

## **A new reference system in town: alkaline phosphatase**



G. Schumann and R. Klauke



### **A reference system for catalytic concentration measurements of ALP**

- ❖ **Reference measurement procedure**
- ❖ **Certified reference material**
- ❖ **Network of reference laboratories**
- ❖ **Reference intervals**
- ❖ **International quality assessment  
for reference laboratories**

## Isoforms of ALP and diagnostic relevance

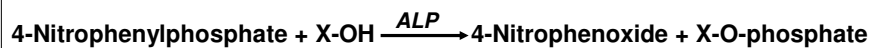
- Liver - ALP 
- Bone - ALP 
- Placenta - ALP
- - - - -
- Small intestine - ALP
- Kidney - ALP
- Other isoforms of ALP

(More than 17 isoforms of ALP are detectable via isoelectricfocusing)

The complexity of ALP isoforms is adversely to reference methodology.

## Reaction principle for ALP measurements

ALP catalyzes transphosphorylation reactions



Various amino-alcohol phosphate acceptors (X-OH) for the transfer:

The diversity of phosphate acceptors was a major adverse effect for international standardization!

- Others were:
- A patent on the metal ion buffer,
  - the measurement temperature 30 °C.

## Reaction Principle for ALP measurements



### Decision for a reference procedure for ALP using AMP



**Reasons for a decision on ALP-AMP**

## IFCC proposal for ALP-AMP at 30 °C

*Clinica Chimica Acta*, (1983) 339F-367F  
Elsevier

339F

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

**This procedure was never endorsed by IFCC.  
Many routine procedures are using AMP.**



**Decision for a reference procedure  
for ALP using AMP**  
(based on the template ALP-AMP, 30 °C)



**IFCC committees:**

**Calibration in Clinical Enzymology (C-CCE)**

**Reference Systems for Enzymes (C-RSE)**

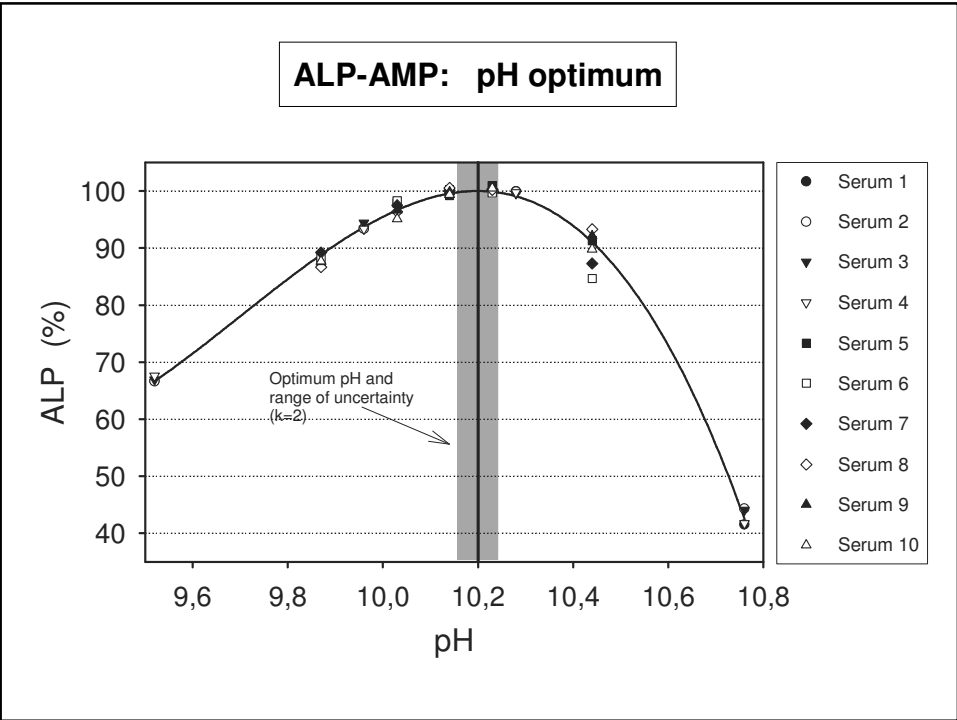
**Work items**

- ❖ Optimization of the reaction conditions
- ❖ Reduction of interferences
- ❖ Linear range of the recorded change of absorbance
- ❖ Stability of reagent solutions
- ❖ Minor compromises

