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

Ferruccio Ceriotti
Chair IFCC C-RIDL
Diagnostica e Ricerca San Raffaele S.p.A
Milano, Italy

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

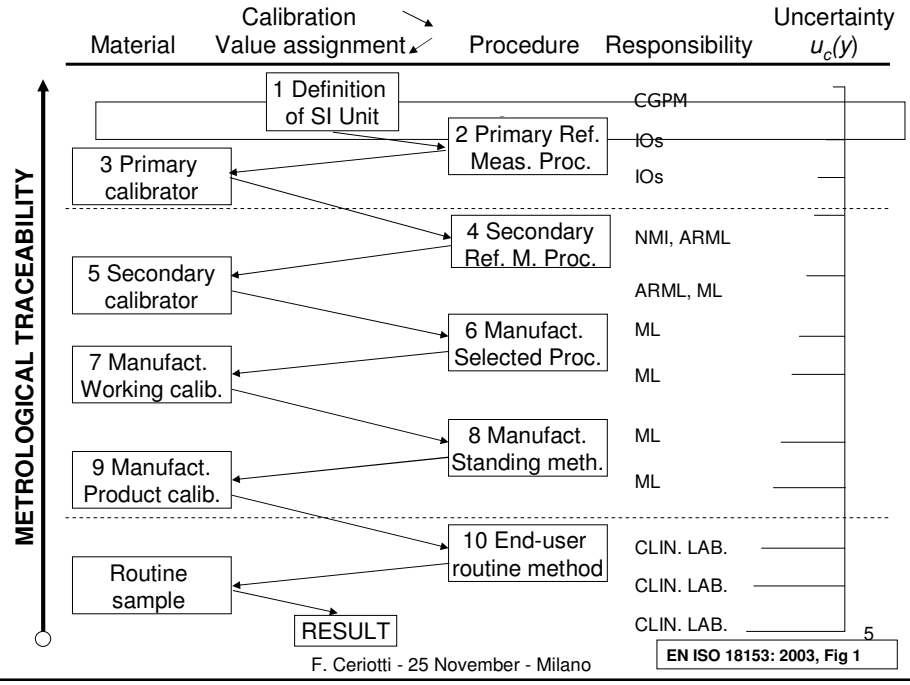
Traceability of the catalytic concentration of enzymes

- 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.

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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**



Requirements for the validity of the metrological traceability chain

- Measurand shall be defined.
- Each level in the calibration hierarchy shall be a measurement procedure or a measurement standard.
- The value assigned to a standard at a given level shall be associated with an uncertainty, the uncertainty added at various step shall be known.
- The quantity shall be the same at all levels.
- Analytical specificity of the routine procedures, as well as the stability and commutability of the calibrators shall be known or investigated.

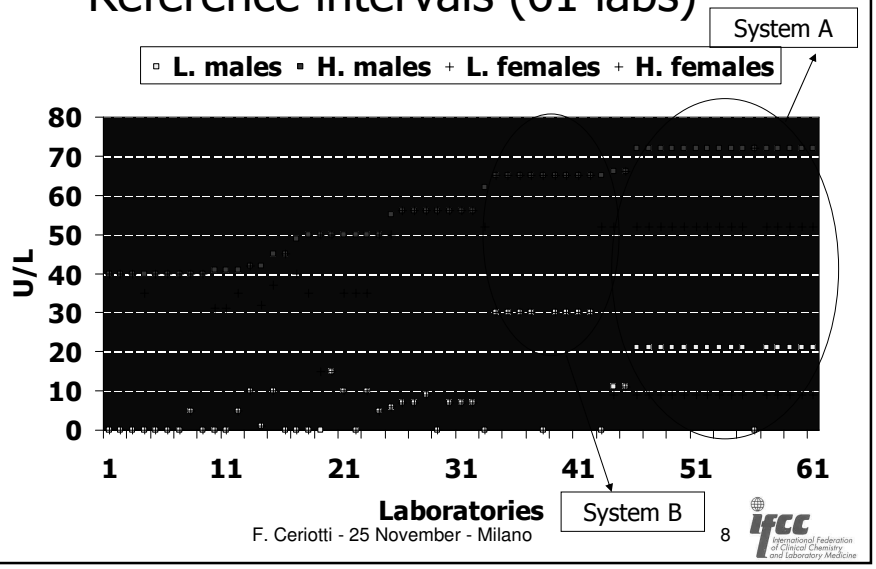
Key for traceability in clinical enzymology

