IFCC Standardization of Enzyme Measurements: Assessment of the Current Situation

IXth Czech National Congress of Clinical Biochemistry

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MHH Medizinische Hochschule Hannover

Area of the Medical University Hannover

Reference Systems for Enzymes

Essential components

- ⇨ Primary reference measurement procedures (IFCC / C-RSE)
- ⇨ Certified reference materials (IRMM)
- ⇨ Official accreditation for reference laboratories (BIPM)
- ⇨ International ring trials for reference laboratories (IFCC / DGKL)
- ⇨ Common reference intervals and decision limits (IFCC / C-RIDL)
- ⇨ Network of reference laboratories (IFCC / C-RSE)

Objectives

- Traceability - how to verify, how to control?
- IFCC - ALP
- IFCC - Lipase

In-vitro-diagnostic Medical Devices (IVD)

Directive 98/79/EC of the European parliament

Appendix 1

General Requirements

Traceability of calibration materials and **control materials** has to be assured by reference measurement procedures and reference materials of higher order.

Time frame

Dec. 1998:	Directive published
Dec. 2003:	End of transition period
Dec. 2005:	**End of transition for putting into service**

Traceability and international standardization

International standardization

= improved inter-laboratory comparability

Traceability

= links to the components of a reference system

= stable relation of results (with and without pathological findings)

= reliable decision limits

= definitive reference intervals

Traceability - how to verify since 2006?

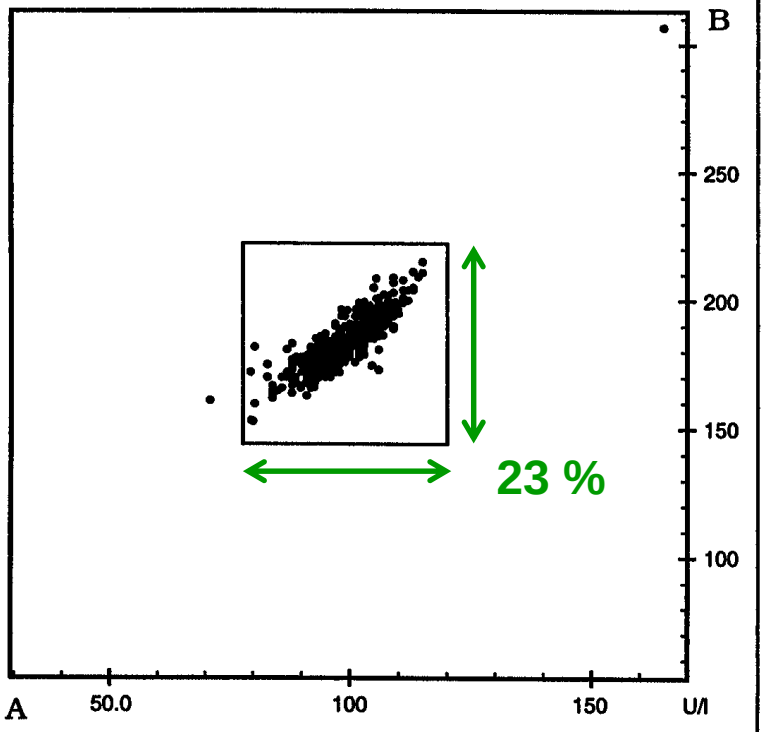
Internal quality control → 100 % recovery

- Manufacturer of a CE-labeled test kit shall have complete responsibility for traceability.

External quality assessment → certificate

- Limits of acceptance are wide
- Commutability of the control material is not guaranteed

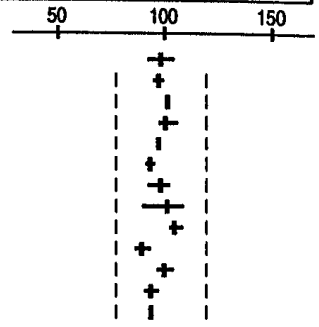
Analyt **AST (GOT)**
Methode **Alle Methoden**



Teilnehmerzahl	631	
Probe/Einheit	A	U/l B
Mittelwert	98.8	183
Standardabweichung	6.81	10.2
Variationskoeffizient	6.90	5.56

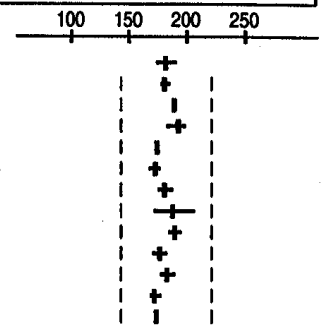
Probe A (RMW = 98.6 U/l)

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		631	71.0	92.8	99.0	105	165
1	4	23	92.0	95.3	98.0	100	107
1	12	6	80.3		102		110
1	13	32	94.0	98.0	101	107	115
1	16	6	92.7		98.0		105
1	28	44	88.0	91.7	94.0	96.0	111
1	30	308	80.4	92.8	99.0	103	165
1	34	11	80.0	90.1	102	109	113
1	38	93	79.6	103	105	109	115
1	40	22	83.0	86.7	90.0	94.0	94.0
1	106	31	94.0	97.0	101	105	109
1	128	31	84.0	91.1	94.3	97.9	99.0
1	228	6	88.0		94.5		97.0



Probe B (RMW = 183 U/l)

M	Kit	N	Min	16.P	50.P	84.P	Max
Alle		631	19.0	175	183	192	307
1	4	23	172	178	182	186	197
1	12	6	160		191		199
1	13	32	177	183	194	200	211
1	16	6	166		176		205
1	28	44	163	168	174	178	208
1	30	308	19.0	176	182	189	307
1	34	11	153	172	189	207	211
1	38	93	153	185	191	196	215
1	40	22	162	171	178	184	186
1	106	31	174	178	184	191	199
1	128	31	165	169	173	179	181
1	228	6	169		175		183

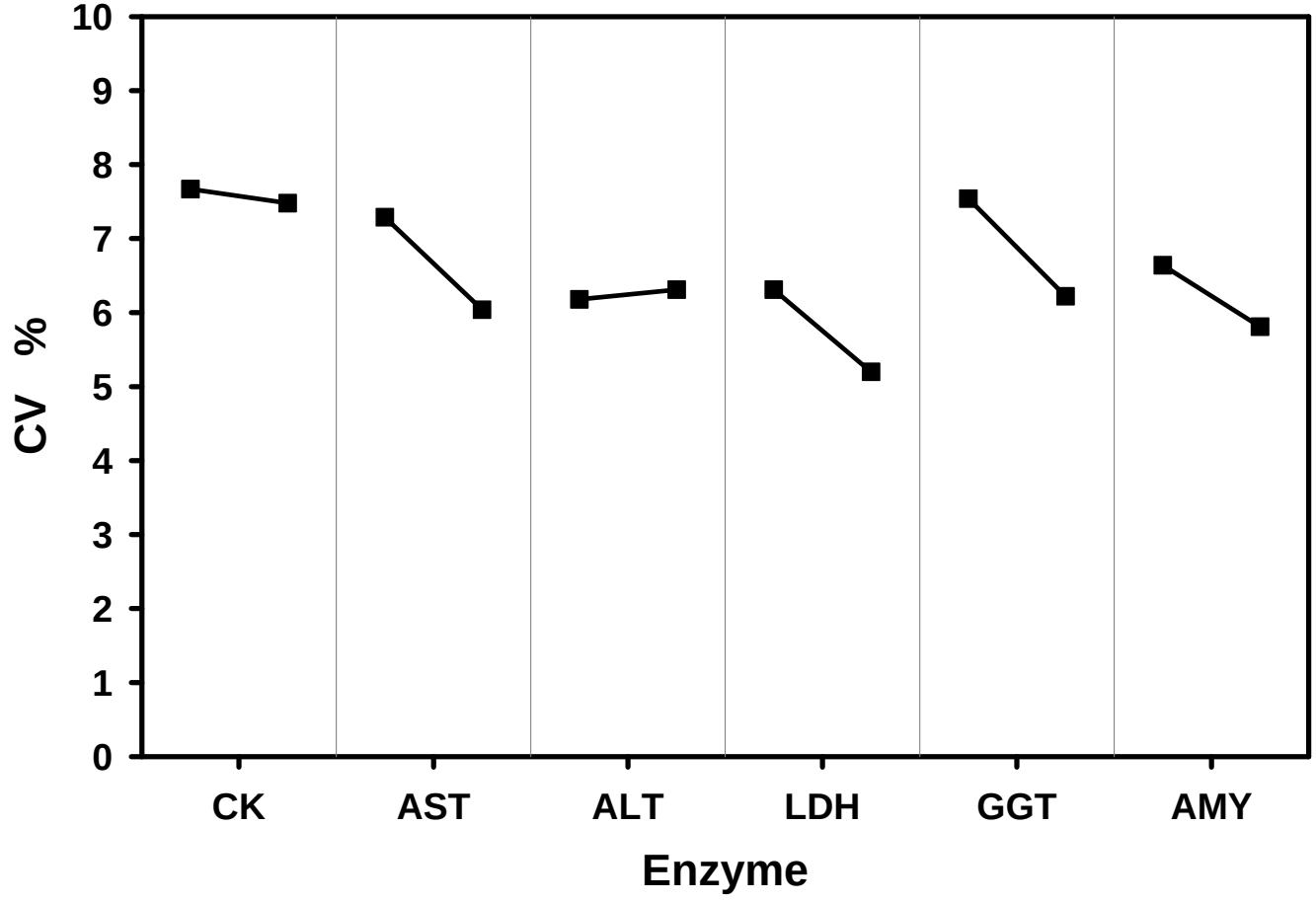


Andere Kits (Anzahl):
1 07(3), 1 20(3), 1 26(2), 1 37(3), 1 39(2), 1 55(1), 1 99(2), 1 108(2),

