Standardization in clinical enzymology: still a challenge?

Ferruccio Ceriotti

Diagnostica e Ricerca San Raffaele

EUROMEDLAB MILANO 2013

19-23 MAY

Laboraf

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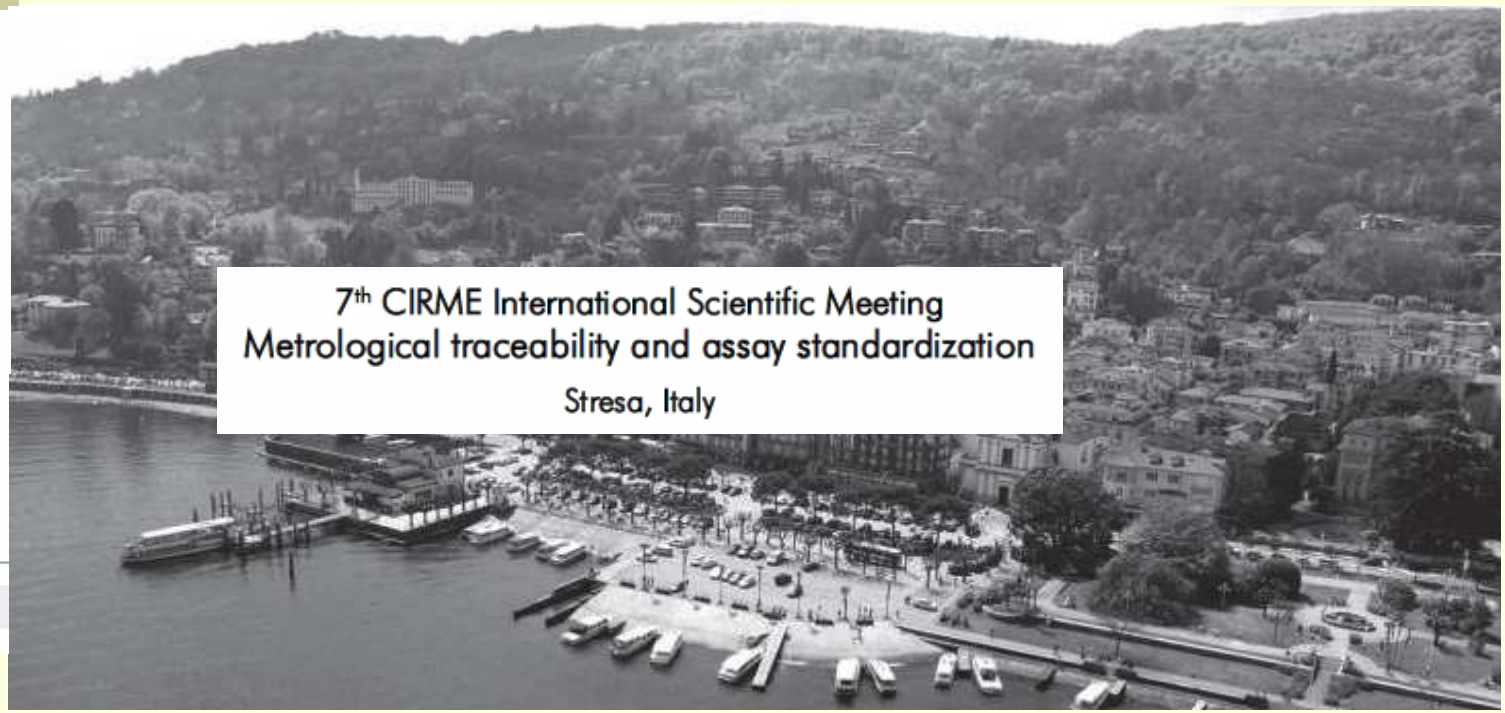


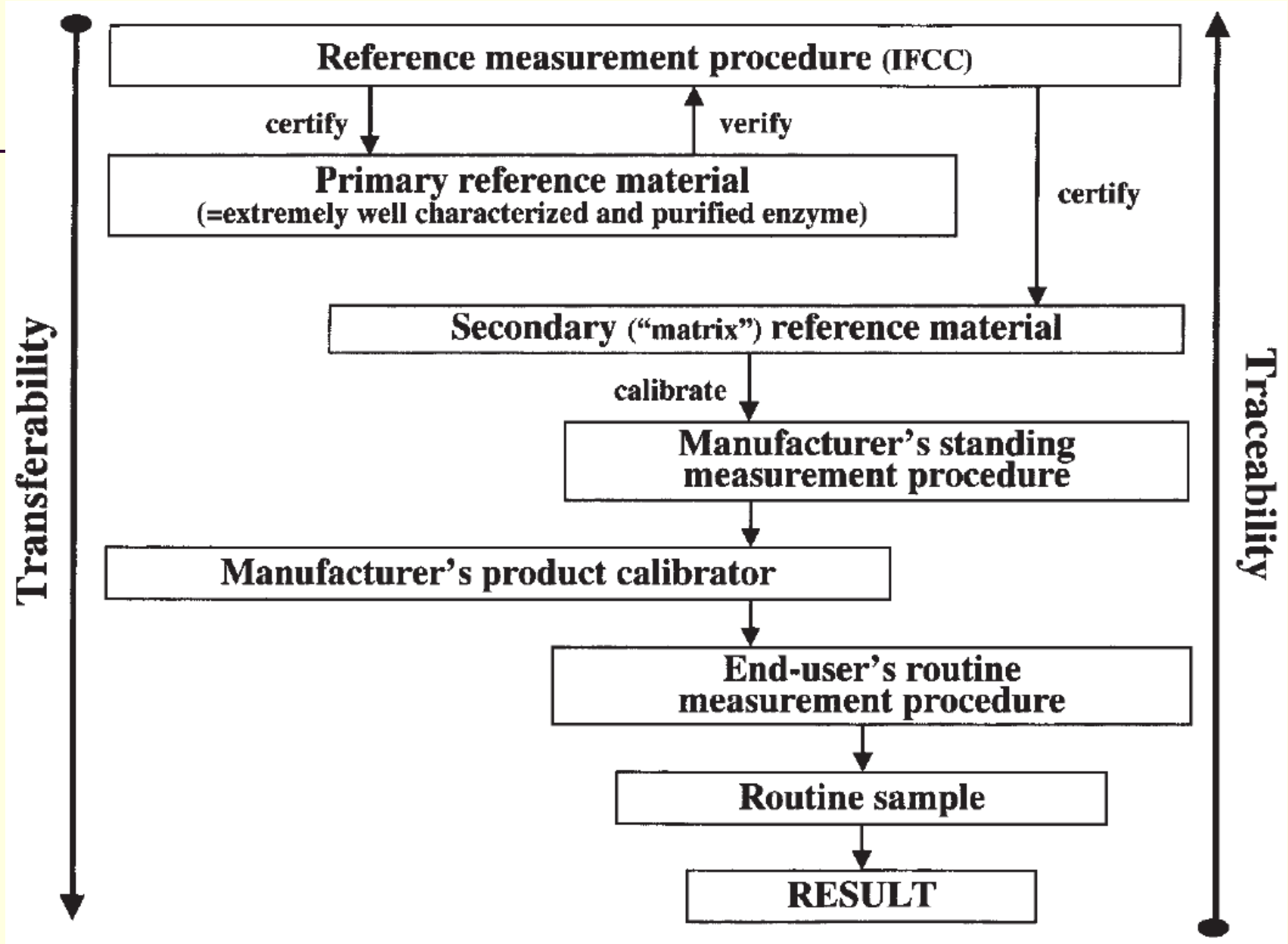
Clin Chem Lab Med 2001; 39(9):795–800 © 2001 by Walter de Gruyter · Berlin · New York