- Specific routine methods
- Commutable calibrators

Improvement in comparability among laboratories

ALT (IFCC method)

Reference intervals (61 labs)



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)

ALT Reference Intervals: examples from the literature

	Males (U/L)	Females (U/L)
Siest*	12.7 – 40	
NHANES**	7 – 48	5 – 35
Leino ('95)	9 – 50	8 – 38
Schumann ('03)	< 45	< 34
NORIP ('04)	10 – 68	8 – 46
Klein ('06)	< 50	< 35

(*) 1975, Phosphate buffer, subjects from 20 to 30 years
(**) white, non Hispanic 20 - 59 years, without P5P

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

IFCC initiatives

- Definition of the theory on how to organize a reference interval study (new CLSI C28-A3 document)
- Organization of a multicenter study for the definition of AST, ALT and γGT reference intervals

Organization of Multicenter Reference Intervals Studies

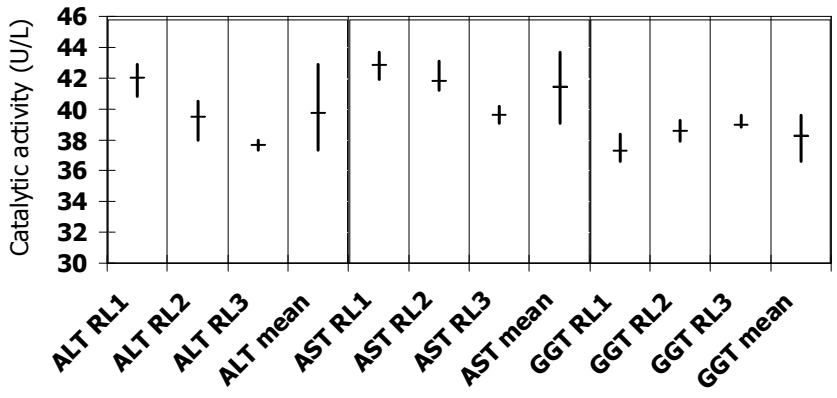
Prerequisites:

- only clinical laboratories using methods for which the traceability to primary reference methods and / or material is clearly stated by the manufactures are eligible for participation;
- only clinical laboratories strictly following the manufacturer's instructions are eligible for participation;
- 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.

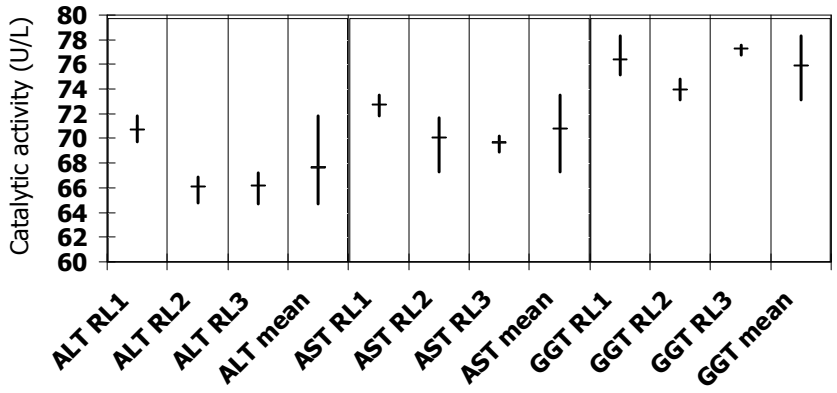
Multicenter reference intervals studies (2)

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);

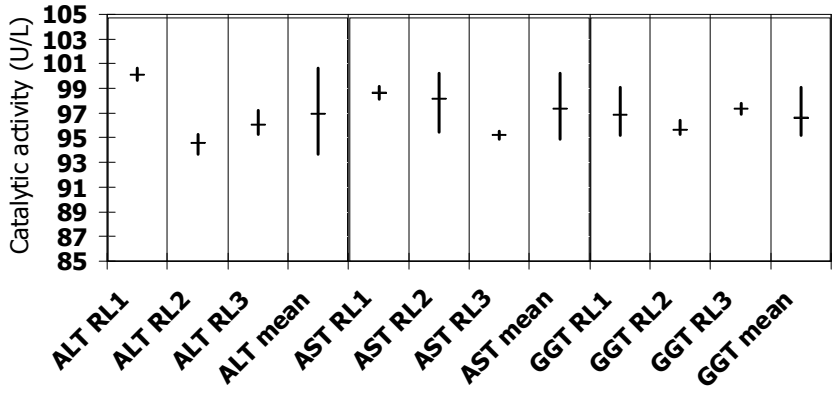
Trueness material Level 1: value assignment