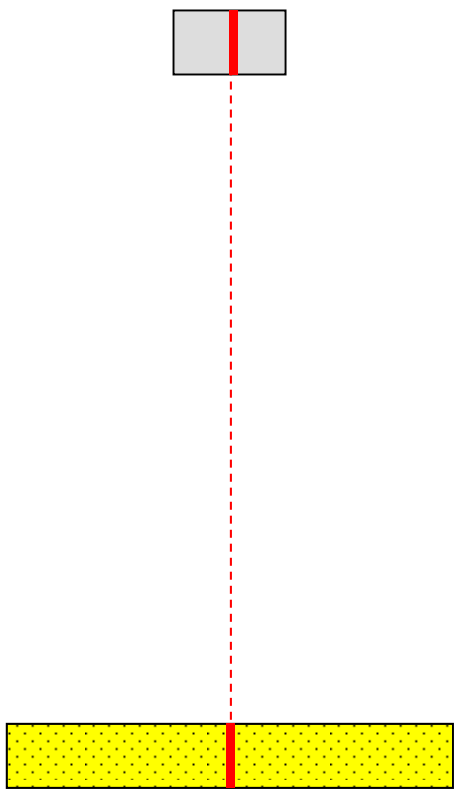
External Quality Assessment

External Quality Assessment

(2006 versus 2008/2009)



Traceability / Uncertainty



True value (human serum)

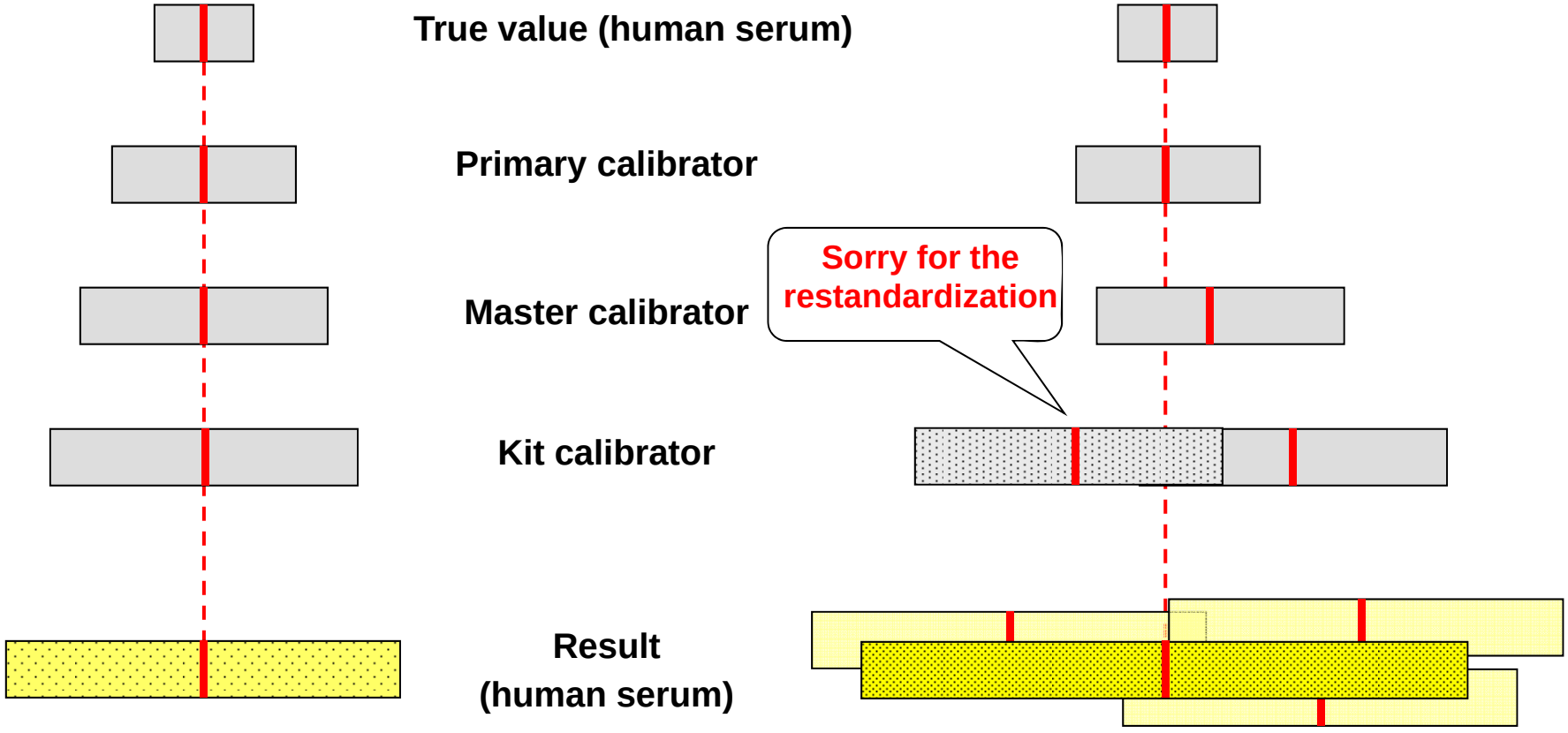
>REFERENCE METHODOLOGY<

Result (human serum)

>ROUTINE LABORATORY<

ideal

Traceability / Uncertainty



ideal

real

Problems with processed, lyophilized calibrators in the calibration hierarchie for enzyme measurements

Very often non-commutable

Secondary Reference Material:

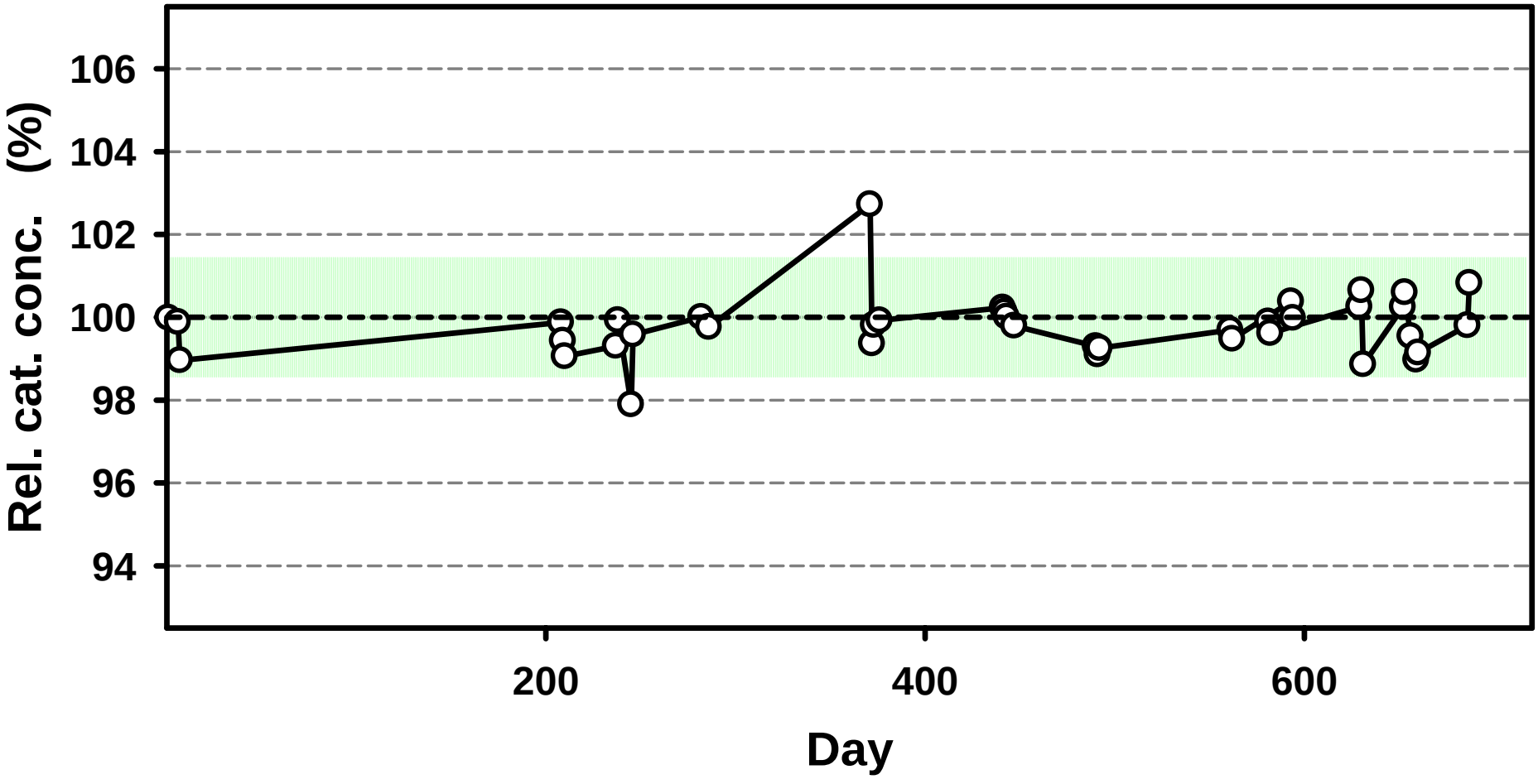
= Pooled human sera

- Collection of human sera
- Tailored target concentrations
- Standardized freezing and thawing of the specimens
- 1 ml aliquots stored below - 75 °C
- Determination of the reference method value

