

(* Italy only, cumulative F 9 – 50, M 13 – 82 U/L)

Conclusions

- Our work is still ongoing and the practical problems to solve are numerous.
- The number of subjects per center has to be sufficiently great, otherwise statistical differences among centers are expected
- GGT upper reference limit is a puzzling problem, clearly a subset of subjects with higher values exists, but are they really "normal"?
- However the possibility of providing reference intervals applicable by any laboratory (able to produce results traceable to the reference measurement procedure) seems quite realistic (at least for Caucasians), but perhaps not for all the enzymes.