W. G. Guder, S. Narayanan,
H. Wisser, B. Zawta

WILEY-VCH

Samples: From the Patient to the Laboratory

The impact of preanalytical variables on the quality of laboratory results

3rd revised Edition

Special Edition: "The Quality of Diagnostic Samples"

CD-ROM included

3rd, revised edition 2003

Samples: From the Patient to the Laboratory

The impact of preanalytical variables on the quality of laboratory results

W. G. Guder · S. Narayanan · H. Wisser · B. Zawta

WILEY-VCH GmbH & Co. KGaA

ISBN: 978-3-527-30981-8

1st Edition, 1996
2nd Edition, 2001
3rd Edition, 2003

Front Cover: Fractal image from Mandelbrot's non linear mathematics.
Stephen Johnson; Tony Stone Bilderwelten, Munich

Library of Congress Card No.:
Applied for.

British Library Cataloguing-in-Publication Data:
A catalogue record for this book is available from British Library.

Bibliographic information published by
Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at <http://dnb.ddb.de>.

Printed in the Federal Republic of Germany.
Printed on acid-free paper.

Composition 4t Matthes + Traut Werbeagentur GmbH, Darmstadt
Printing Druckhaus Darmstadt GmbH, Darmstadt
Bookbinding Litges & Dopf, Buchbinderei GmbH, Heppenheim
ISBN 3-527-30981-0

Preface and Acknowledgements

The authors having known each other for many years decided to summarize their experience of preanalytical variables in 1992 after observing an increasing contribution of these factors on laboratory results. They agreed to summarize their knowledge in a short and understandable form aiming to increase the awareness of these factors among all professions involved in the preanalytical phase of the laboratory diagnostic process. This idea was generously supported by Becton Dickinson, Europe.

After the style and general contents of the book were agreed upon in a first meeting of the authors together with the publisher, the manuscripts were completed by the authors in a short time with the help of many collaborators and colleagues. The authors would especially like to thank Heidrun Dürr and Edith Rothermel, Heidelberg, Klaus Krischok, Munich, Ulrich Wurster, Hannover for providing and designing figures. Thanks also to Ingrid Freina, Ulrike Arnold and Patrick Bernhard, Munich, Carol Pirello, New Jersey, Kerstin Geiger, Marion Wajda and Helga Kallmeyer, Mannheim, Annelies Frim, Stuttgart for their expert secretarial help. David J. Purnell, Plymouth, Wolfgang Heil, Wuppertal, and James Brawley, Gaiberg/Heidelberg greatly supported our work by critically reading the manuscripts. We would like to thank Alois Jochum for translation support.

The present 3rd version includes a special edition of "The Quality of Diagnostic Samples" as CD-ROM, containing all Recommendations of the Working Group on Preanalytical Quality, updated May 2003, kindly provided by Chronolab AG, Zug, Switzerland. Several Figures have been replaced by the newest versions available and references adapted to more recent publications.

In continuation of a 10 years collaboration with the Publisher GIT we thank A. Pillmann (Wiley-VCH) for her experienced support in editing this new version in close collaboration with all contributors.

The authors do hope that the new version will help to continuously increase the awareness of preanalytical variables as a possible source of laboratory errors. As the previous editions it is devoted to all professions involved in the organization and performance of preanalytical steps. The authors would be pleased if this work helps to improve the quality of patient care by increasing knowledge on preanalytical variables in the laboratory diagnostic process.

Walter G. Guder Sheshadri Narayanan Hermann Wisser Bernd Zawta

May 2003

Contents

Samples: From the Patient to the Laboratory

The impact of preanalytical variables on the quality of laboratory results

Preface and Acknowledgements ..... *III*

Contents ..... *V*

Foreword to the First Edition ..... *IX*

Dream and reality – An introductory case *02*

The importance of the preanalytical phase *05*

Biological Influences

Something unavoidable –
Influences of age, gender, race and pregnancy *06*

Changing habits –
Influences that can vary (diet, starvation, exercise, altitude) *08*

May I take a coffee, smoke or drink before blood sampling –
Stimulants and addictive drugs as biological influence factors *12*

Collection of Specimen

When to test? – Timing of sampling *14*

Sampling during infusion therapy? –
The impact of the sequence of diagnostic and therapeutic procedures *16*

Sampling in the supine or upright position? –
Effects of posture and tourniquet *18*

What site for sampling blood? –
Phlebotomy, arterial puncture and sampling from catheters *20*

Blood from the skin – Capillary sampling *22*

Did the lab mix up my sample? –
Techniques of sample identification *24*

A precious sample – Cerebrospinal fluid (CSF) *26*

A sample that is nearly always available –
Urine and saliva as diagnostic probes *28*

Plasma or serum? – Differences to be considered *32*

Take a lavender tube! – Additives and colour codes *34*

Transport and Storage

Fax me a sample –
Effects of time and temperature during transport *36*

Contents

Samples in transit –
Legal standardization for mailing samples 38

How to keep a sample "fresh" –
Storage of samples in the laboratory 40

Preparation of Samples for Analysis

What's has to be done on specimen arrival? –
Specimen processing, centrifugation, distribution 42

Continuous or batchwise? –
Preanalytical workflow and robotics 44

Safety aspects during the preanalytical phase –
Disposal of specimens, needles, tubes and chemicals 46

Special Aspects with each Analyte

What is needed before blood transfusion? –
Special aspects in immunohaematology 50

Why a special tube for coagulation tests? –
Special aspects in haemostasiology 52

Blood cells are sensitive! –
Special aspects in haematological analysis 54

Everything from a drop of blood? –
Special aspects in clinical chemistry 56

Special tubes for hormones? –
Preanalytical factors in immunoassays 58

Blood cells can provide important information –
Special aspects in cellular analysis 60

How to handle genes –
Special aspects in molecular biology 62

When gases evaporate –
Special aspects for blood gases and ionized calcium 66

The right time for drugs… –
Special aspects in therapeutic drug monitoring (TDM) 68

Bacteria and viruses –
Special aspects in microbiology 72

Endogenous and Exogenous Interferences

Can turbid samples be used? – Effects of lipemia *76*

A difficult case – Pitfalls with endogenous antibodies *78*

The serum sample looks reddish – Effects of haemolysis *80*

Does the laboratory have to know all my drugs? – Mechanisms and treatment of drug interference *82*

Everything under control? – Quality assurance in the preanalytical phase *84*

References *88*

Glossary *97*

Index *102*

The Quality of Diagnostic Samples CD-Rom ***Annex***

Serum, Plasma or Whole Blood? Which Anticoagulants to Use?

The optimal sample volume

Analyte stability in sample matrix

The haemolytic, icteric and lipemic sample

Samples and stability of analytes in blood, urine and CSF

Foreword to the First Edition

Laboratory tests generally provide a more sensitive indicator of the state of a patient's health than the patient's account of how he or she feels. This has prompted an increasing emphasis on laboratory tests in the diagnosis and management of the patient's disease. Major decisions about the management of a patient are being made on small changes in laboratory data. Thus, a decision to change the dose of a patient's drug is often made on its plasma concentration.

Laboratories have long been aware that many non-disease factors may affect clinical laboratory test values. These include the potential effect of drugs, either through an effect on the physiological function of various organs, or an interference with an analytical method.

Whereas the laboratorian may be aware of the possibility of an analytical interference, clinicians are largely unaware of these effects and the available resources to help them interpret test values correctly. When this information is not given with the result, clinicians may misinterpret test values and take an inappropriate action with their patients.

Clinical decisions based on laboratory test values are correctly made only when the conditions under which blood or other specimens are properly identified and standardized, or when the lack of standardization is recognized and allowances are made for some lack of comparability with previous test values. While laboratorians are aware of the concepts of intra- and interindividual variation as they affect laboratory data, many colleagues are unfamiliar with all but the most obvious causes of differences in test values, such as gender and age.

An understanding of intraindividual variation of test values is important if appropriate clinical decisions are to be made when serial data are being followed. The new concepts of critical differences or reference changes are now important. For proper interpretation of the typically small differences between laboratory data obtained on successive specimens from patients, the variables affecting the test values need to be standardized wherever possible, but first the pertinent variables need to be identified.

These are the issues that prompt the need to revisit all the factors related to preanalytical variables. It is thus particularly timely for this book to be published. The authors hope to reach a broader audience than the laboratorians who are probably quite familiar with many of the factors affecting test results. Since 1956, when Roger Williams published his pioneering studies on the differences between people in a book entitled "Biochemical Individuality", physiologists have been concerned with the differences between people. Now that we have a broader understanding of the genetic influence on human physiology and behavior and a greater need to extract more information from small changes in laboratory data, the publication of a new book concerned with preanaytical variables which contribute to intra- and interindividual variability is both timely and welcome. This book is intended not just for laboratorians but also for physicians, nurses and everyone involved in the chain of events from the decision to order a laboratory test to the interpretation of its results. Proper application of the information contained in this book should lead to less unnecessary testing, reduced costs and a better understanding of the results.

Philadelphia, April 1996 Donald S. Young M.D., Ph.D.

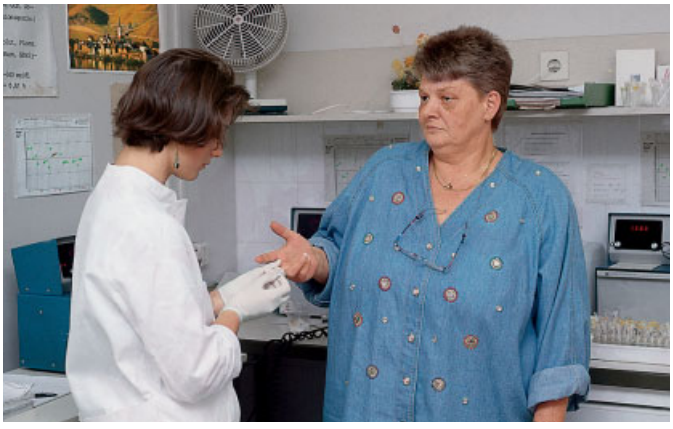
A new patient with diabetes mellitus is encountered

Mrs. Haseltine is a 56-year-old lady who lives in a remote area. She consults her nearby practitioner and reports that over the last two weeks she has urinated more frequently than usual. Also, her body weight has decreased, although she "drinks more soft drinks than ever before". The practitioner finds a positive dipstick result for glucose in her urine. Using a glucometer, he measures glucose from fingertip blood obtained by pricking with a fine lancet. The first drop of blood is washed away with a swab of gauze. In the following drop, glucose is measured by the meter, a process that takes about 30 seconds. The result is 280 mg/dL (15.56 mmol/L), far above the upper limit of the normal range. Mrs. Haseltine is informed that she may have diabetes mellitus and is referred to a diabetologist the next day.

The right sample for the right test at the right time

The diabetologist confirms the result obtained by the practitioner using a capillary blood sample taken 1 hour after breakfast.

Fig. 1-1



Two blood samples are drawn from the patient the following morning (after she has fasted for 12 hours), from the antecubital vein into closed tubes, one, with a lavender-colored stopper, containing EDTA, the other, with a green cap, containing heparin. Mrs. Haseltine is informed that she has type II diabetes mellitus and will have to be placed on a diet in order to treat her disease. She is asked to phone the next day to obtain information on her laboratory results and for further advice.

In the meantime, the heparin blood sample has been centrifuged to separate plasma from the cellular elements. Both tubes are sent to the laboratory by courier in a container especially designed to keep samples at constant temperature. The laboratory receives the samples together with the patient's data and requests for determinations: glycated haemoglobin and blood cell counts from the EDTA blood; potassium and creatinine from the plasma, which has been separated from blood cells, in the closed heparin tube.

The laboratory technician identifies all the samples by comparing the name and bar code number with those on the request sheet. He then enters the request into the lab computer. The samples are put into bar code-reading analyzers for identification and performance of the requested tests. A subsample is taken from the EDTA blood – after slowly mixing it for 3 min on a roller mixer – for the determination of haemoglobin A_{1c} by chromatography. The laboratory report, shown in Tab. 1-1, is sent to the diabetologist the next morning.

Samples: From the Patient to the Laboratory. W. G. Guder, S. Narayanan, H. Wisser, and B. Zawata

ISBN: 978-3-527-30981-8

Tab. 1-1 Laboratory report

Haseltine, Elsa	July 13, 1994	10 a.m.
	Patient Result	Reference Interval
Haemoglobin A_{1C}	8.5%	2.5–6.0
Haemoglobin	14.4 g/dL	12–15
Potassium	3.6 mmol/L	3.5–4.5
Creatinine	1.0 mg/dL	Below 1.1

Mrs. Haseltine is taken into the neighboring county hospital together with a letter from the diabetologist informing the clinician about all tests performed and the results obtained. After two weeks of treatment, Mrs. Haseltine has learned to control her blood sugar using a small glucometer. No further treatment was needed for the next few years.

Fig. 1-2

This is what might happen in reality

Mrs. Haseltine goes to the practitioner with the same symptoms for the same condition. In contrast to the positive urine dipstick result for glucose, the blood sugar is nearly normal (120 mg/dL). The practitioner, to play-it-safe, again refers the patient to a diabetologist.

One week later, Mrs. Haseltine is called in for a glucose tolerance test. The only advice she is given is to fast the night before the test. Mrs. Haseltine wakes up late, however, and misses her morning appointment. She arrives at the doctor's office at noon, having had a snack on the way. She is stressed when the nurse offers her a glucose-containing drink after taking a "fasting" blood specimen.

She feels nauseated while slowly consuming the drink. Whilst waiting for the nurse, she decides not to drink it all and empties the remaining drink down the bathroom sink. Of course, she doesn't report this incident to the nurse when she returns to take a capillary blood sample at one and two hours after the first sample.

When the results are shown to the doctor (Tab. 1-2), he realizes that the glucose concentrations after the first and second hour are not that much different. The diabetologist, unable to arrive at a diagnosis, asks the patient to report the following day at which time two venous blood samples are collected, one with a lavender-colored stopper and the other with a green cap. The tubes are sent to a private laboratory by car. Next day, the results shown in Tab. 1-3 are received by telefax together with the reference values for each test. The glucose value is now normal, potassium elevated and haemoglobin A_{1c}, an indicator of mean blood glucose, elevated to diabetic levels. The diabetologist, concerned by the high potassium level, refers the patient to a clinic. This institution diagnoses that the patient has type II diabetes mellitus, based on their laboratory results.

Tab. 1-2
Results in doctors office: glucose tolerance test

Haseltine, Elsa	July 12, 1994 – 2 p.m.
Fasting Glucose	160 mg/dL
1-Hour Glucose	110 mg/dL
2-Hour Glucose	120 mg/dL

Fig. 1-3

Tab. 1-3 Report from private laboratory Haseltine, Elsa – July 13, 1994 3 p.m.

	Units	Patient's Result	Reference Range
HbA_{1c}	%	8.5	3.5 – 6.0
Haemoglobin	g/dL	13.5	12 – 15
Potassium	mmol/L	5.8	3.5 – 4.5
Creatinine	mg/dL	1.0	Below 1.1
Glucose	mg/dL	105	70 – 110

What happened to Mrs. Haseltine's samples?

Undoubtedly, Mrs. Haseltine was in a diabetic state. Why was the fasting blood sugar nearly normal?

Answer: Fasting may result in near normal values in type II diabetics. In this case, the nurse took the first drop of blood from a fingerprick after "milking" the finger to obtain sufficient blood.

Why was the result of the glucose tolerance test inconclusive?

Answer: The first result was related to patient stress, which leads to increased amounts of glucose being released from liver glycogen stores. Moreover, Mrs. Haseltine had a snack on her way to the doctor because she was hungry. She did not report this to the doctor or the nurse, because she wasn't aware of the possible influence of this snack. For the same reason, she did not report not consuming all of the glucose drink, which had led to a decrease rather than an increase of blood glucose after one hour. The "increase" at the second hour may have been due either to method variation or to a reactive increase brought about by metabolic reactions in the late afternoon. Normally, a glucose tolerance test is performed in the morning, the reference values being valid only for the morning. It should be carried out under standard conditions, as recommended by national and international expert panels.

Why was potassium elevated and glucose normal in the venous specimen?

Answer: The sample was transported in contact with the cells for over two hours in a non- air conditioned car on a hot day. This caused the blood cells to metabolize glucose and release potassium, the concentration of which is approximately 40 times higher in cells than in plasma. This in-vitro influence makes unstabilized blood unsuitable for glucose determination. Potassium can be reliably measured only if plasma is promptly separated from the cells.

All these errors could have been prevented had the preanalytical phase been strictly controlled. Mrs. Haseltine would have been diagnosed earlier with less stress, and fewer costs would have been incurred.

This book is intended to increase awareness of the importance of all steps of the preanalytical phase, including patient preparation, sampling, transport and storage of patient samples.

In each chapter, covering two opposite pages, possible preanalytical variables are explained with regard to mechanisms, effects and preventive actions intended to prevent misinterpretation of laboratory results. In the respective chapters, warnings are given in red and recommendations in green. Like disease mechanisms, biological influences can change the concentration of measured analytes in-vivo, whereas in-vitro changes have to be separated into changes undergone by the measured analyte and interference of the method used to measure the analyte. These definitions are important, because only the latter can be avoided by using a more specific method. The interested reader is referred to the literature summarized on pages 88 – 95 as well as the glossary which defines all the special terms used in this book (p. 97). A detailed information on preanalytical variables of all analytes together with the recommendations on the choice of anticoagulant, the optimal sample volume and the stability of analytes in sample matrix is included in the Annex: The Quality of Diagnostic Samples.

Optimal treatment of patient and his samples is defined as the gold standard

Intrinsic influences such as race, gender and age may influence target analyte concentrations in clinical chemistry and haematology. These variables are individual features of a subject and hence not subject to change. Quite often, intrinsic and external factors are difficult to distinguish.

Age

Age may affect blood and urine analyte concentrations after birth, during adolescence or in old age (Fig. 2-1). Erythrocyte counts and hence haemoglobin are much higher in neonates compared to adults. Within the first few days following birth, increased arterial oxygen provokes erythrocyte degradation. The resulting increase in haemoglobin leads in turn to enhanced concentrations of bilirubin. Since liver function (here in particular glucuronidation) is not fully established in neonates, increased concentrations of bilirubin are observed.

Uric acid concentrations in neonates are in a range similar to adults. However, within days after birth, a significant decrease is observed. Other examples of age-dependence include alkaline phosphatase activity (AP) in serum (which peaks during the growth phase, mirroring bone osteoblast activity) and total and LDL-cholesterol. In addition, age-dependent AP activity and LDL- and HDL-cholesterol in serum are influenced by gender. These gender differences in turn change as a function of age.

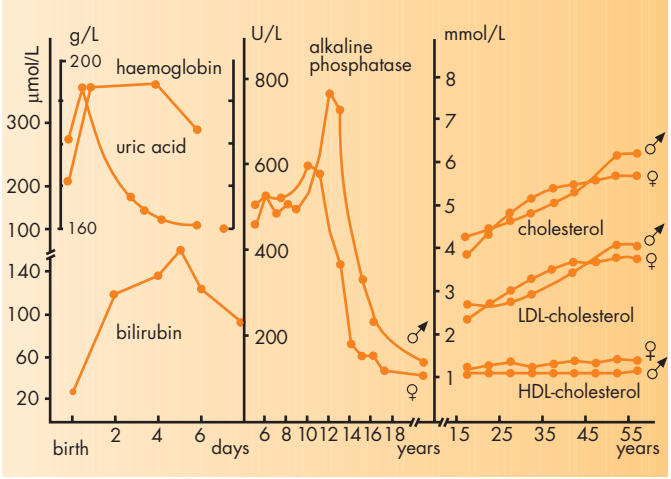


Fig. 2-1
Age dependence of various substrates and enzyme activity (8, 35). Alkaline phosphatase was measured at 30 °C (86 °F) ▼

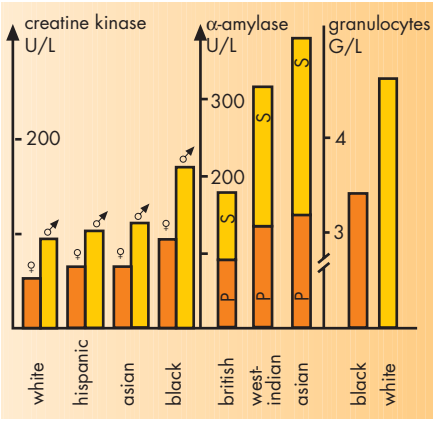


Fig. 2-2
Influence of race on creatine kinase, amylase and granulocytes in blood. P = pancreatic isoenzyme S = salivary isoenzyme ▶

Race

Fig. 2-2 illustrates examples of analytes which are affected by race. Black Americans of both genders have significantly lower white blood cell counts compared to whites. This difference is readily explained by a reduction in the number of granulocytes. In contrast, haemoglobin, haematocrit and lymphocyte counts are almost identical in both groups (97). The monocyte count in whites exceeds that of blacks (11). A significant difference in creatine kinase (CK) activity has been observed for both genders in black and white people. This difference is not due to differences in age, height or body weight (81). A substantial difference in amylase activity has been established between West-Indians and native Britons. Based on the generally accepted threshold value, 50 percent of West Indians had elevated amylase activities (219). Significant racial differences have

ISBN: 978-3-527-30981-8

been reported for serum concentrations of vitamin B_{12} (1.35 times higher concentrations in black people) (183) and Lp(a) (2 times higher concentrations in blacks compared to whites). It is noteworthy in this context that neither atherosclerosis nor mortality is higher in blacks with high Lp(a) (75).