Opinion Paper

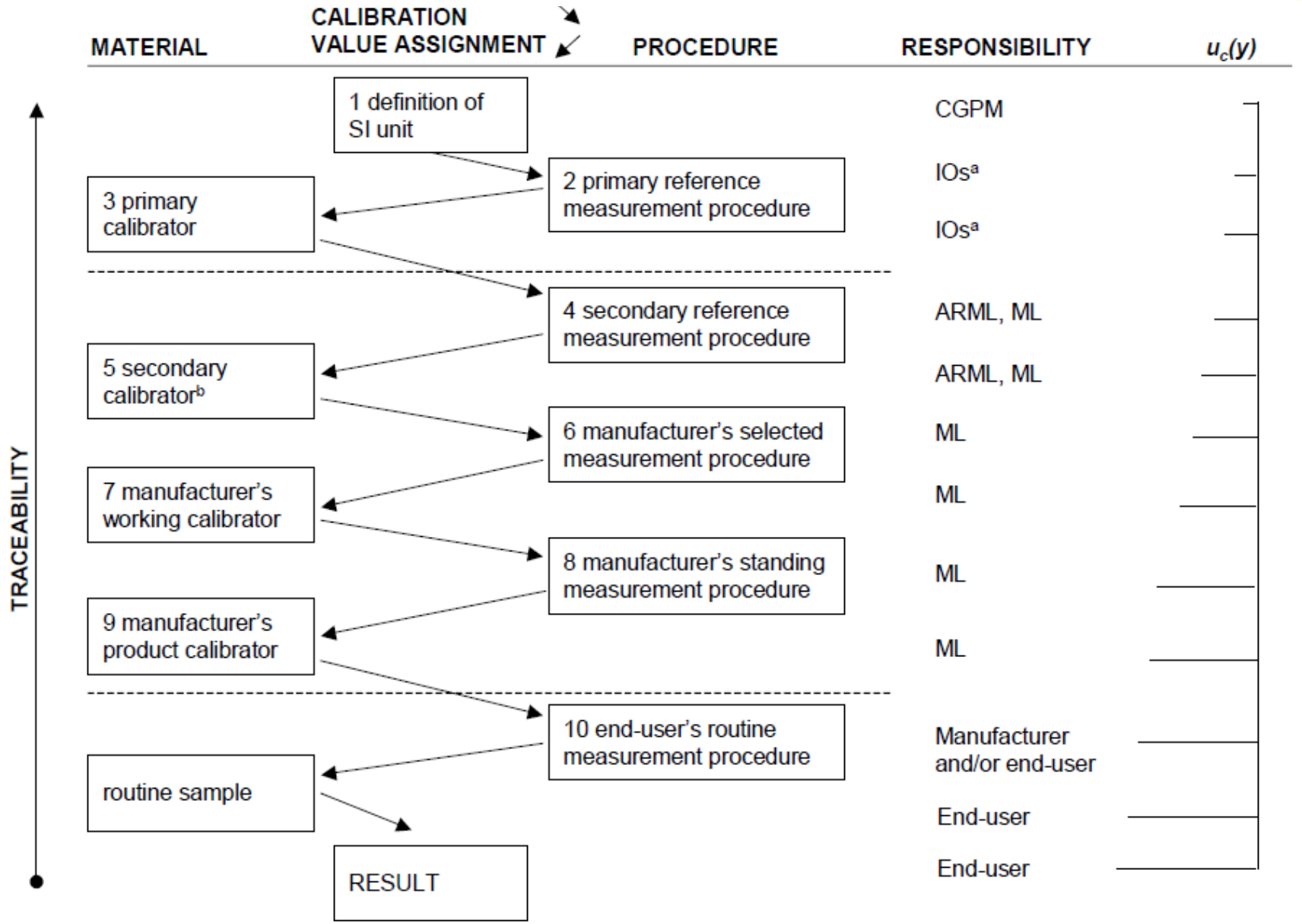
Establishing a Reference System in Clinical Enzymology

Mauro Panteghini[1], Ferruccio Ceriotti[2], Gerhard Schumann[3] and Lothar Siekmann[4]

- Achieving interlaboratory agreement of enzyme activity measurements remains one of the most problematic standardization efforts in clinical chemistry.
- In order to achieve this goal, an approach is needed that would provide a reliable transfer of the measurement values of an uppermost recommended reference procedure to methods which are routinely used in the laboratories.
- Such a structure is presented by the “Reference System” [reference procedures, reference materials, reference laboratories]


Reference measurement procedure (IFCC)
certify
verify
Primary reference material
(=extremely well characterized and purified enzyme)
certify
Secondary ("matrix") reference material
calibrate
Manufacturer's standing measurement procedure
Manufacturer's product calibrator
End-user's routine measurement procedure
Routine sample
RESULT
Transferability
Traceability

ISO 18153:2003



Which is the situation 12 years later?

JCTLM database

JCTLM Database
Laboratory medicine and *in vitro* diagnostics

Enzymes reference measurement procedures

Enzyme	Reference
ALP	*Clin Chem Lab Med* **2011**;49:1439-46
ALT	*Clin Chem Lab Med* **2002**;40:718-24
Amylase	*Clin Chem Lab Med* **2006**;44:1146-55
AST	*Clin Chem Lab Med* **2002**;40:725-33
CK	*Clin Chem Lab Med* **2002**;40:635-42
GGT	*Clin Chem Lab Med* **2002**;40:734-38
LDH	*Clin Chem Lab Med* **2002**;40:743-48
Lipase	----------
Cholinesterase	----------

JCTLM database
Enzymes reference materials (1)

JCTLM
JCTLM Database
Laboratory medicine and *in vitro* diagnostics

Enzyme	Material code	Source	Producer	Cert. Value ± uncertainty
ALP				
ALT	AD454/IFCC	pig heart	IRMM	3.09 µkat/L ±0.07
Amylase	AD456/IFCC	human pancreas	IRMM	9.1 µkat/L ±0.3
	JC ERM 20327		ReCCS	333 U/L ±4
AST	AD457/IFCC	recombinant	IRMM	1.74 µkat/L ±0.05
CK	AD455/IFCC	human heart	IRMM	1.68 µkat/L ±0.07
	JC ERM 20327		ReCCS	420 U/L ±4

IRMM: Institute for Reference Materials and Measurements, European Union
ReCCS (Reference Material Institute for Clinical Chemistry Standards), Japan

JCTLM database
Enzymes reference materials (2)

JCTLM Database
Laboratory medicine and *in vitro* diagnostics

Enzyme	Material code	Source	Producer	Cert. Value ± uncertainty
GGT	AD452/IFCC	pig kidney	IRMM	1.90 µkat/L ±0.04
	JC ERM 20327		ReCCS	149 U/L ±1
LDH	AD453/IFCC	human erythrocytes	IRMM	8.37 µkat/L ±0.12
	JC ERM 20327		ReCCS	398 U/L ±5

