



Effect of traceability – example from a EQA provider.

Harmonization of 7 common enzymes in the Netherlands

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Harmonization 7 common enzymes in the Netherlands

Since 2012 all laboratories measure the enzymes
ALT, AST, ALP, GGT, LD, CK and α -amylase

according to

IFCC primary reference measurement procedures

Patient results are accompanied by

National IFCC reference limits (for adults)

How did we achieve this national harmonization ?



Stichting **K**waliteit **M**edische **L**aboratoria
Dutch Organisator of EQA / proficiency testing

Netherlands **R**eference laboratory for **E**nzymes

Service: IFCC reference measurement procedures for 7 common enzymes



Regional medical laboratory of the Hague
including 5 laboratories
Hospitals + general practitioners in the region

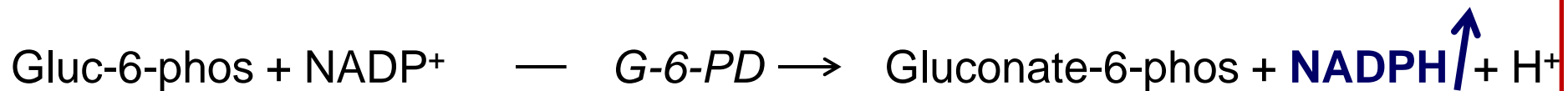
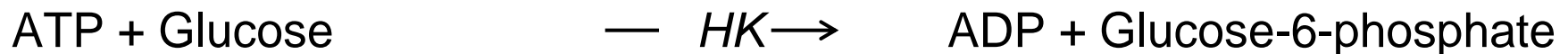
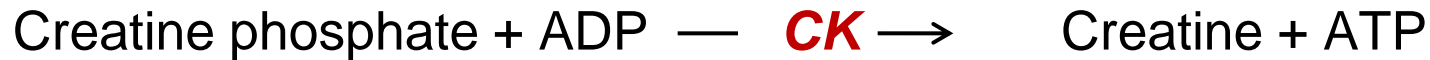
Enzymes present in organs

Enzyme	Organ
Aspartate Amino transferase (AST)	Heart and Liver
Alanine Amino transferase (ALT)	Liver
Alkaline Phosphatase (ALP)	Bile duct / Liver Bone, intestine and other tissues
Gamma Glutamyl Transferase (GGT)	Liver
Creatine kinase (CK)	Muscle. Including cardiac muscle / heart
Lactate Dehydrogenase (LD)	Heart, skeletal muscle, liver, RBC
α Amylase	Pancreas Salivary gland

Enzymes elevated in organ damage

Enzyme	Elevated in organ damage
Aspartate Amino transferase (AST)	Liver disease especially with liver cell damage Myocardial infarction,
Alanine Amino transferase (ALT)	Liver disease especially with liver cell damage
Alkaline Phosphatase (ALP)	Liver disease- biliary obstruction Osteoblastic bone disease-rickets
Gamma Glutamyl Transferase (GGT)	Liver disorder like liver cirrhosis
Creatine kinase (CK)	Myocardial infarction and Skeletal muscle disease / muscular dystrophy
Lactate Dehydrogenase (LD)	Myocardial infarction , other diseases like Liver disease and blood diseases. Hemolysis
α Amylase	Acute pancreatitis Mumps

Clinical enzymology: serial reactions CK



Raise of light absorption of NADPH at wavelength 340 nm

Catalytic activity / concentration: mol/sec = katal / l or mol/min = U / l

Influenced by:

Temp: 25 - 37°C

pH: 6,2 - 9,6

Concentration: substrates, (co) enzymes, inhibitors and ions

35 jaar IFCC standardization of enzymes

- 1980 - 1990: IFCC reference measurement procedures
ALT, AST, CK and GGT at 30°C.
No procedure for ALP, α -amylase en LD.

- 1994: IFCC Committee on Reference Systems for Enzymes (C-RSE).

37°C official incubation temperature of IFCC reference methods.

- Physiological temperature of human body
- In Vitro Diagnostics industry: 37° faster reaction / less substrate. Cheaper
- United Germany: Choice for 37°C. Before 25°C West and 37°C East.

35 jaar IFCC standardization of enzymes

- 1999 - 2009:

Research in transformation IFCC reference methodes from 30° to 37°C.