Gender

As with many macroscopic features and gender-specific hormone patterns, gender differences can likewise be found in clinical chemistry and haematology (Fig. 2-3). The gender difference of serum iron concentrations disappears in patients older than 65 years. Other examples of gender differences are CK and creatinine. The serum activity or concentration depends on muscle mass which is in general more pronounced in males. Certain athletic activities which lead to increased muscle mass may blunt this difference (see p. 9–10).

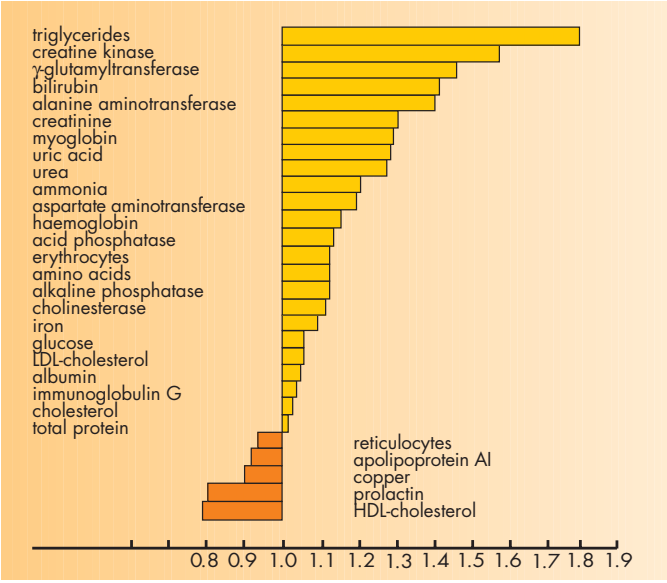


Fig. 2-3
Male – female differences related to the mean value of females as given in (35)

Pregnancy

When interpreting laboratory results during pregnancy, it is necessary to take into account the gestational week at which each sample was taken.

During a healthy pregnancy, the mean plasma volume rises from about 2.6 L to 3.9 L, with probably little change occurring in the first 10 weeks of gestation, and a subsequent progressive rise up to the 35th week, at which time the values level off. Tab. 2-1 describes the mechanism underlying the changes in plasma during pregnancy.

The urine volume may also increase physiologically by up to 25% in the 3rd trimester. There is a 50% physiological increase in the glomerular filtration rate in the last trimester. The well-known changes in hormone production and the plasma concentrations of fertility hormones during pregnancy are accompanied by changes in various analytes, e.g. thyroid hormones, metabolites (amino acids↑, urea↓), electrolytes (calcium↓, magnesium↓, iron↓, zinc↓, copper↑), proteins (especially acute phase proteins↑), and some diagnostically important lipids (triglycerides↑, cholesterol↑), enzymes (alkaline phosphatase↑, cholinesterase↑), factors of the plasma coagulation system and components of the fibrinolytic system. The sedimentation rate is increased five-fold during pregnancy. The concentration changes are caused by different mechanisms as increased synthesis of transport proteins, increased metabolic turnover rate or dilution.

Diet

Diet and drinking are major factors influencing a number of analytes in clinical chemistry. From a practical point of view, one should distinguish acute effects from those observed over a longer period. A critical question in daily routine is whether a standard meal affects target analytes. Fig. 3-1 shows the percentage change in different analyte concentrations as a function of food intake (37, 206). Effects of 5 percent and less may be neglected, since they are clinically irrelevant. Therefore, samples for these analytes do not require strict food deprivation. The extent of food-induced alterations in analytes depends on the composition of the food and the elapsed time between sampling and food intake. The serum concentration of cholesterol and triglycerides are influenced by various factors as food composition, physical activity, smoking, consumption of alcohol and coffee (51). Elevated levels of ammonia, urea and uric acid are observed during a high protein and nucleotide diet. The changes occurring after a standard carbohydrate meal (75 g) are diagnostically helpful in testing glucose tolerance. On the other hand malnutrition and starvation may alter analyte concentrations in a clinically relevant fashion. Early indicators of low protein diet are reduced serum concentrations of prealbumin and retinol-binding protein. Some alterations in clinical chemical analytes induced by starvation over 48 hours are summarized in Fig. 3-2. Metabolic acidosis with a decrease of both pH and bicarbonate results from an increase in organic acids, mainly the ketone bodies (acetoacetic acid, 3-hydroxybutyric acid).

Fig. 3-1
Change of the serum concentration of different analytes two hours after a standard meal (37, 206)
▼

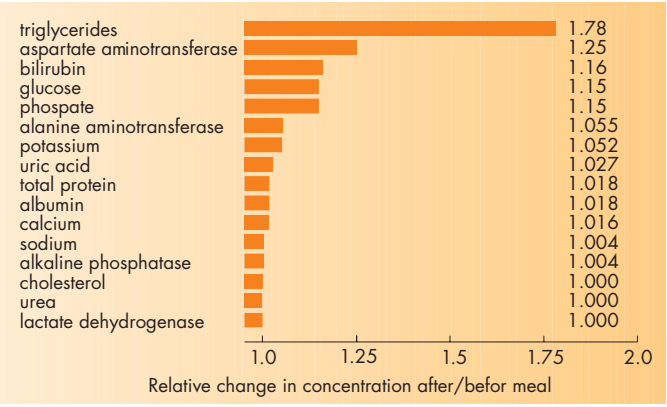
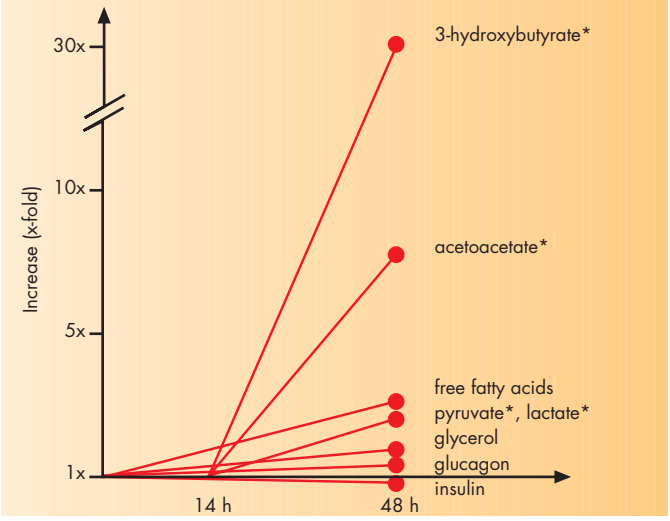


Fig. 3-2
Variation of several analytes after 40-48 h starvation (113).
** Starting point after 14 h starvation*
▼



Starvation

Changes in analyte concentrations induced by long-term starvation (4 weeks) are shown in Fig. 3-3 at the end of the starvation period in comparison to the initial values. The concentrations of blood cholesterol, triglycerides and urea are reduced. In contrast, creatinine and uric acid concentrations are elevated. The increase in uric acid concentration during starvation periods even requires treatment. The latter is due to reduced clearance of uric acid as a result of ketonemia (44). It is readily apparent that long-term starvation is closely associated with reduced energy expenditure; hence, as a result, T_4 and, to a larger extent, T_3 concentrations are reduced in serum. Besides such alterations, urinary

excretion of several compounds is likewise affected by long-term starvation. Urinary excretion of ammonia and creatinine is increased whereas that of urea, calcium and phosphate is reduced (231). Changes in analyte concentrations brought about by long-term starvation are similar to those observed following surgical procedures or in patients with a catabolic status.

In measuring quantitative urinary excretion rates, excreted amounts per day are preferable to those per liter in order to eliminate variations in drinking habits and water excretion.

Mechanisms

Changes may be due either to an increase in reabsorption of the measured analyte (triglycerides, glucose, amino acids), intestinal or liver metabolism of reabsorbed metabolites (VLDL, urea, ammonia) or regulatory changes due to food intake or deprivation (uric acid, γ-glutamyltransferase, cholinesterase, thyroxine, retinol-binding protein, ketone bodies).

Recommendation

In order to avoid misinterpretation of laboratory results, sampling after 12 h fasting and reduced activity is recommended as a standard procedure.

Exercise

Before considering the influence of exercise on target analytes in clinical chemistry, two types of exercise have to be distinguished. First, static or isometric exercise of brief duration and high intensity which utilizes the energy (ATP and creatine phosphate) already stored in muscle and, second, dynamic or isotonic exercise of lower intensity and longer duration (e.g. running, swimming, cycling) which utilizes ATP produced by aerobic or anaerobic pathways. In addition, the effect of physical training and muscle mass should be mentioned. Acute changes of analytes during exercise are due to volume shifts between the intravasal and interstitial compartments, volume loss by sweating and changes in hormone concentrations (e.g. increase in the concentrations of ephinephrine, norepinephrine, glucagon, somatotropin, cortisol, ACTH and decreased concentrations of insulin) (4, 177). These changes in hormone levels may in turn alter the leukocyte count to more than 25 G/L as well as increasing glucose concentrations. Fig. 3-4 shows

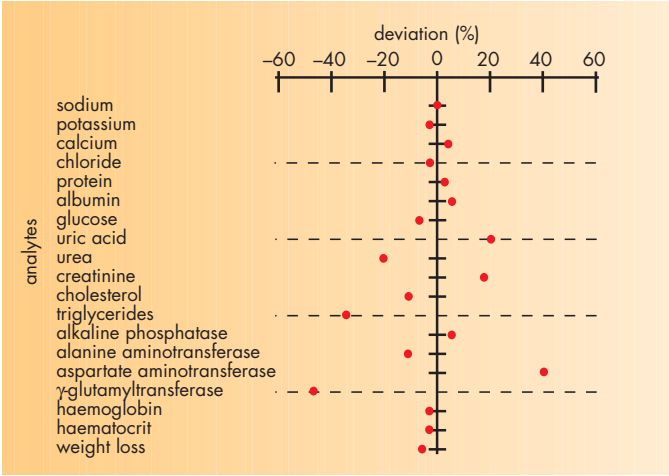


Fig. 3-3
Change (%) of clinical chemical analytes after 4 weeks starvation and a daily supply of 33 g protein, vitamines and electrolytes (44, 232)

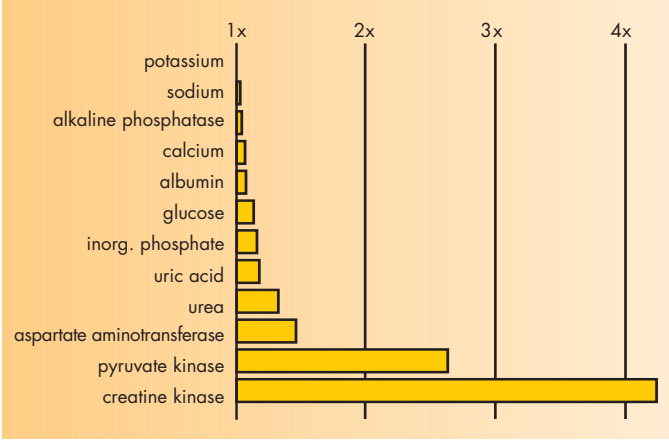


Fig. 3-4
Increase of various analyte concentrations after a marathon race. Blood was drawn one day before and 45 min after the race (203)

changes in analyte concentrations induced by marathon running (203). The extent of change depends on a variety of individual and/or environmental factors (e.g. training status, air temperature and intake of electrolyte- and carbohydrate-containing liquids during the actual run).

The changes observed (e.g. increased albumin) can in part be attributed to the above-mentioned volume shift from intravasal to the interstitium or to loss of volume by sweating. The increased uric acid concentration in serum is a consequence of reduced urinary excretion due to increased lactate concentrations. Hypoxia-mediated creatine kinase (CK) increase depends on the training status and hence shows a high degree of individual variability. The less physically fit an individual is the more pronounced the increase in CK. Training increases both the number and the size of mitochondria which is associated with increased capacity of the oxidative enzyme system. This effect in turn increases the capacity of the muscle to metabolize glucose, fatty acids and ketone bodies in aerobic pathways. As a consequence, mitochondrial CK-MB increases to more than 8 percent of the total CK activity without evidence of altered myocardial function. Well-trained individuals have a higher percentage of total activity in terms of the CK-MB of skeletal muscle compared to untrained persons. Several other analyte concentrations likewise depend on muscle mass and training status. Thus, plasma creatinine, urinary creatinine and creatine excretion increase and lactate formation after exercise decreases in trained compared to untrained athletes. Vigorous exercise may cause erythrocytes or other blood cells to be excreted in urine. These exercise-induced changes, however, usually disappear within a few days.

Altitude

Some blood constituents exhibit significant changes at high altitude compared to findings at sea level.

Significant increases with altitude are observed, for example, for C-reactive protein (CRP) (up to 65% at 3600 m), β_2-globulin in serum (up to 43% at 5400 m), haematocrit and haemoglobin (up to 8% at 1400 m) and uric acid. Adaptation to altitude takes weeks and return to sea level values takes days. A significant decrease in values with increasing altitude is found in the case of urinary creatinine, creatinine clearance, estriol (up to 50% at 4200 m) serum osmolality, plasma renin and serum transferrin (239).

Caffeine

Caffeine is found in many constituents of food ingested daily. Despite its widespread use, the influence of caffeine on various analytes in clinical chemistry has not been investigated in detail. Caffeine inhibits phosphodiesterase and hence cyclic AMP degradation. Cyclic AMP in turn promotes glycogenolysis, thereby increasing blood glucose concentrations. In addition, the glucose concentration increases due to gluconeogenesis via epinephrine. Activation of triglyceride lipase leads to a threefold increase of non-esterified fatty acids (141). Quantification of hormones and drugs bound to albumin is hampered by the fatty acid-induced displacement effect. Three hours after the intake of 250 mg of caffeine, plasma renin activity and catecholamine concentrations have been found to be elevated (175).

Studies intended to investigate these analytes should take caffeine consumption into account.

Effects of smoking

Smoking leads to a number of acute and chronic changes in analyte concentrations, the chronic changes being rather modest. Smoking increases the plasma/serum concentrations of fatty acids, epinephrine, free glycerol, aldosterone and cortisol (239). These changes occur within one hour of smoking 1-5 cigarettes. Alterations in analytes induced by chronic smoking include blood leukocyte count, lipoproteins, the activities of some enzymes, hormones, vitamins, tumor markers and heavy metals (Fig. 4-1) (239).

The mechanism underlying these changes has not been fully elucidated. A large number of pyridine compounds, hydrogen cyanide and thiocyanate are found in tobacco smoke. They can account for concentration changes by direct or indirect effects. Decreased angiotensin converting enzyme activity (ACE) in smokers is believed to result from the destruction of lung endothelial cells with a subsequent reduction in the release of ACE into the pulmonary circulation and/or enzyme inhibition (76). The extent of changes also depends on the amount, kind (cigarettes, cigars, pipes) and technique of smoking (with or without inhalation). Moreover, smoking-induced changes are influenced by age and gender (204).

Fig. 4-2 shows the concentrations of cotinine, thiocyanate and carboxyhaemoglobin, used as markers for the qualitative and quantitative assessment of smoking habits. Cotinine has the advantage of having a longer half-life (20–28 h) than nicotine, the parent compound (12–15 min) (189).

Fig. 4-1
Deviation (%) of blood analyte concentrations between current smokers and non smokers, chronic effects (239) ▶

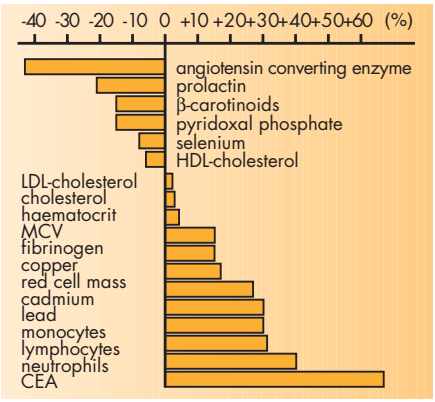
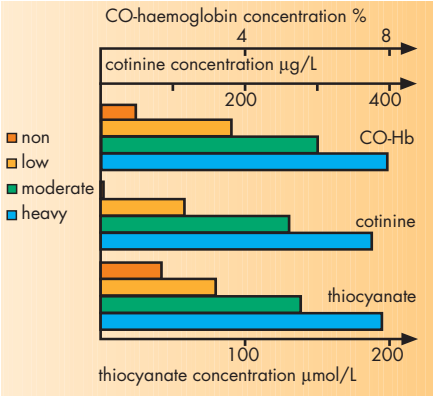


Fig. 4-2
Effect of smoking on different blood analytes caused by smoke constituents (141, 239) ▶



Alcohol

Alcohol consumption, depending on its duration and extent, may affect a number of analytes. These alterations are used in part for diagnosis and therapeutic monitoring. Among alcohol-related changes, acute and chronic effects should be considered separately.

The acute effects (within 2–4 hours) of ethanol consumption are decreased serum glucose and increased plasma lactate due to the inhibition of hepatic gluconeogenesis. Ethanol is metabolized to acetaldehyde and then to acetate. This increases the formation of hepatic uric acid formation (67). Together with lactate, acetate decreases serum bicarbonate, resulting in metabolic acidosis. Elevated lactate reduces urinary uric acid excretion. Consequently, after acute alcohol ingestion, the serum concentration of uric acid increases (204).

The long-term effects of ethanol ingestion include an increase in the serum activity of liver enzymes. The increase of g-glutamyltransferase activity is caused by enzyme induction. Glutamate dehydrogenase as well as aminotransferases (AST, ALT) activities increase due to a direct liver toxic effect (57). The increase in desialylated forms of proteins in blood (i.e. carbohydrate deficient transferrins) is due to an inhibition of enzymatic glycosylation during post-translational processing of these proteins in the liver. In chronic alcoholism, serum triglycerides increase due to decreased plasma triglyceride breakdown. The increased MCV may be related to a direct toxic effect on the erythropoetic cells or a deficiency of folate (173).

The data in Fig. 4-3 do not take into account either the dose or the time-dependency, which underly both the acute and the chronic effects. Enhanced diuresis is also a result of the decreased release of vasopressin followed by increased secretion of renin and aldosterone (17, 115).

