

Quality of Diagnostic Samples, Kind and Amount of Sample, Stability during Transport and Storage.

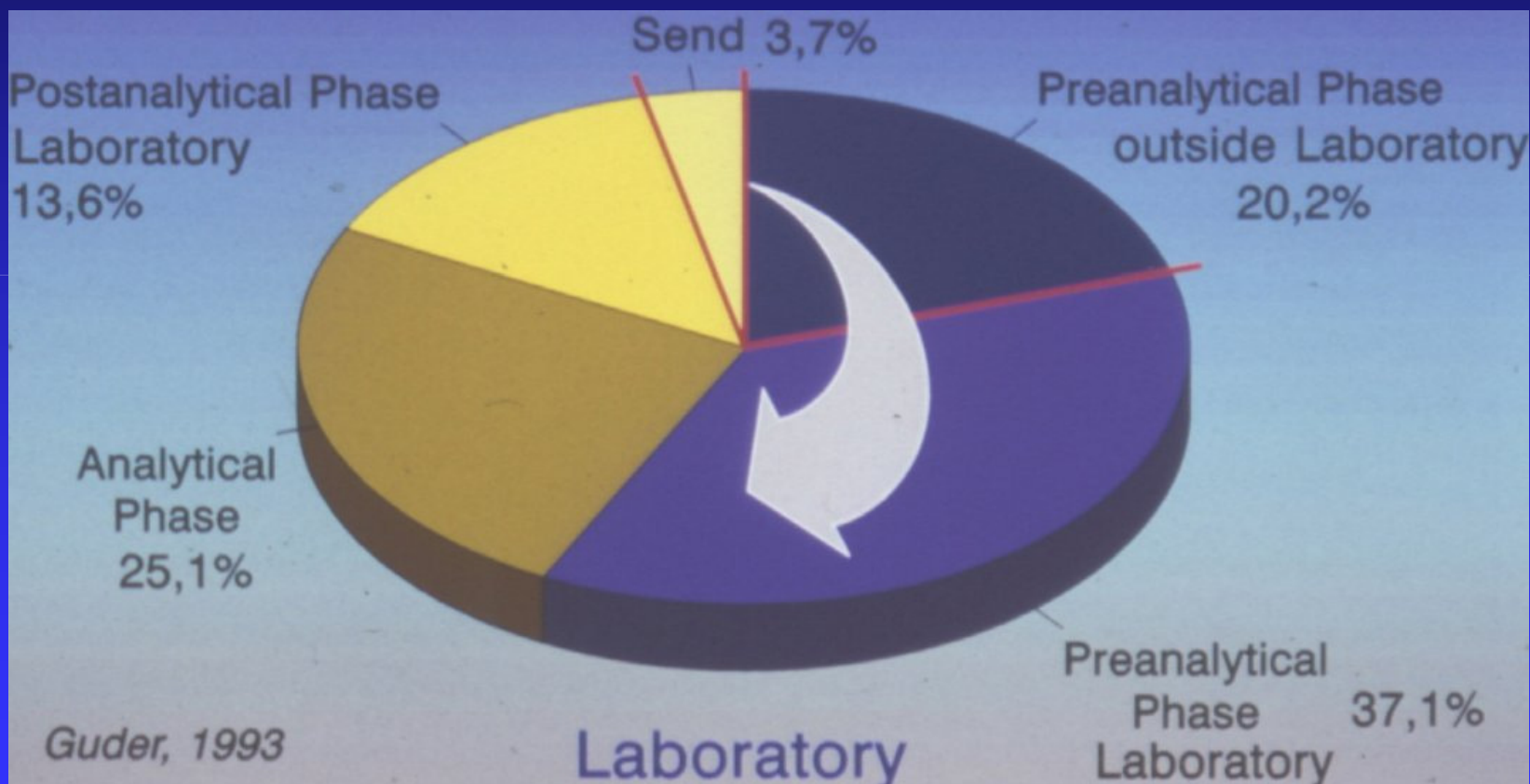
Walter G. Guder



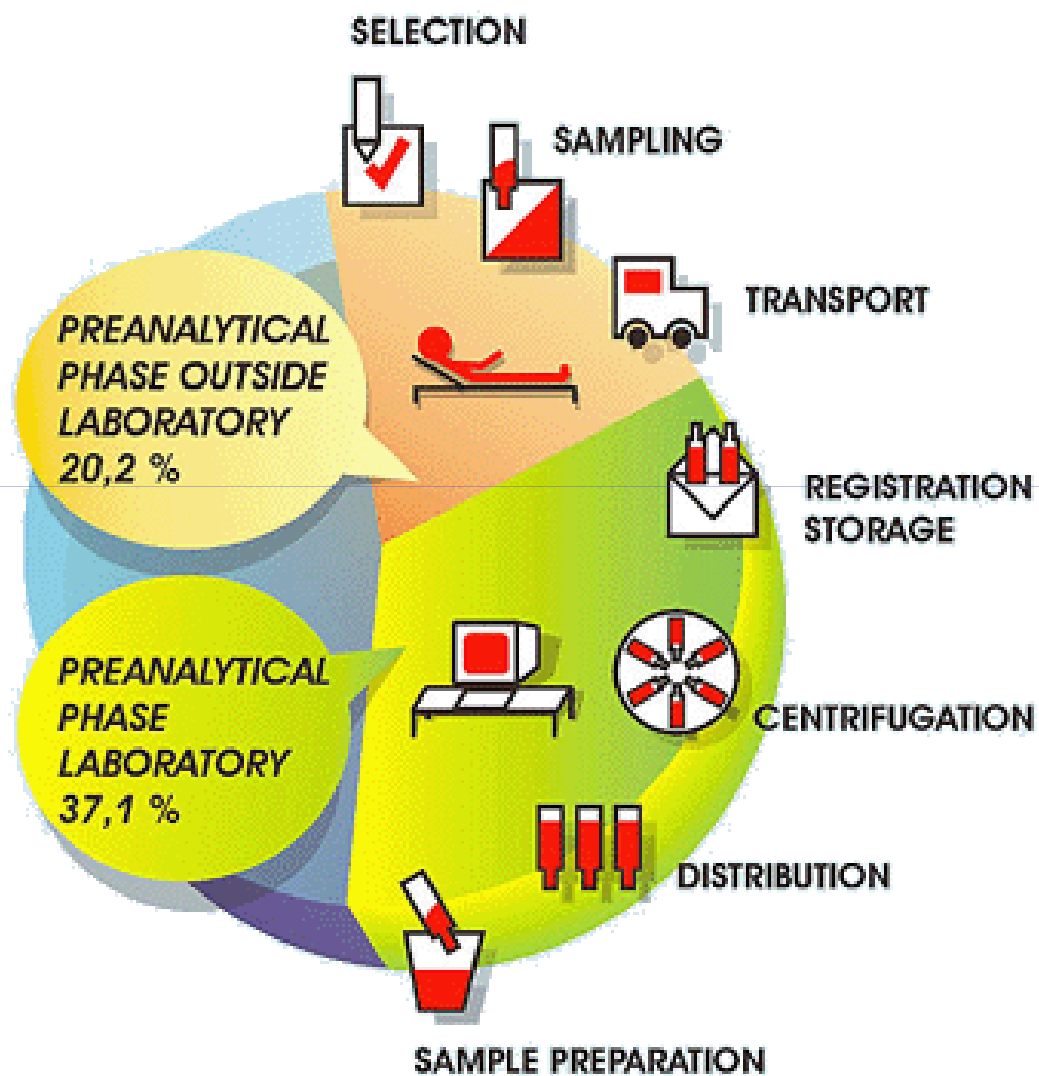
**Former Chief of
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The Preanalytical Phase in the Diagnostic Process