IFCC C-RSE and worldwide network of reference laboratories (n=10),

Network laboratories:

Reference institutes

University- and general hospitals, including our laboratory in the Hague

In vitro diagnostics manufacturers

35 jaar IFCC standardization of enzymes

- 2002 - 2011:

Publication new Primary IFCC reference methods 37°C with (preliminary) reference limits for normal caucasian population.

Clin. Chem. Lab. Med: Part 1 tm 9 including ALP, α -amylase and LD

- 2002 – 2016:

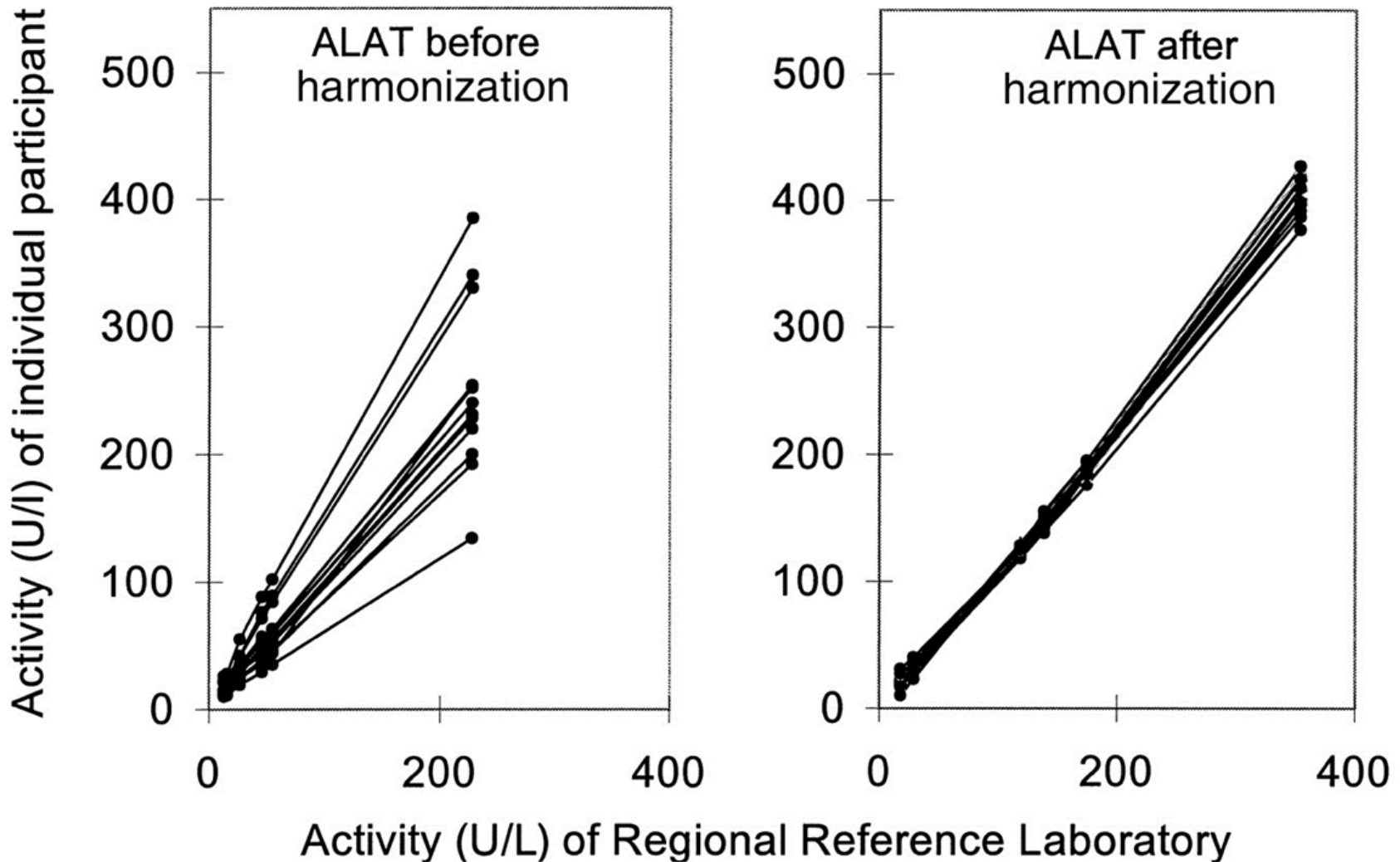
Primary Certified Reference Materials (CRM) developed and assigned with target values of IFCC reference methodes by the network