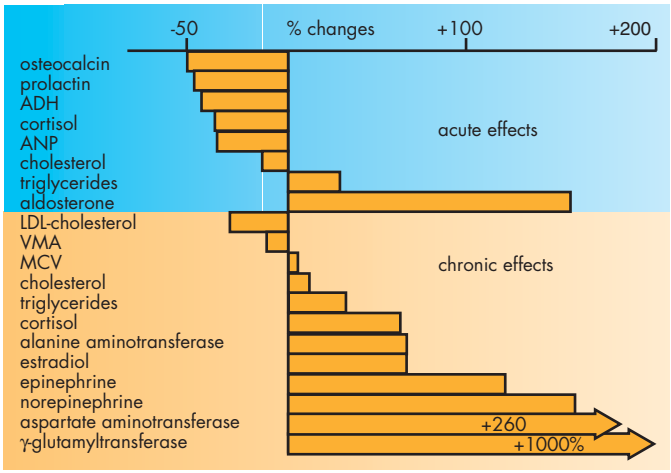


Fig. 4-3
Acute and toxic effects of alcohol ingestion on clinical chemical analytes (115, 168, 239)

Addictive drugs

Addictive drugs such as amphetamine, morphine, heroin, cannabis and cocaine can influence the results of laboratory tests. Morphine causes spasms of the sphincter of oddi, thus elevating levels of enzymes such as amylase and lipase. The biological effects of addictive drugs on selected laboratory tests are listed in Tab. 4-1.

Tab. 4-1 Biological effects of addictive drugs on plasma concentrations of selected analytes (241)

Addictive drug	Increased/Decreased in plasma		
1. Amphetamine	Increased: free fatty acids.	3. Heroin	Increased: pCO_2, T_4, cholesterol,
2. Morphine	Increased: α-amylase, lipase, AST,		potassium due to severe rhabdomyolysis.
	ALT, bilirubin, alkaline phosphatase,		Decreased: pO_2, albumin.
	gastrin, TSH, and prolactin.	4. Cannabis	Increased: sodium, potassium, urea,
	Decreased: insulin, norepinephrine,		insulin, chloride.
	neurotensin, pancreatic polypeptide.		Decreased: creatinine, glucose, uric acid.

Changes brought about in specimens due to the time factor should be taken into account in the preanalytical phase. Three questions are essential in this context:

- When should a sample be taken?
  – Time of day
  – Time after last sample
  – Time after last meal
  – Time after drug etc.
- When do I require the result of the specimen taken now?
- Can results be compared with the results obtained at a different time in daily, monthly and yearly rhythms, either from the same patient or from a reference population?

For the sake of clarity, we can differentiate between linear time, going from the past to the future, and cyclic time; both of these can influence the results of laboratory tests (Fig. 5-1).

Influence of circadian rhythm (217)

Several analytes tend to fluctuate in terms of their plasma concentration over the course of a day (Tab. 5-1).

Thus, the concentration of potassium is lower in the afternoon than in the morning, whereas that of cortisol increases during the day and decreases at night (Fig. 5-2).

The cortisol rhythm may well be responsible for the poor results obtained from oral glucose tolerance testing in the afternoon.

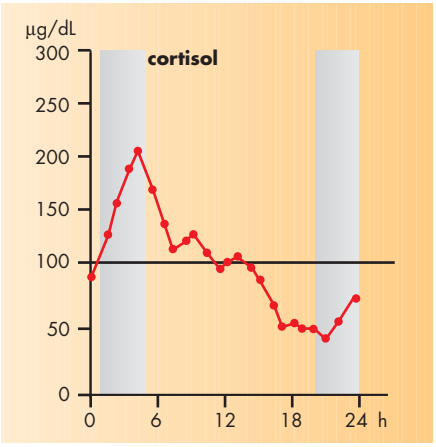


Fig. 5-2
Daily variation of plasma concentrations of cortisol (shaded areas = sleep period)

For this reason, reference intervals are actually obtained between 7 and 9 a.m. The circadian rhythm can also be influenced by individual rhythms concerning meals, exercise and sleep. These influences should not be confused with real circadian changes. In some cases, seasonal influences also have to be considered. Thus, triiodothyronine (T_3) is 20% lower in summer than in winter (82) whereas 25-OH-cholecalciferol exhibits higher serum concentrations in summer (162).

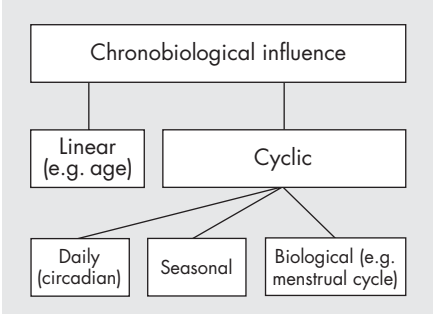


Fig. 5-1
Linear and cyclic chronobiological influence

Analytes may change during the menstrual cycle (239)

Analytes can also exhibit statistically significant changes due to the biological changes that occur in the hormone pattern during menstruation. Thus, the aldosterone concentration in plasma is twice as high before ovulation than in the follicular phase. Likewise, renin can

ISBN: 978-3-527-30981-8

Tab. 5-1 Diurnal variation of selected analytes (S = serum; U = urine) (235)

Analytes	Maximum (time of day)	Minimum (time of day)	Amplitude (percentage of daily mean)	Analytes	Maximum (time of day)	Minimum (time of day)	Amplitude (percentage of daily mean)
ACTH	6–10	0–4	150–200	Norepinephrine (S, U)	9–12	2–5	50–120
Cortisol (S,U)	5–8	21–3	180–200	Haemoglobin	6–18	22–24	8–15
Testosterone	2–4	20–24	30–50	Eosinophils	4–6	18–20	30–40
TSH	20–2	7–13	5–15	Iron (S)	14–18	2–4	50–70
T_4	8–12	23–3	10–20	Potassium (S)	14–16	23–1	5–10
Somatotropin	21–23*	1–21	300–400	Phosphate (S)	2–4	8–12	30–40
Prolactin	5–7	10–12	80–100	Sodium (U)	4–6	12–16	60–80
Aldosterone	2–4	12–14	60–80	Phosphate (U)	18–24	4–8	60–80
Renin	0–6	10–12	120–140	Volume (U)	2–6	12–16	60–80
Epinephrine (S)	9–12	2–5	30–50	Body temp.	18–20	5–7	0.8–1.0 °C

* Start of sleeping phase

show a pre-ovulatory increase. Even cholesterol exhibits a significant decrease during ovulation. In contrast, phosphate and iron decrease during menstruation.

Why has blood to be taken 12 hours after the last meal? (49, 239)

As mentioned under diet (see p. 8–9), several metabolic products of food can increase in venous blood or become altered due to post-absorptive hormonal effects. Other analytes may be disturbed by turbidity due to chylomicronemia present in postprandial blood samples.

In order to eliminate these variables, a 12 hour period of starvation is recommended before blood is sampled for the analysis of these analytes (Tab. 5-1).

Timing with regard to diagnostic and therapeutic processes

As described in the next chapter, a number of diagnostic procedures may influence laboratory results. In order to prevent this, the timing of sampling has to be organized to take place before interfering diagnostic procedures. Likewise, interfering drugs should be administered after taking a blood sample. On the other hand, in drug monitoring (see p. 68) the exact timing of sampling is essential for correct interpretation of the drug level.

Important rules for the timing of sampling:

- *If possible, samples should be taken between 7 and 9 a.m.*
- *Sampling should be carried out 12 hours after the last meal.*
- *Samples should be taken before interfering diagnostic and therapeutic procedures are performed.*
- *In drug monitoring, consider the peak after drug administration and the steady state phase before the next dose.*
- *Always document the exact time of sampling in the charts and requests.*

But:

- *A sample taken at the wrong time can be worse than taking no sample.*
- *A sample whose analytical results arrive too late is a wasted sample.*

Implausible laboratory results after diagnostic and therapeutic intervention?

The following diagnostic and therapeutic measures can result in both in-vivo (frequent) and in-vitro (less common) effects on laboratory tests (79, 99, 232):

- Operations
- Infusions and transfusions
- Punctures, injections, biopsies, palpations, whole-body massage
- Endoscopy
- Dialysis
- Physical stress (e.g. ergometry, exercise, ECG)
- Function tests (e.g. oral glucose tolerance test)
- Immunoscintigraphy
- Contrast media, drugs
- Mental stress
- Ionizing radiation

Operations

Changes in serum enzyme activities are frequently so great that specific targeting of an organ is no longer possible. The elevation in acute phase proteins (e.g. C-reactive protein (CRP), fibrinogen) at the beginning of the postoperative phase is accompanied by a decrease in albumin; this cannot be explained alone by haemodilution.

Transient elevations in urea concentration in serum/plasma (up to 60 mg/dL or 10 mmol/L) as well as a decrease in cholesterol are very frequent in the first postoperative days whilst the creatinine concentration remains normal. This may be due to protein breakdown subsequent to gastro-intestinal tract surgery as well as to bleeding in the lumen of the bowel, e.g. in the case of a stress ulcer.

Tab. 6-1 Infusions/transfusions as interfering factors and/or contaminants of laboratory diagnostic tests

Infusion/Transfusion	Analyte affected	Trend	Comments, Mechanism
Dextran	Thrombin time, reptilase time	↓	5 – 10 sec slower
	von Willebrand factor	↓	
	Total protein in serum, plasma	↑	Biuret, method-dependent (turbidity, flocculation, greenish coloration)
	Urea, serum	↓	
	Blood grouping serology		Pseudoagglutination
γ-globulin	Serological determinations during virus-mediated and bacterial infections		False positive
Electrolytes	Potassium, sodium, magnesium	↑	Contamination
Glucose	Glucose	↑	Contamination
Glucose	Inorg. phosphate, potassium,	↓	Insulin
	Amylase, bilirubin	↓	Up to 15 %, particularly in neonates
Fructose	Uric acid	↑	Metabolic effect
Citrate (blood transfusion!)	pH value in blood	↓	
	Coagulation tests	↓↑	Inhibition

Infusions, transfusions

Haemolysis and hence the concentrations of free haemoglobin and potassium, as well as the activity of lactate dehydrogenase in plasma obtained from conserved blood, increase with the age of the transfused conserved material.

Contamination of laboratory samples by infusion solutions is the commonest and often the most relevant form of preanalytical interference in the hospital (228, 242) (Tab. 6-1).

Blood should never be collected proximal to the infusion site.

Specimens should be collected from the opposite arm. A certain period of time should be allowed to elapse following infusion therapy (Tab. 6-2)

Tab. 6-2 Recommendations for scheduling infusions and blood sampling

Infusion	Earliest time of blood sampling in hours after cessation of infusion
Fat emulsion	8
Carbohydrate-rich solutions	1
Amino acids and protein hydrolysates	1
Electrolytes	1

It is recommended that the laboratory be informed of when and what type of infusions were carried out and when blood samples were taken.

Sampling from catheters

If samples are to be taken from intravenous and intraarterial infusion catheters, the cannula should be rinsed with isotonic saline commensurate with the volume of the catheter. The first 5 mL of blood should be discarded before a blood sample is taken.

Sampling for coagulation tests from heparin-contaminated catheters is particularly critical. For heparin-dependent methods (thrombin time, APTT), it is recommended that an amount of blood equivalent to twice the volume of the catheter be discarded; the blood first taken after this should be used for non-haemostaseological investigations and the subsequently obtained citrated blood only used for determining heparin-insensitive analytes: Prothrombin time, reptilase time, fibrinogen according to Clauss, AT III, fibrin monomers. It is important that before transferring blood to the sampling vessel containing sodium citrate solution there is no lengthy pause during which the blood in the catheter is allowed to "stand".

Mental stress

The importance of mental stress on laboratory results is frequently underestimated (anxiety prior to blood sampling, preoperative stress, etc.). Increased secretion of hormones (aldosterone, angiotensin, catecholamines, cortisol, prolactin, renin, somatotropin, TSH, vasopressin) and increased concentrations of albumin, fibrinogen, glucose, insulin, lactate and cholesterol have been observed.

Fig. 6-1

Sampling in the supine or upright position?

Posture

It is a well-known fact that body posture influences blood constituent concentrations. This is caused by different mechanisms. First, the effective filtration pressure (e.g. the difference between capillary pressure and colloidal osmotic pressure in plasma) increases in the lower extremities when changing from the supine to the upright position. As a consequence, water is moved from the intravasal compartment to the interstitium; this reduces the plasma volume by about 12 percent in normal individuals. Blood particles with a diameter of more than 4 nm are restrained by membranes and cannot follow this volume shift. A change from the upright to the supine position leads to a decrease in the effective filtration pressure and hence to a volume shift in the reverse direction (176).

A change in plasma volume leads to an apparent concentration change in cells, macromolecules and protein-bound small molecules. Most low molecular weight compounds show no change in their apparent concentrations when changing from the upright to the supine position. As osmolality is mainly mediated by such compounds, the first is only modestly affected by changes in plasma volume (1–2%). Because of partial protein binding, the concentrations of free and bound calcium are affected in a different manner. Whilst the concentration of free calcium is independent of posture, total calcium increases by 5–10 percent when changing from the supine to the upright position (172). Other changes are due to altered blood pressure which in turn causes secretion of vasoactive compounds. In addition, the metabolic consequences of regulatory changes due to postural changes may alter body fluid composition.

The effects of posture on analytes in venous anticubital blood are shown in Fig. 7-1. As expected from the described mechanism, most cellular and macromolecular analytes decrease between 5 and 15% compared to the supine position. These effects can be more pronounced in patients with a tendency to edema (cardiovascular insufficiency, liver cirrhosis). Reduction in plasma volume induces a decrease in blood pressure which in turn leads to increased secretion of renin, aldosterone, norepinephrine and epinephrine. A fall in blood pressure causes decreased secretion of atrial natriuretic peptide which leads to decreased plasma concentrations (211). An example of the metabolic changes brought about due to posture is the urinary excretion of calcium which increases during long-term bed rest (see Fig. 12-2, p. 28).

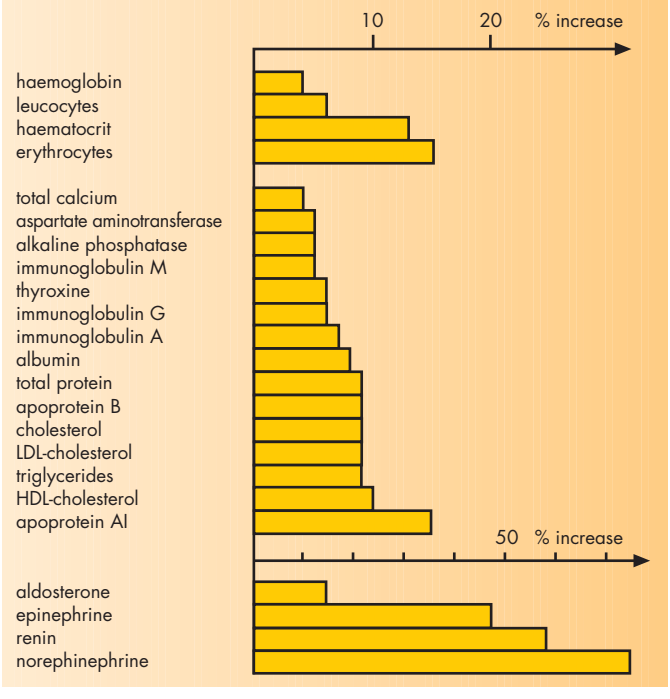


Fig. 7-1
Increase (%) of plasma concentration of various analytes when changing from supine to an upright position (55, 131, 232, 239)

Tourniquet

What happens when a tourniquet is kept on during sampling? A tourniquet

is usually applied to facilitate finding the appropriate vein for venipuncture (see p. 20). Using a pressure below the systolic pressure maintains the effective filtration pressure inside the capillaries. As a consequence, fluid and low molecular compounds are moved from the intravasal space to the interstitium. Macromolecules, compounds bound to protein and blood cells, do not penetrate the capillary wall so that their concentration apparently increases while the concentration of low molecular substances is unchanged.

Fig. 7-2 shows the changes of different analyte concentrations (93). The alterations of low molecular weight analytes observed following 6 min. of constriction are in the range of ±3 percent and therefore within the range of analytical imprecision. However, it has been shown that constriction of the forearm muscles causes an increase in serum potassium concentration. Therefore, during venipuncture for potassium determination, repeatedly clenching and unclenching a fist should be avoided and a superficial vein selected (45, 197). A venostasis of two minutes did not change the blood lactate concentration (mean +2.2 %), but decreased the pyruvate blood concentration significantly (mean –18 %) (108). The extent of changes in high molecular weight analytes depends on the duration of constriction. Fig. 7-3, for example, demonstrates changes of lactate dehydrogenase activity and total protein concentration. The greatest effects are observed within 5 minutes, little change taking place thereafter (58). Constriction times of one minute with subsequent release of the tourniquet have no consequences on plasma serum analyte concentrations and coagulation factors.

To reduce the intra- and interindividual variance of laboratory results a standardised sampling procedure is an important prerequisite. The following conditions should, whenever it is possible, be fulfilled: a preceding phase of rest and fasting, the same body position, daytime and tourniquet application time, avoidance of repeatedly clenching and unclenching a fist. The tourniquet application time should not be longer than one minute. During a running infusion the phlebotomy should be performed on the opposite arm, in any case not proximal from the running infusion. If it is necessary to repeat sampling for any other reason, the phlebotomy should also be performed on the other arm (176, 177).

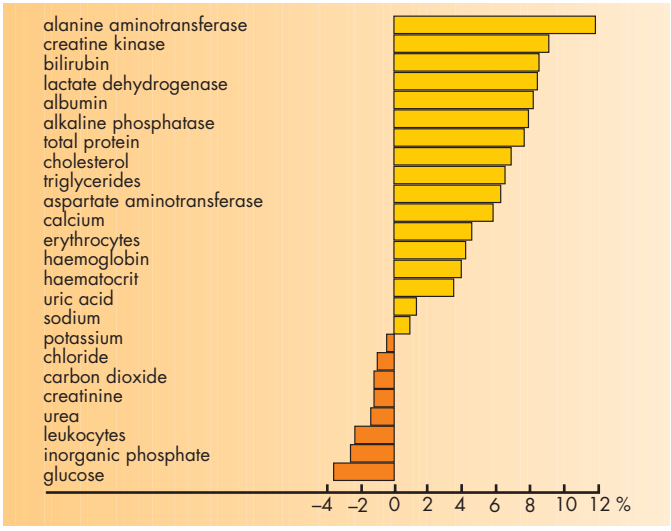


Fig. 7-2
Change (%) in serum concentration of various analytes after a tourniquet application time of 6 min (93)

In comparing laboratory results, reference intervals should be obtained under identical conditions with regard to body posture (176).

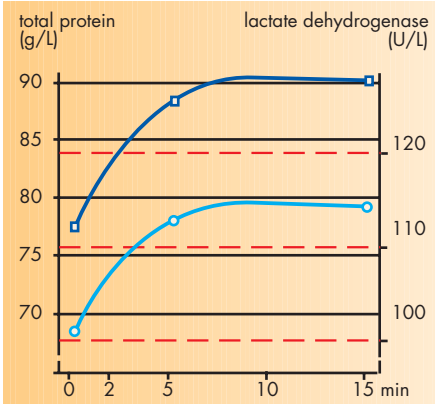


Fig. 7-3
Change in total protein concentration (○) and lactate dehydrogenase activity (□) in serum during a 15 min tourniquet application time (58)

What site for sampling blood?

The site of collection such as vein, capillary or artery has a bearing on the procedural aspects of specimen collection (205).
Specific NCCLS documents address procedures for the collection of blood specimens by venipuncture (156), skin puncture (157) and arterial puncture (158).

Phlebotomy

Critical steps in phlebotomy involve not only preparation of the patient for blood collection and proper collection of the specimen, but also other steps amongst which are proper identification of the patient. This avoids patient mix-up and ensures patient safety. Fig. 8-1 visualizes the steps to be taken in blood collection using evacuated tubes.

- ***Identification:*** *Match the patient's test order form with the patient number, bar code or wrist band number.*
- ***Position:*** *Position of the patient (sitting or recumbent) for venipuncture.*
- ***Materials:*** *Ensure blood collection equipment (needle, collection holder, tubes) is at hand.*
- ***Inspection:*** *Inspect patient's arm, select a vein while the patient's fist is clenched.*
- ***Disinfection:*** *Clean the venipuncture site.*
- ***Vein Exposure:*** *Apply a tourniquet.*
- ***Puncture Collection:*** *See Fig. 8-1.*
- ***Mixing:*** *Mix the blood in tubes containing additives or clot activators.*
- ***Prevention of Bleeding:*** *Apply gauze to the venipuncture site while removing the needle. Apply a bandage to the patient's venipuncture site.*
- ***Disposal:*** *Dispose of the needle in a safety disposal unit.*

The order of collection of tubes is important when multiple tubes of blood are collected (Tab. 8-1).