Ward / Practice



Errors in Laboratory Medicine



Quality Criteria

Analytical Phase

Precision

Accuracy

Plausibility

Preanalytical Phase

Adequate Selection and
Request

Adequate Kind and
Amount of Sample

Adequate Timing

Quality Assurance in the Preanalytical phase

10 Questions to Start with

A

1. Do you know the transport time of samples entering your lab?
2. When and how do you react to inappropriate samples/samples without request/requests without sample?
3. How do you handle hemolytic/lipemic/icteric samples?
4. Do you refuse requests?
5. Do you know the doctor's responsible for selection and requests?

Quality Assurance in the Preanalytical phase

10 Questions to Start with

B

6. Can you decide about format and program of your request system?
7. Do you offer consulting to clinicians?
8. How long is your intralaboratory preanalytic time?
9. How do you identify/document samples?
10. Do you document times of entering, processing, analyzing and release of samples and results?

EN ISO 15189

Requirements for Quality and Competence in Medical Laboratories

■ 5.4 Pre-examination procedures

- 5.4.1 Informations on sample identification (Patient and requester) and type, transport and time of arrival.
- 5.4.2 Specific instructions on proper sampling and handling of primary samples ,
- 5.4.3 Information on available examinations, indications and preparation of patients ,
identification, storage and elimination of samples, including time sequences
- 5.4.4/5 Documentation and traceability of primary and secondary samples
- 5.4.6 Informations regarding date and time of sampling, transport
and receipt of samples including temperature .
- 5.4.7/8 Documentation of sample receive and criteria for rejection of samples
- 5.4.9/10 Criteria of sample volumes and types of analytical samples
- 5.4.11 Documentation of emergency tests and intralaboratory transport,
- 5.4.12 Traceability of secondary samples
- 5.4.13 oral requests
- 5.4.14 Storage conditions and stability criteria.

Quality Assurance

Methods	Example: Double Requests
A. Awareness of possible problem	Same spectrum twice a day
B. Documentation of reality	Ward specific statistics
C. Definition of quality standards	Criteria of half life, ward specific reason for dupl.
D. Quality manual	Documentation and publication of criteria
E. Implementation of internal QA	Information of wards and consequences (cancel test request not meeting the approved standards)
F. Enter external QA program	

Quality of Selection and Request

Example: Lipoproteins

Traditional	Alternative
Cholesterol	Risk Assessment
Triglycerides	Coronary Risk Analysis
HDL – Cholesterol	Lipoprotein Analysis
LDL – Cholesterol	Follow Up
Lipoprotein Electrophoresis	Confirmation
Apoprotein B	
Apoprotein A	
Lp(a)	
Ultracentrifugation	

Original article

Ann Clin Biochem 1993; 30: 52-59

**The role of expert systems in improving the test
requesting patterns of clinicians**

Margaret Peters and P M G Broughton

From the Wolfson Computer Laboratory, Queen Elizabeth
Medical Centre, University of Birmingham, Birmingham
B15 2TH, UK

Wahl de optimalen Probenvolumens

[Selecting the Optimal Sample Volume]

Reference Guder WG, da Fonseca-Wollheim F, Heil W, Müller-Plathe O, Töpfer G, Wisser H, Zawta B.

Wahl des optimalen Probenvolumens (In German).
Deutsche Gesellschaft Klinische Chemie
Mitteilungen 1996;27:106-7.

**Volumen of analytical sample used in measurement procedures I:
Clinical Chemistry, Hitachi 717 analyzer**

Analyte	Analytical portion (μl)	Dead volume (μl)
Na, K, Cl	20	12
Ca	10	12
Phosphate	5	12
Glucose	20	12
Creatinine	10	12
Urea	4	12
ALAT / ASAT	20	12
Pseudocholinesterase	3	12
Alkaline phosphatase	4	12
γ-GT	7	12
Creatinine kinase	7	12
α-Hydroxybutyrate dehydrogenase	7	12
Triacylglycerol	3	12
Cholesterol	3	12
Uric acid	7	12
Subtotal	150	192
Total		342

Calculation of required volume of the laboratory (blood) sample for the measurement of a single quantity, e.g. Plasma-cholesterol concentration


	Analytical sample		Laboratory blood sample
	1 st analysis	2 nd analysis	
Volume of the analytical sample used (μl)	15	15	60
Dead volume of the primary sample tube (μl)	100		200
Dead volume of the secondary sample cup (μl)	150		300
Total (μl)			560

Required volume of the laboratory blood sample:

$$2 \times ([\{V_{\text{analytical portion}} + D_{\text{pipettor}}\} \times R] + D_{\text{primary tube}} + D_{\text{secondary cup}})$$

V, volume; D, dead volume; R, replicates; the factor of 2 considers a packed cell volume of 0.5.