Traceability and daily practice

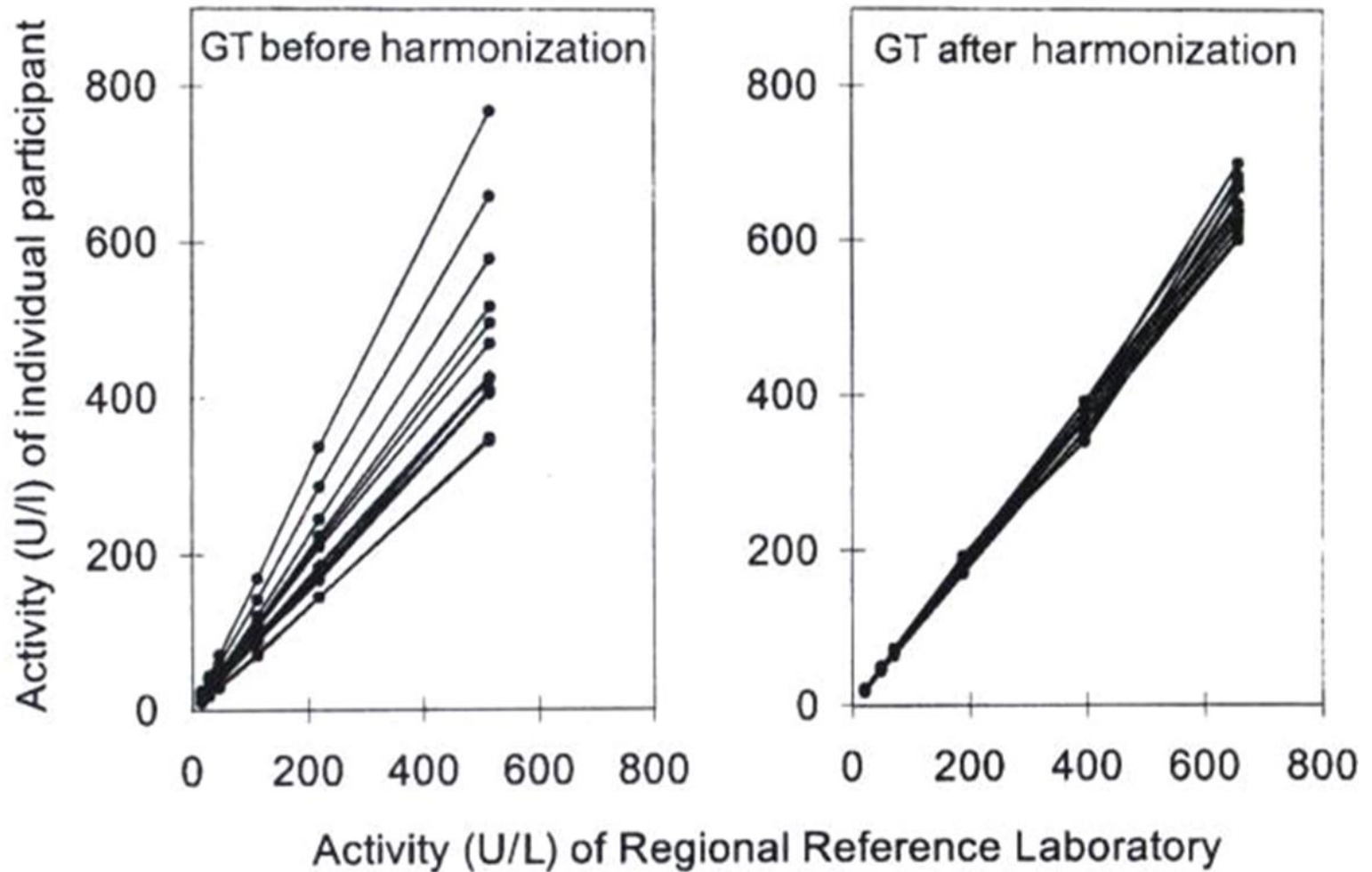
- Reference method is the golden standard but is not practical.
- Reference materials are practical.
 - Can be used on analytical device.
 - Results compared to target values assigned by reference methods.
- Patient results are *traceable* to reference methods.