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

**Concentrations in the Final Complete Reaction Mixture:**

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

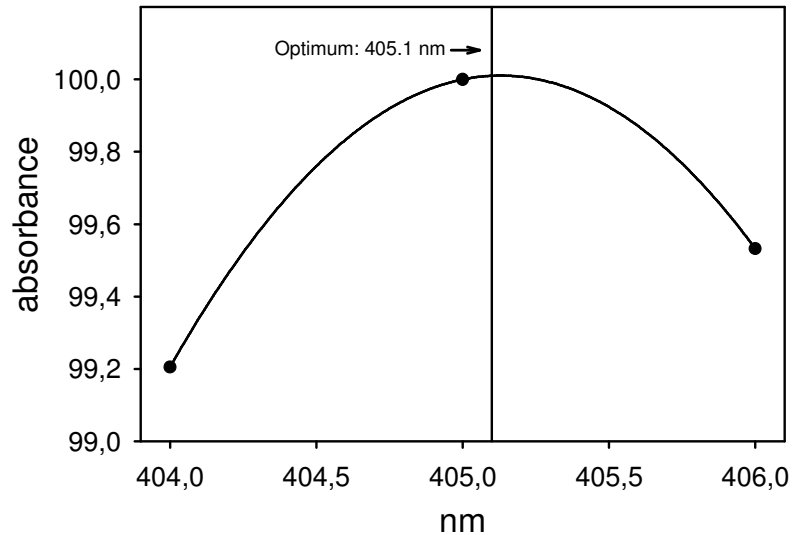


**Measurement parameters of the proposed IFCC reference measurement procedure for ALP (2)**

**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: detector signal optimum



### ISO 18153 (“traceability standard for enzymes”)

The enzyme measurand is described by the specified measurement procedure

- kind of substrate and its concentration,
- activators and their concentrations,
- direction of the catalyzed reaction,
- indicator component,
- buffer system and pH,
- measurement temperature,
- pre-incubation time,
- material used for starting the reaction,
- lag time (delay time),
- reaction time (measurement interval),
  - wavelength, band width, light pass,
  - volume fraction of sample

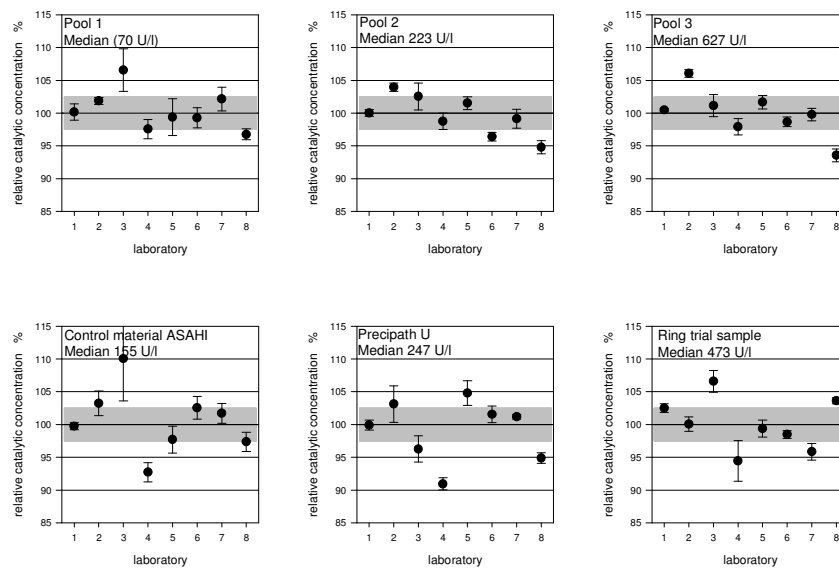
*Don't change measurement parameters of the reference procedure !*