Optimal Sample Volume	
Vol a (ml plasma/serum)	<input type="text"/>
Da (ml plasma/serum)	<input type="text"/>
Dp (ml blood)	<input type="text"/>
Ds (ml plasma/serum)	<input type="text"/>
N	<input type="text"/>
<input type="button" value="Calculate"/>	
Vol b (ml blood) =	

Optimal Sample Volume	
Vol a (ml plasma/serum)	<input type="text" value="0.020"/>
Da (ml plasma/serum)	<input type="text" value="0.100"/>
Dp (ml blood)	<input type="text" value="0.500"/>
Ds (ml plasma/serum)	<input type="text" value="0.100"/>
N	<input type="text" value="4"/>
 <input type="button" value="Calculate"/>	
Vol b (ml blood) =	

Optimal Sample Volume	
Vol a (ml plasma/serum)	<input type="text" value="0.020"/>
Da (ml plasma/serum)	<input type="text" value="0.100"/>
Dp (ml blood)	<input type="text" value="0.500"/>
Ds (ml plasma/serum)	<input type="text" value="0.100"/>
N	<input type="text" value="4"/>
<input type="button" value="Calculate"/>	
Vol b (ml blood) = 1.66	

Proposed standardized volumens of laboratory blood samples:
Recommendations of the Working group “Preanalytics” of the
German Society of Clinical Chemistry

Application	Sample volume (additive)
Clinical chemistry	
- serum	4 - 5 ml
- plasma	3 – 4 ml (heparin)
Hematology	2 – 3 ml (K ₂ -EDTA)
Coagulation testing	2 – 3 ml (sodium citrate)
Immunoassays, proteins	1 ml / 3 – 4 Immunoassays
Blood gas analysis	1 ml (heparin)
Blood sedimentation rate	2 – 3 ml (sodium citrate)

Measures to reduce the sample size for in vitro diagnostics

- Barcode reading of the primary sample tubes
- Avoidance of secondary sample cups and aliquotation
- Use of sample tubes with low diameter
- Use of measurement procedures with low analytical portions
- Storage of the laboratory sample in primary sample tubes (use of separators as barrier between cells and plasma/serum)
- Use of plasma instead of serum (~ 10 % higher yield of analytical sample)

The Preanalytical Phase

Patient
Preparation



Specimen
Sampling



Sample
Transport



Analysis
Storage

Influence Factors

are biological,
related to patient,
occur in vivo,
affect conc. of analyte,
stable (sex) or
changing (age)