Deviation of ALT results in 1996

Laboratories in the Hague and Leiden
6 patient pool sera



Deviation of GGT results in 1996



From "Multicenter harmonization of common enzyme results by fresh patient-pool sera."
Paul Franck, et al. Clinical Chemistry 44:3 614-621 1998

Harmonization versus Standardization

- **Harmonization:**

comparable, reproduceable, precise results

but not yet

- **Standardization:**

comparable, reproduceable, precise **TRUE** results



Certified Reference Materials (CRM)

- Primary CRM / standards:

IRMM and IFCC (C-RSE and network of reference laboratories)



- Secondary standards, calibrators and proficiency materials

- Calibrators for analytical devices produced by diagnostica industry
- Verifiers like  Enzyme Trueness vericator . Checking calibration
- EQA / PT materials like  General Chemistry EQA scheme

Certified Reference Materials

Name EC number	Primary CRM IFCC / IRMM IFCC target value	Organ tissue or Human type Enzyme Recombinant E.coli in BSA	Trueness Verificator SKML IFCC target value	Human Pool Serum spiked with Human type Enzyme Recombinant E.Coli
ALT Alanine Aminotransferase EC 2.6.1.2	ERM AD 453k / IFCC 103,8 U/l	Recombinant Human Liver	Batch 2011.237 183 U/l	Recombinant Human Liver
ALP Alkaline Phosphatase EC 3.1.3.1	Under developement		Batch 2011.237 242 U/l	Recombinant Human Liver
α - amylase Alfa Amylase EC 3.2.1.1	IRRM-IFCC AD 456 546 U/l	Human pancreas α – amylase isoenzyme	Batch 2011.237 297 U/l	Recombinant Human Pancreas Human Saliva
AST Aspartate Aminotransferase EC 2.6.1.1	ERM AD 457 / IFCC 104,6 U/l	Recombinant Human Liver	Batch 2011.237 128 U/l	Recombinant Human Liver
CK Creatine Kinase EC 2.7.3.2	ERM AD 455k / IFCC 314 U/l	Recombinant Human Muscle MM isoenzyme	Batch 2011.237 394 U/l	Recombinant Human Muscle
GGT Gamma Glutamyltransferase EC 2.3.2.2	ERD AD 452 / IFCC 114,1 U/l	Pig kidney	Batch 2011.237 159 U/l	Recombinant Human Liver
LD Lactate Dehydrogenase EC 1.1.1.27	ERM AD 453k / IFCC 330 U/l	Recombinant Human erythrocyte LD1 iso enzyme.	Batch 2011.237 701 U/l	Human erythrocyte .