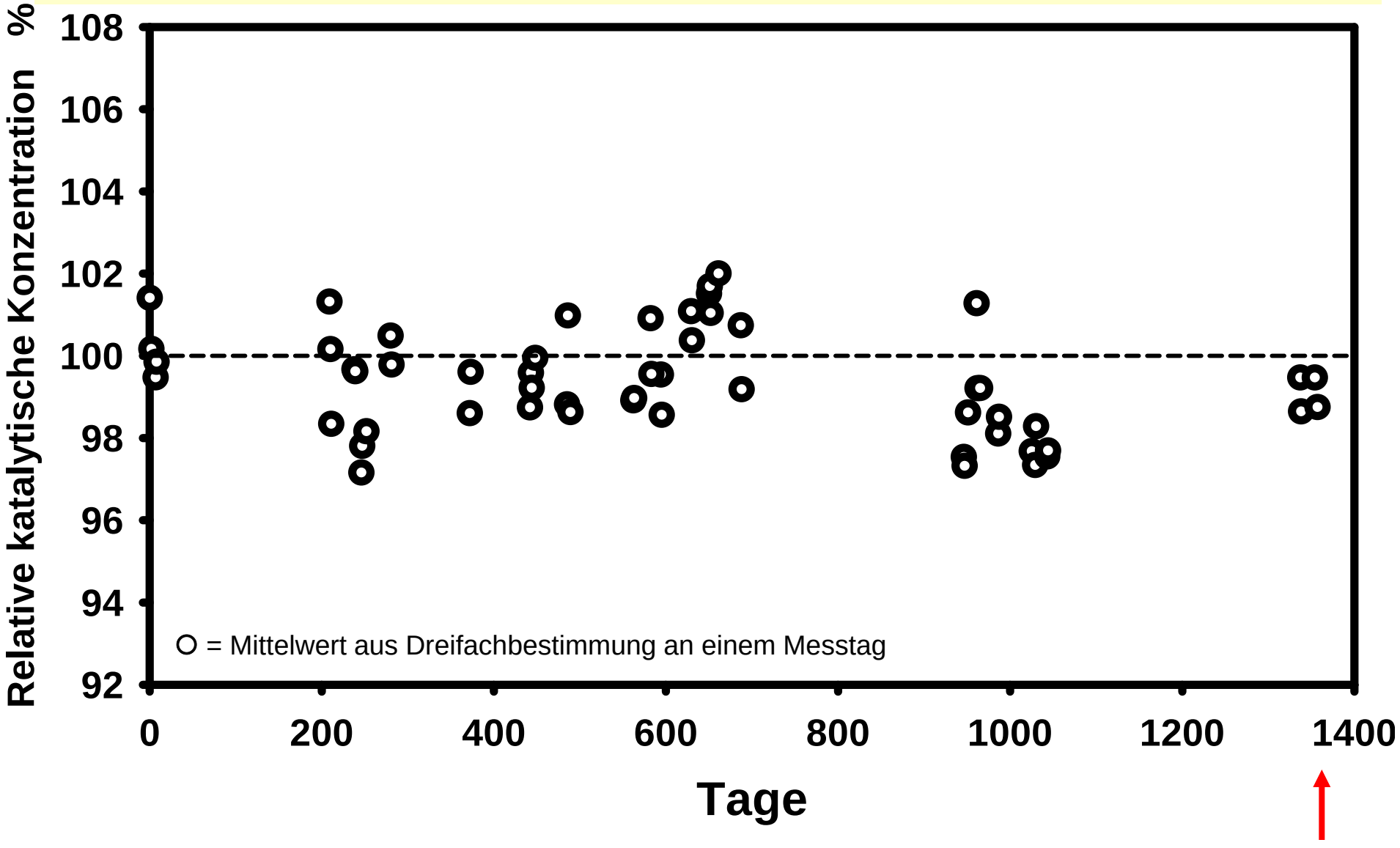
Considerations for the composition of pooled sera

- **The more single sera in the pool the better**
- **Exclusion of sera with intensiv lipemia, hemolysis and bilirubinemia**
- **Exclusion of sera with elevated concentration of monoclonal immunoglobulins**
- **Avoid unusual composition of isoenzymes (e.g. CK-BB)**
- **No high concentration of the “wrong” isoenzyme (e.g. salivary α-amylase)**

Stability of the secondary reference materials (example: AST)



Time course of the relative catalytic concentration of AST in pooled human serum



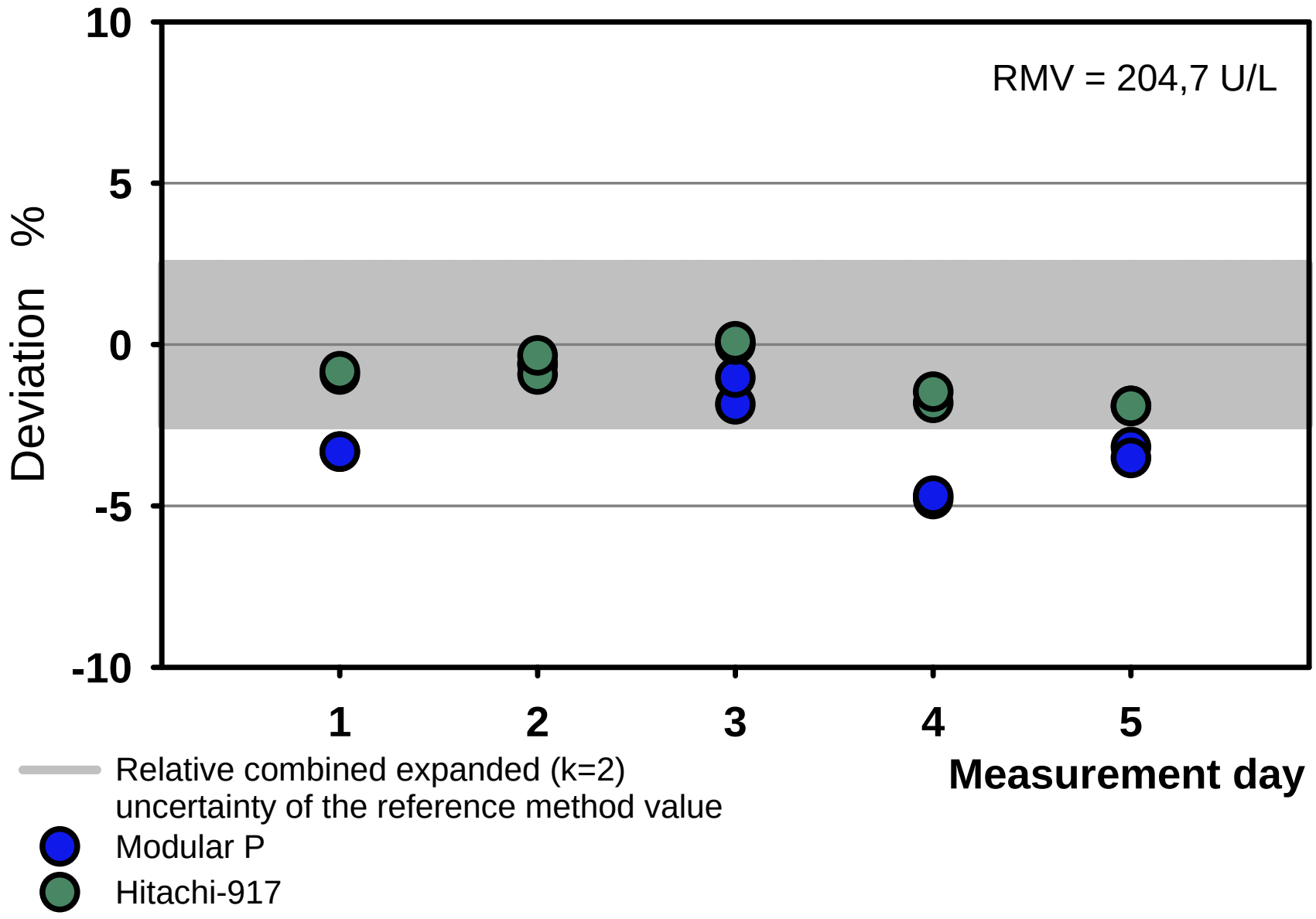
Control material:

Pooled human sera with certified RMVs

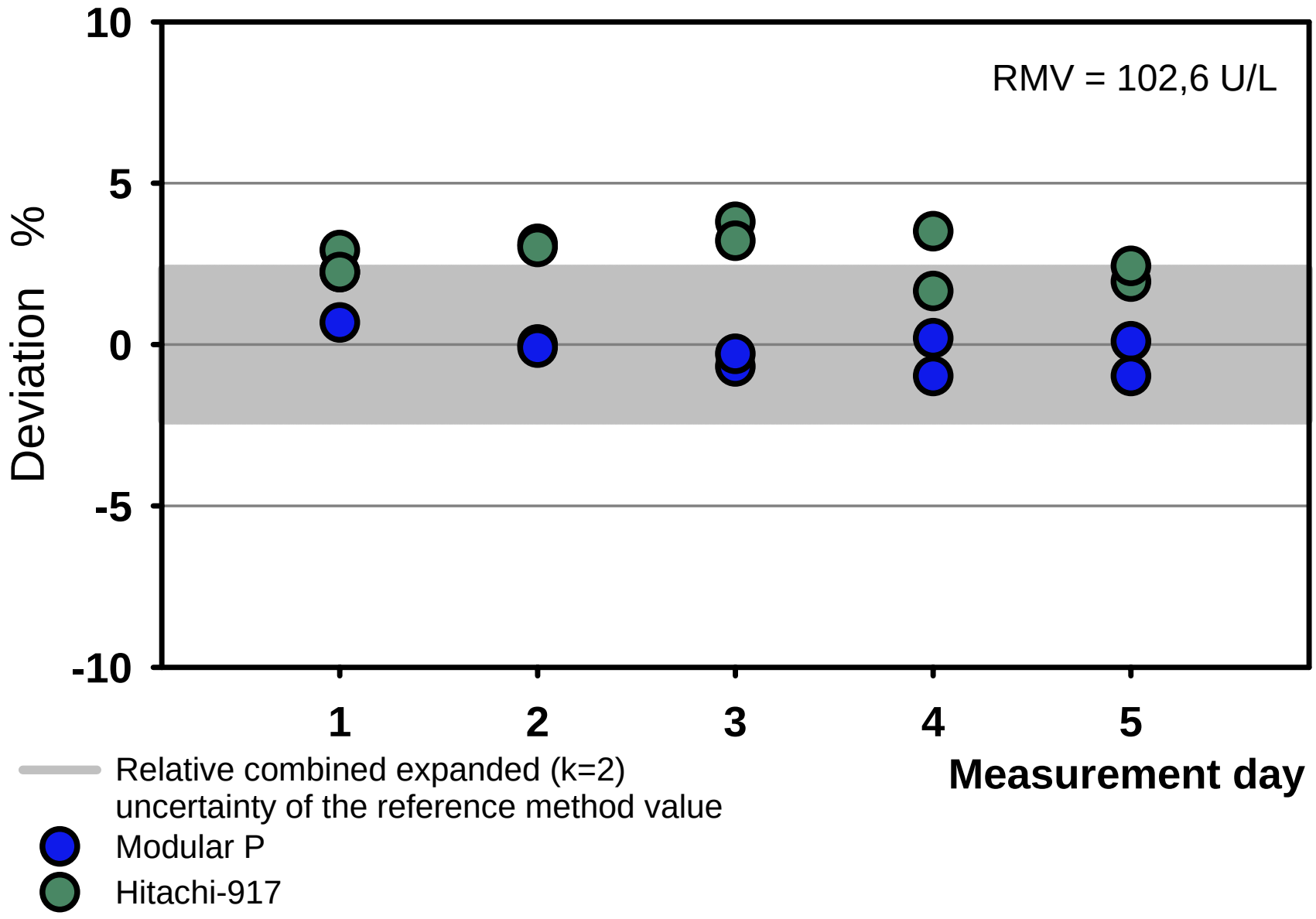
applied to routine procedures from Roche Diagnostics (Modular, Hitachi 917)