Quality of sample:

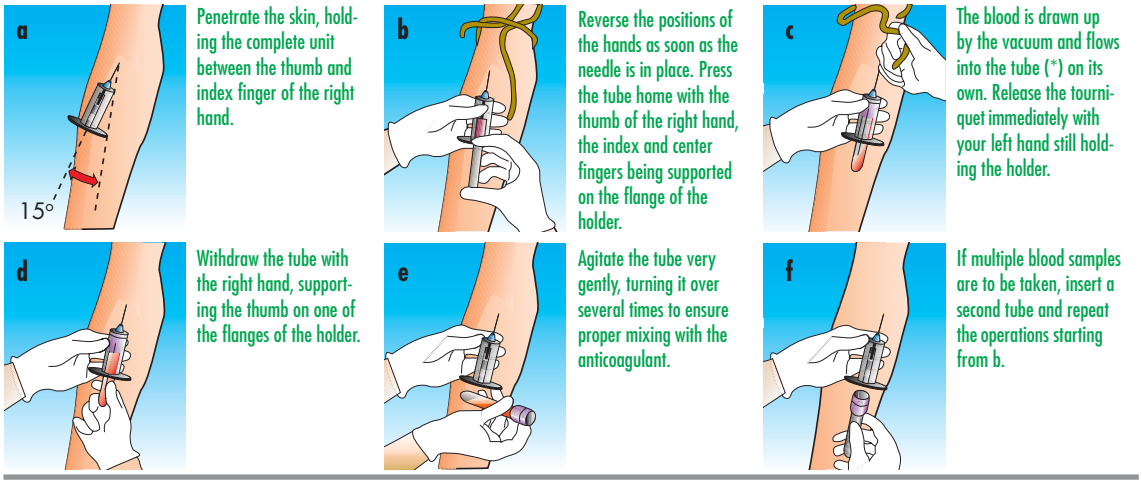
Examine to see whether the tubes are overfilled or underfilled.

Overfilling tubes intended for haematology determinations can cause spurious results due to the absence of a bubble at the very top which will prevent proper mixing of the specimen on rocker type mixing devices (166).

For certain procedures, the anticoagulant to blood ratio is critical (see p. 52).

If multiple blood collection tubes are to be collected (156), the following order of collection is recommended:

Fig. 8-1 Steps in blood collection



* If the blood fails to flow into the tube, the needle should be revolved to exclude that it is occluded against the wall of the vein. If this does not result in blood flow, it has not found a vein. Before withdrawing the needle, disengage the tube so as to preserve the vacuum for further use. Recommence operations from a. Note: The phlebotomist in above steps (b to f) is wearing gloves

Tab. 8-1 Recommended sequence of collecting various blood specimens

1. Blood culture	Blood
2. Non-additive tube	Serum
3. Citrate	Plasma
4. Heparin	Blood
5. EDTA	Blood/plasma
6. Glycolytic inhibitor	Glucose/lactate

If a blue stoppered citrate tube intended for coagulation testing is to be drawn first, a 5 mL discard tube should be filled to eliminate possible thromboplastin contamination from the venipuncture site.

How much blood is needed?

The volume of blood collected from a patient should be kept to a minimum to avoid blood loss that could render the patient anemic. The term investigational anemia has been coined to refer to blood loss due to repeated venipunctures. Often, too much blood is drawn as is apparent from a Mayo Clinic study where it was reported that for routine collections, an average of 45 times the required volume of specimen (range 2 to 102 times!) was withdrawn, while for microcollection an average of 7 times the required volume of specimen (range 1 to 20 times) was withdrawn (40). In an earlier study it was reported that 47% of patients who required blood transfusions had a phlebotomy-related red blood cell loss of more than 180 mL, an amount equivalent to 1 unit of packed red blood cells (198).

It was recommended (71, Annex) to collect twice the amount of blood needed for analysis. The amount of blood needed may be calculated from the analytical portion needed, the dead space of the analytical system and the sample tube using the following formula:

Blood volume needed = 2 x [number of repetitive tests x (analytical volume + dead space of the analytical system) + dead space of the secondary tube] + dead space of primary tube. This formula assumes that 50 % of blood volume can be used as analytical volume (71, Annex).

Sampling from artery

Particular care should be taken when blood is collected from an artery (158). The common sites of arterial puncture are the femoral artery, brachial artery and radial artery. Other sites include scalp arteries in infants and the umbilical arteries during the first 24 to 48 hours of life. Arterial puncture is necessary when venous blood does not permit the measurement of the relevant concentration of the desired analyte (e.g. blood gases, pH). Arterial puncture can be performed either singly by inserting a short-beveled, sharp needle into an artery. A syringe can be attached to the needle either directly or by way of tubing with an adapter such as in a winged infusion set. Arterial blood can be collected without suction when using 23-gauge or larger needles, allowing the pressure in the artery to force the blood into the syringe.

Sampling with catheter

Continuous or repeated sampling of arterial blood can be performed by leaving a permanent needle, cannula or catheter in the artery or central vein. Care should be taken to ensure that no clot is formed at the tip or in the lumen of the catheter. Between samples, an anticoagulant (preferably heparin) should be used to flush the needle.

When sampling with a venous catheter, the coagulation tube containing sodium citrate should be filled before a heparin-containing tube is collected. The first few mL of blood, representing 1 to 2 volumes of the catheter, should be discarded to avoid contamination with anticoagulant.

Blood from the skin

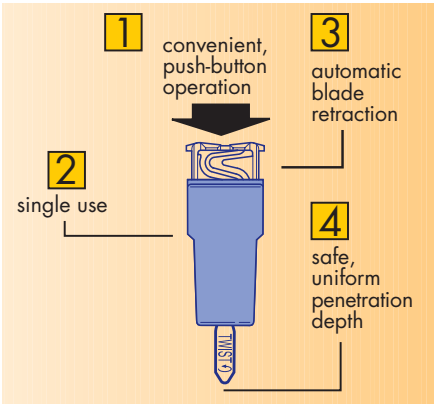


Fig. 9-2
Genie™ lancet
▶

Skin puncture is the procedure of choice if a small amount of blood is to be taken from a pediatric subject. In adults, capillary blood is used for blood gases, glucose and lactate. There are differences between capillary and venous blood, especially in oral glucose tolerance testing.

Blood obtained by skin puncture is composed of a mixture of blood from the arterioles, venules and capillaries; it may also be diluted with interstitial and intracellular fluid.

The relative composition of skin puncture blood will depend on variables such as blood flow to the skin during collection. Warming the puncture site prior to blood collection in effect arterializes the blood in the skin (157).

Sites for blood collection are illustrated in Fig.9-3. They include the palmar surface of the distal phalanx of the finger and the lateral or medial plantar surface of the heel (157).

Finger puncture should not be performed on infants since there is a likelihood of injuring the bone. There is a linear relationship between the volume of blood collected and puncture site penetration depth (7). Therefore, the lancet should be selected according to the site punctured and the amount of blood needed (Fig. 9-1).

The depth of incision made to an infant's heel is critical since puncturing deeper than 2.4 mm on the plantar surface of the heel of small infants especially can damage the calcaneus or heel bone. This can be avoided by using semi-automatic disposable lancets (Fig. 9-2). After selection of the skin-puncture site, the site should be cleaned with a 70% aqueous solution of isopropanol prior to puncture (125). After drying the site with a sterile gauze to ensure that residual alcohol has been removed (since it would otherwise cause haemolysis), skin puncture should be performed with a disposable lancet. The use of other disinfectants to clean the puncture site should be avoided as this would spuriously elevate the results for uric acid, phosphorus or potassium (225).

The first drop of blood that flows after skin puncture should be discarded by wiping with gauze, since this drop is

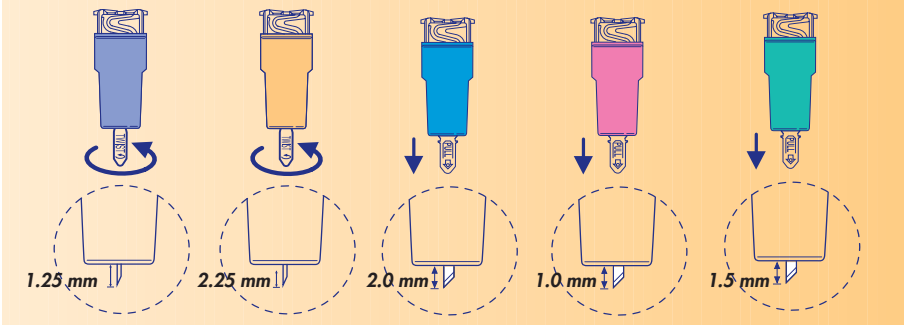
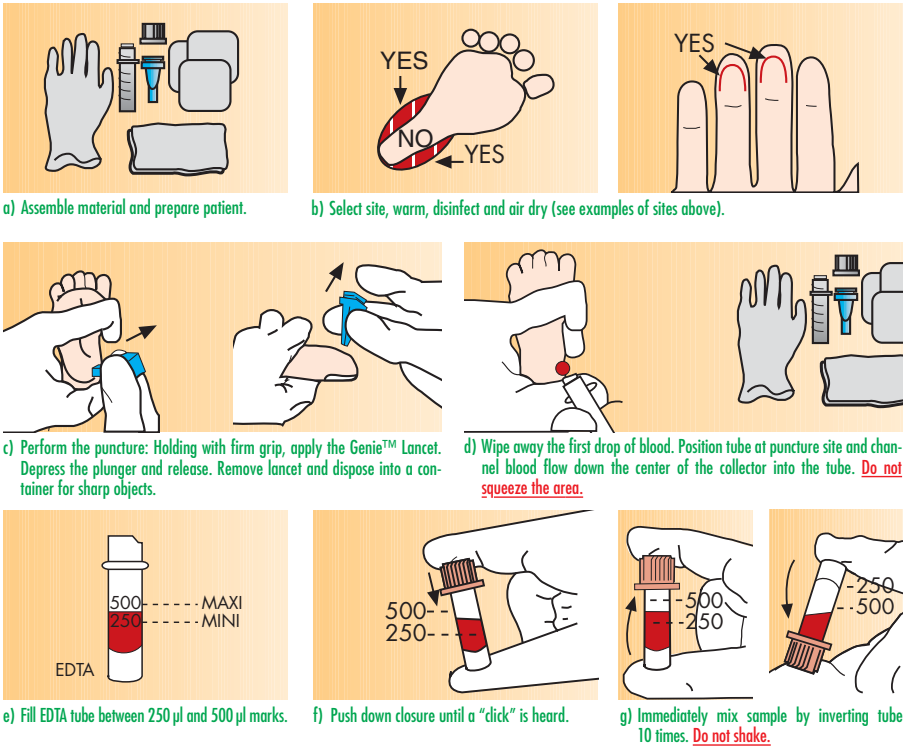


Fig. 9-1
Penetration depth of Genie™ lancet



a) Assemble material and prepare patient.

b) Select site, warm, disinfect and air dry (see examples of sites above).

c) Perform the puncture: Holding with firm grip, apply the Genie™ Lancet. Depress the plunger and release. Remove lancet and dispose into a container for sharp objects.

d) Wipe away the first drop of blood. Position tube at puncture site and channel blood flow down the center of the collector into the tube. Do not squeeze the area.

e) Fill EDTA tube between 250 µl and 500 µl marks.

f) Push down closure until a "click" is heard.

g) Immediately mix sample by inverting tube 10 times. Do not shake.

Fig. 9-3
Capillary blood collection with a typical micro collection device

likely to be contaminated with tissue fluids. By application of gentle pressure but without milking or massaging the area around the puncture site, free flowing drops of blood should be collected by touching the drops with the tip of a collector top and letting them flow by capillary action into a properly labeled microcollection device.

Fig. 9-3 illustrates a typical microcollection technique. In the event that drops do not flow freely from the collector top into the microcollection tube, the tube may be gently tapped to facilitate the flow of drops of blood into the tube. Upon completion of blood collection, the tube should be firmly secured. Additive-containing tubes should be well mixed after specimen collection by gently inverting the tube approximately 10 times.

Collection of blood specimens into heparinized capillary tubes intended for blood gas analysis should be made after warming the puncture site with a towel soaked in running water at a temperature not greater than 42 °C to bring about arterialization of the puncture site. The capillary tubes should be free of air bubbles after collection.

On conclusion of specimen collection, the puncture site should be pressed with a sterile gauze or swab and held in place until bleeding stops. As a safety measure, it is advisable not to apply adhesive bandages over the puncture site of infants and children, not only out of concern that the adhesive may cause irritation but also due to the fact that the bandage could become loose and be swallowed by the child.

The safety lancets used for skin puncture should be deposited in an appropriate puncture-resistant safety container intended for such a purpose.

Did the lab mix up my sample?

Everyone concerned with diagnostic procedures is aware of the problem of sample identification. The mixing up of patients' names, requests, samples or test results can have serious effects on patient treatment and should therefore be considered as maltreatment. This chapter briefly summarizes the present state of the art of patient and sample identification during the preanalytical phase (18).

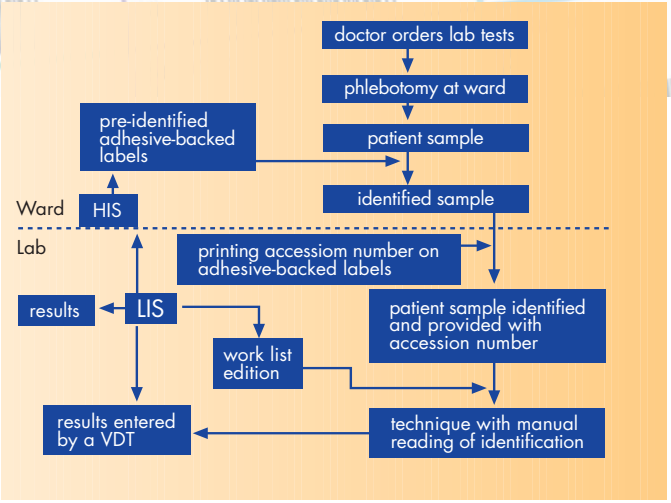


Fig. 10-1
Information flow during a laboratory test in a hospital. HIS = hospital information system LIS = laboratory information system

Name or number?

When admitted to a hospital, a patient has to be identified by many people including nurses, doctors, technicians and other persons. The same is true for any sample taken from the patient and transferred to an in-house or external laboratory. As can be seen in Fig.10-1, communication between all parts of the diagnostic process requires transfer of this patient identification. A name used to identify an individual has proved to be insufficient in transferring the identity. Name, prename and date of birth is the most frequently used combination of information for identification. In order to reduce the amount of information involved, a patient number is allocated in many hospitals. This number cannot be used for any other individual. Ideally, this number is printed together with the name on any request, sample and report. Ward, room and bed numbers may be of additional help. This is especially useful if sampling is not done by the same person as the one ordering laboratory tests.

Since such a large amount of information cannot be entered on small labels, a separate request form usually contains all the basic information together with the test orders linked to the sample by a number or code only.

Fig. on the back of the above text illustrates an example of a request form containing a sufficient number of labels for all possible samples, used on sample tubes (Fig. 10-2). Alternatively, a package of preprinted, self-adhesive labels with the patient identification may be provided on admission to hospital (Fig.10-3).

As a minimal requirement the sample entering the lab should contain the following information:

- *Name, prename*
- *Date of birth*
- *Identity number of sample, or patient or request*
- *Ward number (sender, name of ordering physician)*
- *Time of sampling (day, hour, min)*

Techniques of identification

Upon arrival in the laboratory, the patient has to be identified from the information provided. This can be done visually by reading the name and ward from the label and request form, transferring this information to the laboratory chart and all subsamples. The latter requires the generation of a sub-numbering system which is linked to the individual and the day of analysis (usually no more than three digits long).

Many institutions, however, use electronic means to transfer identification data: this can be done on-line using a hospital information system which transfers the basic data of the patient together with the request directly to the laboratory computer. In this case, the samples arriving in the laboratory have to be linked to a given request by bar-code or alternative code system fixed to the

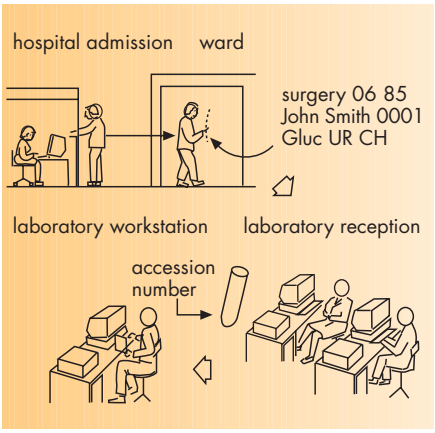


Fig. 10-2
Request number on adhesive labels for sample tubes

Fig. 10-3
Patient's label provision at hospital admission and their use in the laboratory

samples. In the example given in Fig. 10-2, the number on the sample is identical to the number of the request-form which is then electronically linked to the patient by the laboratory computer (Fig. 10-3).

Recently, more sophisticated systems have been suggested and will be widely used in future: a two-dimensional bar code (Fig. 10-4), or transponder-chips fixed to the sample can contain all the necessary information including requests and patient information.

Direct versus indirect sample identification in the laboratory

The identity of the patient is unequivocally linked to the sample container at the collection point of the sample, providing the primary sample is maintained throughout the analytical process. This needs serum/plasma separators to keep the analytical sample (plasma/serum) in the primary sample (blood) tube so that it can be identified directly by the reader of the analytical instrument.

If such a reader is not available or subsamples have to be distributed, a sampling device may be used to automatically define the positions of each sample in each analyzer used. This kind of identification is called indirect identification (by location) (128). Whereas direct identification mechanisms allow the sample to be identified at any position and any time, indirect identification by position can be done only with the aid of a position list or a computer system. In addition, any sub-sampling has the inherent danger of additional sample mix-up.

- Any sample should be identified unequivocally before being analyzed.
- Direct identification procedures from primary samples should be used in preference to indirect identification and subsampling, to reduce misidentifications.
- Any sample whose identity and origin is not sufficiently documented should be processed to a stable form (serum, plasma, closed in refrigerator) and missing information added before analysis is performed.

Fig. 10-4 Two dimensional bar-code in comparison to unidimensional bar-code.

How to collect CSF?

Puncture sites

CSF and serum form an inseparable unit in modern diagnostics; for this reason CSF and serum should be collected as closely together as possible (103). The puncture site is generally lumbar, but also ventricular, suboccipital or via a shunt and should be noted.

Fig. 11-1 Sampling of cerebrospinal fluid

The puncture site should be marked, and the area disinfected. Treatment of weals with a local anesthetic is desirable for the patient. Puncture should be saggital and sloping upwards (20°) (Fig. 11-1).

Amount required

The quantity taken depends on the clinical situation and is not so critical in adults; CSF is very rapidly replenished (50 mL/day in adults). Particularly when searching for tumor cells, it is important to obtain as much CSF as possible (up to 30 mL being optimum in adults) (Tab. 11-1). The sampling time should be as long as possible, using a needle as fine as possible, to avoid headache following puncture.

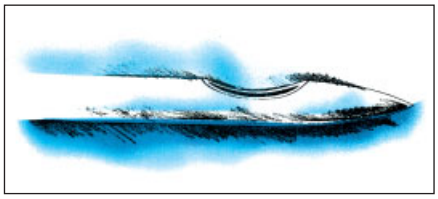


Fig. 11-2 "Atraumatic" pencil – shaped needle

Which specific aspects of CSF sampling are important?

The fasting patient should be seated or asked to lie on his/her side on a flat support. The patient's back should be bent forwards and the position secured by an assistant. The musculature should be as relaxed as possible (Fig. 11-1). The time at which the sample is taken should be noted, together with information on any initial treatment (e.g. in bacterial meningitis).

It is recommended that an "atraumatic" pencil-shaped needle (0.7 mm outer diameter), as designed by Sprotte and Whitacre (Fig.11-2), be used instead of a 22G needle with a Quincke cut so as to avoid post-puncture syndrome (headache) (24).

In order to avoid contamination CSF should be obtained and transported in closed tubes.