IRMM: Institute for Reference Materials and Measurements, European Union
ReCCS (Reference Material Institute for Clinical Chemistry Standards), Japan

JCTLM database

Enzymes reference measurement services

JCTLM Database
Laboratory medicine and *in vitro* diagnostics

Enzyme	Service providers
ALP	CIRME, Italy
ALT	CIRME, Italy; Instand and RfB-DKL, Germany; LREC, Spain
Amylase	RfB-DKL, Germany; LREC, Spain
AST	CIRME, Italy; Instand and RfB-DKL, Germany; LREC, Spain
CK	CIRME, Italy; Instand and RfB-DKL, Germany; LREC, Spain
GGT	CIRME, Italy; Instand and RfB-DKL, Germany; LREC, Spain
LDH	CIRME, Italy; Instand and RfB-DKL, Germany; LREC, Spain

Analita: *AST (GOT)*	UI/L

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	534	4	87.0	8.7	7.54	86.0
Tuo Metodo	30	1	94.1	4.3	4.09	94.0
Tuo Met / Sis	30	1	94.1	4.3	4.09	94.0

Campione	2 *(Scad. 06/03/2013)*
Tuo risultato	101

	Diff. S	Diff. %
Tutti	1.85	16.09
Tuo Metodo	1.69	7.33
Tuo Met / Sis	1.69	7.33

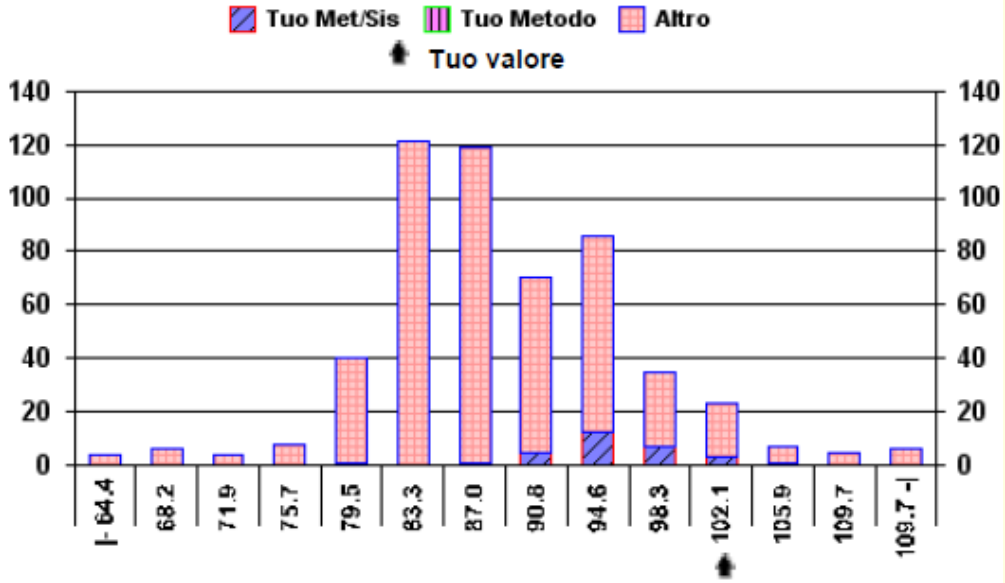
Campioni											
1	2	3	4	5	6	7	8	9	10	11	12
O	O										

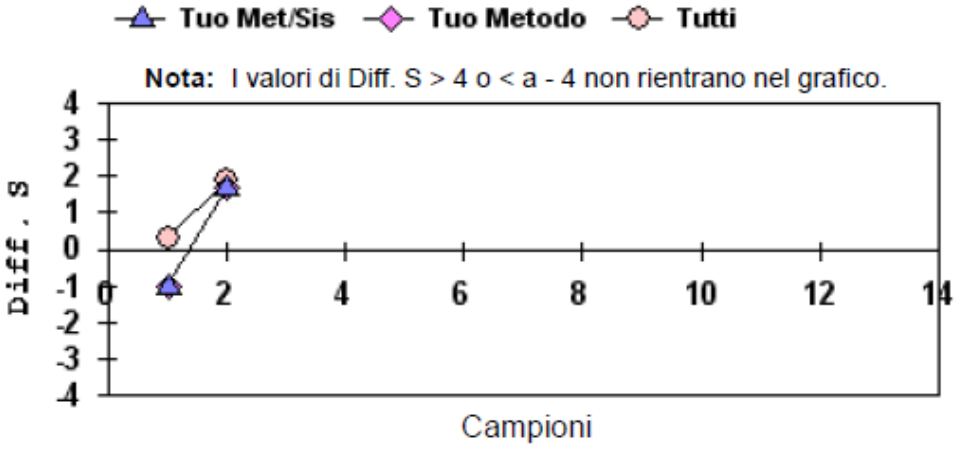
O = Interno X = Esterno rispetto ai limiti dichiarati

N. risultati numerici	534
N. risultati semiquantitativi/qualitativi	

Riepilogo x Metodo risultati numerici (> 7 Centri)				
Metodo	N.	Out	M.	C.V.
IFCC	258	2	88.5	9.3
IFCC/ROCHE COBAS 6000-8000	52	1	83.6	4.4
IFCC/ARCHITECT	48	1	83.4	4.2
HENRY BECKMAN (LX/CX/DX)	41	2	80.5	4.0
IFCC / P-5-P	30	1	94.1	4.3
IFCC/ROCHE INTEGRA	25	1	82.9	4.6
IFCC DIMENSION	23	0	92.1	8.4
IFCC DRY CHEMISTRY	21	0	89.5	5.0
IFCC/ROCHE MODULAR	15	0	82.6	2.5
IFCC/P-5-P ARCHITECT	11	0	95.5	9.1
NON COMUNICATO	10	0	87.3	8.0