Commutable stable proficiency materials

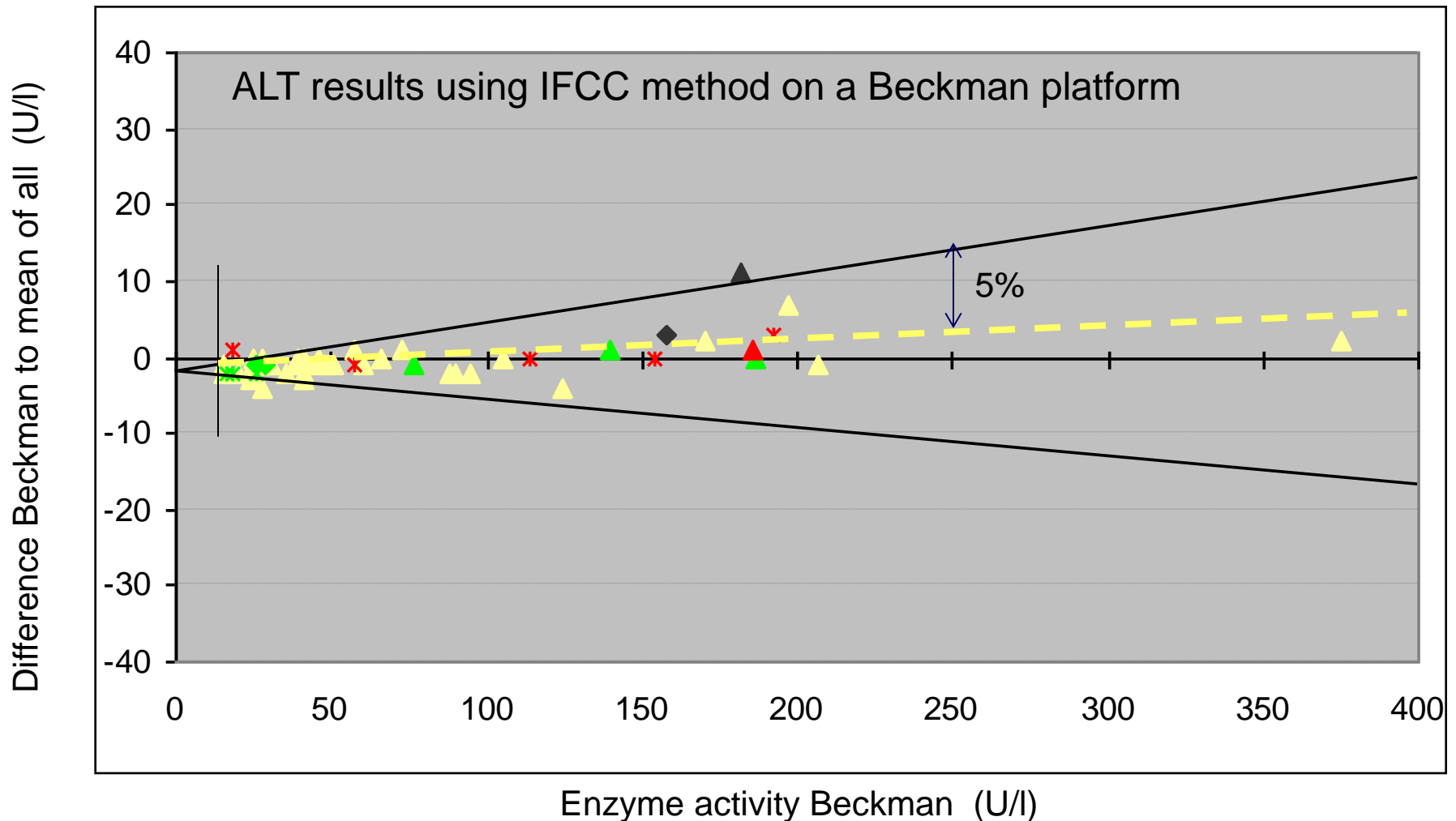
- Perform like patient samples.

Guideline CLSI EP30-A: Prerequisites for commutability

- SKML: Fresh frozen patient pool sera
 - Stable at -70°C
 - No additives.
 - Samples covering the clinical relevant range
 - Spiked with human recombinant enzymes
 - Target values assigned by IFCC reference method