The CSF should be placed, under aseptic conditions, in separate colorless polystyrene tubes with stoppers (sterile for microbiology and dust-free for cytology or clinical chemistry). For cytology, the use of additives such as EDTA and fluoride should be avoided. After a sample has been obtained, the needle should be removed and the wound covered with a plaster. Patients should be kept lying on their stomachs for at least 30 min to avoid subsequent leakage.

Tab. 11-1 Sequence and quantity of CSF recommended

Fraction	Adults	Infants
	Discard the first 0.5 mL and all artificially sanguineous CSF	
Microbiology*	~ 2 mL	~ 1 mL
Cytology	> 10 mL	> 1 mL
Supernatant used for clinical chemistry	(tumor cells!)	(tumor cells!)
Total	12 mL	2 mL

*Before commencing chemotherapy, or 2–3 days after its withdrawal.

What about storage and transport?

Most important of all:
As soon as possible after the sample has been taken, it should be sent to the laboratory by courier.

CSF is not particularly "cell friendly". Transport in an icebath over 200 km by car (approx. 3 hours) is possible. Stability data of individual components are given in the Annex. Further recommendations are given in Tab. 11-2.

Tab. 11-2 Recommendations for transport and storage of CSF

Up to 1 hour	Do not cool
Up to 3 hours	Transport on ice
	Never deep freeze
	No additives
	No partial fixation
Long-term storage	After centrifuging off cells, immediately cool to –70 °C in glass or polypropylene vessels that can be tightly closed.

For cytological investigations, instead of original CSF, send cytocentrifugation preparations to the laboratory. This should be prepared close to the patient using a special centrifuge (centrifugation for 20 min at 180 *g*). These samples are stable for 4 to 6 days at room temperature or – when fixed in acetone – for 3 to 12 months at –70 °C (immunocytology depending on the antigen) (237).

Special hints for CSF sampling

- Sampling in several portions means that the concentration gradients that always exist (e.g. for albumin and IgG) are not taken into account. This can be avoided if the entire volume is first collected in one tube, well mixed and then divided into aliquots.

- Gloves dusted with talcum powder should not be used when withdrawing CSF, as CSF cytology is thereby invalidated.

- The presence of up to 6000 leukocytes/µL should not alter the lactate concentration within 3 hours at room temperature (120).

- Deep-freezing of CSF is better carried out at –70 °C than at –20 °C since, for example, oligoclonal protein bands slowly disappear after storage over 6 months at –20 °C.

A sample that is nearly always available

Urine

A review of the ten most popular errors in the analysis of urine revealed that some of the problems were of a preanalytical nature: samples were too old, sampling vials had been contaminated, the samples were not homogeneous and preanalytical factors had not been adequately taken into account when interpreting test results (59, 109). The quality of the collection vial is a critical factor in this context. The ideal container for any urine specimen is a wide-mouthed bottle of appropriate size (Fig.12-1). Urine containers used should either be disposable or, if not, detergents have to be reliably removed prior to use. If the urine is to be analyzed bacteriologically, containers have to be sterile. In protein and hormone analysis, analyte absorption to the vessel wall should be avoided. The different kinds of sampling are given in Tab. 12-1. For pediatric and newborn patients, urine specimen collection bags with hypoallergenic skin adhesive should be used. First, the pubic and perineal areas should be cleaned with soap and water. Then, the adhesive should be pressed all around the vagina or the bag fixed over the penis and the flaps pressed to perineum. The container should be checked every 10–15 min (153). Urine samples obtained in the morning offer several advantages. A high degree of osmolality indicates an intact concentrating ability on the part of the kidney. Urine samples obtained in the morning are particularly useful in identifying mycobacteria. Deviations due to diet, physical activity and posture are diminished. Fig.12-2 shows the

Fig. 12-1
Containers for urine specimens for qualitative and quantitative testing

Tab. 12-1: Different types of urine specimens and their use in the laboratory

1.	Random or spot urine	Qualitative chemical determinations
2.	First morning urine	Cellular constituents and casts
3.	Second morning urine (7 – 10 a.m.)	Quantitative determinations related to creatinine
4.	24 h urine	Quantitative determinations

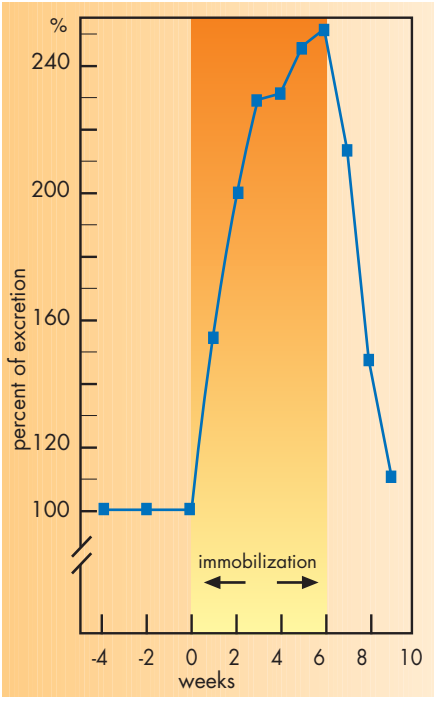


Fig. 12-2
Urinary excretion of calcium during a six-week immobilization period

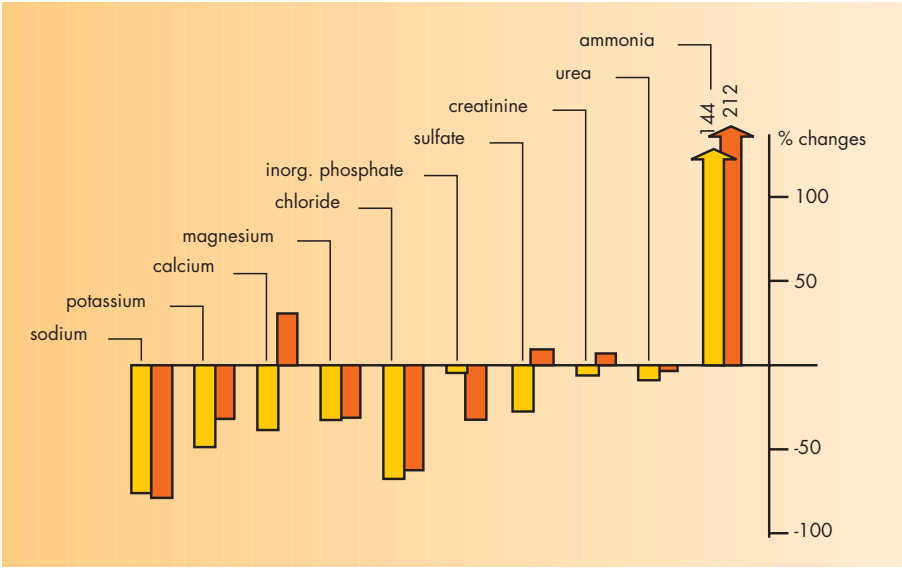


Fig. 12-3
Influence of 4-day starvation on urinary excretion of electrolytes and substrates. Change (%) during (■) and one day after the voluntary starvation (■) related to the starting value (106)

increase of the urinary excretion of calcium to 240% during a six week immobilization period (240).

Fig.12-3 gives an example of the influence of diet on the urinary excretion of electrolytes and some substrates. Under these special conditions, five healthy volunteers received only distilled water during a four-day voluntary period of starvation. Most analytes showed a reduced level of excretion. Only ammonia excretion is increased as a consequence of the metabolic acidosis induced by starvation (106).

For quantitative analysis, one should use timed urine, preferably in the form of 24-hour urine collection (104). Mid-stream catheterized or suprapubic puncture urine specimens are recommended for bacteriological investigations (59, 109).

In Fig.12-4, the collection of a mid-stream urine (clean-catch urine) is shown schematically. Mid-stream urine containing bacteria $>10^5$/mL is considered to indicate significant bacteruria. Analysis of urine samples should ideally proceed within one hour following sample generation (except in the case of 24-hour urine samples). A short processing time is of particular importance once a morphological evaluation of the labile urinary sediment constituents has to be performed. Thus, the stability of erythrocytes and leukocytes in urine is dependent on both pH and osmolality. A pH above 7.5 and an osmolality below 300 mosm/kg results in rapid degradation of these cells (165). Other

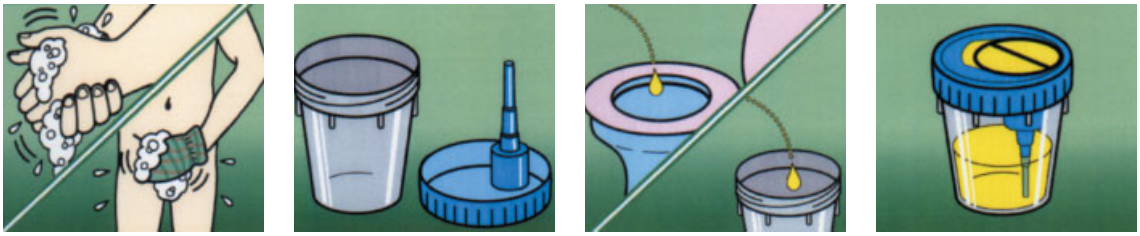


Fig. 12-4
Collection and sampling of mid-stream urine (clean-catch urine)

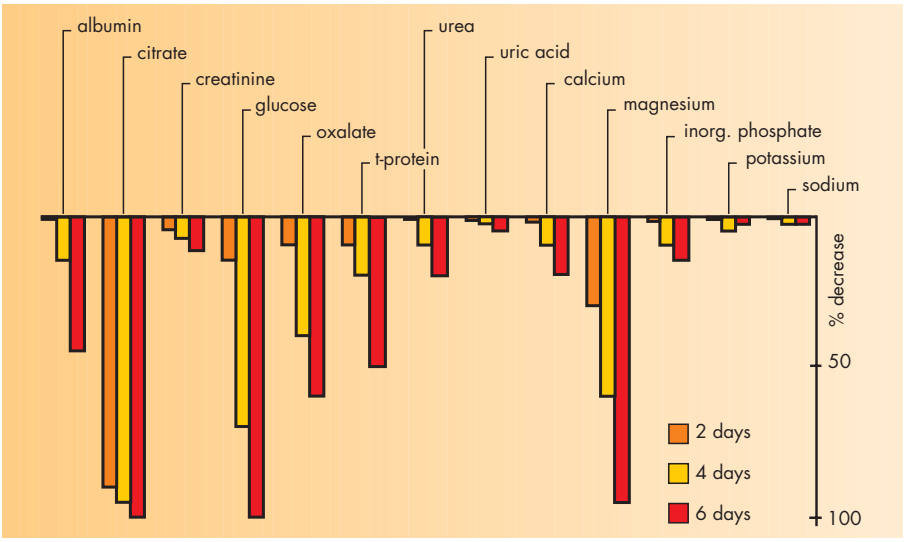


Fig. 12-5
Influence of storage time on the recovery of various analytes in urine samples without additives and storage at room temperature (% changes from the starting point) (186)

analytes tend to form crystals at physiological pH (calcium, oxalate, uric acid) or are decomposed if not adequately stabilized (glucose, urea, citrate). Fig.12-5 shows the changes (%) of various analytes after a storage of two, four and six days at room temperature without additives. Citrate, glucose, oxalate and magnesium are unstable. Addition of sodium azide in a final concentration of 10 mmol/L urine for the same time and temperature stabilized all these constituents for 6 days at room temperature (186). Tab. 12-2 lists a number of additives which are used to stabilize compounds in urine.

Tab. 12-2: Urine preservatives (59, 91)

Preservative	Analytes stabilized
Thymol, 5 mL of a 10% solution in 2-propanol	Most constituents
Sodium azide, 10 mmol/L urine	Glucose, urea, uric acid, citrate potassium, calcium, oxalate
Hydrochloric acid, 25 mL 6 mol/L per 24-hours urine volume	Catecholamines and metabolites, 5-hydroxyindolacetic acid, calcium, magnesium, phosphate
Sodium carbonate, 2 g/L urine	Porphyrins, urobilinogen
Urine pH > 8.0	Uric acid

No single additive is available that stabilizes all classes of compounds.

For quantitative determination, storage with a stabiliser at room temperature is recommended. If the urine samples are stored at 4–6 °C for calcium, magnesium, oxalate and phosphorus determination, acidification (pH 1.5–2.5) and, for uric acid, alkalization (pH > 8.0) is necessary. For special analytes, different types of preservatives are necessary (see Tab. 12-2).

Saliva

Saliva can be used for analyzing various compounds such as steroids and drugs. Samples obtained can be derived from one gland only (gland-specific saliva) or be a mixture of products from different glands (mixed saliva) (Fig. 12-6). The latter specimen is used for routine procedures. Different techniques are employed to obtain saliva from the oral cavity (77). Several collection devices have been developed to standardize the collection of saliva for hormone and drug monitoring. Tab. 12-3 provides a selected listing of the presently available devices and adsorb-

Tab. 12-3: Commercially available saliva collection devices using adsorbing materials

Name	Manufacturer	Adsorbing material	Sampling device
Salivette	Sarstedt	Cotton roll with citric acid or polyester roll	Centrifugal device
Omni-Sal	Saliva Diagnostic Systems	Adsorbing pad with fluid volume indicator	Separation by pressure through a filter into a buffer containing tube
Orapette	Trinity Biotech	Rayon ball sampling	Expulsion by screwing a piston
Ora Sure = Epi Screen	Epitope	Pad on "lollipop" stick	Centrifugal tube with antimicrobial buffer
Accu Sorb™	Avitar Technologies	Polymer pad fixed on the closure	Squeezing manually (milking) into a plastic tube
Oral Screen	Avitar Technologies	Polymer foam cube sampling	Squeezing by pressure on the surface
Abusa-stick	Chem-Elec	Saliva swab	
Alcoscan	Lifescan	Saliva swab	
Q.E.D. (used for saliva alcohol test)	SCT Technologies	Cotton swab on a stick	Squeezing by pressure on the surface

ing materials. No one device can be considered ideal for all purposes. Devices using materials adsorbing saliva are either contaminated with substances interfering with the measuring procedure or the drug itself is adsorbed by the materials to varying degrees depending on the device used. Recoveries of drugs have recently been reported to range from 59 to 107% (78).

Saliva has several advantages in drug monitoring compared to blood. Gorodischer has reported that 85% of parents and 50% of children indicated a preference for saliva sampling over venous blood withdrawal (64). The ease of collection of saliva makes it ideal for self-testing at home and in cases where blood sampling is difficult (e.g. in newborns). Limitations in sampling posed by saliva are related to viscosity and in some cases to the difficulty in obtaining sufficient volume. The saliva/plasma ratio of drugs of abuse has been reviewed (78).

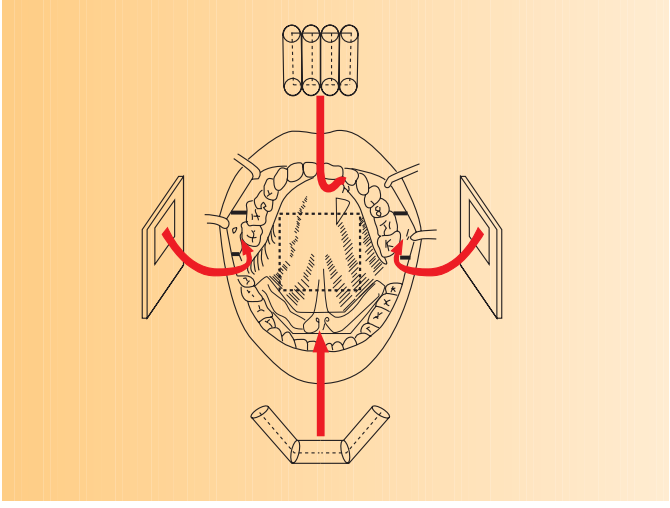
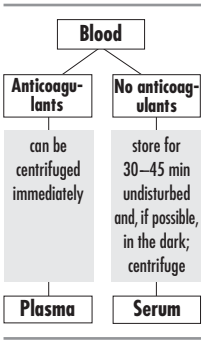


Fig. 12-6 Collection of saliva from different locations of the oral cavity. In the upper part 4 cotton rolls connected by a thread were placed above the tongue. At the bottom 3 shortened cotton rolls connected by a thread were placed below the tongue. On the left and right side metalline strips are shown which are wrappped in polyethylene foil with a rectangular opening in the area of the orificium of the ductus parotidis. The 4 parts were placed into the oral cavity and left there for 9 minutes without moving the tongue. Saliva was collected from the strips and the cotton rolls by centrifugation in Salivettes® (78)

Plasma or serum?



Serum is obtained from whole blood by centrifugation after completion of the platelet and clotting factor coagulation process. Serum must therefore be regarded as an artifact. By definition, it is devoid of clotting factors but is enriched with the cellular components of platelets and metabolic products.

Plasma is the virtually cell-free supernatant following centrifugation of whole blood, the coagulability of which is inhibited by the addition of anticoagulants during or immediately after sampling. Anticoagulants inhibit clot formation through various mechanisms (72, Annex). The required concentration of anticoagulants and their composition is described in an international norm (89).

For some analytes, there are diagnostically relevant differences between the results obtained from serum and those obtained from plasma (see Tab. 13-1 and Fig. 13-1). A linear correlation has been shown between the difference in serum-plasma potassium and phosphate and the number of platelets in blood (Fig. 13-2). Some constituents found in high concentrations in platelets can therefore not be correctly determined in serum, e.g. acid phosphatase activity, neuron specific enolase, dopamine and serotonin (69).

Differences between the values obtained in serum and plasma are due to the following physiological and technical reasons:

- The analyte may be consumed during clotting: fibrinogen, platelets, glucose.
- The analyte may be released from cells during clotting: potassium, lactate dehydrogenase, phosphate, ammonia, lactate.
- The anticoagulant may interfere with the assay or contaminate some assays:

Tab. 13-1: Analytes with diagnostically relevant serum/heparinized plasma concentration differences and their main causes (226)

Analyte	% change in comparison to the mean in plasma	Main cause of the serum/plasma difference
Potassium	+6.2	Lysis of the cells, particularly the platelets*
Inorganic phosphate	+10.7	Release from cellular elements
Total protein	–5.2	Effect of fibrinogen
Ammonia	+38	Thrombocytolysis, hydrolysis of glutamine
Lactate	+22	Release from cellular elements

** Also causally responsible for high potassium values in serum are – in addition to thrombocytolysis – haemolysis and extreme leukocytolysis (when leukocyte counts are > 50 G/L). Concerning the platelet influence in whole blood, the following applies: an increase in the platelet count by 100 G/L means an average increase of 0.11 mmol/L in the difference between the serum and plasma potassium values.*

Fig. 13-1
Plasma serum differences obtained in 4 mL separator tubes (226). Ratio of the median difference between serum and plasma and the coefficient of variation of the analytical procedure used ▶

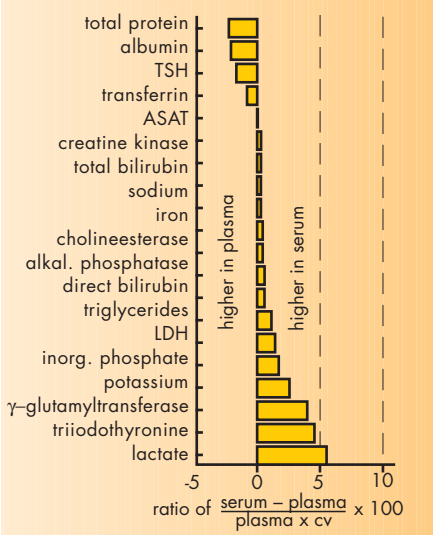
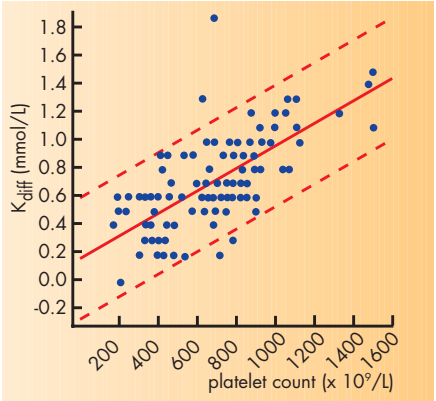


Fig. 13-2
Dependence of plasma-serum difference in potassium on platelet count in blood (121)



γ-glutamyl transferase, lithium in flame photometry, when calibrated with lithium.