Tuo Metodo (Met)	Tuo Sistema (Sis)
IFCC / P-5-P	*AUTOMATICO*

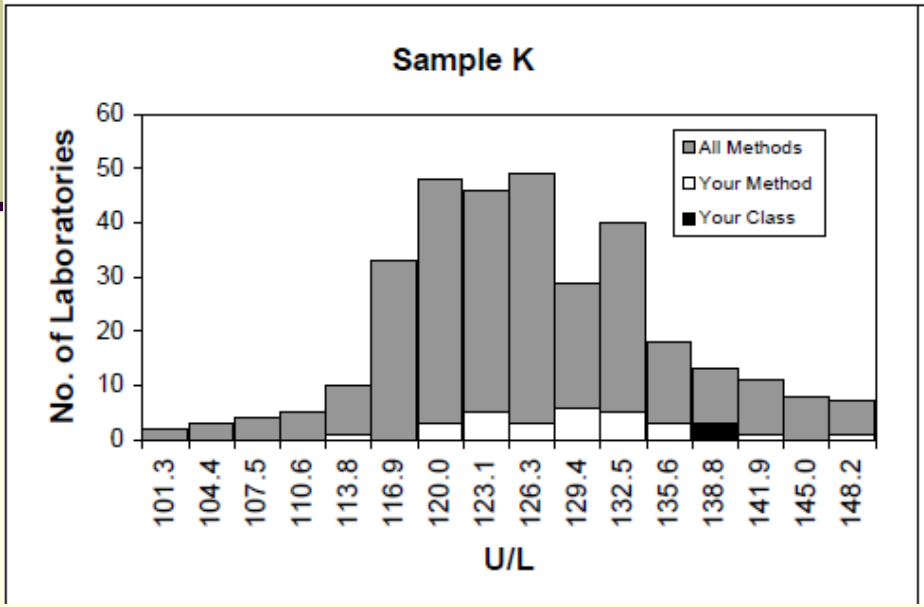


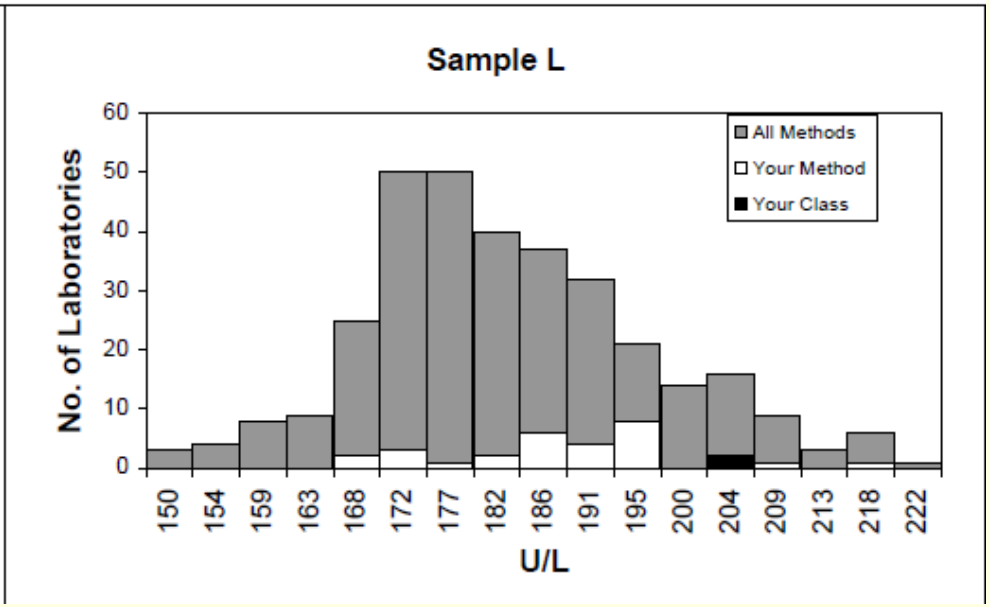


	Your result	Ref. Method Value	Target value	Total Error of Measurement	TE_a	Score
Sample K	**140**	134.5	129.6	**8.0%**	± 15.2%	53
Sample L	**205**	197.1	189.1	**8.4%**	± 15.2%	56

	Sample K					***Sample L***				
Output Group	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z
IFCC (PLP added)	31 (0)	129.6	7.3	5.6%	1.4	30 (0)	189.1	10.6	5.6%	1.5
IFCC (no PLP)	273 (3)	126.3	9.1	7.2%		274 (1)	182.0	13.9	7.6%	
Henry	4 (0)	120.7	2.7	2.2%		4 (0)	171.6	4.6	2.7%	
Dry VITROS	31 (0)	120.3	4.4	3.6%		29 (2)	175.7	4.7	2.7%	