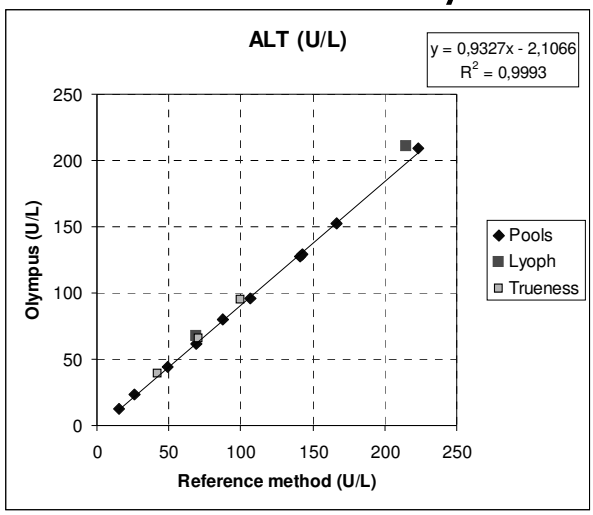
Trueness material Level 2: value assignment



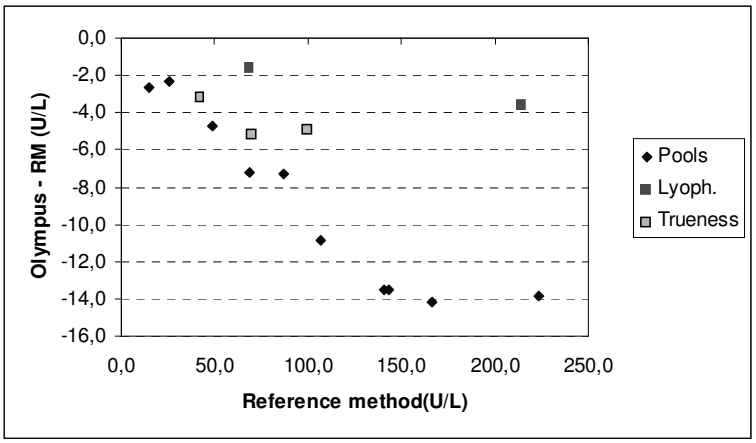
Trueness material Level 3: value assignment