Commutability EQA samples and Reference Materials

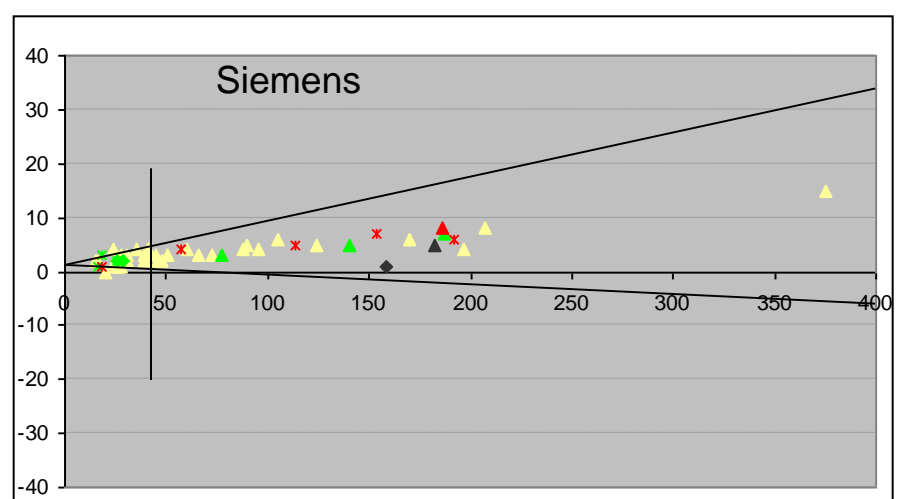
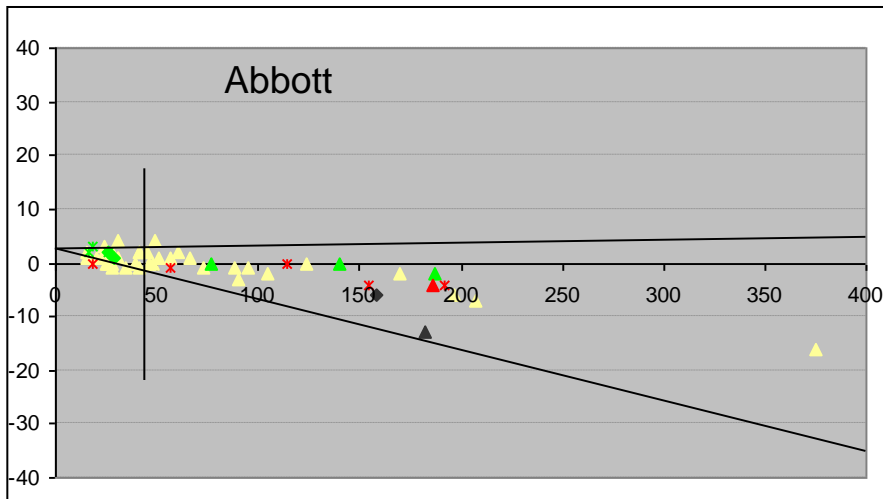
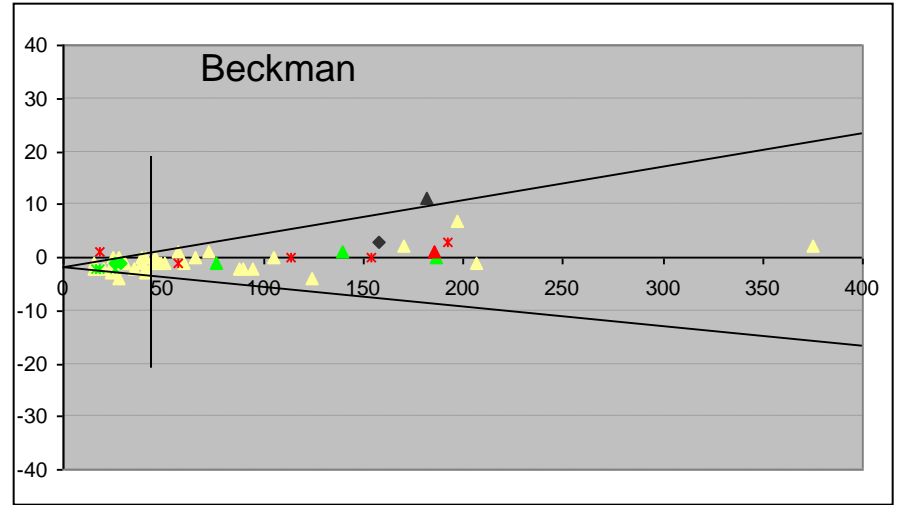
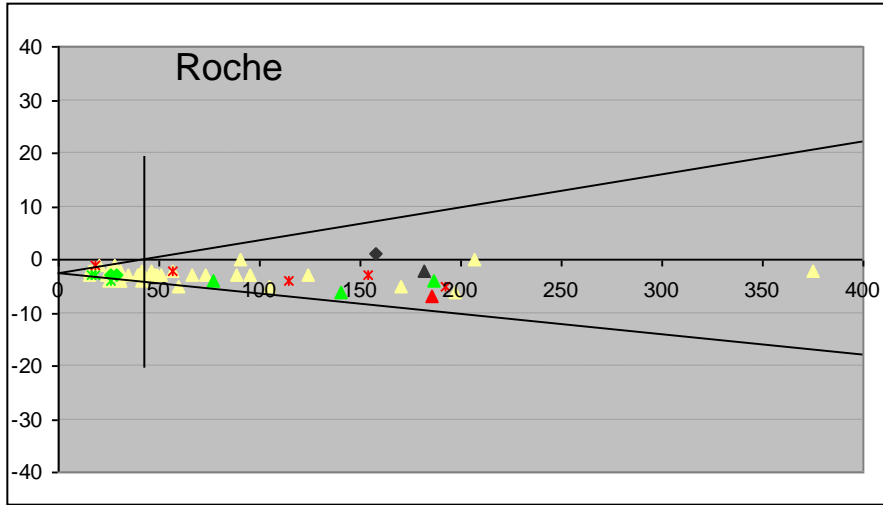
- ▲ Patient Sera ◆ Poolserum ▲ Spiked Single Serum all enzymes ✖ EQA samples
▲ IRMM Ref. Material ◆ Asahi Kasei Ref. Material ▲ Trueness Verificator