● Methodology of the respective determination, including for example the type of measuring instrument used (monochromatic/bichromatic measurement) (80) may cause interference. This includes interference of fibrinogen with some heterogeneous immunoassays.

What advantages does plasma have over serum?

The recommendations published by the Working Group on Preanalytical Quality (72, Annex) has described the advantages and disadvantages of using plasma versus serum:

1. Time saving. Waiting for blood to clot is eliminated. The centrifugation period can be reduced considerably by increasing the rotation speed.
2. Higher yield. Approx. 15–20% more plasma than serum can be obtained from whole blood.
3. Virtually no interference due to subsequent coagulation. Post-centrifugal coagulation can occur in serum. This effect does not occur in plasma.
4. Results from plasma are more representative of the in vivo state compared to serum (Fig. 13-1).
5. Lower risk of haemolysis and thrombocytolysis. In healthy persons, free haemoglobin is about 10 times less concentrated in plasma than in serum. In plasma, the platelets remain intact in vitro; there is hence no pseudohyperkalemia here, as is found in serum (121).

What are the disadvantages of plasma relative to serum?

1. Protein electrophoresis is altered. Fibrinogen appears as a peak in the region of the γ-globulins and can simulate or mask an M-gradient.
2. Method-dependent interference. Anticoagulants can – as potential complexing agents and enzyme inhibitors – lead to method-dependent interference. Every new procedure should therefore be tested for anticoagulant interference.
3. Cation interference. When heparinates are used, lithium or ammonium may interfere with the methods for determining them.

The annex summarises the present state of knowledge on the use of anticoagulants in the individual analytes.

If serum or plasma separator tubes are used, renewed centrifugation following storage of the sample in the refrigerator should be avoided as this would lead to noticeable increases in the concentration of cellular constituents like potassium, inorganic phosphate and lactate dehydrogenase.

Types of plasma

Presently various different types of plasma are recommended for individual analytes and test procedures. Thus buffered sodium citrate is recommended for coagulation testing. Various types of plasma obtained from citrated blood are used:

Tab. 13-2 Different types of plasma

Plasma	Relative centrifugal force (*g*)	Centrifugation time (min)
Platelet-rich	150–200	5
Platelet-poor	1000–2000	10
Platelet-free	2000–3000	15–30

K_2-EDTA is recommended for haematological cell analysis and analytes sensitive to metalloproteinase degradation. Heparin plasma is recommended for nearly all extracellular constituents.

When obtaining a blood sample, it is imperative that attention be paid to ensuring thorough mixing of the blood with the anticoagulant; foaming should be avoided.

Not more than 2 minutes should elapse between the beginning of stasis and the mixing of the blood with anti-coagulant.

Take a lavender tube!

Fig. 14-1
Colour codes for anticoagulants and additives described by ISO 6710 (89)

Additives

In addition to anticoagulants such as EDTA, heparin, citrate and oxalate, other additives have been used for blood collection.

To insure that there is no confusion on the part of the phlebotomist in identifying the proper tube, the stoppers and closures of anticoagulant and additive-containing tubes are colour-coded. Thus, for anticoagulant-containing tubes, lavender is the stopper colour code for EDTA, green is for heparin and blue is for citrate. Glycolytic inhibitors such as fluoride or iodoacetate either alone or in combination with an anticoagulant such as heparin or EDTA have been colour-coded gray. Additional letter codes have been included in the ISO norm (89).

Fig.14-1 shows pictures of tubes used in blood collection and identified by their proper colour code.

Tubes containing acid-citrate-dextrose (ACD A or B formulation) are used for the preservation of cells and are colour-coded yellow.

Tab. 14-1 Additives of colour coded tubes

Tube	Application	Colour/letter code
1. Plain (no additive), yields serum	Clinical chemistry and serology	red/Z
2. Heparin (12–30 U/mL)	Plasma chemistry	green/LH or NH
3. K_2- or K_3-EDTA (1.2–2.0 mg/mL)	Haematology and selected chemistry determinations on plasma	lavender/K2E or K3E
4. Sodium citrate (0.105–0.129 mol/L)	Coagulation	light blue/9NC
5. Sodium fluoride (2–4 mg/mL)/potassium oxalate (1–3 mg/mL)	Glucose, lactate	gray/FX

Heparin

Heparin, convenient for use in blood collection tubes, functions by accelerating the inhibition of factor Xa by antithrombin III. In contrast to low molecular weight heparin preparations, the conventional high molecular weight heparin used in blood collection tubes also possesses antithrombin activity.

While the lithium salt of heparin is widely used to obtain plasma for clinical chemical analysis, blood collection tubes containing salts of sodium or ammonium heparin are also commercially available. Ammonium heparin may limit the blood urea nitrogen assay when ammonium ions are being measured (144).

EDTA

This compound functions as an anticoagulant by binding calcium. EDTA is discussed in the haematology chapter (p. 54).

Citrate

Sodium citrate also functions as an anticoagulant by chelating calcium. Sodium citrate is discussed in the coagulation chapter (p. 52).

Glycolytic inhibitors

Both sodium fluoride and lithium iodoacetate have been used in blood collection tubes to preserve glucose. Mannose and fluoride have also been suggested. When combined with an anticoagulant such as Na_2-EDTA (1 mg/mL of blood) or potassium oxalate (2 mg/mL of blood), the nominal concentration of fluoride used to inhibit glycolysis is 2.5 mg/mL of blood (60 mmol/L).

Fluoride inhibits the enzyme enolase in the glycolytic pathway and thereby prevents the degradation of glucose (34a).

In contrast to fluoride, iodoacetate acts on glyceraldehyde-3-phosphate dehydrogenase. After an initial loss of glucose during the first 3 hours of blood collection (an average loss of 9 mg/dL in healthy subjects), both fluoride or iodoacetate are effective in preserving glucose for at least 3 days (34a, 193). It should, however, be recognized that blood specimens with a high white blood cell, red blood cell or platelet count will have more rapid glucose consumption, before inhibitors such as fluoride or iodoacetate become effective (34a). In newborns, particularly because of the high haematocrit, as much as 68% of glucose can be consumed in blood specimens collected without glycolytic inhibitors and stored at room temperature for 5 hours (126). To overcome this problem, inhibitors which inhibit the first enzyme hexokinase in the glycolytic pathway have been suggested. Such an inhibitor is mannose, which is used in combination with fluoride to better preserve glucose (117). The inhibition by mannose, which is a competitive inhibitor, is short-lived, inhibiting glycolysis only up to 4 hours after blood collection. However, beyond 3 hours of specimen collection, fluoride would have become effective; thus, a mixture of mannose (2 mg/mL of blood) and sodium fluoride (2 mg/mL of blood) would have been effective in minimizing the loss of glucose which would otherwise have occurred had blood been collected using either sodium fluoride or lithium iodoacetate alone.

Fig. 14-2 depicts the efficacy of glycolytic inhibitors in preserving glucose (49, 220).

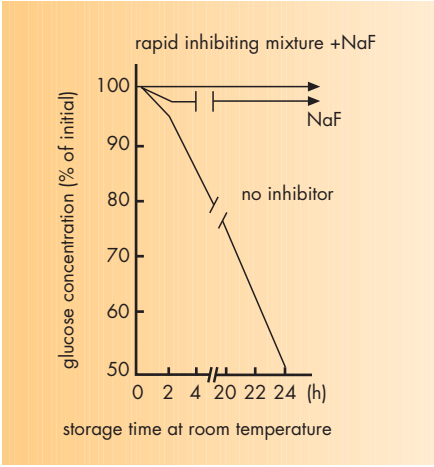


Fig. 14-2
Preservation of glucose with glycolytic inhibitors (49)

Preservation of cells

Anticoagulant – additive mixtures such as ACD (anticoagulant-citrate-dextrose or acid-citrate-dextrose) have been used in blood collection tubes to preserve red blood cells. ACD is available in 2 formulations, A and B. The difference between the 2 formulations is the blood to additive mixture ratio.

Tab. 14-2

The composition of ACD formulations

	ACD A	ACD B
Sodium citrate	2.2 g/dL	1.32 g/dL
Anhyd. citric acid	0.73 g/dL	0.44 g/dL
Dextrose (glucose)	2.45 g/dL	1.47 g/dL
ACD/blood (v/v)	1/5.67	1/3
pH	5.05	5.10

In the ACD A formulation, an additive to blood ratio of 1:5.67 is used whereas in the ACD B formulation the additive to blood ratio is 1:3.

ACD preserves red blood cells for 21 days when blood is stored between 1 and 6°C (22).

Effects of time and temperature during transport

The transfer of samples to the laboratory can be accomplished in different ways. Normally, transfer times are short when the laboratory is located close to or in clinics and represent no problem. The time from drawing the blood sample to centrifugation, however, should not exceed one hour. Some analytical procedures require special additives such as sodium fluoride/ oxalate for quantification of lactate (9) or sodiumborate/serine EDTA for quantification of ammonia (56). Determination of free haemoglobin in plasma requires gentle handling of the EDTA blood sample. Transfer to the laboratory may proceed either by courier or a pneumatic tube delivery system. State-of-the-art systems of the latter type ensure gentle transfer thereby avoiding haemolysis. Such samples can be used to determine target analytes in clinical chemistry, haematology or to perform blood gas analysis (80). If for some technical reason a long distance transfer of the sample is required (e.g. by mail or laboratory courier), then whole blood samples should be avoided. Fig.15-1 demonstrates the influence of temperature and duration of storage of clotted blood samples on some target analytes (84, 163).

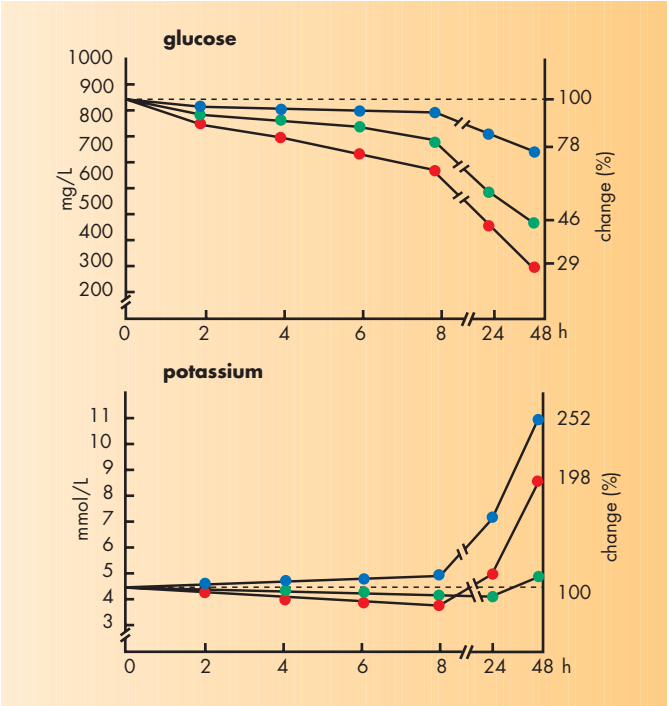


Fig. 15-1
Temperature and time effect of storage of clotted blood without anticoagulant on various serum analytes (163). ( ) 4 °C, ( ) 23 °C, ( ) 30 °C

Release of potassium from erythrocytes is minimal at room temperature due to the temperature-dependent activity of the Na+, K+-ATP-ase. This effect increases both at 4°C and above 30°C. Glucose concentrations decrease with increasing temperature, whereas the opposite phenomenon is observed with inorganic phosphate because the activities of phosphatases in serum and red blood cells increase this compound. As demonstrated in Fig.15-1, duration of storage at a given temperature is an influencing factor. If a whole blood sample is stored for two hours at 23°C, glucose concentrations decrease by about 10 percent (Fig. 15-1).

Pathological samples may show deviations from the usually observed effects of time and temperature. Time-dependent decrease of glucose in whole blood samples is enhanced in leukocytosis. Similarly, the time-dependent increase in ammonia is enhanced in samples with elevated γ-glutamyltransferase activities (56). Antibodies may alter haematological cell counts depending on the temperature dependence of the antibodies (see p. 78-79).

Many clinical chemical analytes such as electrolytes, substrates or enzymes are not affected by a mail transport time of up to four days. The haemoglobin concentration and the erythrocyte count are also stable. Major differences

ISBN: 978-3-527-30981-8

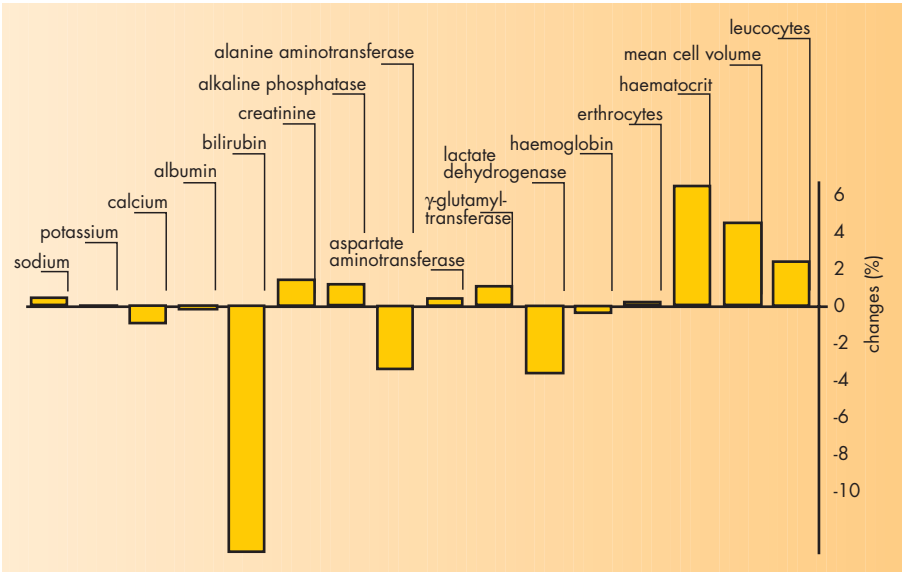
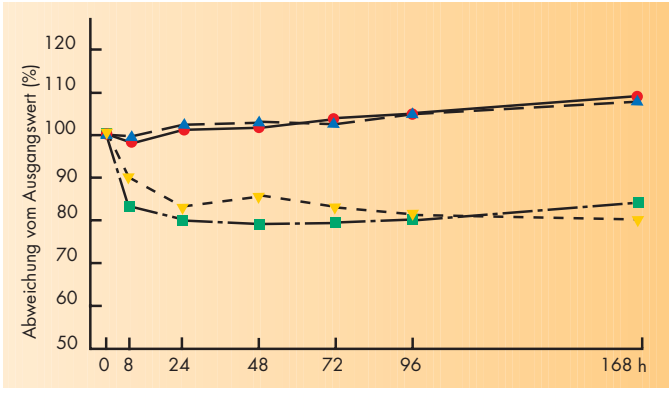


Fig. 15-2
Stability of various analytes during mail transport (13, 122)

are observed for the haematocrit, the mean cell volume and bilirubin (Fig. 15-2). A reliable examination of a differential leukocyte count requires preparation of the blood smear within three hours of sampling. The included Annex "The Quality of Diagnostic Samples" may be used to find additional details on analyte stability in full blood and serum/plasma.

Numerous studies related to the stability of clinical chemical analytes exist compared to the few investigations on the stability of haemostasiological parameters (83, 216). As shown by Heil et al. the stability of coagulation factors in patient samples is dependent on whether they are on heparin therapy or not. For the thrombin time this is clearly demonstrated in figure 15-3. The causes for the changes of stability are manyfold, i.e. loss of the haemoglobin buffering system after plasma separation from the red blood cells, an in vitro fibrinolytic effect, or drug influences on platelets. The results of the study (83) can be summarised as follows: citrated blood samples of patients without heparin therapy for the determination of prothrombin time, APTT, thrombin time, protein C and factor V are stable for eight hours at room temperature, provided that the plasma is not separated from the erythrocytes. This is not valid for factor VIII and protein S. The sample stability of patients with heparin therapy, stored at room temperature or in the refrigerator is below eight hours for the global tests and the determination of single coagulation factors. Therefore, all patient samples should be analysed within four hours at room temperature after sampling. If this is not possible, platelet poor plasma should be stored at –20° C.

Fig. 15-3
Thrombin time (TT) determination in plasma samples of patients with and without heparin therapy, stored at room temperature (RT) and 6° C (83)
(●) without heparin at RT
(■) with heparin at RT
(▲) without heparin at 6° C
(▽) with heparin at 6° C



Samples in transit

Fig. 16-2
Infectious substance label for package containing etiologic agents
▶

When blood or other body fluids from human subjects are mailed to a distant laboratory, several safety regulations have to be complied with. In addition, the integrity of the sample has to be preserved to insure accurate analysis by the investigator. Specimens that are mailed must "withstand leakage of contents, shocks and pressure changes, and other conditions incident to ordinary handling in transportation" (50, 159, 196).

The persons dispatching diagnostic samples have to assure, that the contents are packaged in a way insuring arrival in an undisturbed state. No risk should occur for humans, animals and the environment during transport.

A biohazard label as shown in Figure 16-1 should be affixed to the package.

Regulations concerning transport by post are reported in national standards (43). Here samples with infectious substances have to be treated differently compared to materials with a low risk of infection (like most samples of blood, serum, urine, stool, swabs, slides and filter papers). For posting of diagnostic samples even if non volatile the package can be sent by letter post. Packages with infectious materials have to be labelled with the remark: DIAGNOSTIC SAMPLE/INFECTIOUS HAZARD. For international traffic and posting the description in French language: "Matières Biologiques Périsablés" is required. The responsibility for the posting of infectious materials is with the dispatching physician's site.

Fig. 16-1
Biohazard Label for air-shipped specimens

In Europe the standard package EN 829 (Fig. 16-1) is accredited (50). No glass is allowed as sample material to reduce the risk of breakage and possible harm to people involved in transport.

The package for biological materials consists of the following parts:

- The inner package for the sample material,
- absorbing material,
- the outer package ensuring specimen records and laboratory forms,
- the box or mailing bag.

Instead of the outer package several specimen containers up to 500 mL may be packaged in one box consisting of card board, wood, suitable plastic or metal according to the regulations for biohazard transport.

Remarks: In any case where infectious substances are contained in the package, additional secondary container is needed to prevent any leak of material by any mechanical challenge.

Always remove injection needles when mailing blood sampling systems.

Package glass slides adequately to ensure they do not get damaged if knocked, dropped or if pressure is applied.

Ship stool specimens in leak-proof, screw-capped containers.

When mailing dried blood specimens on filter paper, place in a strong paper envelope and then seal in plastic-lined, padded post bag. This provides protection against potentially infectious dried blood specimens and ensures the integrity of the specimens during transport.

For shipping frozen and refrigerated specimens, an insulating material such as a polystyrene container is adequate. Dry ice should be used for freezing.

Caution should be taken to insure that the container packed with dry ice is able to release carbon dioxide gas so as to avoid a build up of pressure that could cause the package to explode.

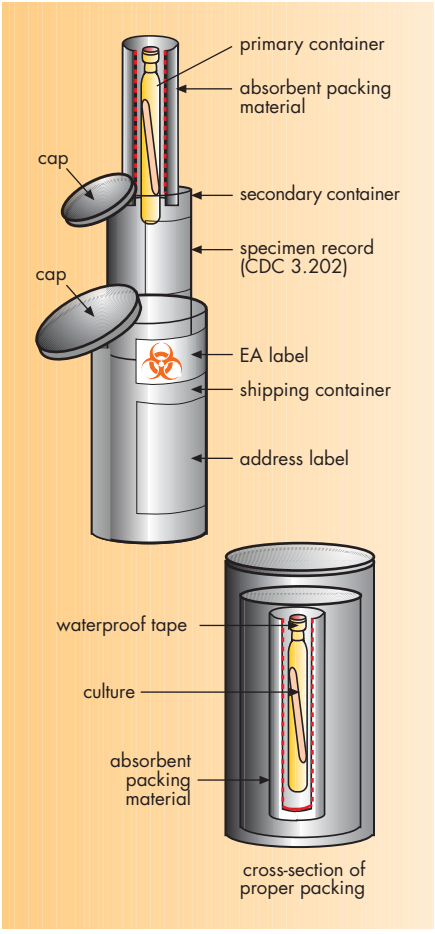


Fig. 16-3
Packaging of specimens for transport according to NCCLS (159)

NCCLS document H18-A2 describes procedures for the handling and transport of diagnostic specimens and etiologic agents (159).