CK (U/L)	45,7	101,1	204,7	975,8				
GGT (U/L)	18,7	51,1	102,6	179,2	277,9	521,2		
AMY (U/L)	24,1	121,1	601,7					
ALT (U/L)	14,9	43,9	108,0	192,4	299,6	677,2		
AST U(/L)	16,6	38,1	109,5	172,9	264,2			
LDH (U/L)	121,5	138,8	195,6	279,4	286,8	404,1	509,2	545,0
ALP (U/L)	72,6	237,1	639,3					

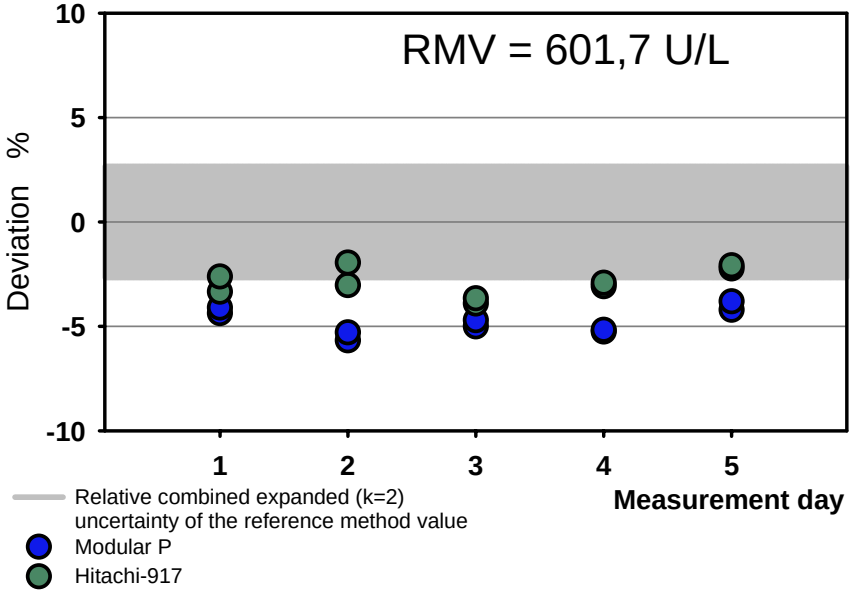
CK in lot 6, level 3: deviation from the reference value



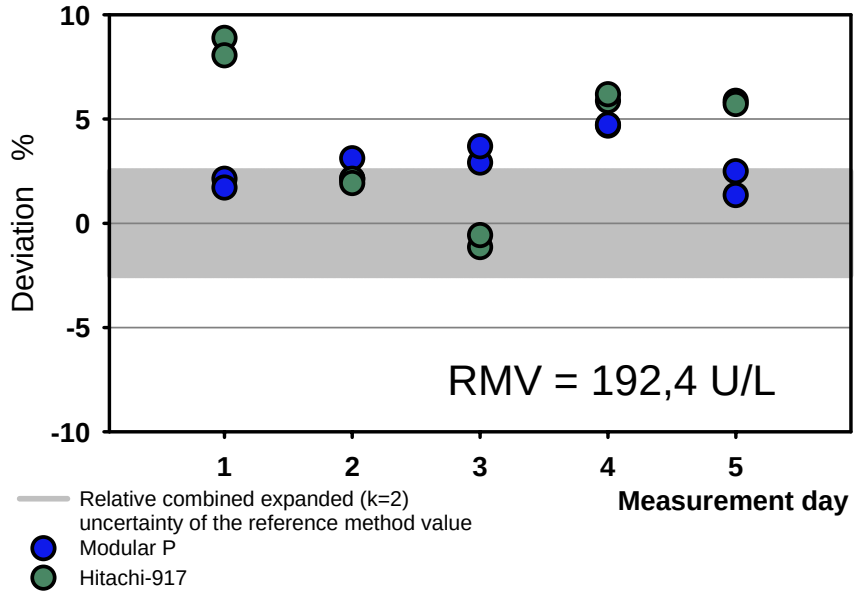
GGT in lot 6, level 3: deviation from the reference value



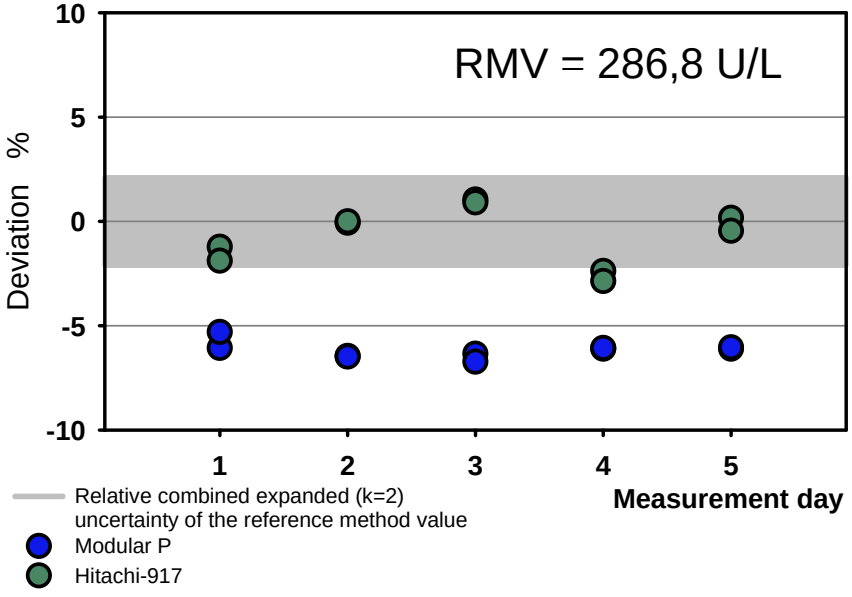
Amylase in lot 5, level 3: deviation from the reference value



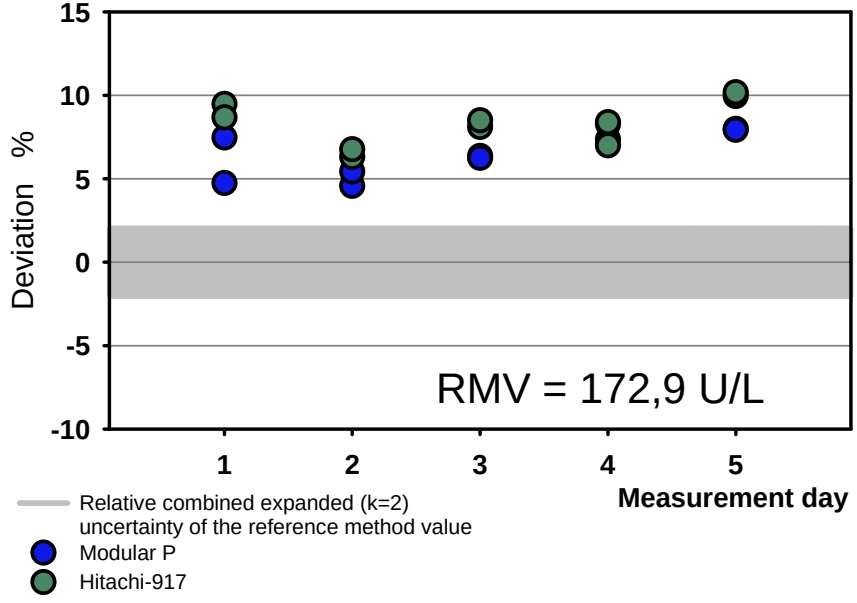
ALT in lot 6, level 4: deviation from the reference value



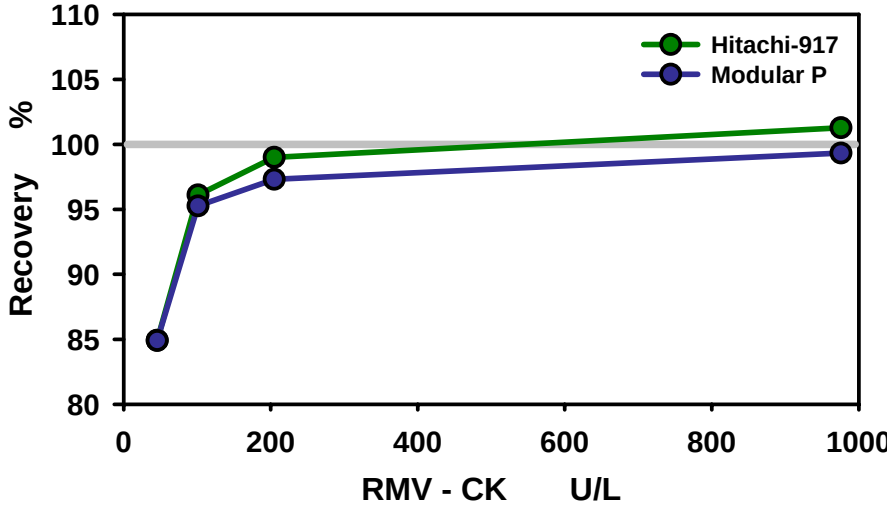
LDH in lot 7, level 3: deviation from the reference value