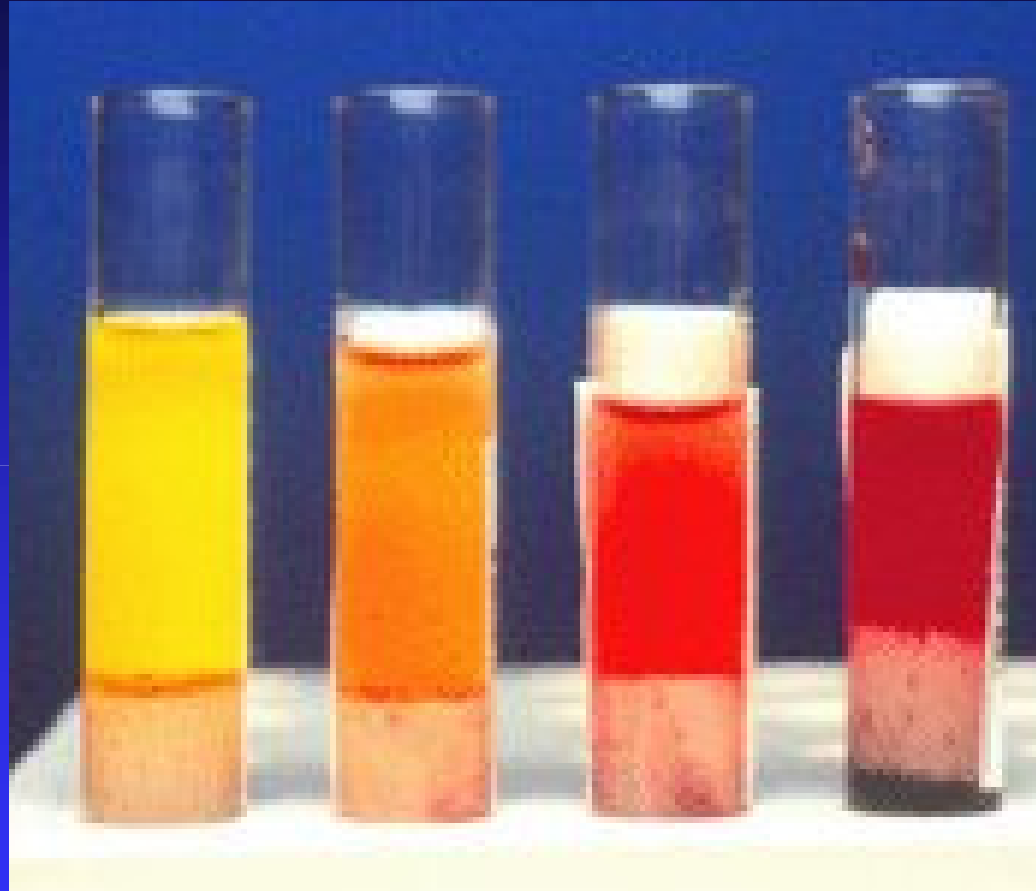
Interference Factor

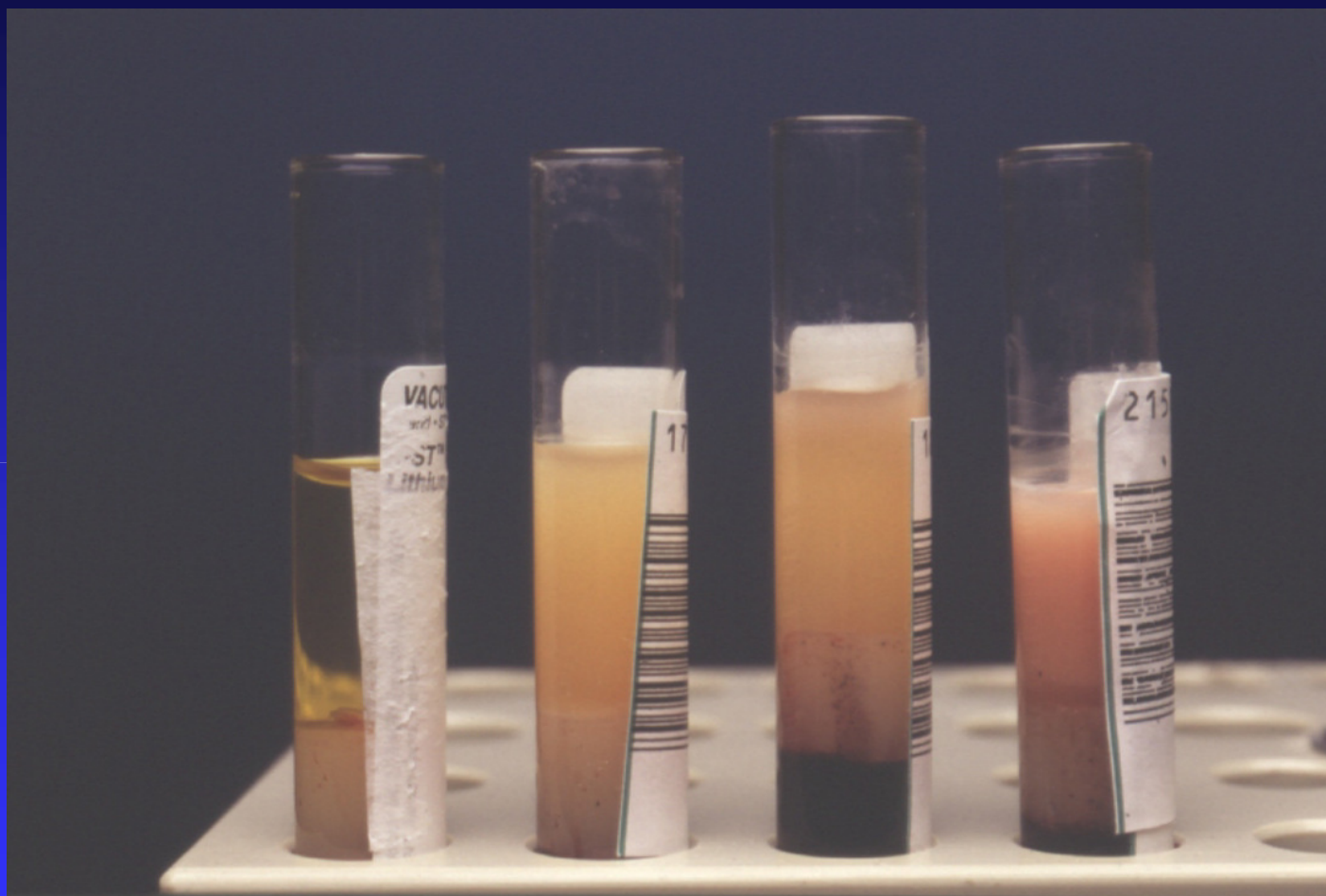
are related to sample,
different from analyte,
endogenous (turbidity) or
exogenous (drugs),
act in vitro
act method dependent

if analyte changes in vitro, one may define it as in vitro influence

**Standardization of the
preanalytical phase,
Consultation**

**Evaluation of mechanism,
choice of a specific method,
Consultation**





The role of consulting

Hemolysis

Turbidity

- Estimation of degree

- Comparison with other samples of the same patient

- Information of clinician

- Questions regarding mechanism

- Questions regarding needs

Discard sample

New sample

undisturbed

Analyse
without sample
treatment

Method

disturbed

Analyse after
sample
treatment
or

enter: Result
disturbed by
interference

Quality of timing in the Preanalytical Phase

Timing of sampling for biological/medical reasons

Examples:

sensitive parameter (catecholamines in blood)

circadian and feeding rhythms (cortisol, triglycerides)

drug monitoring (steady state or maximum)

menstrual cycle (LH, FSH)

Quality of timing in the Preanalytical Phase

Process:

■ Time Request - Sampling

■ Timing of Sampling

Over 24 hours

Timing after Patient's Preparation

Distance from Previous Sampling

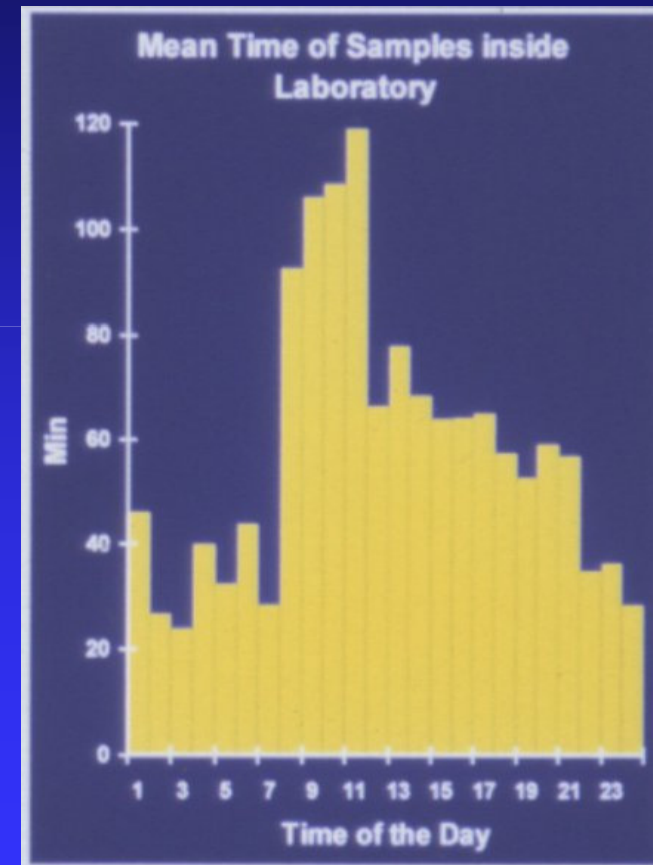
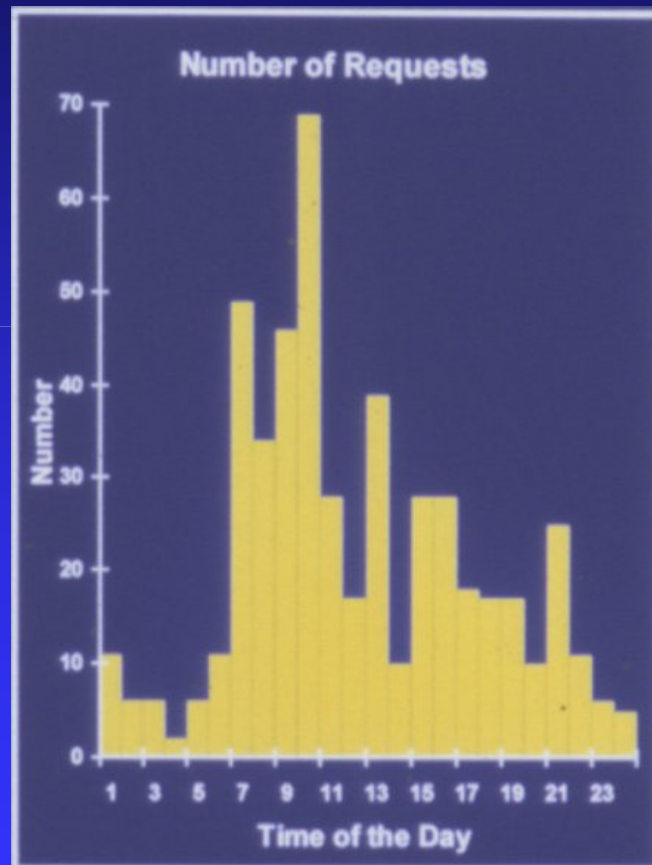
■ Timing of Transport and Processing

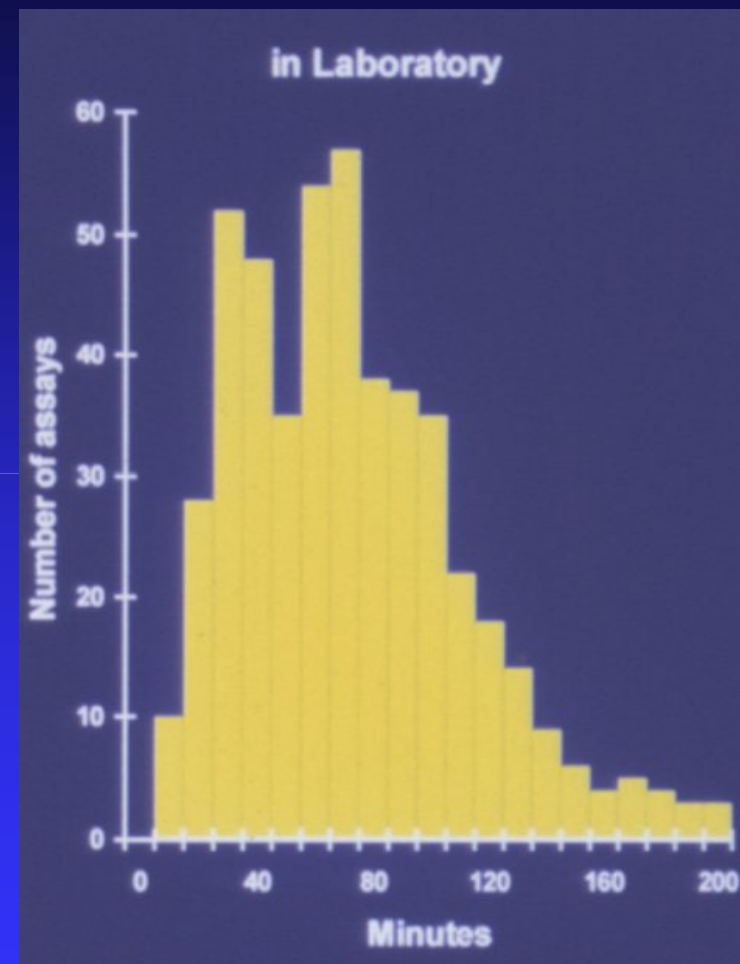
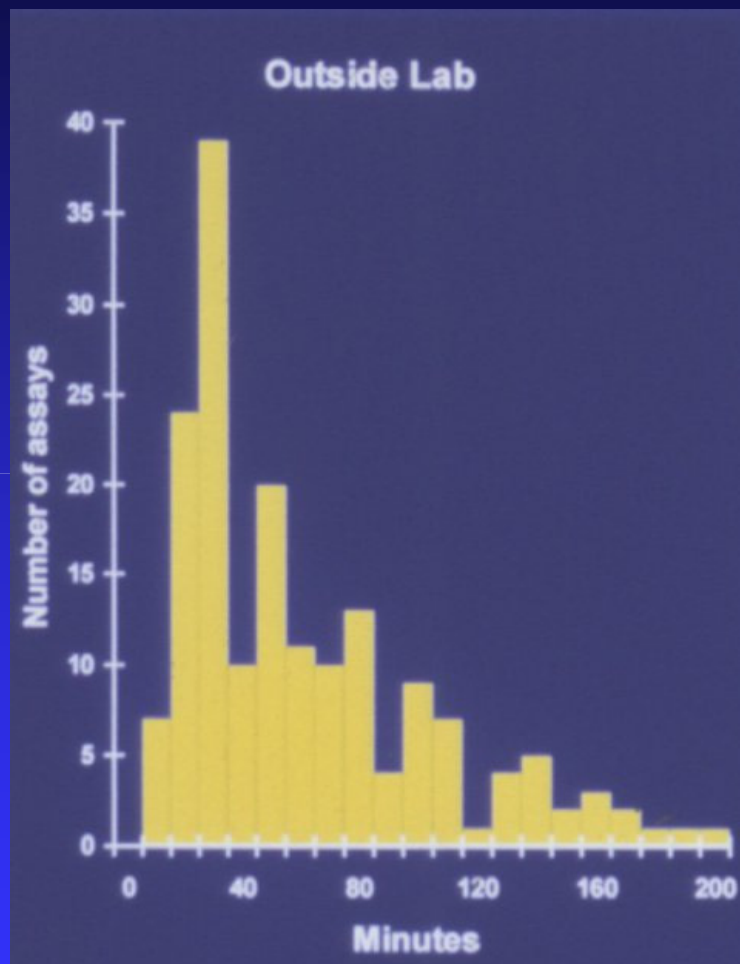
Time Sampling - Transport