## 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

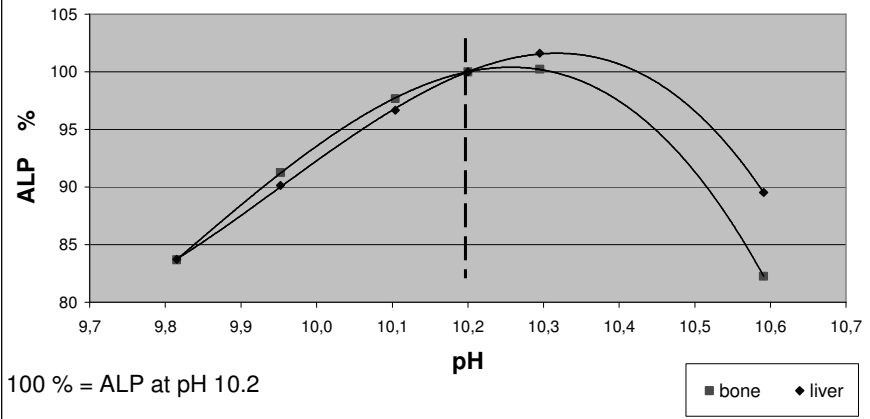
Investigation of pooled human sera,  
processed control material,  
a candidate reference material

## Preliminary results of the feasibility study

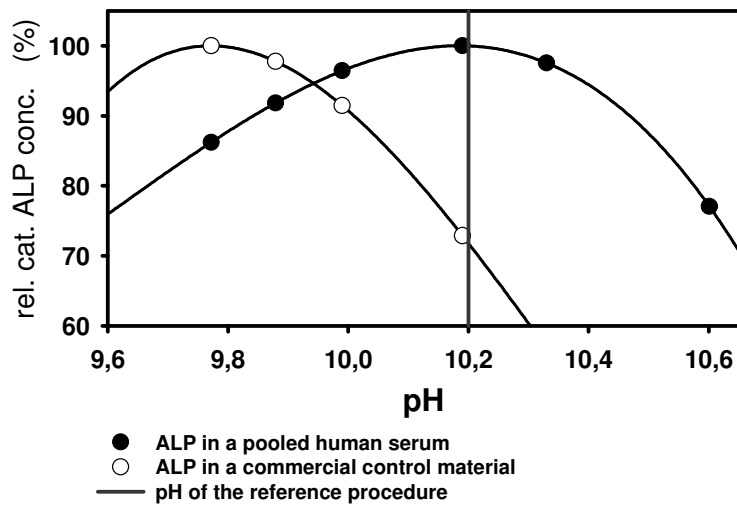


### ALP-AMP reference procedure

#### pH optimum for the liver and the bone isoenzyme

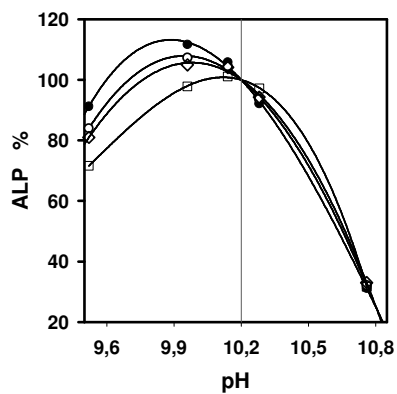
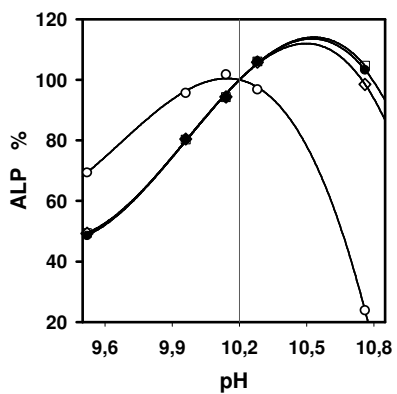