In Europe details are regulated by European Standard prEN 829 for in vitro diagnostic systems (50). Here detailed information is found together with the requirements and testing procedures regarding transport packages, samples, absorbing materials and protective vessels. The label to be used is shown in Fig. 16-4.

Fig. 16-4
Label to be used for transport of medical and biological specimen according to EN 829 (German version)

How to keep a sample "fresh"

10 rules and some recommendations

1. The procedure is governed by the stability of the constituents of the sample. The most important causes for alterations to the quality of specimen are:

- Metabolism of the blood cells
- Evaporation/sublimation
- Chemical reactions
- Microbiological decomposition
- Osmotic processes
- Effect of light
- Gas diffusion

2. Rapid transport and short storage times improve the reliability of laboratory results.

3. Specimens and samples are preserved longer the cooler they are stored (but note exceptions!).

4. Specimens and samples should always be stored in closed vessels (evaporation!).

5. The danger of evaporation also exists in refrigerators (condensation of moisture on the cooling elements).

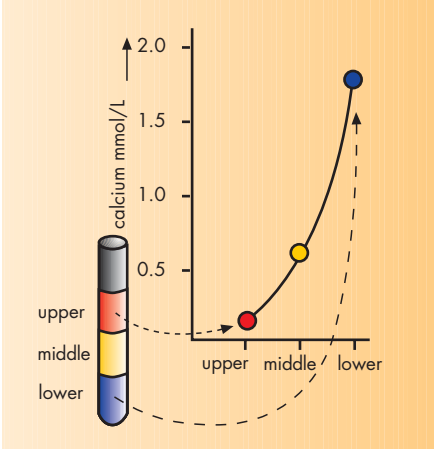


Fig. 17-1
Formation of concentration gradients of calcium in plasma samples after rethawing without mixing

6. Storage problems are reduced if disposable sampling systems are used.

7. Separating agents (e.g. gel separators) improve the serum/plasma yields and enable serum to be left in the original tubes above the blood (111).

8. Avoid shaking the sample vessels (pneumatic tube dispatch systems!): risk of haemolysis.

9. Always store sample vessels containing blood vertically; the clotting procedure is accelerated.

10. Label infectious material and handle it with particular care.

8 special rules and some more useful recommendations

1. Avoid storing of whole blood. Information on sensitive analytes is given in the Annex (see enclosure).

Blood samples should reach the laboratory within 45 min of collection in order to ensure that centrifugation and separation of the sample is carried out within 1 hour (49, 112, 159).

2. Avoid glycolysis to keep glucose, lactate and pH stable. Glycolysis can be avoided by the addition of an inhibitor in conjunction with an anticoagulant (171, 220).

3. Avoid the effect of light otherwise there will be a fall in the values of bilirubin, vitamin C, porphyrins, creatine kinase (CK) and folic acid.

4. Reduce contact with air as far as possible. If this is not done, evaporation/sublimation will result in an apparent increase in the concentration/activity

of all non-volatile components. This is particularly the case when the volume of the sample is relatively small and the surface area is relatively large.

5. Whole blood should not be stored in the refrigerator. When urine is cooled, salts may precipitate out of the solution (calcium and magnesium phosphate, uric acid).

6. For certain analytes, the specimens/samples should not be deep frozen. Failure to observe this can result in deviating results for the following analytes:

Tab. 17-1 Examples of blood and urine constituents which should not be stored frozen

Sample	Analytes
Serum/plasma:	Lipoprotein electrophoresis
	Apolipoprotein A-I and B
	LDL-cholesterol (prevented by the addition of glycerol)
	Fibrin monomer positive plasma*
EDTA-Blood	Haematology
Urine	IgG
	Sediment
	Uric acid (precipitations!)

*Negative test result, prolonged PTT, shortened thrombin time, shortened reptilase time (215).

7. Correct thawing
A very common source of error is the inadequate mixing of deep-frozen samples after they have been thawed. Concentration gradients are produced during thawing as the concentrated solution first melts and then runs down the sides of the vessel (see Fig. 17-1).

After thawing, the sample should therefore be inverted several times, avoiding the formation of foam. Look for undissolved material and, if necessary, bring into solution by careful warming.

8. Store samples after analysis in such a way as to permit the confirming of results, checking the identity of samples or performing additional tests for medical or legal reasons:

Tab. 17-2 Recommended storage time and conditions for analytical samples

Samples for	Storage time	Temperature
Clinical Chemistry:	1 week	Refrigerator
Immunology:	1 week	Refrigerator
Haematology:	2 days	Room temperature
Coagulation:	1 day	Refrigerator
Toxicology:	6 weeks	Refrigerator
Blood grouping:	1 week (at least)	Refrigerator

Fig. 17-2
Storage of samples should enable easy reidentification for confirmatory tests

Centrifugation

Centrifugation of clotted blood to obtain serum should be performed after making sure that the blood has clotted. Normally, the waiting time for blood to clot is approximately 30 min. However, patients who are on anticoagulant therapy, or those with coagulation defects will have delayed clotting.

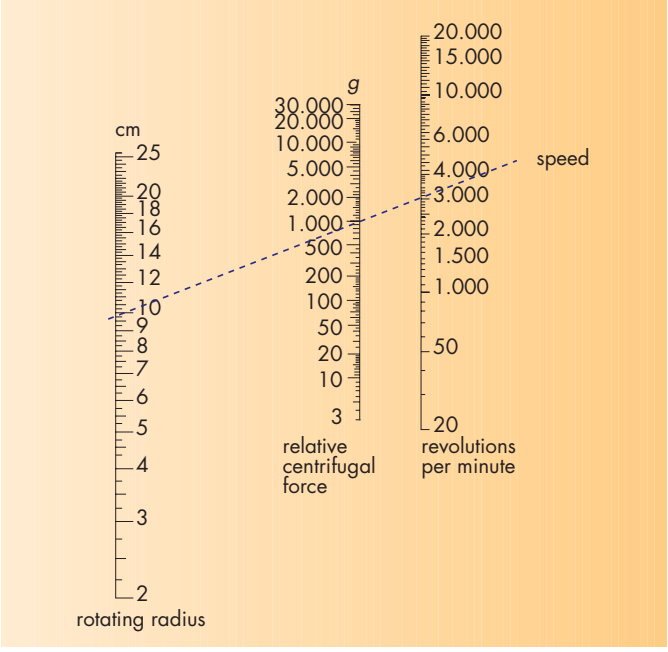
Centrifugation of clotted blood to obtain serum or anticoagulated blood to obtain plasma is typically performed at 1000 to 1200 *g* for 10 to 15 min. For the production of platelet-free plasma a centrifugation for 15 – 30 min at 2000 – 3000 *g* is necessary (see Tab. 13-2). In coagulation procedures, citrated whole blood should be centrifuged at 2000 *g* for 15 min (159).

The relative centrifugal force can be calculated either by using an empirical equation or by using a nomograph as shown in Fig. 18-1. The equation used to calculate the relative centrifugal force (rcf) is as follows:

$$rcf = 1.118 \times 10^{-5} \times r \times n^2$$

Where r is the mean radial distance from the axis of rotation in centimeters and n is the speed of rotation in revolutions per minute (rpm). 1.118×10^{-5} is a constant which is derived from the centrifugal force as multiple of *g*.

Fig. 18-1
Nomograph for calculation of relative centrifugal force



Centrifugation is usually performed between 20 to 22°C. However, analytes that are labile during centrifugation at ambient temperature, especially if the temperature increases during centrifugation, should be centrifuged at refrigerated temperature (4°C). However, refrigeration can lead to leakage of potassium from the cell, thus spuriously increasing its value (see Fig. 15-1).

Specimens should not be recentrifuged after sampling the serum or plasma. In doing this the ratio of plasma water to cell volume may be altered thus causing alterations in analyte concentrations. Specimens with a gel barrier material should never be recentrifuged.

The time and centrifugal force applied to the sediment of heparinized blood should be such as to leave no platelets in the plasma layer. Failure to completely sediment platelets will result in spurious increases in potassium, lactate dehydrogenase, acid phosphatase and inorganic phosphate from platelets remaining in the plasma (Fig. 18-2) of the sample (69). Fig. 18-3 shows the visual control of plasma after centrifugation at various speeds.

Microcollection tubes with or without anticoagulants can be centrifuged to obtain either serum or plasma in a microcentrifuge with an adapter that ac-

ISBN: 978-3-527-30981-8

cepts such tubes. The centrifugation of such microcollection tubes is performed at speeds ranging from 6,000 to 15,000*g* for a minimum of 90 sec.

A preventive maintenance program for centrifuges should include a schedule for periodic checking of centrifugation speeds attained at a specified speed settings using a tachometer.

Sample handling after centrifugation

After centrifugation, samples may be transferred directly to the analyzer. Ideally, the analyzer needle takes the analytical sample by piercing the closed stopper after the sample has been mixed. In most laboratories, however, the stopper has to be removed and the samples distributed. To prevent evaporation, this should be done shortly before analysis. Subsamples should follow the same rules as the primary specimens regarding identification, storage conditions and safety aspects.

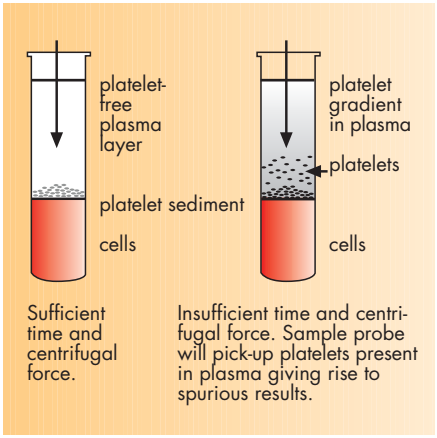


Fig. 18-2
Effect of platelet contamination due to insufficient time and centrifugal force applied to heparinized blood

To avoid contact with blood containing other potentially infectious materials, subsampling should be avoided as far as possible.

This is facilitated by the use of separators in tubes. Alternatively, distribution can be performed by mechanical devices (Fig. 19-1, p. 44).

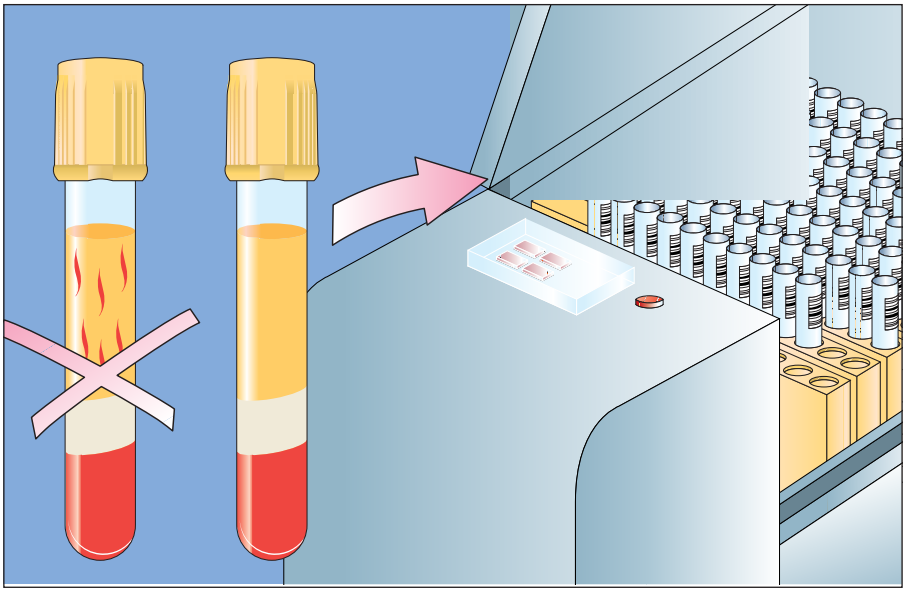


Fig. 18-3
Visual control of adequacy of centrifugation before analysis of serum

Workflow can be defined as the steps carried out from the time a specimen arrives in the laboratory to the time the results are reported to the physician. This workflow then determines the turnaround time (TAT) of laboratory results. A broader definition of workflow would encompass steps from the time the physician orders a test to the time the results reach him.

The importance of preanalytical time in terms of total TAT can be appreciated from a study by Godolphin which is detailed below (62).

Tab. 19-1 Contribution of the preanalytical phase to total turnaround time (TAT)

	% of TAT
Preanalytical	57.3%
Analytical	25.1%
Postanalytical	17.6%

In recent years, automated sample sorter systems have been introduced to cope up with the workload of large reference laboratories and automated aliquoting systems have provided a simplified alternative to time-consuming and cumbersome manual aliquoting procedures. Fig.19-1 illustrates an automated sample sorter system (127).

Fig. 19-1 Automated sample sorter system (with kind permission of P. Mountain, Autolab, Toronto, Canada)

Consolidation of work areas such as routine, special chemistry and haematology sections should facilitate workflow. Efficient workflow is dependent on streamlining sample processing, aliquoting and distribution steps (61).

Discontinuous steps such as centrifugation and labeling samples adversely affect total turnaround time.

Robotics

Robots are devices that can be programmed to perform specific tasks. As such, robotics is a term used to describe the utilization of robots to perform specified repetitive mechanical tasks that are programmed and hence under electronic and computer control.

Robots of varying flexibility are available (139). Robots with three degrees of freedom that can move about in a three-dimensional space, but are incapable of rotation are called **Cartesian robots** and have applications ranging from sampling devices on automated analyzers to pipetting stations for handling liquids (54).

Robots with four degrees of freedom are called cylindrical robots and are capable of moving in and out of plane with a wrist roll. By the use of robotic arms, these **cylindrical robots** have been used for sample preparation tasks such as in blood typing or in multiple steps such as sample extraction, separation of aqueous and organic phases and sample injection associated with high performance liquid chromatographic procedures.

Highly flexible robots with five degrees of freedom are called **jointed robots** and are very versatile with their wrist rotatory motion and ability to reach the remote positions on an instrument. Fig.19-2 illustrates robots of varying degrees of flexibility.

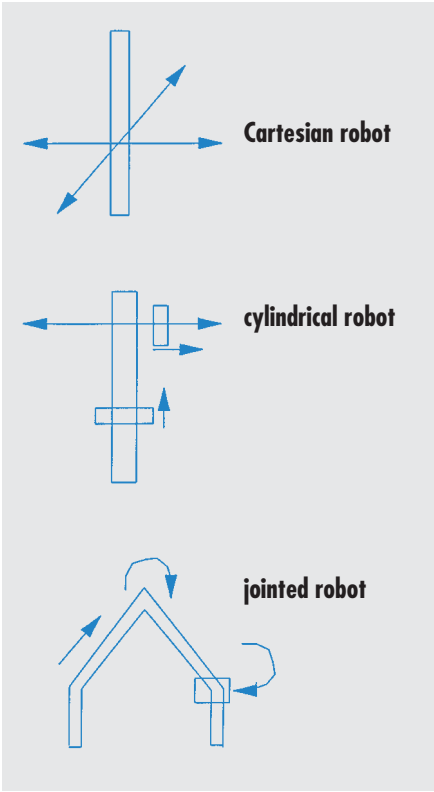


Fig. 19-2
Robots of varying degrees of flexibility

Robots are also used to transport specimens to the laboratory across taped tracks (118).

M. Sasaki, a pioneer in the use of robotics, used a conveyer belt system to transport samples to the robotic analyzers which in turn were connected to the conveyer belt (Fig.19-3). Sasaki has used robotic analyzers to perform a variety of tests that include serological aggregation tests including AIDS tests, blood transfusion tests such as ABO blood typing, cross-matching, Rh factor testing and hormone analysis (181, 182).

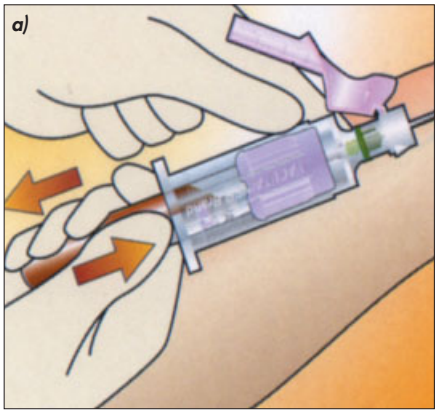
Fig. 19-3
Conveyer belt system to transport samples to robotic analyzers (with kind permission of M. Sasaki, Kochi, Japan)

In the meantime different systems of advanced analytical systems including robotic regulation have been developed and incorporated into working laboratories (85, 86, 207).

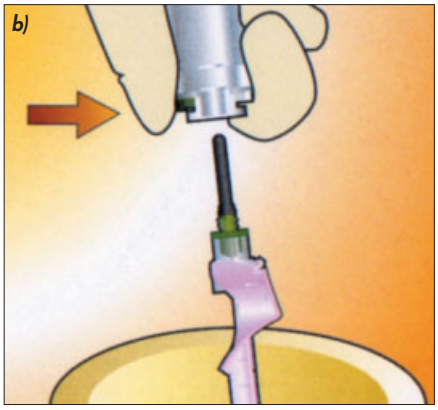
While robotics can contribute to the efficiency of workflow, by their very nature they require special considerations. This is due to the fact that the movements of robotic arms are under the control of complex electronic circuits. As such, any fluctuation in line voltage can disrupt the operation of the robot. Hence, having access to an uninterrupted filtered power supply with back-up batteries is essential for robotic operations.

Finally, cost and space considerations have to be considered in the process of determining the suitability of robotic operations in a clinical laboratory.

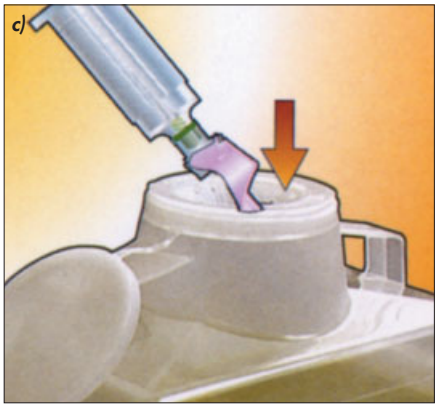
Fig. 20-1
Disposal of needle into sharps container



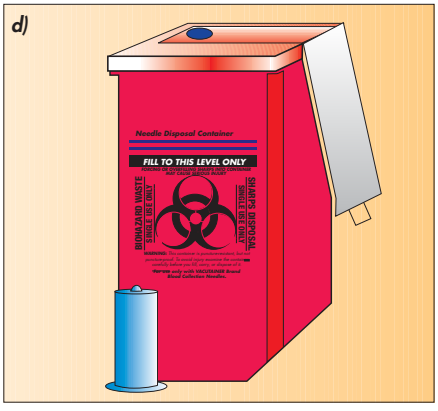
a) Sampling with a safety needle



b) Quick release holder; discard the needle into sharps container by pressing the release button. The holder then can be reset ready for reuse.



c) Single use holder: immediately after sampling, discard the needle holder combination into a sharps container.