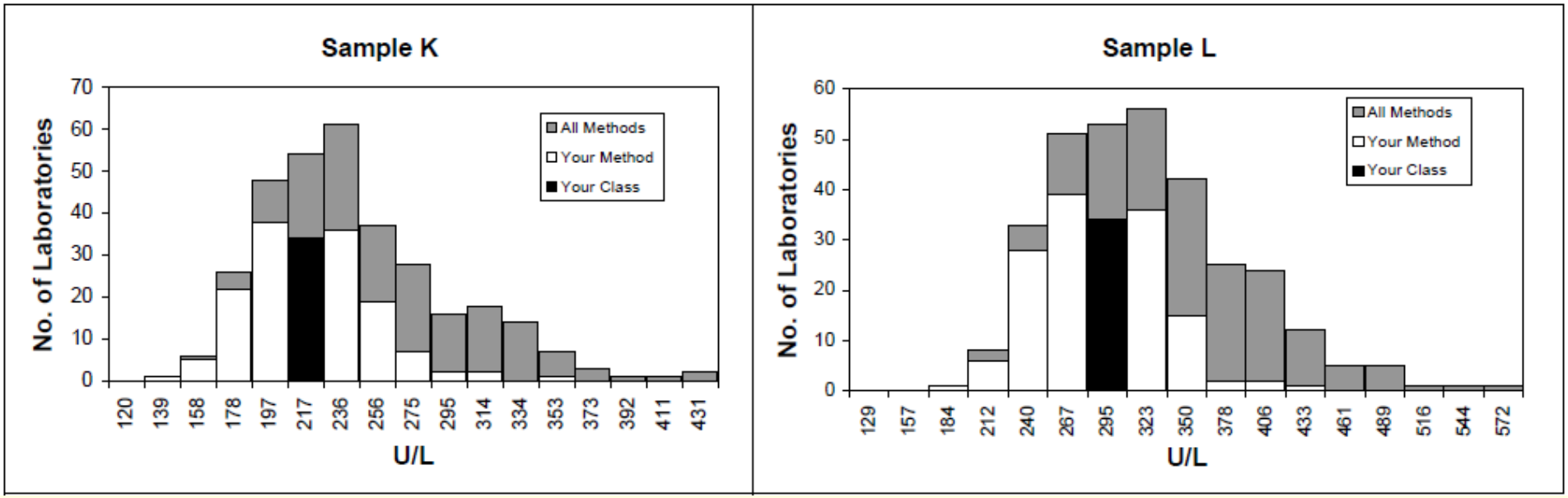
AST





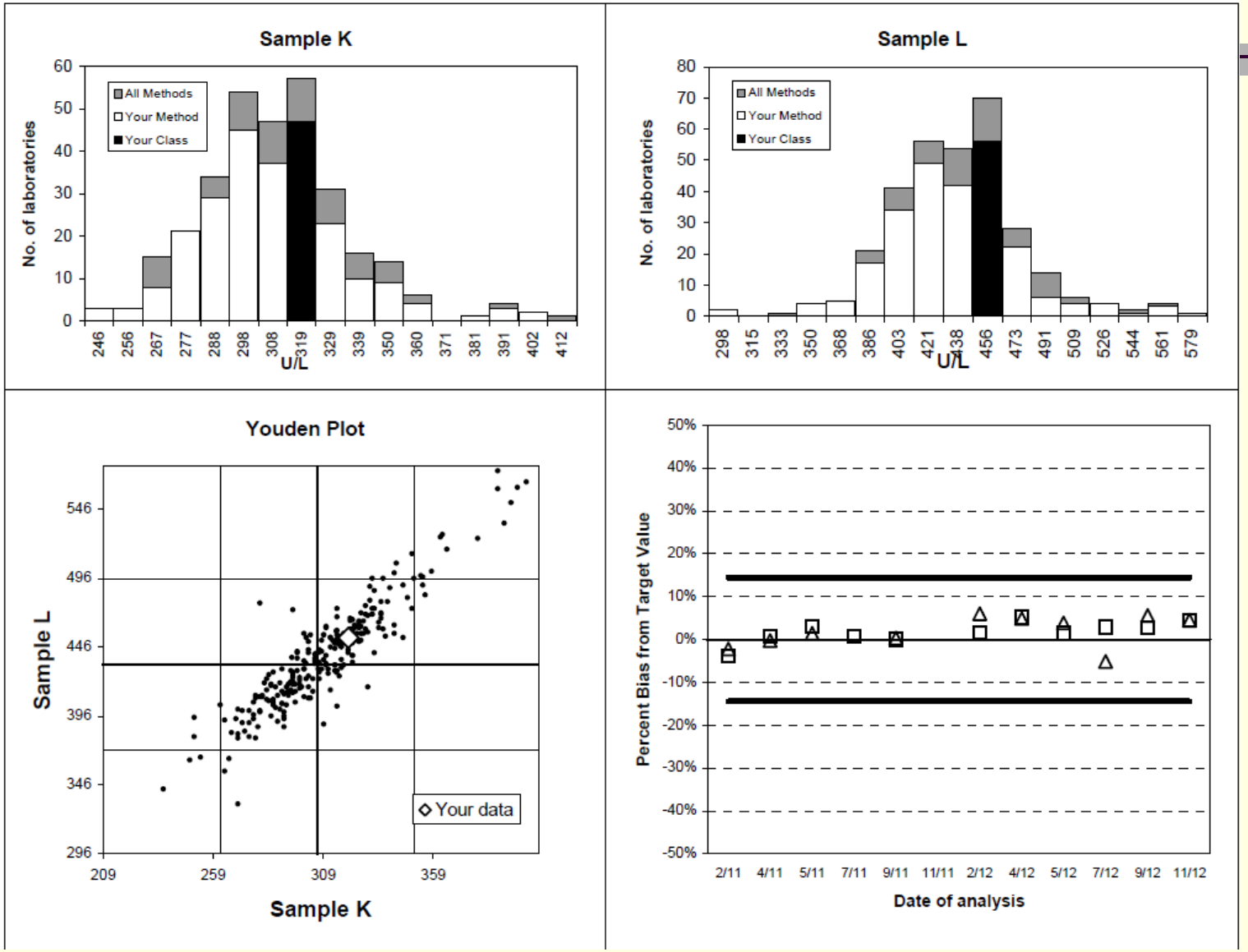
	Sample K					Sample L				
Output Group	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z
DEA SCE	27 (0)	287.2	57.4	20.0%		27 (0)	368.9	53.9	14.6%	
Vitros	30 (0)	232.2	13.7	5.9%		29 (0)	318.1	23.0	7.2%	
DEA DGKC	76 (0)	297.1	40.3	13.6%		76 (0)	389.1	56.2	14.4%	
AMP	170 (0)	217.2	32.1	14.8%	0.1	167 (0)	289.4	44.7	15.5%	0.2
AMP SFBC	3 (0)	280.2	95.5	34.1%		3 (0)	377.3	76.7	20.3%	
AMP Dimension	18 (0)	210.1	17.4	8.3%		17 (0)	279.0	23.5	8.4%	

ALP



	Your result	Ref. Method Value	Target value	Total Error of Measurement	TE_a	Score
Sample K	**320**	308.8	306.1	**4.5%**	± 14.4%	32
Sample L	**453**	430.6	433.6	**4.5%**	± 14.4%	31

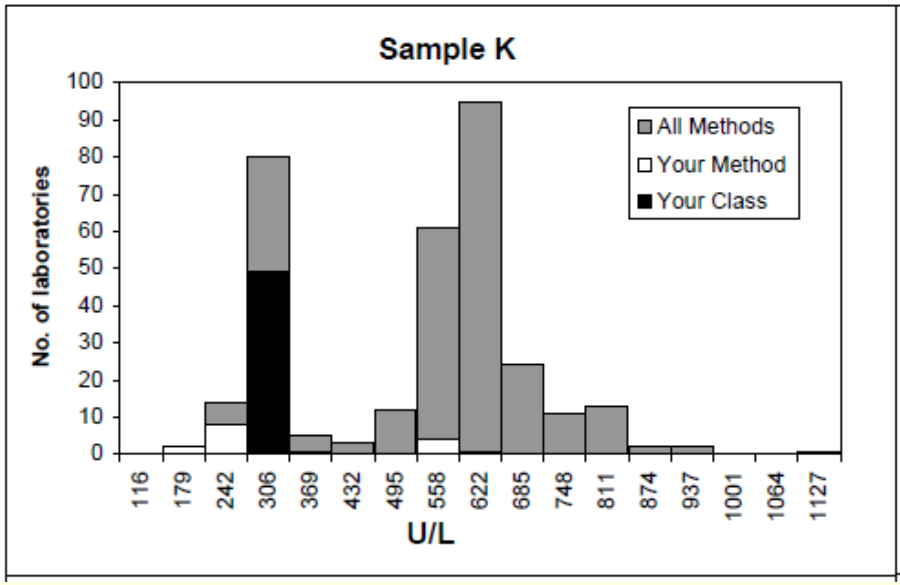
CK

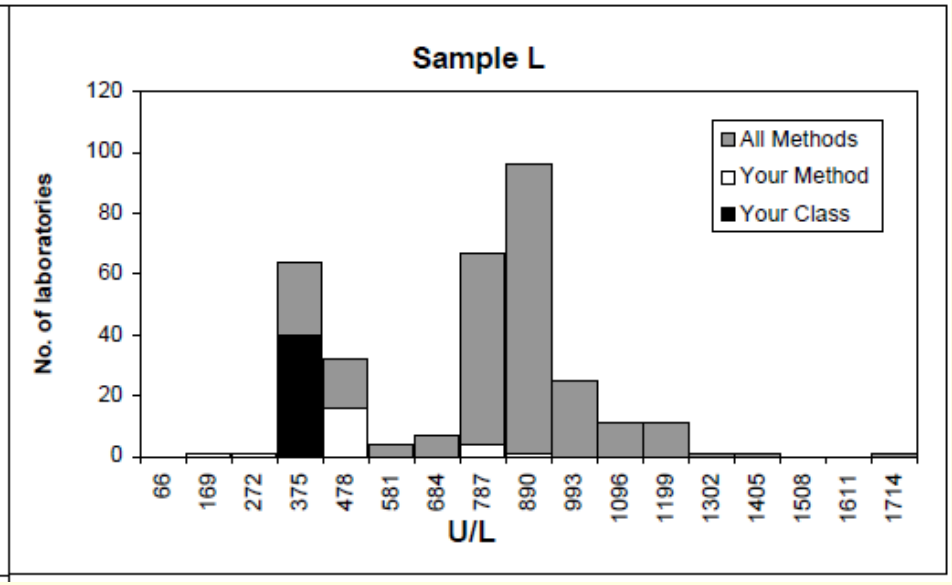


	Your result	*Ref. Method Value*	*Target value*	*Total Error of Measurement*	*TE_a*	*Score*
Sample K	**282**	288.6	290	**-2.9%**	± 11.4%	25
Sample L	**411**	409.2	415	**-0.9%**	± 11.4%	8