#### Control material with a low pH optimum compared to human liver-/bone-ALP





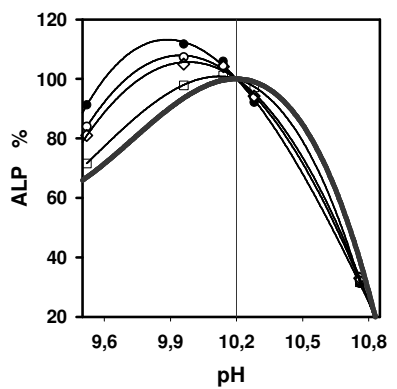
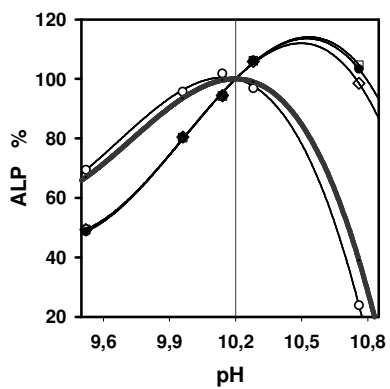
### pH optima of ALP isoforms



- commercial calibrator
- commercial control material 1
- commercial control material 2
- ◇ commercial control material 3

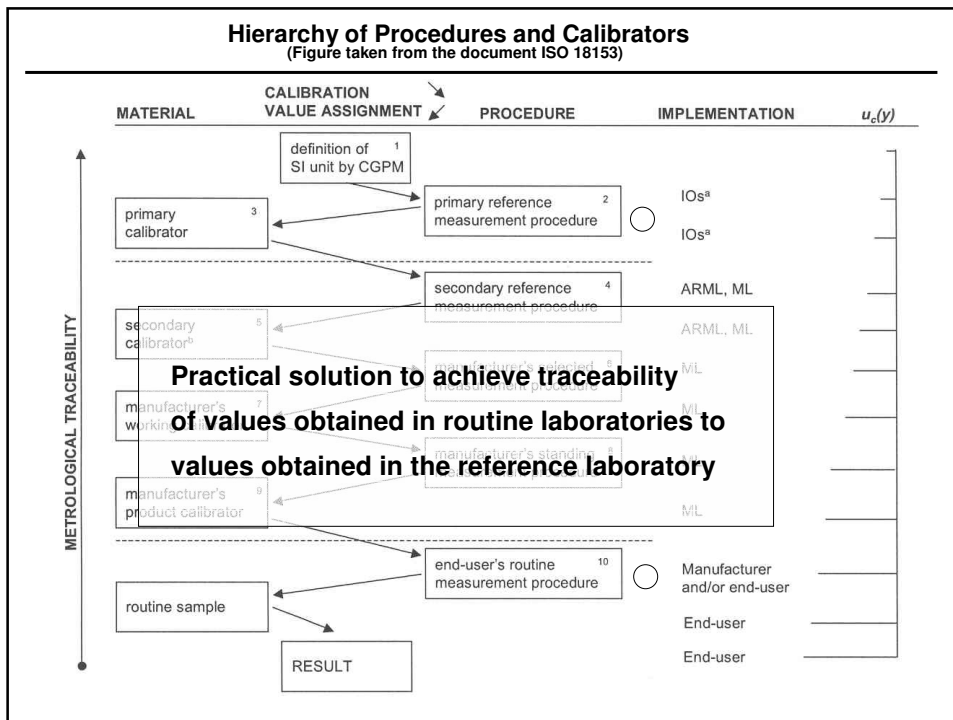
- commercial control material 4
- ring trial material 1
- ring trial material 2
- ◇ ring trial material 3

### pH optima of ALP isoforms



- commercial calibrator
- commercial control material 1
- commercial control material 2
- ◇ commercial control material 3
- human serum

- commercial control material 4
- ring trial material 1
- ring trial material 2
- ◇ ring trial material 3
- human serum