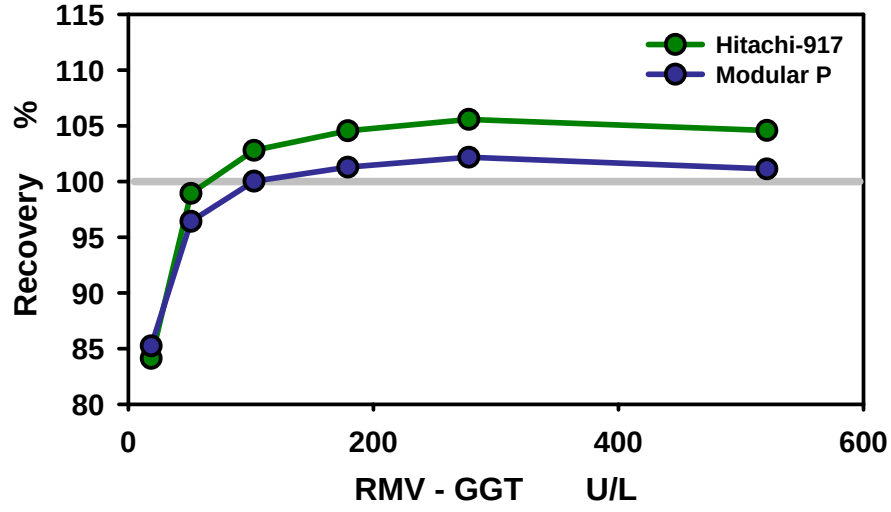


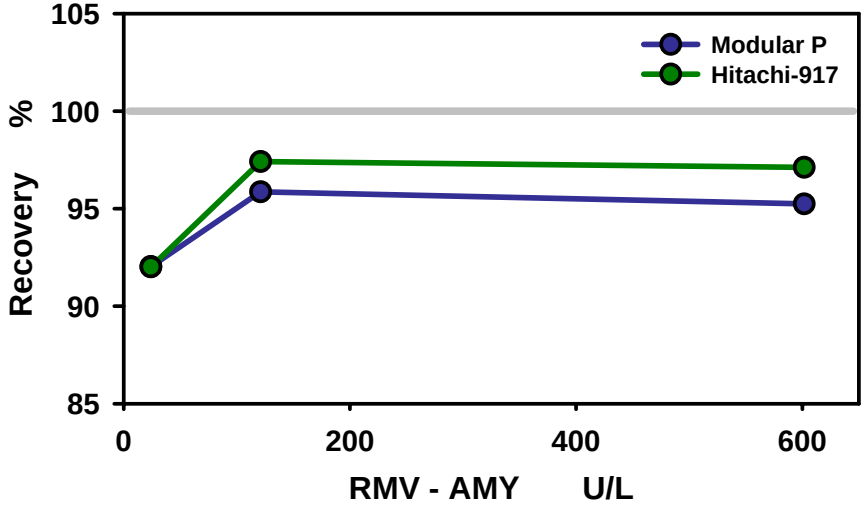
AST in lot 6, level 4: deviation from the reference value

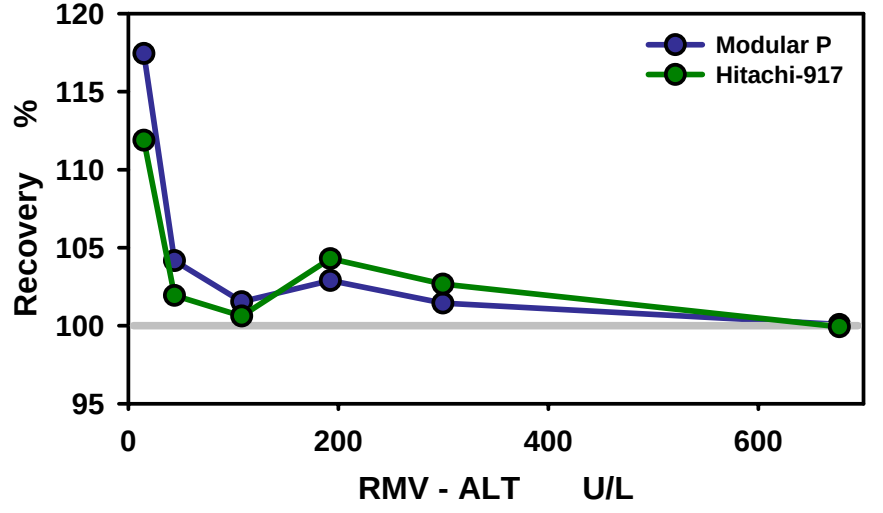


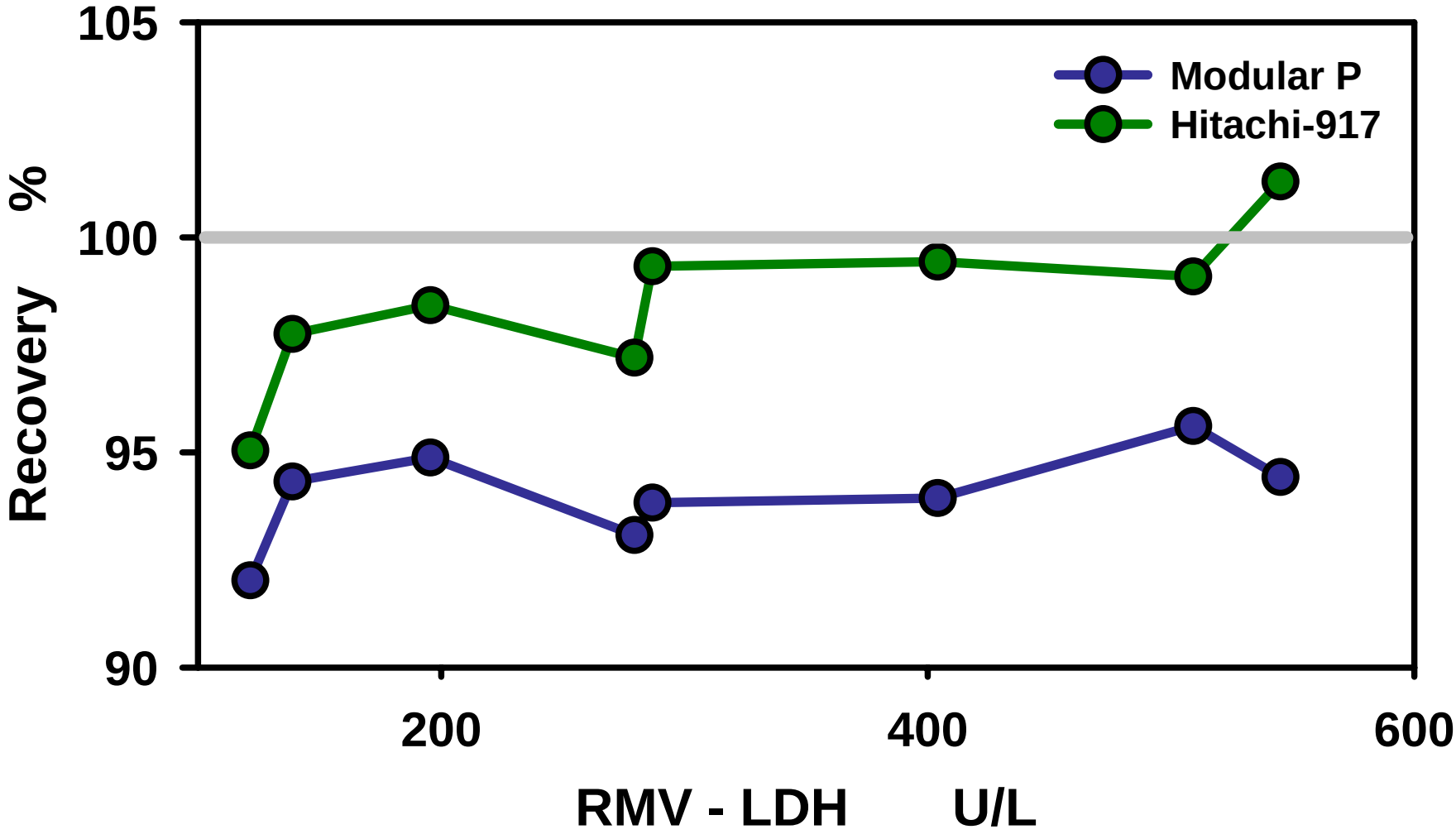
Recovery experiments: 5 measurements days, 3-6 pooled sera