Commutability for ALT with 4 Manufacturers

▲ Patient Sera ◆ Poolserum ▲ Spiked Single Serum all enzymes ✖ EQA samples

▲ IRMM Ref. Material ◆ Asahi Kasei Ref. Material ▲ Trueness Verificator

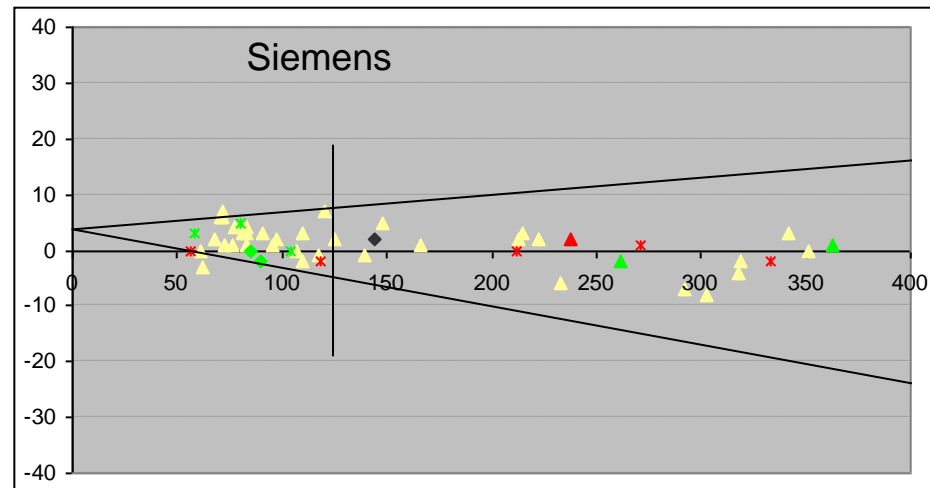
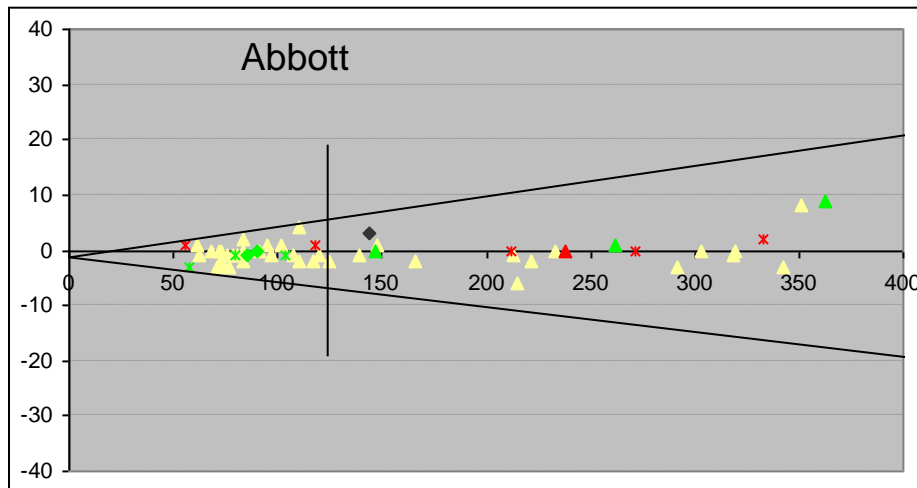
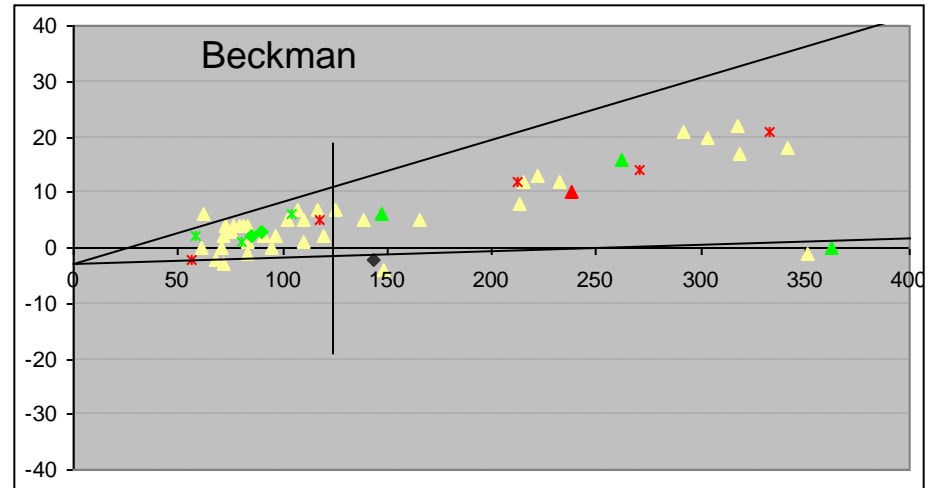
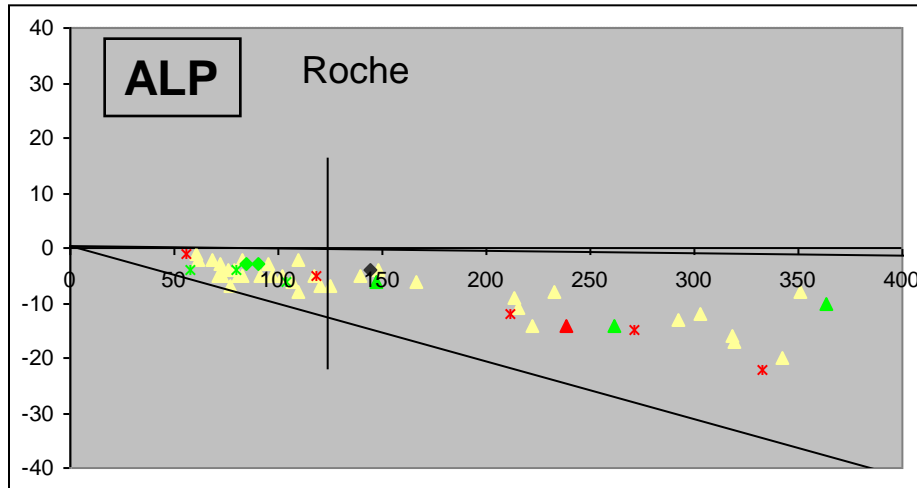