### Secondary reference material: Pooled human serum

- ❖ Serum residues collected after completion of routine measurements

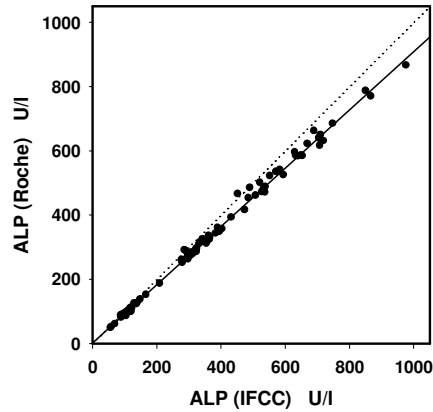
- ❖ Pooling of sera to obtain tailored catalytic concentrations

- ❖ Pooled sera aliquoted (1,25 ml) and deep frozen (below -75 ° C)

- ❖ Determination of reference method values from frozen aliquots

### 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)

### ALP: Value assignment to pooled human sera

Name	RMV	<i>U</i>	<i>Urel</i>
Cal ALP 01-01	70,2 U/l	1,5 U/l	2,2 %
Cal ALP 02-01	223,8 U/l	5,9 U/l	2,7 %
Cal ALP 03-01	631,4 U/l	13,8 U/l	2,2 %

## ALP: Value assignment to pooled human sera and calibration

### Reference method values

Name	RMW	U	U <sub>ref</sub>
Cal ALP 01-01	70,2 U/l	1,5 U/l	2,2 %
Cal ALP 02-01	223,8 U/l	5,9 U/l	2,7 %
Cal ALP 03-01	631,4 U/l	13,8 U/l	2,2 %

### Results of the Modular (n = 3)

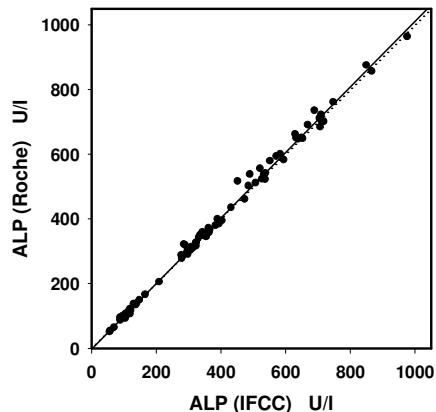
Material	Day	Date	Value 1	Value 2	Value 3	Mean	SD	CV	Deviation from RMV		Calibration calibration line
									absolute	relative	
Cal ALP 01-01	1	28.3.2008	64,0 U/l	64,1 U/l	64,1 U/l	64,1 U/l	0,1 U/l	0,1 %	-6,1 U/l	-8,7 %	slope 0,894
Cal ALP 02-01	1	28.3.2008	203,9 U/l	204,6 U/l	205,3 U/l	204,6 U/l	0,7 U/l	0,3 %	-19,2 U/l	-8,6 %	intercept 2,7
Cal ALP 03-01	1	28.3.2008	567,1 U/l	565,1 U/l	567,1 U/l	566,4 U/l	1,2 U/l	0,2 %	-65,0 U/l	-10,3 %	
Cal ALP 01-01	2	1.4.2008	64,5 U/l	65,0 U/l	64,8 U/l	64,8 U/l	0,3 U/l	0,4 %	-5,4 U/l	-7,7 %	slope 0,899
Cal ALP 02-01	2	1.4.2008	206,5 U/l	206,4 U/l	207,1 U/l	206,7 U/l	0,4 U/l	0,2 %	-17,1 U/l	-7,7 %	intercept 3,2
Cal ALP 03-01	2	1.4.2008	570,6 U/l	571,0 U/l	569,8 U/l	570,5 U/l	0,6 U/l	0,1 %	-60,9 U/l	-9,6 %	

### Calibration line from all values

Number	6
Slope	0,895 ± 0,004
Intercept	3,816 ± 1,361
s <sub>y,x</sub>	2,30
Correlation	0,9999