ALT, trueness materials commutability



ALT, trueness materials commutability

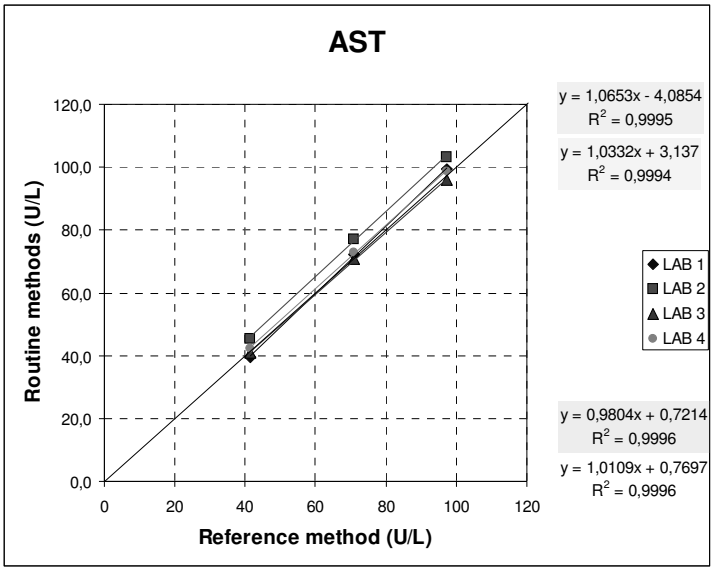


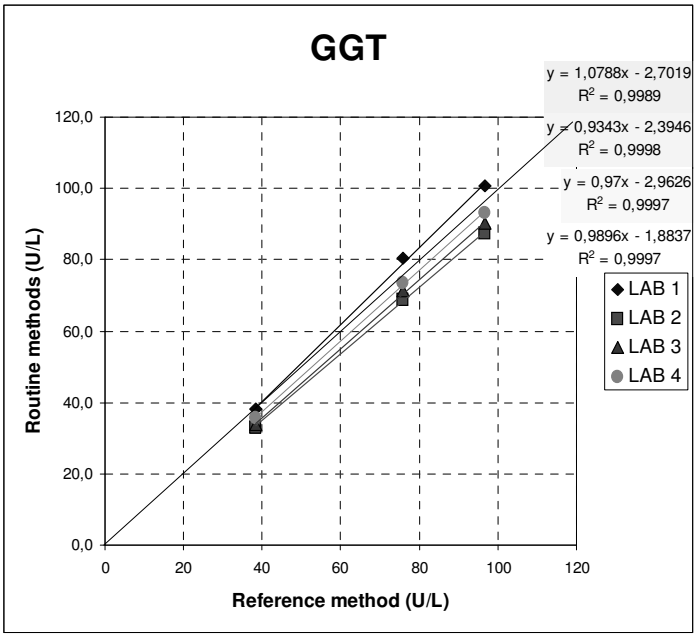
Multicenter reference intervals studies (3)

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)
0,0
20,0
40,0
60,0
80,0
100,0
120,0
0
20
40
60
80
100
120
y = 1,0653x - 4,0854
R2 = 0,9995
y = 1,0332x + 3,137
R2 = 0,9994
y = 0,9804x + 0,7214
R2 = 0,9996
y = 1,0109x + 0,7697
R2 = 0,9996
LAB 1
LAB 2
LAB 3
LAB 4


GGT
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
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

Multicenter reference intervals studies (4)

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

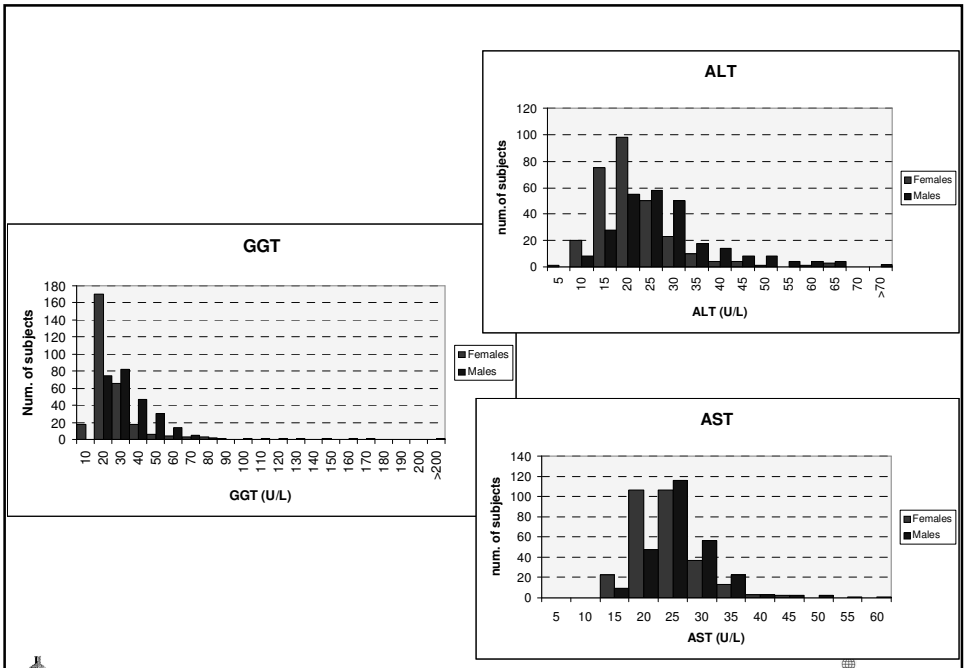
In total 1000 subjects: 240 African, 460 Caucasian and 300 Asian subjects. [Four laboratories in Europe, 3 in Asia and 3 in US collecting preferentially samples from African – American people].