Commutability for ALP with 4 Manufacturers

▲ Patient Sera ◆ Poolserum ▲ Spiked Single Serum all enzymes ✕ EQA samples

◆ Asahi Kasei Ref. Material

▲ Trueness Verificator



IFCC reference method harmonization / standardization

SKML EQA / PT results enzymes in 2012-2013

<u>Expressed as:</u>	Overall	ALT	AST	Amyl	ALP	CK	GGT	LD
Compliance %	95%	97	98	87	96	96	96	97
Recovery %	99%	100	102	99	96	100	98	98
Inter-lab CV %	4%	4	4	4	6	5	4	3
Clinically Allowable Total Error %		28	17	16	12	30	22	11

IFCC (preliminary) patient reference limits

Name EC number	IFCC Reference Methods	IFCC Reference Upper limit U/l Caucasian population	the Hague / Leiden Upper limit U/l Harmonization 1998
ALT Alanine Aminotransferase EC 2.6.1.2	Clin Chem Lab Med. 2002; 40(7) :718-724.	F < 34 M < 45	F < 42 M < 46
ALP Alkali n Phosphatase EC 3.1.3.1	Clin Chem Lab Med. 2011 ; 49(9): 1439-46	F < 98 M < 115	F < 120 M < 124
α - amylase Alfa Amylase EC 3.2.1.1	Clin Chem Lab Med. 2006; 44(9): 1146-55.	F < 107 M < 107	
AST Aspartate Aminotransferase EC 2.6.1.1	Clin Chem Lab Med. 2002 ; 40(7): 25-733.	F < 31 M < 35	F < 38 M < 43
CK Creatine Kinase EC 2.7.3.2	Clin Chem Lab Med. 2002; 40(6): 635-642.	F < 145 M < 171	F < 168 M < 200
GGT Gamma Glutamyltransferase EC 2.3.2.2	Clin Chem Lab Med. 2002; 40(7): 734-738.	F < 38 M < 55	F < 35 M < 50
LD Lactate Dehydrogenase EC 1.1.1.27	Clin Chem Lab Med. 2002; 40(6): 643-648.	F < 247 M < 248	


Summary

- **Reference method:** conclusively accepted by international committee

- **Reference materials:**

Primary CRM / standards for reference methods. Commutable?

Secondary standards and proficiency materials. Commutable

- Calibrators of in vitro diagnostics industry
- Trueness verifiers for checking calibration
- EQA proficiency materials like  General Chemistry EQA scheme
Results are compared to target values assigned by reference methods.

- **Patient results:** *traceable chain* from reference limits to reference methods



The End

Thank you for your attention