	Sample K					***Sample L***				
Output Group	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z
SCE (Pyruvate - Lactate)	40 (0)	599	65	10.9%		40 (0)	861	97	11.2%	
DGKC 1 (Pyruvate - Lactate)	116 (10)	596	26	4.3%		116 (10)	846	44	5.2%	
SFBC (Pyruvate - Lactate)	34 (0)	587	80	13.7%		34 (0)	832	109	13.1%	
Vitros	30 (0)	762	84	11.1%		30 (0)	1101	103	9.4%	
IFCC (Lactate - Pyruvate)	61 (6)	290	15	5.0%	0.6	59 (7)	415	21	5.0%	0.2
Beckman (Pyruvate - Lactate)	3 (1)	755	39	5.1%		2 (2)	1050	9	0.8%	
Lactate-Pyruvate Dimension	17 (0)	288	16	5.5%		16 (0)	410	18	4.5%	

LDH





Analita: *AMILASI*	UI/L

N° Risposte totali: 430

Sottogruppo: *AMILASI*	UI/L

	N.	Out	Media	C.V.	S.D.	Med.na
Tutti	381	8	213.0	6.9	14.7	212.0
Tuo Metodo	22	1	207.7	7.1	14.8	207.0
Tuo Met / Sis	22	1	207.7	7.1	14.8	207.0

Campione	2 *(Scad. 06/03/2013)*
Tuo risultato	219

	Diff. S	Diff. %
Tutti	0.41	2.82
Tuo Metodo	0.76	5.44
Tuo Met / Sis	0.76	5.44

Campioni												
1	2	3	4	5	6	7	8	9	10	11	12	
O	O											

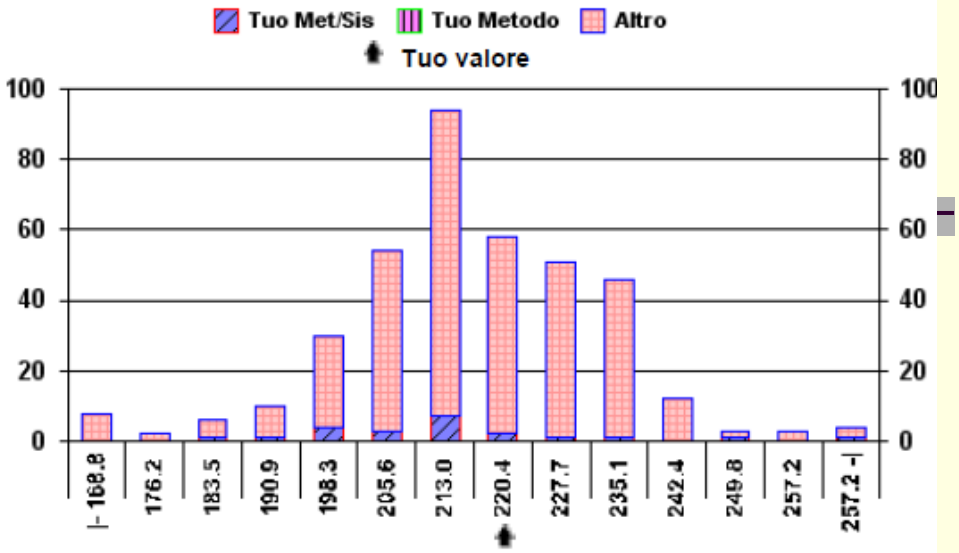
O = Interno X = Esterno rispetto ai limiti dichiarati

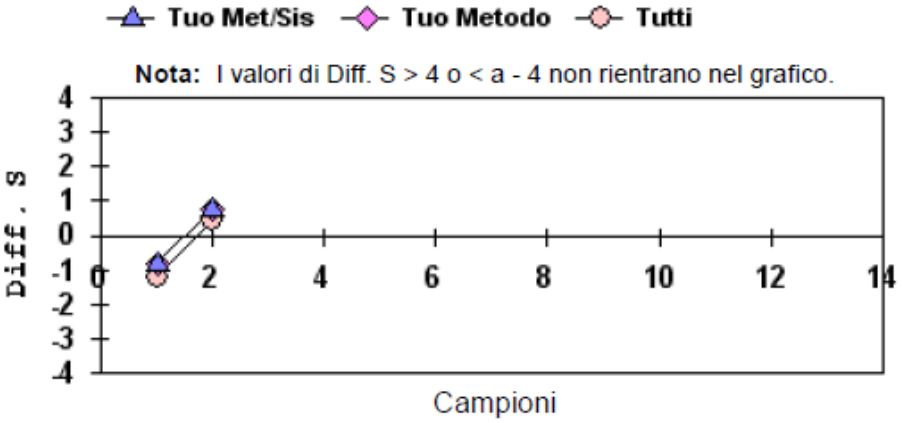
N. risultati numerici	381
N. risultati semiquantitativi/qualitativi	

Riepilogo x Metodo risultati numerici (> 7 Centri)

Metodo	N.	Out	M.	C.V.
2-CL-PNP/MALTO TRIOSIDE	84	2	212.4	8.3
G7PNP/EPS-IFCC/ROCHE COBAS 6-8000	47	2	205.4	2.8
2-CL-PNP-MALTO TRIOS/ ARCHITECT	41	1	225.2	2.7
G7PNP BLOCCATO	38	1	204.9	9.5
G7PNP	30	0	209.3	6.8
2-CL-PNP/MALTOTRIOSIDE DIMENSION	25	1	225.5	4.8
G7PNP/EPS BLOCCATO	22	1	207.7	7.1
G7PNP/EPS-IFCC/ROCHE INTEGRA	19	0	207.4	4.5
G7PNP/EPS-IFCC	15	0	213.9	4.5
G7 EPS / BECKMAN (LX/CX/DX)	13	1	222.1	3.8
4-NITROF. -MALTOHEP -ETILIDENE	11	0	213.3	11.5
G7PNP/EPS-IFCC/ROCHE MODULAR	11	0	207.5	2.4
AMYLOPECTINA DRY CHEMISTRY	21	1	144.2	5.9
MALTOTETR.S PGM BECKMAN (LX/ CX/DX)	16	0	217.1	4.0