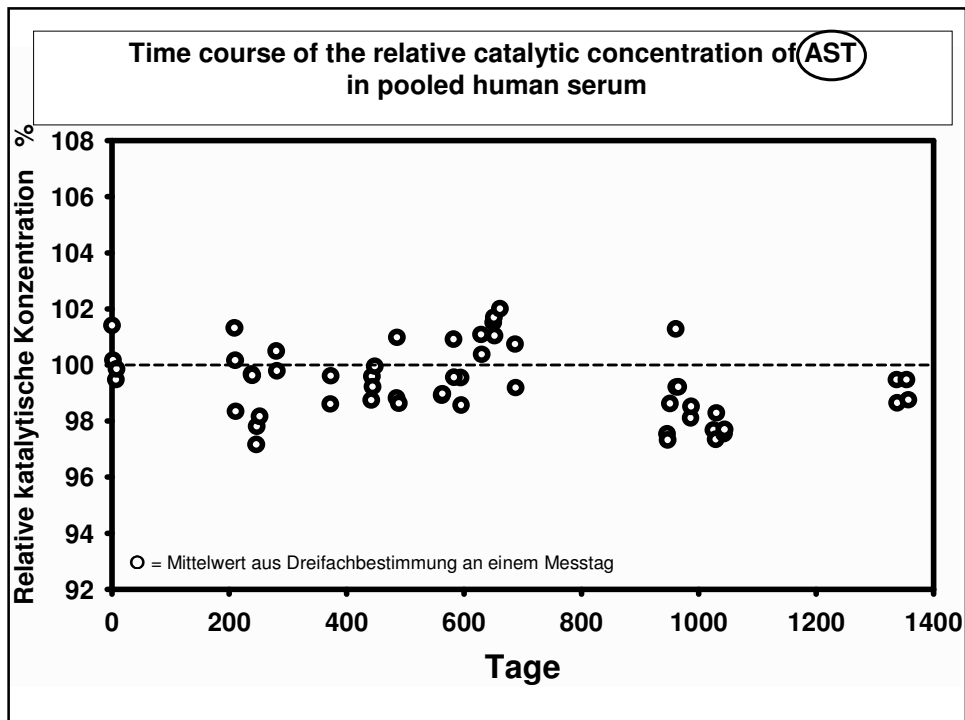
## Method comparison ALP (recalibrated):

Reference procedure vs Roche Diagnostics (Modular P)



Number of values	99
Slope	1,014
Slope, lower limit 95 %	1,002
Slope, upper limit 95 %	1,024
Intercept	-2,34
Intercept, lower limit 95%	-4,34
Intercept, upper limit 95%	0,22
Coefficient of correlation	0,9982
Ratio mean	1,007

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

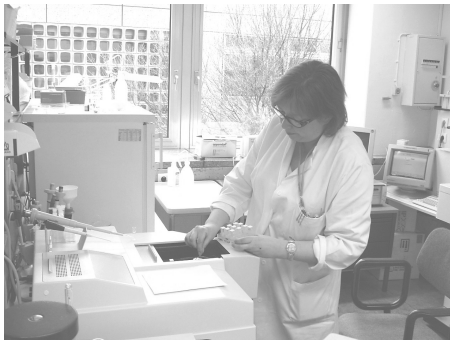


### Factors influencing performance and measurement uncertainty of the reference procedure

- **Small sample volume (50  $\mu$ l)**
- **Non-linearity of the reaction rate – requires exact timing**
- **Aging of the substrate (NPP) – requires control**
- **pH decrease due to intake of  $\text{CO}_2$**
- **Viscosity of the buffer solution (750 mol/l AMP) and layering complicates temperature equilibration to 37 °C within one minute.**

## Summary

- ✓ The reference measurement procedure is ready for publication
- ✓ A reference material is available.  
A certification campaign is required.
- ✓ The network has produced good results in a feasibility study.
- ✓ C-RIDL is working together with C-RSE to provide reference intervals for ALP.
- ✓ Method comparisons have shown that the implementation of the new IFCC reference procedure in routine use seems to be relatively easy.



Renate Strache

Officially accredited  
**Calibration laboratory**  
(Reference laboratory)

DKD-K-20602  
DKD-K-20603

**DE** DGKL  
Deutsche Vereinigung Gesellschaft  
für Klinische Chemie und Laboratoriumsmedizin e.V.

Rainer Klauke