Transport Time

Time Registration - Analysis

Workload and Intralaboratory Time over 24 Hours



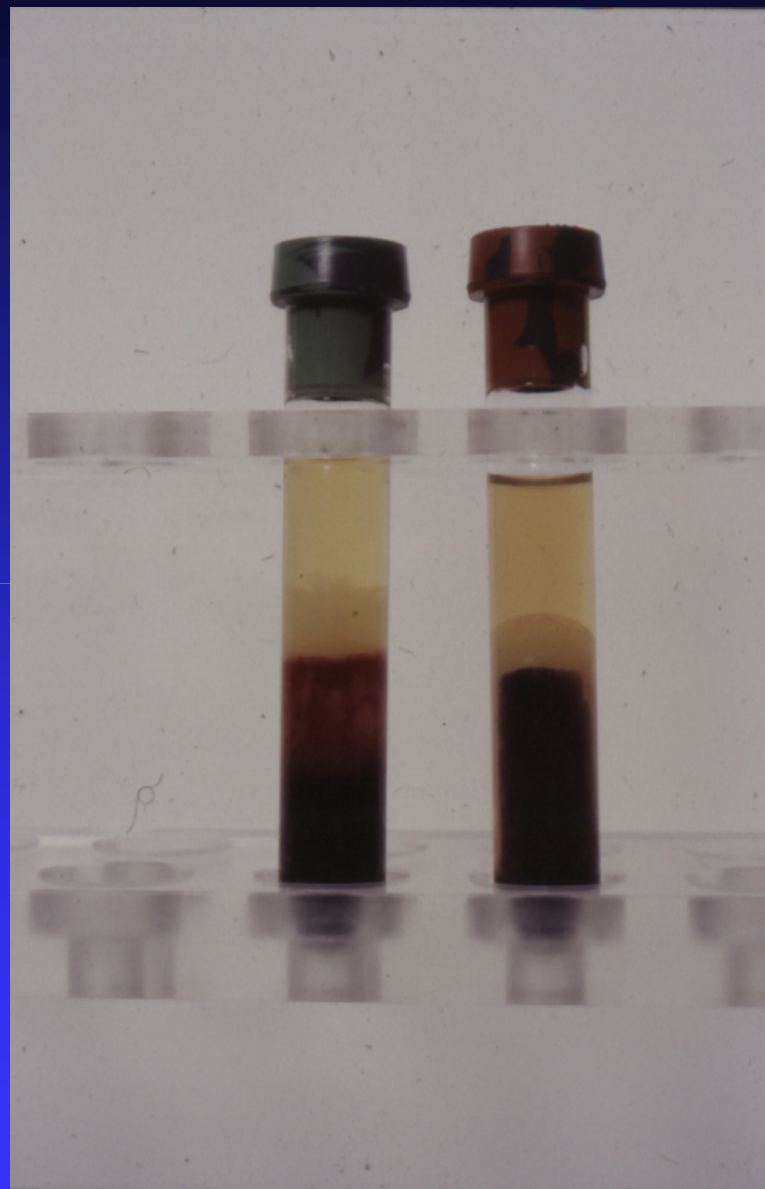


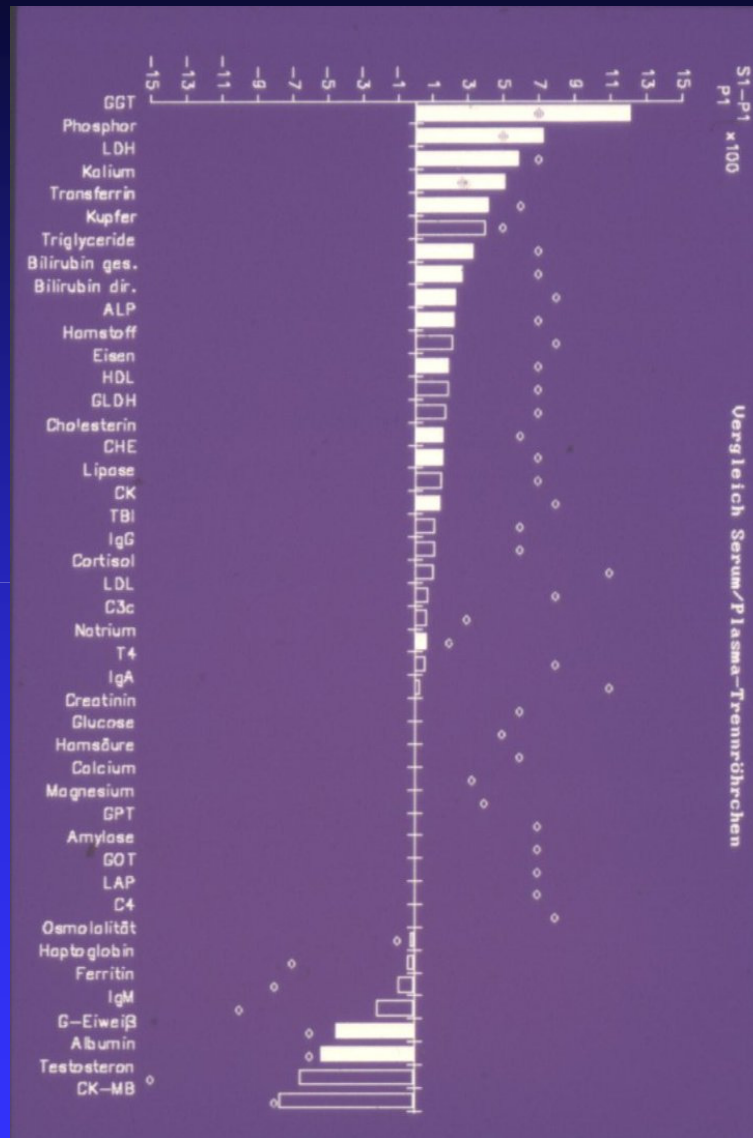
Average Pre-Analytical Specimen Processing Times*