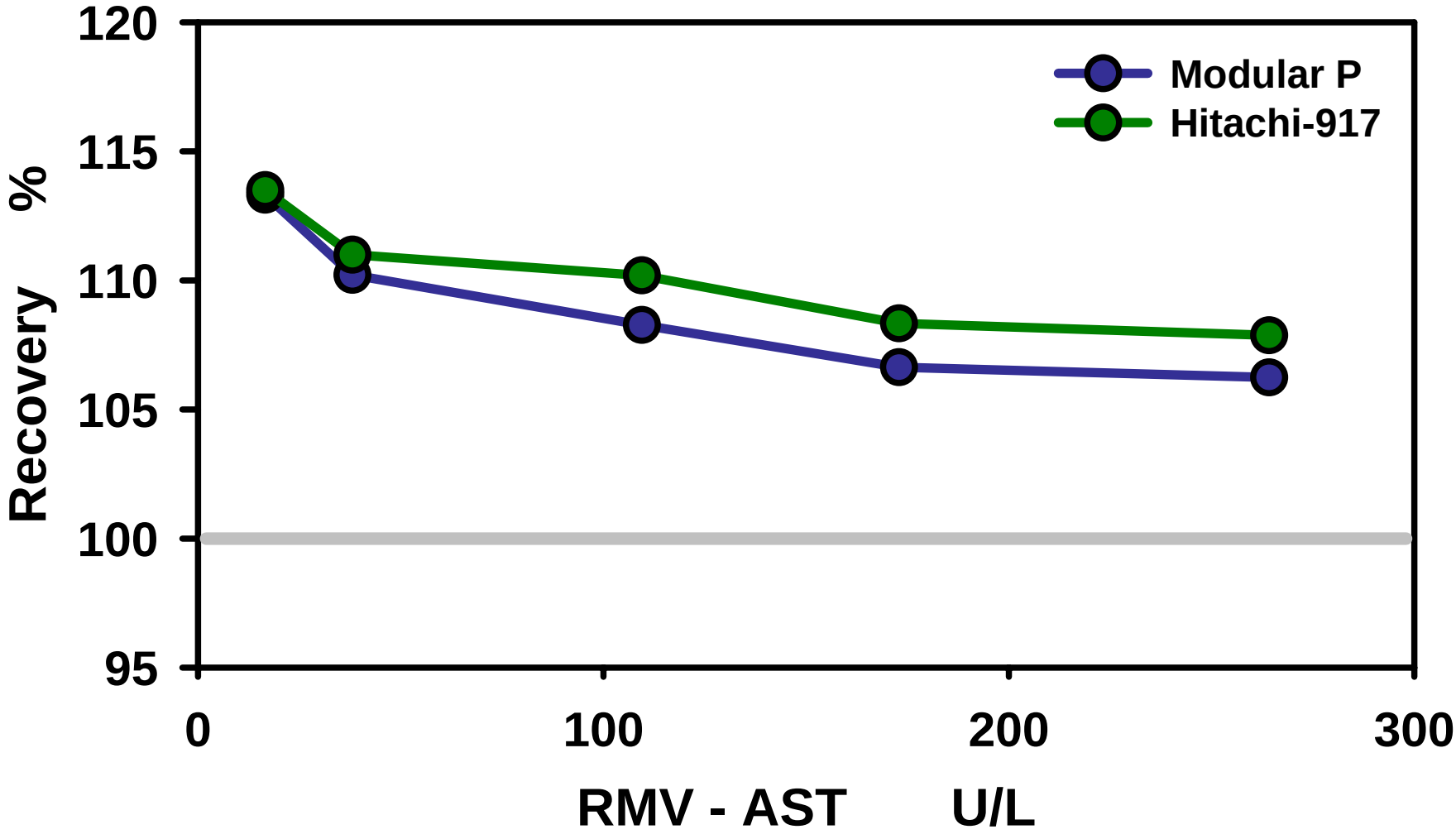


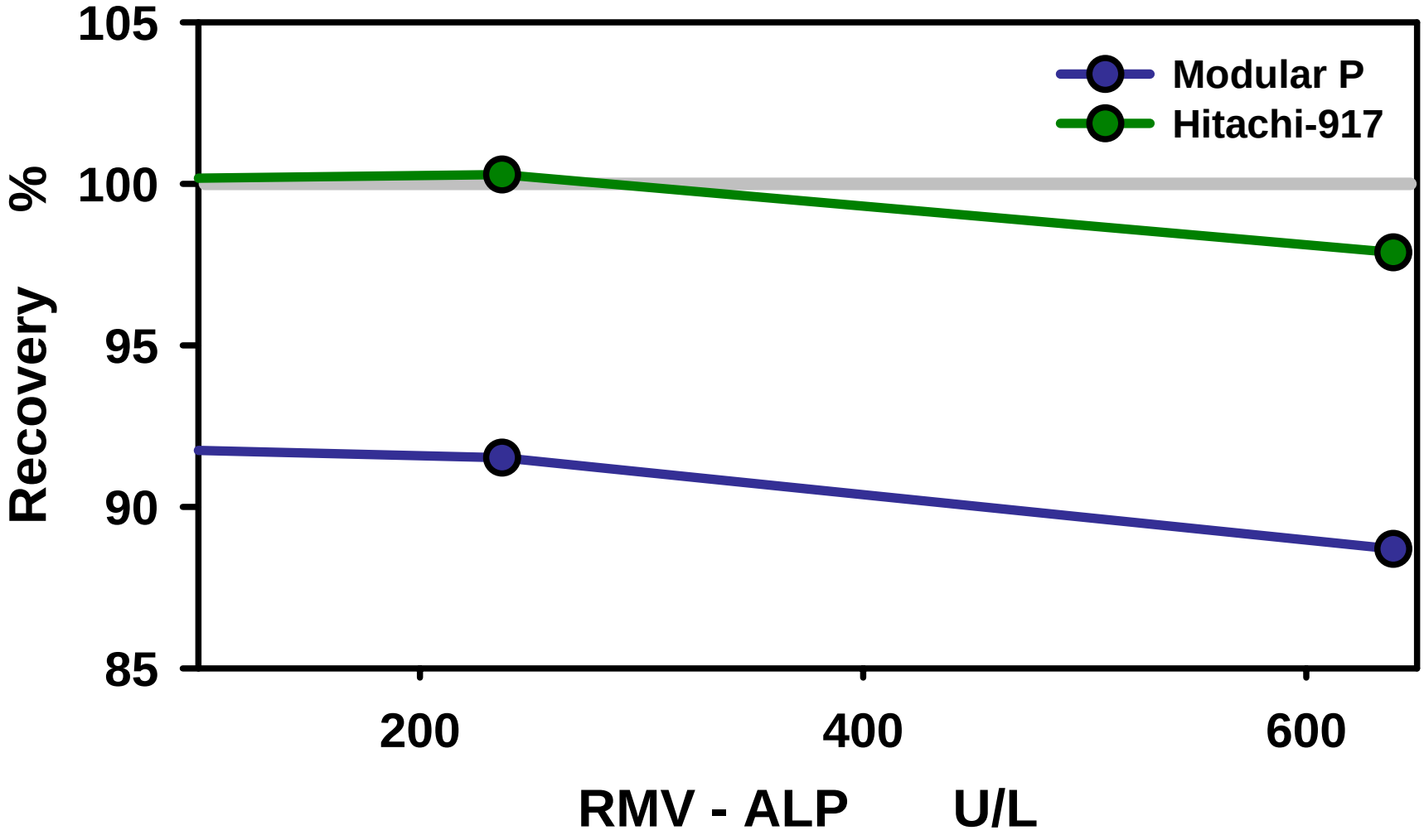







105
100
95
90
Recovery %
Modular P
Hitachi-917
200
400
600
RMV - LDH U/L


120
115
110
105
100
95
Recovery %
Modular P
Hitachi-917
0
100
200
300
RMV - AST U/L


105
100
95
90
85
Recovery %
Modular P
Hitachi-917
200
400
600
RMV - ALP U/L

Allowable bias considering intra individual and inter individual biological variation

S-Amylase	**7,4 %**
S-ALT	**12,0 %**
S-AST	**5,4 %**
S-CK	**11,5 %**
S-GGT	**10,8 %**
S-LDH	**4,3 %**

*) C. Ricos et. al. Current databases on biological variation: pros, cons and progress. Scand J Clin Lab Invest 1999; 59: 491-500.

Pooled human sera with certified RMVs

applied to routine procedures from Roche Diagnostics (Modular, Hitachi 917)

	Mean recovery (Modular)		Mean recovery (Hitachi 917)	
CK	94,2 %		95,3 %	
GGT	97,7 %		100,1 %	
AMY	94,4 %		95,5 %	
ALT	104,6 %		103,6 %	
AST	108,9 %	5,4 %	110,2 %	
LDH	94,0 %	4,3 %	98,4 %	
ALP	90,7 %		99,4 %	

Pooled human sera with certified RMVs

Use for manufacturers:

- Calibration concepts based on commutable calibration material
- Control material
  - ◆ sufficient number of aliquots
  - ◆ sufficient stable at – 75 °C

Benefit for routine laboratories:

- Control of commercial routine procedures
  - ◆ easy handling
  - ◆ airtight results

Reaction Principle for ALP measurements

Decision for a reference procedure for ALP using the substrate AMP

4-Nitrophenylphosphate + AMP $\xrightarrow{ALP}$ **4-Nitrophenoxide + AMP-phosphate**

4-Nitrophenylphosphate + H_2O $\xrightarrow{ALP}$ 4-Nitrophenoxide + Phosphate

Reasons for a decision on ALP-AMP

IFCC proposal for ALP-AMP at 30 °C

Clinica Chimica Acta, (1983) 339F–367F
Elsevier

INTERNATIONAL FEDERATION OF CLINICAL CHEMISTRY

SCIENTIFIC COMMITTEE, ANALYTICAL SECTION

EXPERT PANEL ON ENZYMES

IFCC methods for the measurement of catalytic concentration of enzymes

Part 5. IFCC method for alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1)

IFCC Document Stage 2, Draft 1, 1983–03 with a view to an IFCC Recommendation

Many routine procedures are using AMP.

However, this procedure was never endorsed by IFCC.

Measurement parameters of the proposed IFCC reference measurement procedure for ALP (1)

Concentrations in the Final Complete Reaction Mixture:

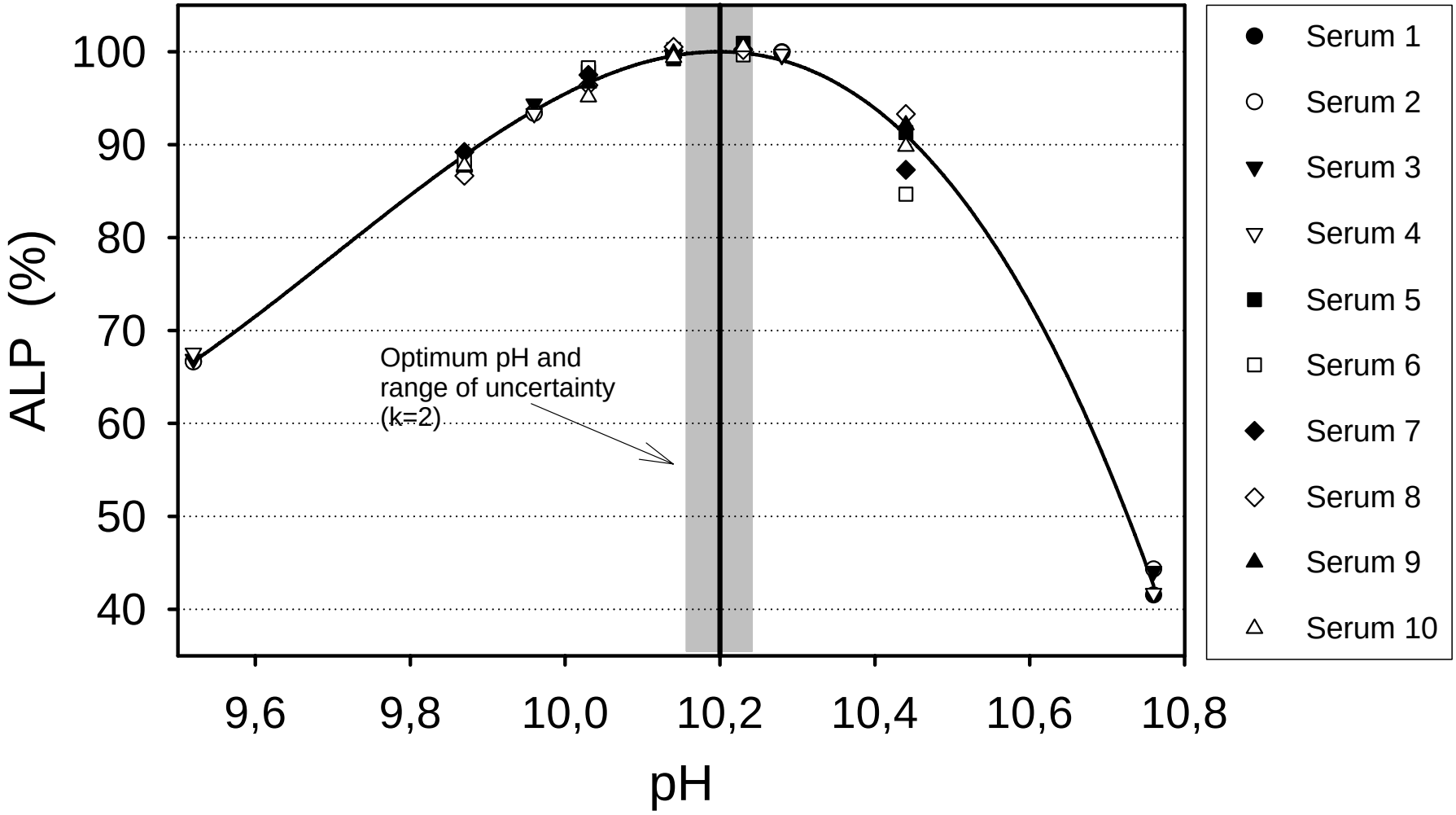
2-Amino-2-methyl-1-propanol	750 mmol/l
pH (37 °C)	10.20 ± 0.05
4-Nitrophenylphosphate	16 mmol/l
Zinc sulfate	1 mmol/l
Magnesium acetate	2 mmol/l
HEDTA	2 mmol/l
Volume fraction of sample	0.0222 (1 : 45)

Measurement parameters of the proposed IFCC reference measurement procedure for ALP (2)

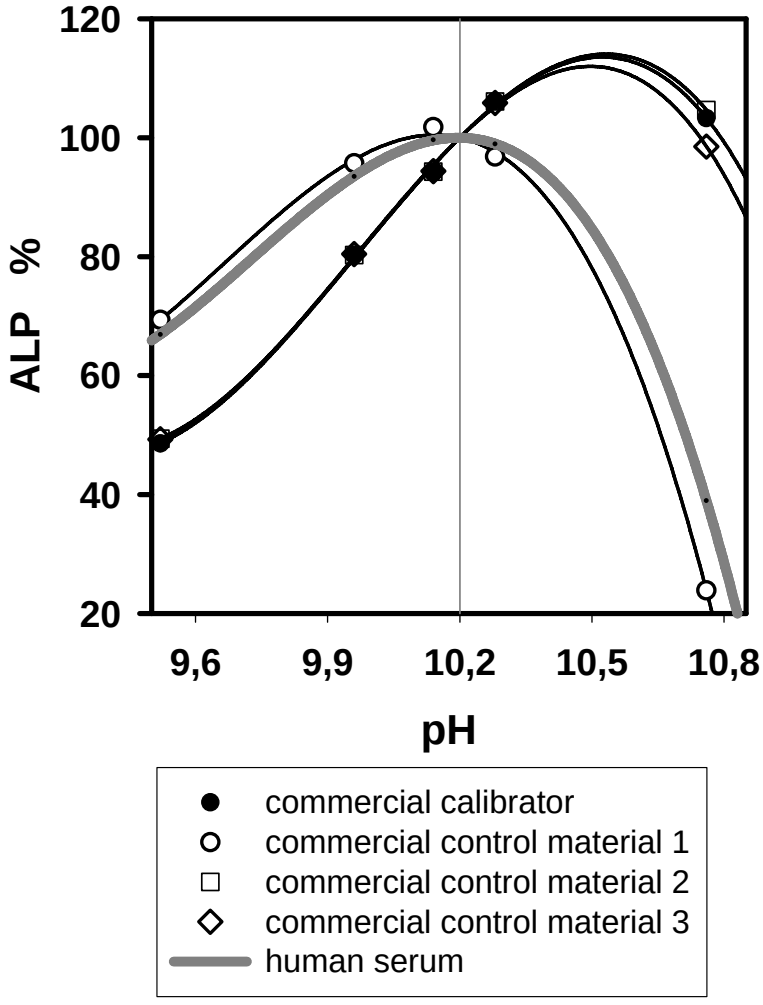
Measurement Conditions:

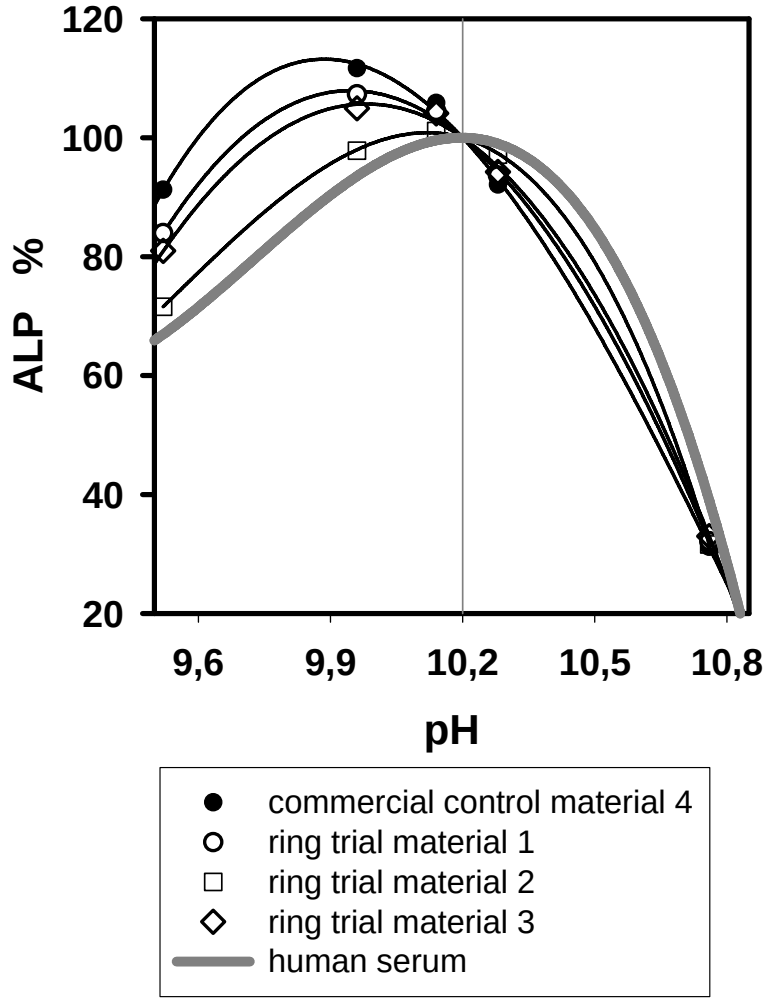
Temperature	37.0 °C ± 0.1 °C
Wave length	405 nm ± 1 nm
Band width	≤ 2 nm
Light path	10.00 mm ± 0.01 mm
Incubation time	60 s
Delay time	60 s
Measurement interval	120 s
Readings (measurement points)	≥ 6

ALP-AMP: pH optimum



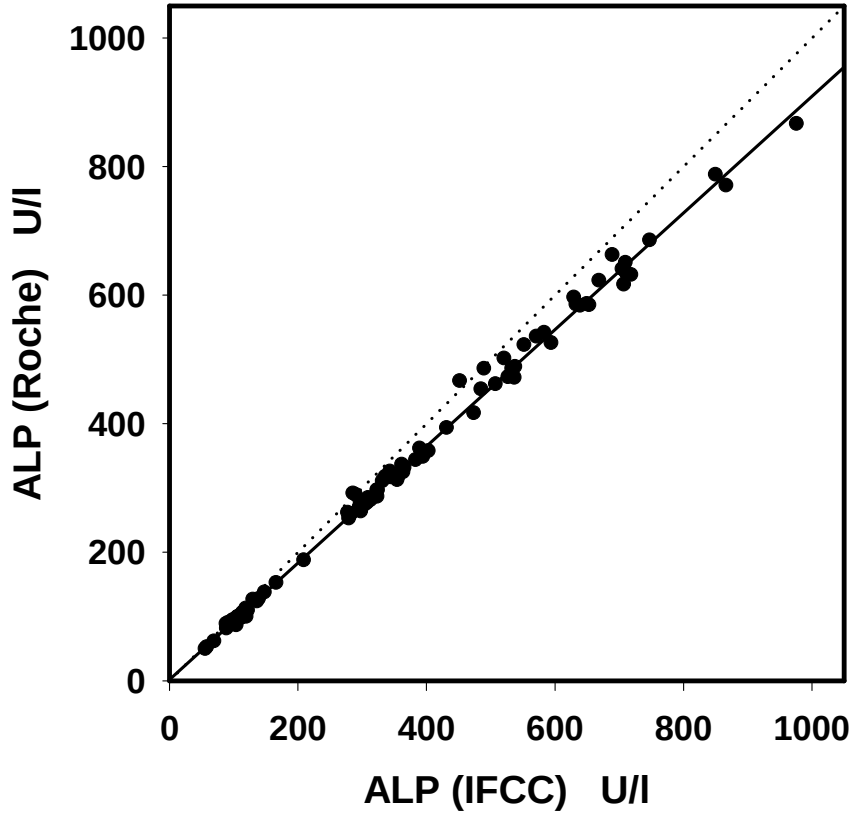
pH optima of ALP isoforms





Method comparison ALP:

Reference procedure vs Roche Diagnostics (Modular P)



Number of values	99
Slope	0,908
Slope, lower limit 95 %	0,898
Slope, upper limit 95 %	0,917
Intercept	1,75
Intercept, lower limit 95%	-0,03
Intercept, upper limit 95%	4,07
Coefficient of correlation	0,9982
Ratio mean	0,921

ALP measurements
y = x
Regression line (Passing/Bablok)

Nearly no intercept. Recalibration is only a matter of the slope

Network of reference laboratories performing a feasibility study

Francesca Canalias	Spain
F Ceriotti	Italy
PFH Franck	Netherlands
FJ Gella	Spain
PJ Jørgensen	Denmark
R. Klauke	Germany
R Nagel (Roche Diagn.)	Germany
M Panteghini	Italy
G Schumann	Germany

Investigation of pooled human sera,
processed control material,
a candidate reference material

Co-operation of two IFCC committees

Committee Reference Systems for Enzymes (C-RSE)

Committee Reference Intervals and Decision Limits (C-RIDL)

Publication of the IFCC reference measurement procedure and reference intervals in preparation

Reference Procedure for Lipase

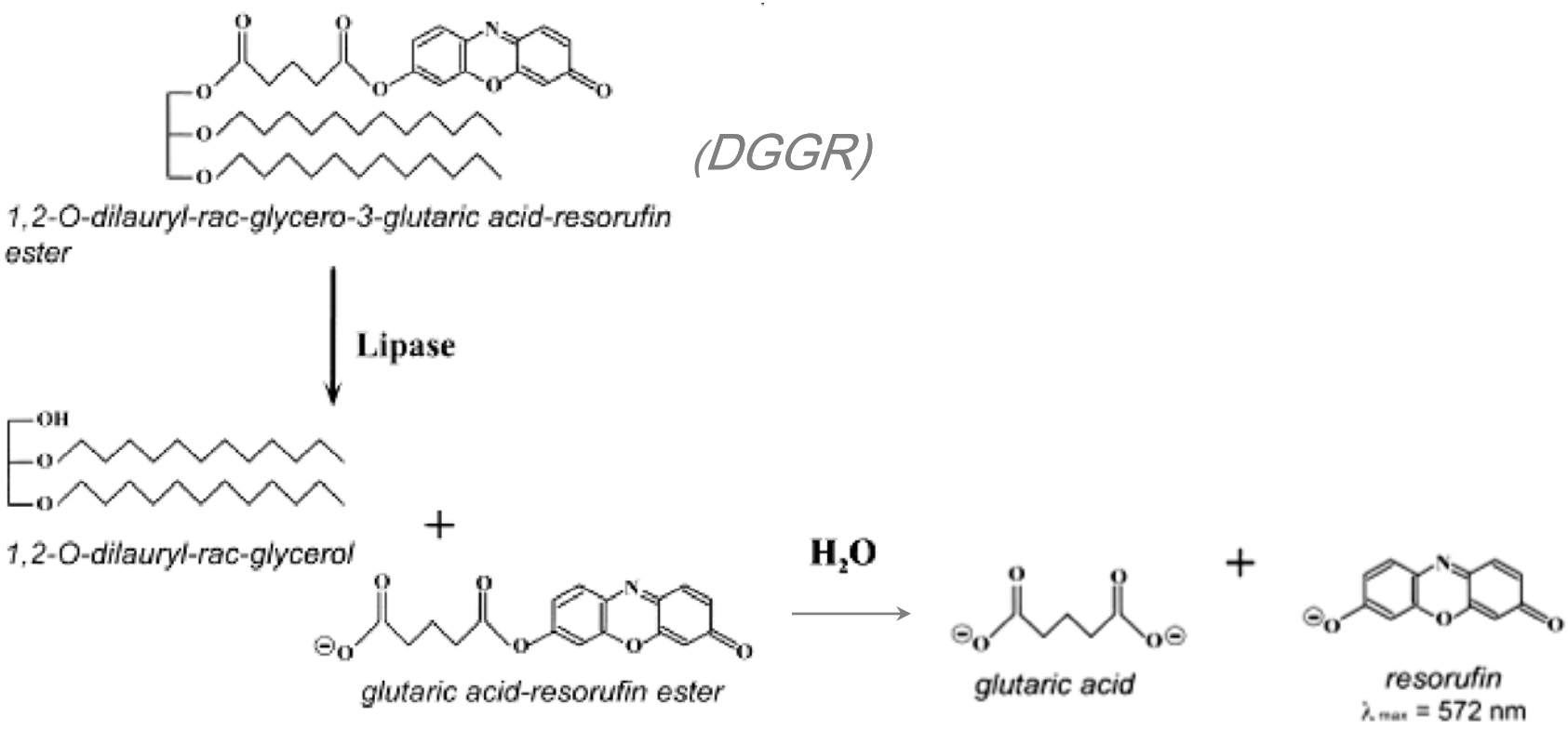
Measurement principle

- ~~Titrimetry~~
- Spectrometry

↓

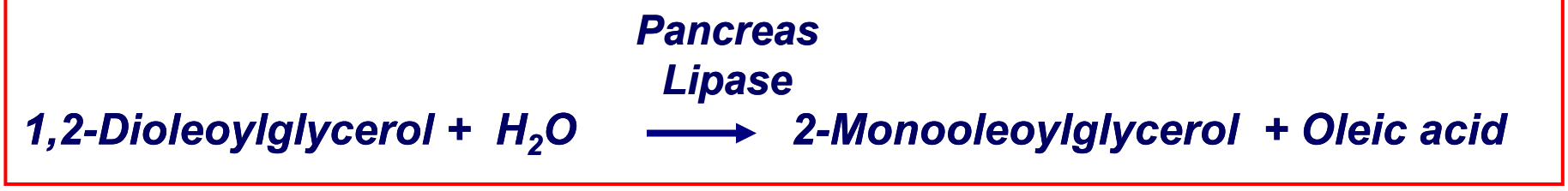
Reference measurement procedure closer to routine procedures

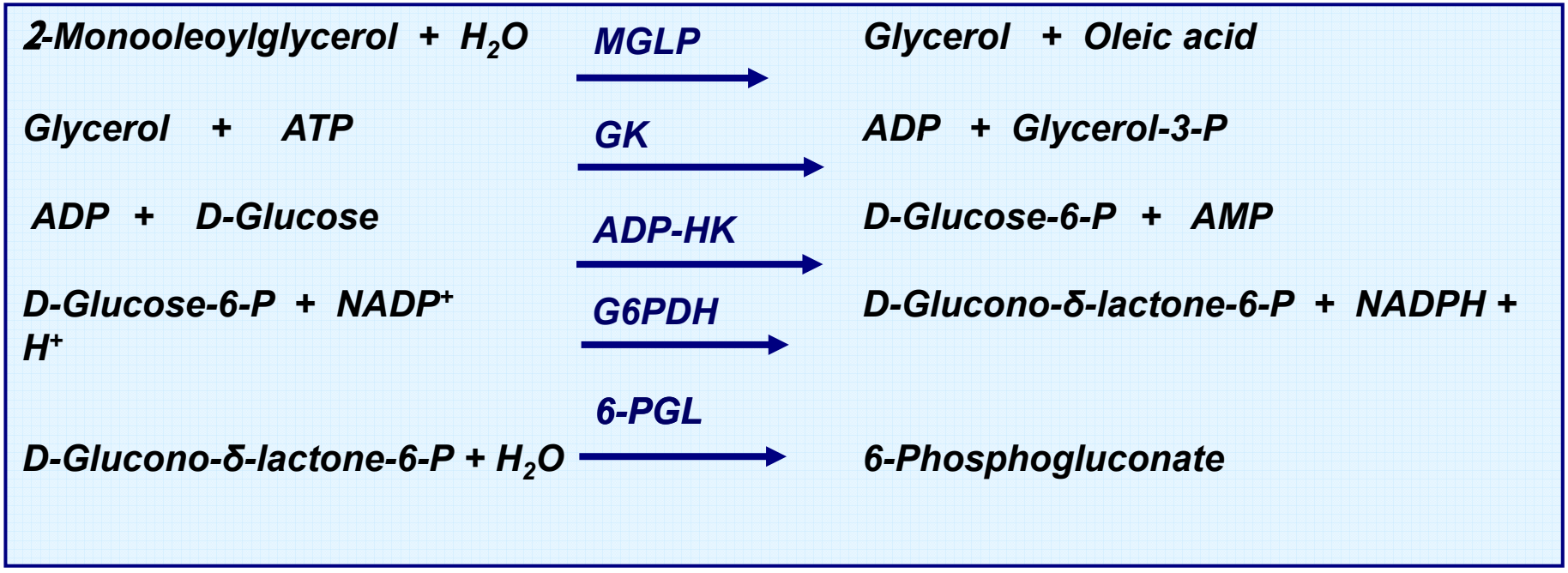
Candidate for a Reference Procedure for Lipase



(Beisson *et al.*, Eur. J. Lipid Technol., 2000)

Candidate for a Reference Procedure for Lipase





MGLP: Monoglyceride lipase, GK: Glycerol kinase , ADP-HK: ADP-dependent hexokinase, G6PDH: Glucose -6-phosphate dehydrogenase, 6-PGL: 6-Phosphogluconolactonase

Reference measurement procedure for pancreatic lipase

C-RSE is working on a concept for the development of a reference measurement procedure (RMP) for lipase.

C-RSE is in favour of spectrophotometry as the measurement principle for the RMP for lipase.

The decision on a single substrate (see two choices) for the RMP needs consensus with the corporate members of IFCC.

C-RSE needs common consent or even a mandant from the corporate members of IFCC for the choice of a suited measurement principle and the selected substrate for reference methodology.

Renate Strache

DGKL
Deutsche Vereinte Gesellschaft
für Klinische Chemie und Laboratoriumsmedizin e.V.

Rainer Klauke

Calibration laboratory
(Reference laboratory)

DKD-K-20602