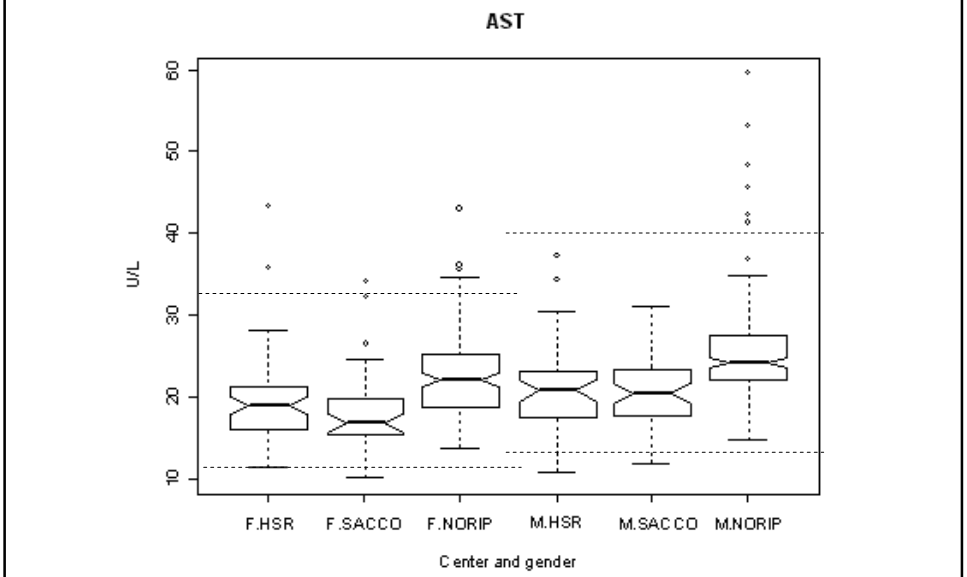
Exclusion criteria

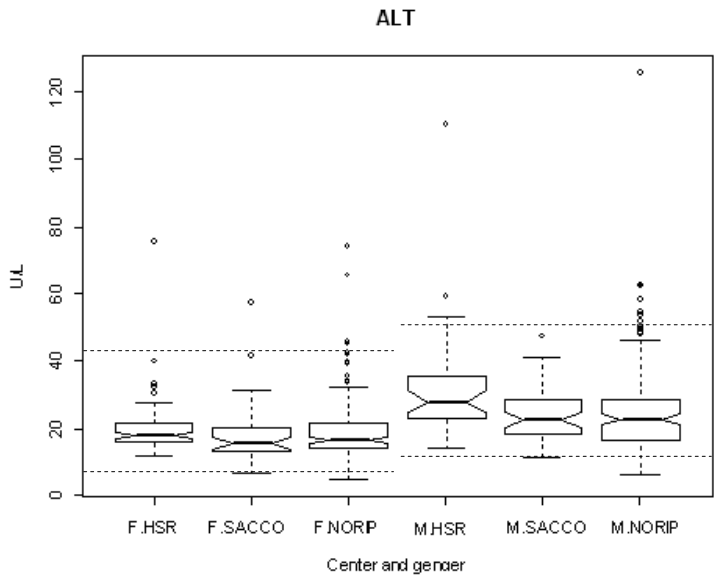
- Diabetes mellitus type 1 and type 2
- Myopathies
- Burns and muscle traumas
- Hypothyroidism
- Chronic nephropathies
- Acute and chronic infection
- Hepato-biliary diseases
- Therapeutic drugs with influence on serum and plasma enzyme concentration (e.g. warfarin, antiepileptics, diphenylhydantoin,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

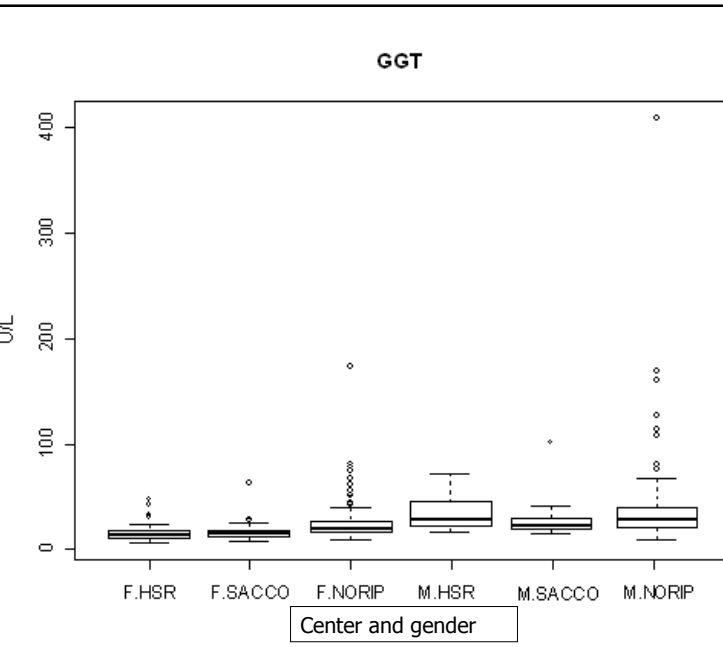
Exclusion criteria – laboratory tests

- 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
ALT (U/L)
Females
Males
GGT
Num. of subjects
GGT (U/L)
Females
Males
AST
num. of subjects
AST (U/L)
Females
Males


AST
U/L
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 still 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).