Processing step	Time, min.	
	Urgent	Routine
Arrival, wait and sort	2,4	10,9
Wait for log it	0,3	1,1
Log it	1,7	5,3
Wait for labeling	0,3	4,9
Labeling	1,0	4,3
Wait for centrifuge	0,9	1,0
Centrifuge cycle (load, run, unload)	15,3	16,0
Wait for aliquoting	1,3	6,7
Aliquot	4,0	11,1
Total	27,2	61,3

*Observed with usual batch size and conventional centrifugation in laboratory 3 in Table 1
(observation period, 24 h)

Godolphin et al, Clinical Chemistry, Vol.36, No.9, 1990, 1554





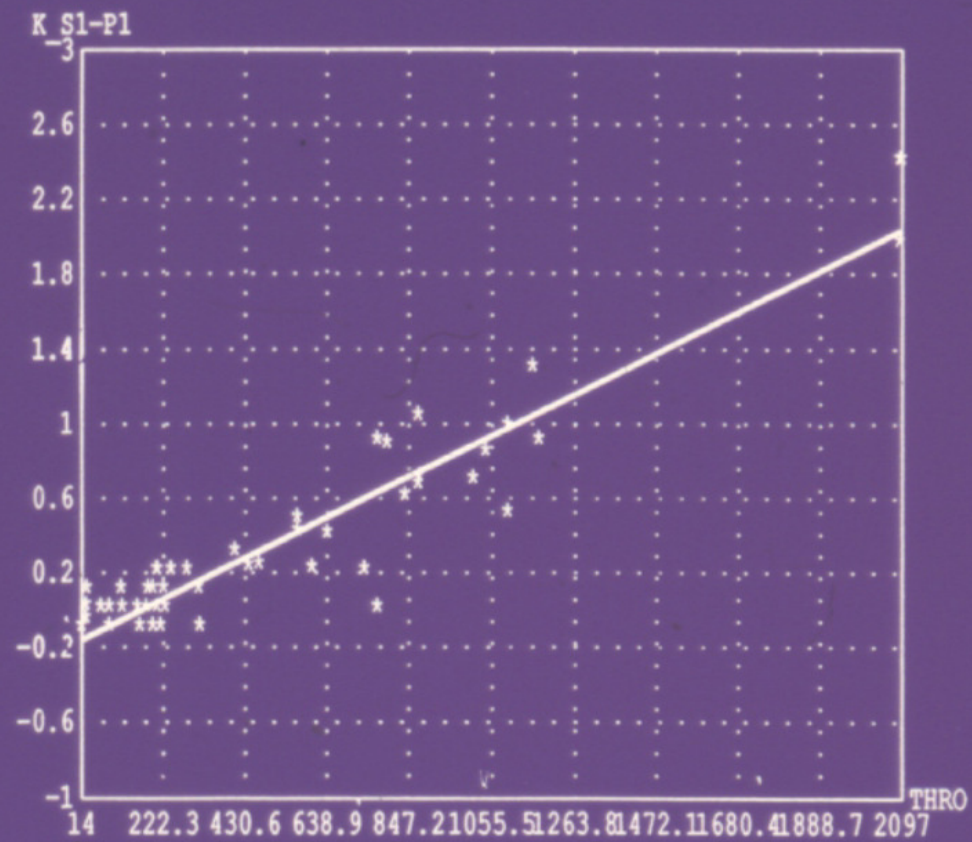


Fig.21 Abhängigkeit der Serum-Plasma-Kaliumdifferenz (mmol/l) von der Thrombozytenzahl ($10^3/\text{mm}^3$). $r=0,95$.

The effect of Thrombocytosis on Serum Potassium and Phosphorus Concentrations

Dave M, Lutomski, MS, Robert H. Bower,
MD†*

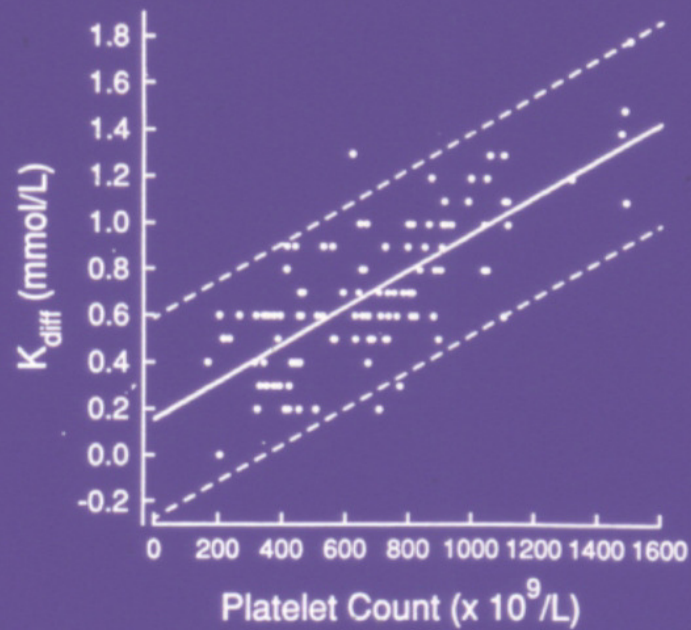


Figure 1. Relationship of serum minus plasma potassium concentration (K_{diff}) to platelet count with the 95% confidence interval for the points ($y = 0.0008x + 0.16$; $r^2 = 0.55$).