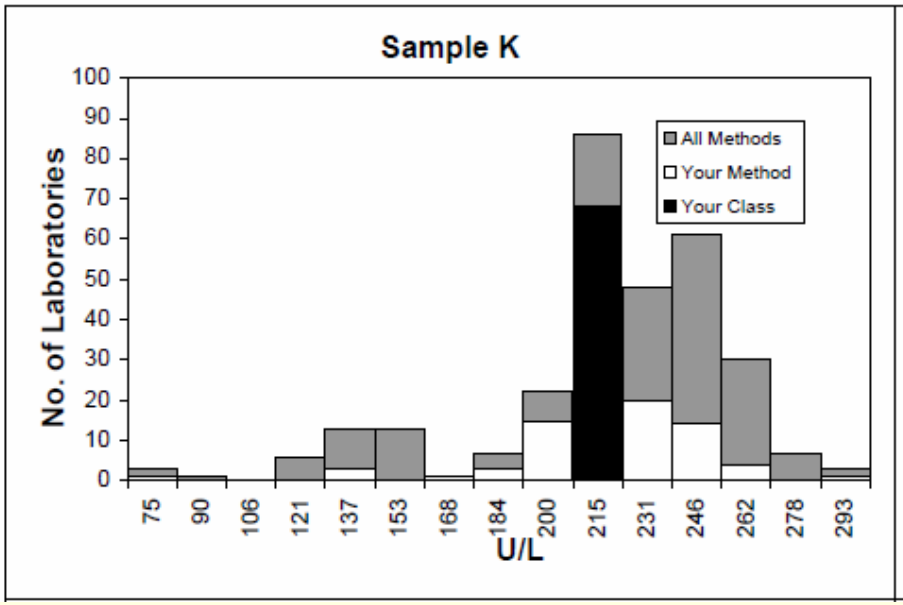
Tuo Metodo (Met)	Tuo Sistema (Sis)
G7PNP/EPS BLOCCATO	*AUTOMATICO*

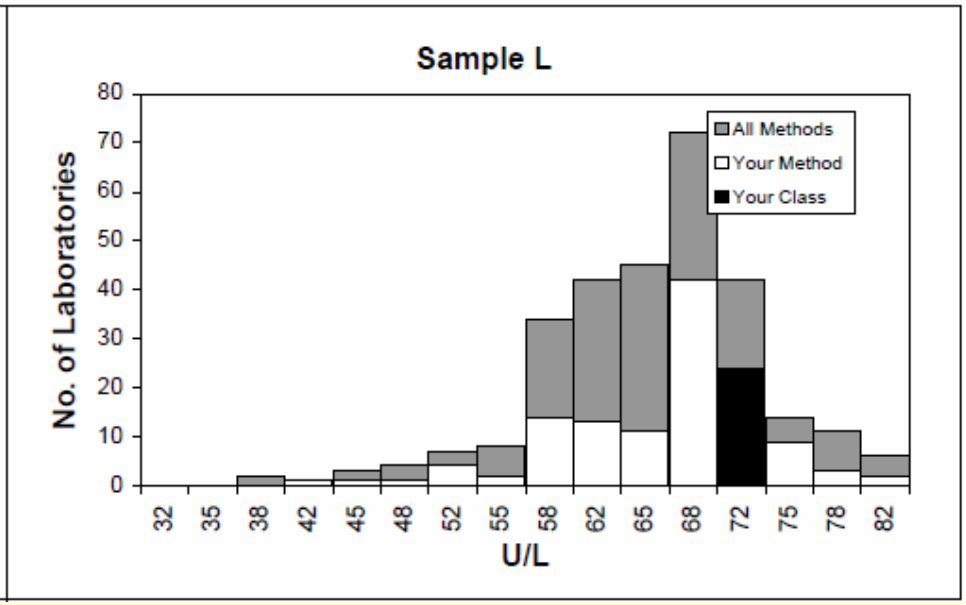




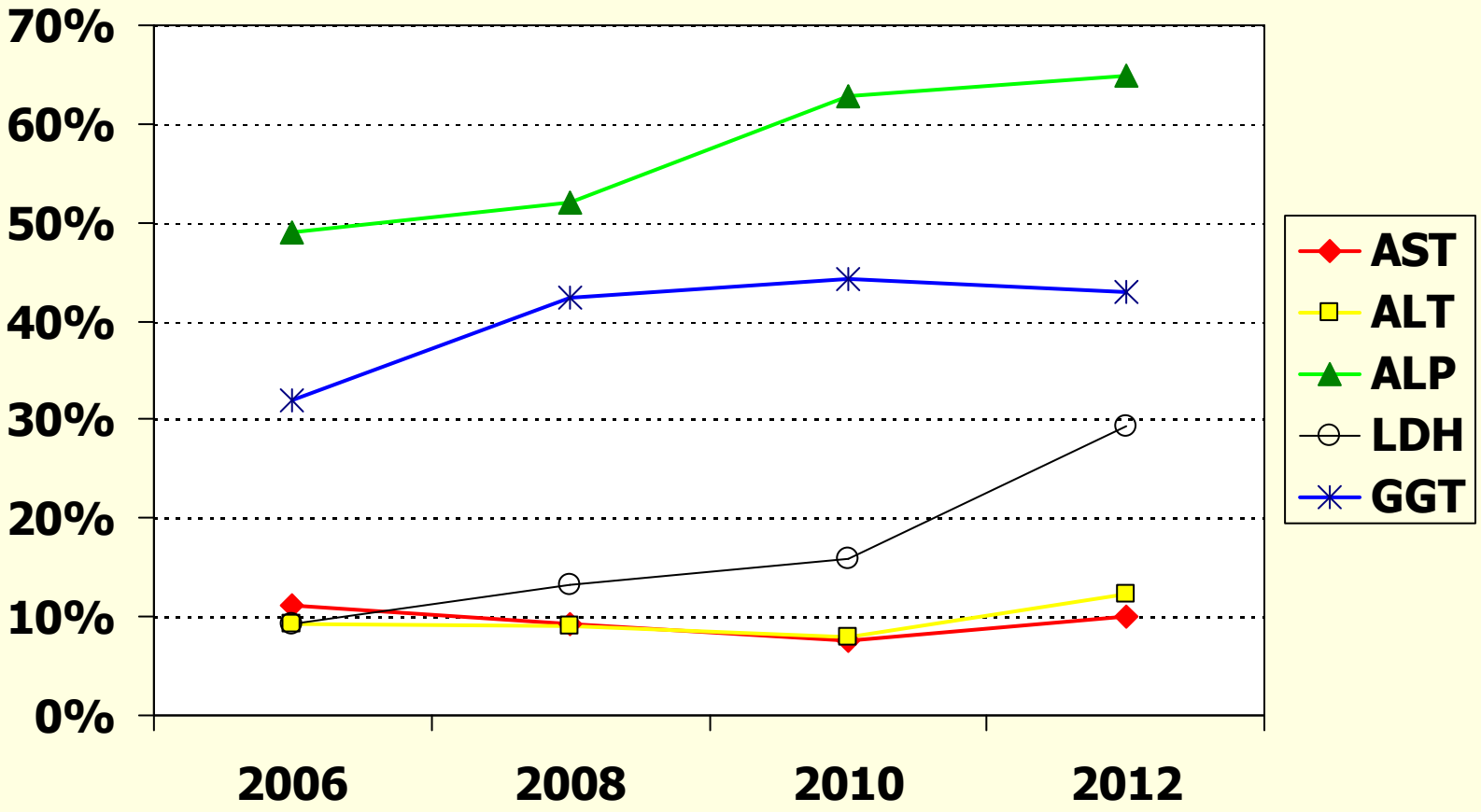
	Sample K					Sample L				
Output Group	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z
CNPG3	120 (3)	242.8	21.1	8.7%		118 (4)	65.2	7.0	10.8%	
New Etiliden PNP-G7	130 (2)	218.4	12.3	5.6%	0.1	131 (2)	67.5	5.6	8.3%	0.6
Etiliden PNP-G7 1st	5 (0)	232.0	46.0	19.8%		5 (0)	58.8	14.0	23.8%	
PNP-G7	5 (0)	243.0	12.7	5.2%		5 (0)	67.6	9.0	13.3%	
Benzyliden PNP-G7	2 (0)	220.0	12.8	5.8%		2 (0)	59.0	8.0	13.6%	
VITROS	29 (0)	143.4	13.8	9.6%		28 (1)	67.9	5.1	7.6%	

Amylase





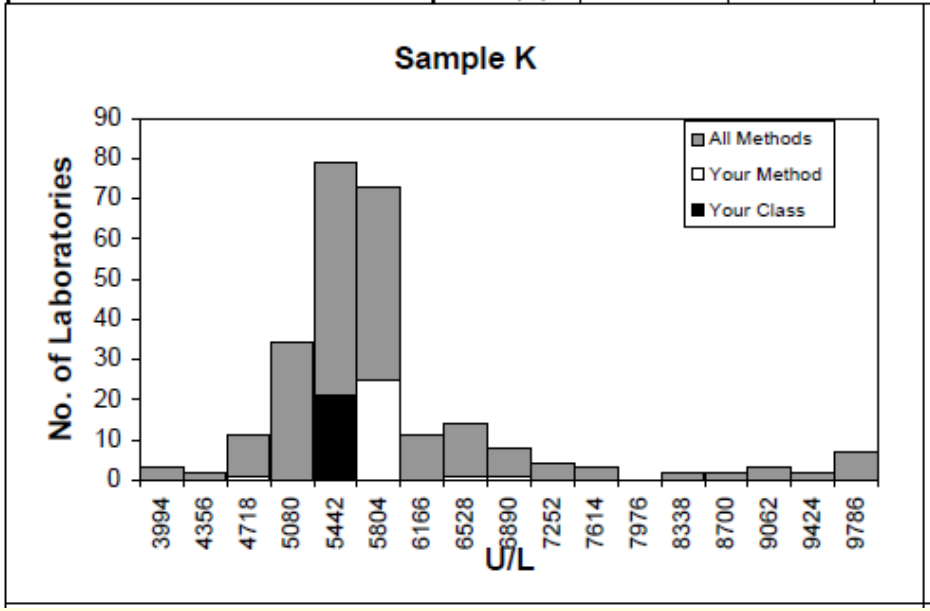
Percentage of Italian laboratories using methods traceable to IFCC reference methods

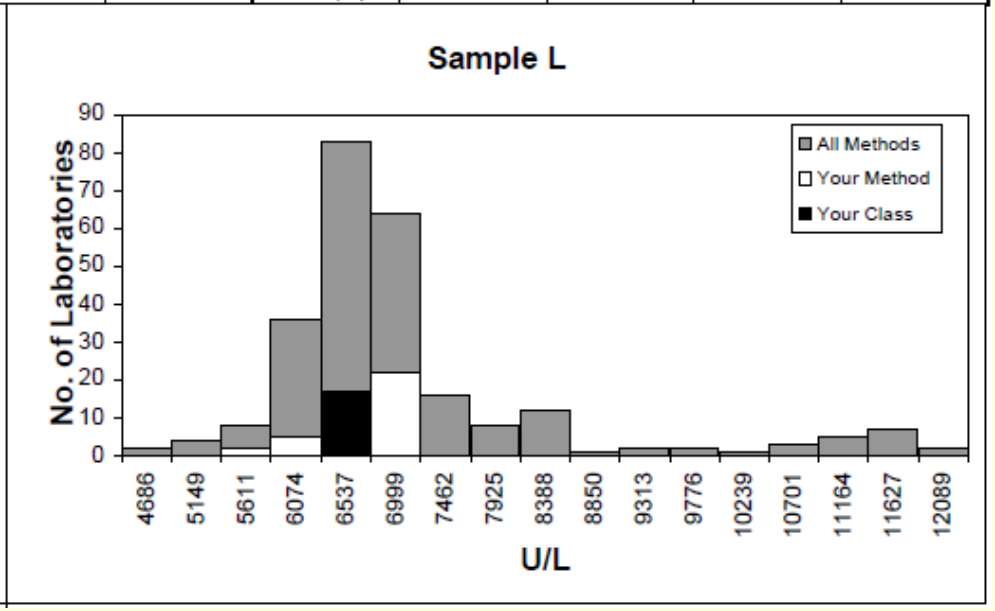


Data from PROLARIT EQAS

Cholinesterase

	Sample K					Sample L				
Output Group	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z	N (outliers)	Mean (U/L)	SD (U/L)	CV%	Your z
BTC DTNB opt	24 (0)	5909	662	11.2%		24 (0)	7086	672	9.5%	
BTC DTNB Hitachi	7 (0)	5664	113	2.0%		7 (0)	6884	121	1.8%	
DGKC '94	45 (1)	5634	607	10.8%		45 (1)	6777	706	10.4%	
BTC DTNB	61 (0)	5661	520	9.2%		61 (0)	6776	707	10.4%	
DGKC '94 Olympus	24 (0)	5417	237	4.4%		23 (0)	6496	262	4.0%	
BTC DIP	16 (1)	9567	565	5.9%		16 (0)	11394	407	3.6%	
BTC DTNB Beckman	2 (0)	6013	121	2.0%		2 (0)	7257	167	2.3%	
Choline Oxidase	10 (1)	2891	213	7.4%		10 (1)	3410	191	5.6%	
Butirrylthiocholine Hexacyanoferra	50 (1)	5660	180	3.2%	0.3	47 (3)	6725	240	3.6%	1.0
VITROS	26 (0)	5216	243	4.7%		25 (1)	6382	201	3.1%	





Why are we still not there?

- Legislation
- Industry
- Profession

Legislation

- IVD Directive 98/79 gives only generic indications on traceability (and the new version will not improve this part)
- JCTLM lists have no legal value, are just one of the possible higher order reference, not THE reference
- Accreditation programs not active in Italy and do not specifically require traceability to JCTLM reference system

Industry

- Some requirements (e.g. P5P in transaminases reagents) are more difficult to fulfill
- In absence of mandatory requirements and of clear requests from the profession they have no interest in new investments in a “mature” field
- No perception of a competitive advantage in offering IFCC traceable enzyme results
- To fulfill the request of a global market most of them continue to offer different reagents for the same enzyme

Profession

- The advantages of standardization are not fully perceived, nor by laboratorians, neither by clinicians
- Changes require efforts: new reference intervals, explanations to clinicians and patients, etc..
- Instead of requesting manufacturers to change, most of us just waits for the new proposals from industry

How to complete the standardization in clinical enzymology?

- Some ideas
  - Collaboration with clinicians to make them aware of the importance of standardized results (as for creatinine, cholesterol or HbA1c) inserting standardization requirements into clinical practice guidelines
  - New EQAS using commutable materials with reference method assigned value and evaluation of laboratory's performance as bias from the target
  - Development of common reference intervals only for standardized methods
  - Ask manufacturers to abandon old fashion methods and to promote only the standardized ones

Conclusions

- The reference measurement systems is almost complete (only ALP reference material is still missing an Lipase is on the [long] way)
- Unfortunately still only a minority of clinical laboratories is adopting standardized methods (at least in Italy)
- The challenge now is to transform a theoretical design in an real advantage for the patients' care

Thank you!