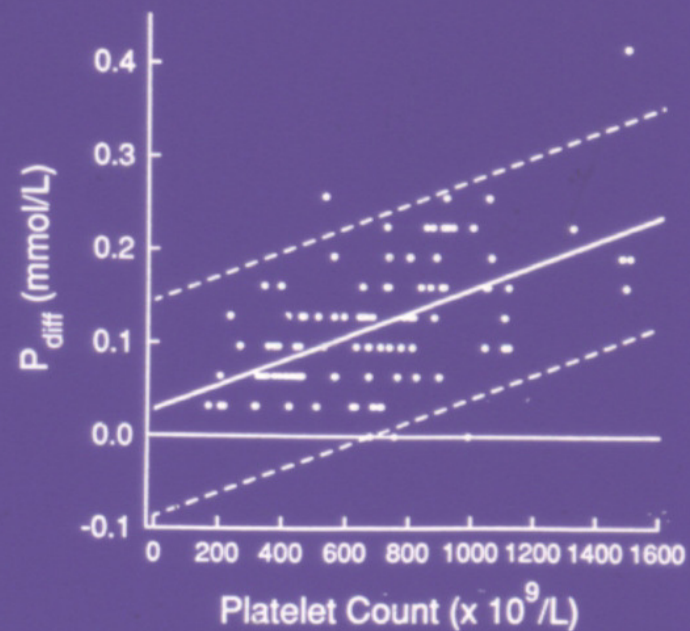
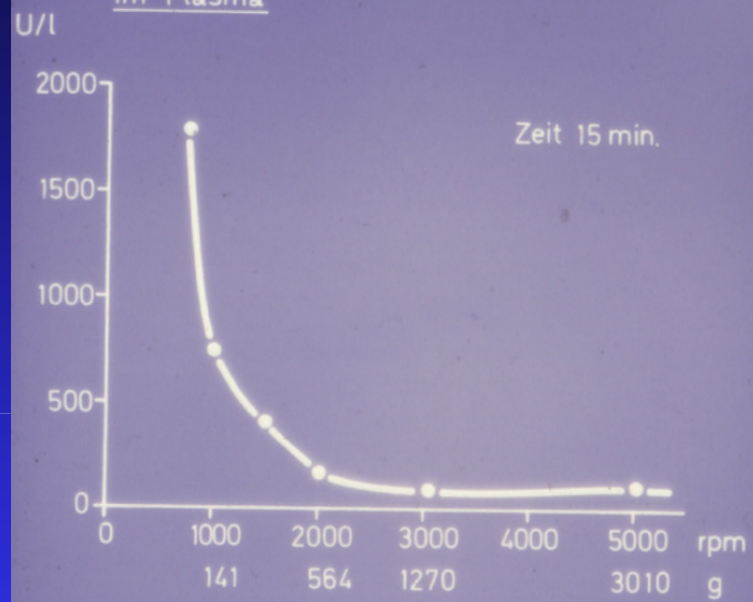


Figure 2. Relationship of serum minus plasma phosphorus concentration (P_{diff}) to platelet count with the 95% confidence interval for the points ($y = 0.00013x + 0.029$; $r^2 = 0.31$).

Einfluß der Zentrifugation auf die LDH-Aktivität
im Plasma



Rothwell, D.J. et al.
Clin. Chem. 22, 1024 (76)

Future Aspects

*Ordered by their Estimated Importance, answers
from 25 European Countries*

1. Diagnostic Request Strategies
2. Diagnostic Validation of Tests
3. Preanalytical Phase
4. Interpretative Results
5. Economic Controlling
6. Biochem. Mechan. of Disease

Further topics:

**Informatics, Sample Handling, Consulting in Wards,
Methodology**

European Questionnaire

Guder 1995

Sampling and Transport

International Standards and Recommendations

Process	NCCLS	ECCL S	ISO/EN
Venipuncture	H4-A3 1991	1987	
Skin puncture	H4-A3 1991	1986	
Arterial puncture	H11-A2 1992		
Single Urine	GP8-P 1985		
Blood for Coagulation Tests	H21-A2 1991		
Timed Urine	GP13-P 1987		
Blood containers	1982	1984	6710/14254
Handling and Transport	H5-A2 1985		15189, 829
Processing of Blood	H18-A 1990		
Specimen for Toxicology	H31-P 1986		

WHO/DIL/LAB/99.1 Rev.2
Original: ENGLISH
Distr.: GENERAL



WORLD HEALTH ORGANIZATION

**USE OF ANTICOAGULANTS IN DIAGNOSTIC
LABORATORY INVESTIGATIONS**

2002

Recommendations of the Working Group on Preanalytical Variables of the German Society for Clinical Chemistry and Laboratory Medicine (DGKL)

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www.diagnosticsample.com

Die Qualität diagnostischer Proben. 5.Auflage 2005

Table: The Use of Various Analytical Samples in Analyzing Blood Constituents

⊕ Recommended sample, + Can be used without changes of result, (+) Can be used with limitations (see comments, in case of citrated plasma this indicates the need to consider dilution by citrate (74))., – Not recommended, Decreased (↘) or increased (↗) values may be measured in comparison to recommended samples. Blank field means no data were found in literature. Greek letters refer to the information provided by diagnostic companies, umbers in brackets to the references.

Analytes	Serum	Heparinate Plasma	EDTA Plasma	Citrated Plasma	Whole blood			Remarks / Comments
					Hep	EDTA	Citrate	
Acetaminophen see Paracetamol								
Acetylsalicylate	+	+β	+ β	(+)β				
α ₁ -Acid glycoprotein (orosomucoid)	+	+ γ	+ γ, γγ	(+)				
Adenovirus antibodies	+		(+)					Complement fixation test, ELISA IgG, IgM
Alanineaminotrans-ferase (ALAT, ALT, GPT)	+	+	+	(+)				
Albumin	+	+*	(+)↘	(+)				*Bichromatic assay recommended for colorimetric assay (72),
Aldosterone	+	+	⊕					

Guder WG, Hagemann
P, Wisser H, Zawta B

Fokus Patientenprobe

Kompendium
Präanalytik