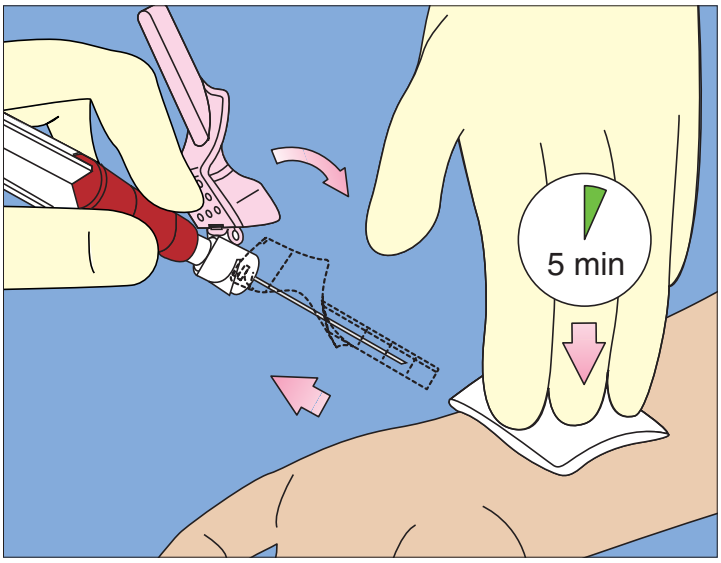
d) Container

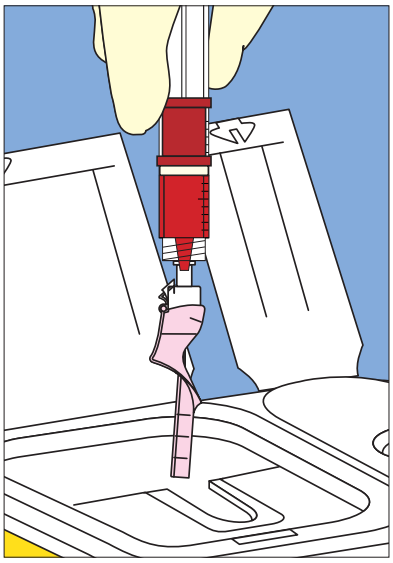
Steps to ensure safety are paramount to the protection of the health care worker. The NCCLS document GP17-T provides tentative guidelines on clinical laboratory safety (154). This document outlines steps for the maintenance and inspection of the laboratory, the general requirements for personal and laboratory safety, warning signs and labels needed, fire prevention and control, electrical and radiation safety, handling of compressed gases and carcinogens, chemical and microbiological hazards and hazardous waste disposal (154).

Specifically, the disposal of specimens, needles, tubes, and chemicals are discussed in this chapter.

Disposal of needles and other sharp objects

The disposal of all sharp objects such as needles is accomplished by placing them in leak-proof, puncture-resistant containers with appropriate labels and color coding, an example of which is illustrated in Fig. 20-1.





Immediately after sampling, activate needle safety mechanism and discard the needle into sharps container.

Fig. 20-2
Safety needle for arterial collection and nestable sharps container

Reshielding of sharps and the bending and breaking of needles should be avoided.

However, should recapping become necessary, either a one-handed scoop or, preferably, a reshielding device should be used.

Simple reshielding devices are available, which allow the phlebotomist to reshield the needle with minimal risk of needle prick injury (Fig.20-2).

Specific safety devices have also been developed for the disposal of micro blood collection sets (Fig. 20-3).

Tube and sample disposal

Specimen collection tubes containing blood and which are intended for disposal should be placed in biohazard bags that can withstand autoclaving. These bags should be placed in leak-proof containers and then tightly closed.

Bulk body fluids such as urine, vomit, feces and other body fluids may be disposed of by flushing down the toilet.

Containers of fluids such as blood bags should be incinerated after placing in biohazard waste containers.

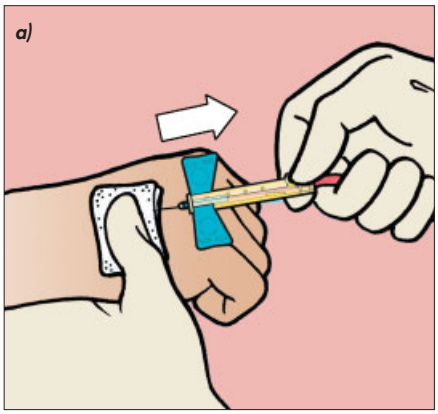
Chemicals

Chemical waste can be ignitable, corrosive, reactive and toxic (152).

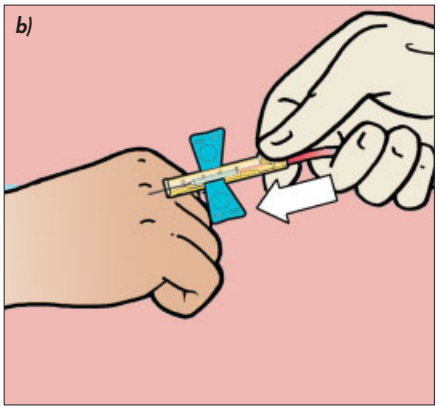
Examples of ignitable chemical waste include volatile flammable liquids such as organic solvents (e.g., alcohols, acetone, xylene, toluene, etc.), oxidizers such as peroxides and nitrate salts and flammable gases such as butane, silane and hydrogen. These materials should be labeled with the appropriate hazardous chemicals label shown in Fig. 20-4.

Disposal of flammable solvents via a sink or flush toilet is not recommended. If such solvents are readily dissolvable in water, they could, in small amounts, be disposed of by pouring down a sink,

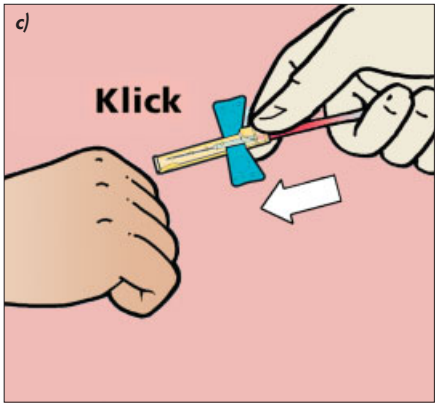
Fig. 20-3
SAFETY LOK™ System for reshielding needle of blood collection set



a) When sampling is complete apply gauze pad on puncture site. Grasp the yellow shield between your thumb and forefinger while using your remaining fingers to hold the tubing against the palm of your hand.



b) With the tubing held taut, advance your thumb and forefinger to slide the safety shield forward until an audible "click" is heard.



c) The click confirms that the shield is locked into place, covering the needle. Dispose the blood collection set in a suitable container.

followed by copious amounts of water. It is best to collect flammable solvents in safety cans or drums and store in a storage cabinet prior to collection by a disposal collector or company. Ether and chlorinated solvents should be collected in separate cans. Other solvents may be combined in one can.

Examples of corrosive solvents are strong acids such as sulfuric, hydrochloric, and phosphoric acid and bases such as ammonia (ammonium hydroxide).

Toxic, corrosive and inflammable chemicals should not be used as preservatives in the preanalytical phase.

Never add urine to concentrated acids. Such acids should be diluted by adding them slowly down the sides of the urine container. Disposal of strong acids and corrosive materials should preferably be done by pouring down the sink, followed by copious amounts of running cold water from a tap.

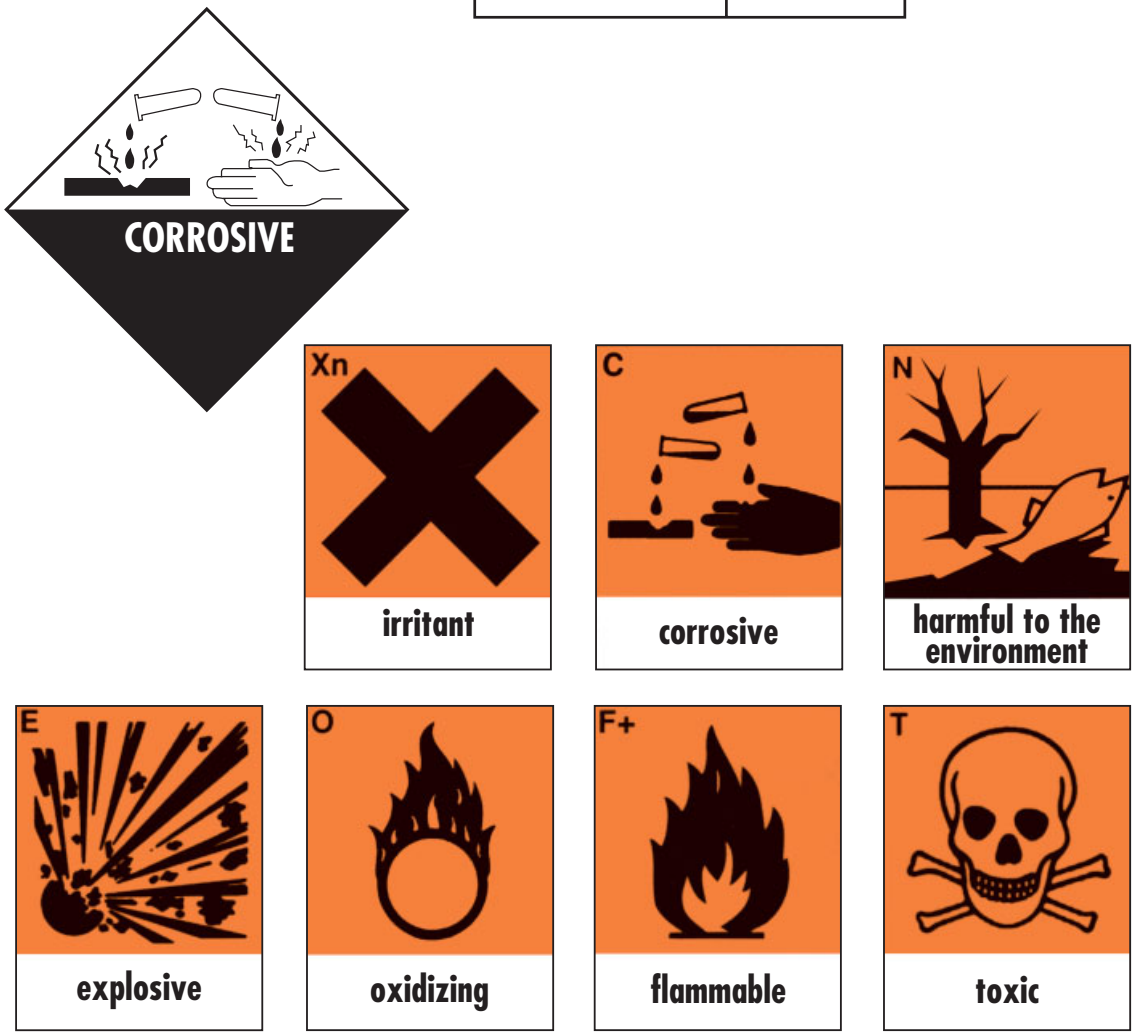
Thus when urine is collected in hydrochloric acid, the first portion should be collected before acid is added.

Toxic chemical waste such as toxic metals can pose a threat to ground water.

The Environmental Protection Agency in the United States of America (EPA) lists chemicals that constitute toxic waste (222). A European list of maximal allowable concentrations of chemicals in air, water and foodstuffs is also available (42).

Finally, to be knowledgeable about chemical hazards, each laboratory should have a file of material safety data sheets (MSDS) that list the hazardous properties of each chemical, and which can be readily consulted in cases of emergency or when there is doubt as to their hazardous nature.

Fig. 20-4
Safety labels of United States and European origin



Ensuring patient and sample identity

While processing blood and blood products 41 % of the reported defects were shown to be related to the preanalytical phase, 55 % to the postanalytical phase and only 4 % to the analytical phase according to a study of Boone et al. (20). The group at highest risk are patients issuing 10 or more units per day.

Most haemolytic transfusion reactions result from discrepancies in patient or sample identity. The frequency of administration of a wrong transfusion by means of mismatching the samples is 1:60000 transfusions. Therefore, request forms for blood transfusion must contain the first name, last name, the date of birth and, if possible, an identification number unique to the patient.
Before blood sampling is performed, active control of patient identity has to be thoroughly ascertained by asking the subject for his or her last name, first name and date of birth. Blood samples must be collected in correctly labeled tubes. The name of the person who has drawn the sample must also be documented.

The right sample

- Serum is used for immuno-haematological tests. Anticoagulants such as EDTA or citrate prevent complement activation. Consequently, complement-activating antibodies are not detectable in such plasma samples.
- The use of haemolytic samples is normally not allowed because antibody-induced haemolysis can be masked.
- The patient sample for pretransfusion testing should not be taken less than 72 hours prior to the test.
- Each patient sample for immuno-haematological examination must be stored at 4–6°C for one week following the test.
- For cold agglutinin determination, the blood sample must be kept at 37°C until serum has separated.
- Citrate blood is necessary if identification of erythrocyte-bound antibodies requires elution.
- EDTA blood samples are used for the detection of in-vivo complement binding by erythrocytes. For special erythrocyte antigen testing, citrate-anticoagulated blood should be used.
- Samples of blood contaminated with Wharton's jelly may agglutinate spontaneously. The cells should be separated by washing three times with 0.9% sodium chloride solution.
- Interferences of the agglutination reaction involve either pseudo- or polyagglutination. Pseudoagglutination (rouleaux) can be caused endogenously or exogeneously (Fig. 21-1).
- Exogenous factors are infusions of dextran, polyvinyl-pyrolidon or fibrinogen; among endogenous variables are disproteinemias in immunocytoma, liver cirrhosis or hyperfibrinogenaemia.
- Polyagglutination is a condition in which red blood cells are agglutinated by a high proportion of group-compatible sera. The same phenomenon occurring in vitro is called polyagglutination (28). This can occur during viraemia or bacteriaemia (endogenously) or after exogenous bacterial contamination of patient erythrocytes. Bacterial enzymes modifiying erythrocyte antigens released are one of the causes of in vitro and in vivo polyagglutination. T-polyagglutination is caused by a microbial neuraminidase, the TK-polyagglutination by a β-galactosidase or the acquired B-antigen by deacetylase.

Non-microbial polyagglutinations are also known. These are caused by somatic mutation of a pluripotent haemopoetic stem cell (Tn) or congenital genetic defects.

Samples: From the Patient to the Laboratory. W. G. Guder, S. Narayanan, H. Wisser, and B. Zawata

ISBN: 978-3-527-30981-8

● All immuno-haematological results should be observed and interpreted by two independent persons who should sign for the correctness of the procedure.

Storage of blood for transfusion

Blood units have to be stored in special refrigerators equipped with an automatic temperature recording system and an optical or audible alarm system. The low alarm activation should be set at 3.5 ± 0.5°C and the high alarm activation at 5.5 ± 0.5°C. The surface of the blood container must be clean and dry. Any item that could result in the puncture of blood containers should be removed from the storage area. Products for transfusion must be stored separately from reagents or used pilot tubes. The storage refrigerator should be kept clean. A standard procedure for cleaning the storage refrigerator is mandatory (5).

What has to be done before transfusion?

Both on delivery of the blood unit and prior to transfusion, all information on the container and the accompanying transfusion form have to be checked for identity. The ABO-status of the donor must be evaluated immediately before transfusion by means of a bedside test. In the case of autologous transfusion, ABO status has to be checked for both the blood unit and the recipient by bedside testing. The residual blood of the transfused unit has to be kept for at least 24 hours after infusion at 2–8°C (230).

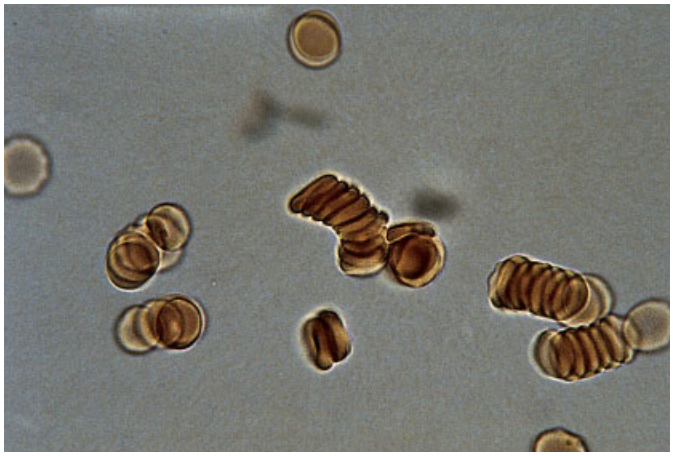
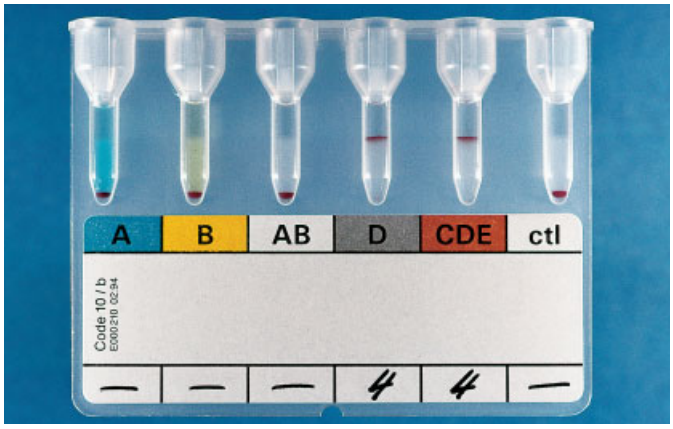
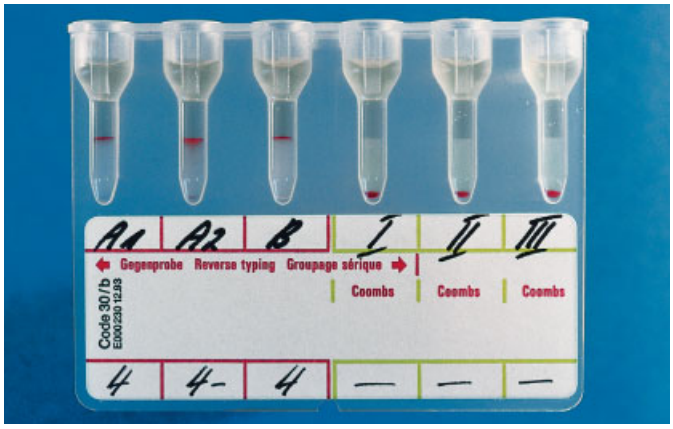


Fig. 21-1
Pseudoagglutination (rouleaux)

Fig. 21-2
Blood typing by gel filtration test





Why a separate tube for the coagulation test?

If several evacuated blood collection tubes are used, the specimen for coagulation testing should be collected in the second or third tube in order to minimise contamination with tissue thromboplastin.

Specimen collection

The results of coagulation screening tests such as the prothrombin time (PT, Quick Test) and the activated partial thromboplastin time (APTT) are decisively influenced both by the anticoagulant used, its concentration and ratio to blood, and by the manner of collection and further processing of the specimen. Citrate can be regarded as standard anticoagulant (105).

Concentration of the anticoagulant

Strict observation of a ratio of 1+9 (citrate solution to blood) for determination of the APTT and other coagulation screening tests is recommended.

Prothrombin time (PT) reported as the international normalised ratio (INR) is sensitive to the concentration of citrate. INR values, especially with a responsive PT reagent similar to the WHO thromboplastin, with an international sensitivity index (ISI) equal to 1, are generally higher with a 0.129 mol/L (3.8 %) than with a 0.109 mol/L (3.2 %) sodium citrate (2, 142). The differences in INR between the two concentrations of citrate can vary from 0.7 to 2.7 INR units (2).

Hence a laboratory should not interchange the two concentrations of citrate when PT is reported in INR units on patients who are undergoing oral anticoagulant therapy.

In addition to maintaining the nominal anticoagulant to blood ratio (1+9), the amount of headspace above the blood is also a variable that can affect the APTT result (2, 194). Apparently, the increase in surface area in a tube favours platelet activation with the subsequent release of platelet factor 4, which in turn neutralises some of the heparin causing a shortening of the APTT value (2, 194).

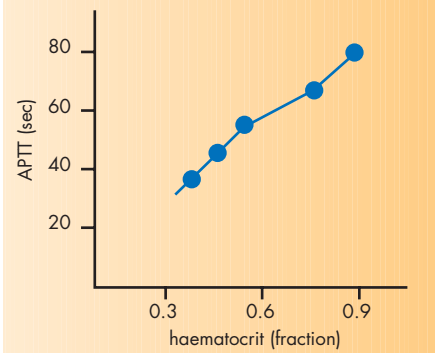


Fig. 22-1
Effect of haematocrit on the APTT using a buffered citrate solution (0.129 mol/L)
▶

The recommended citrate concentration is 0.105 mol/L (3.2 %) (89, 155). A citrate solution buffered to pH 5.5 is preferable to an unbuffered solution as in the buffered solution the pH of the specimen is closer to the physiological range (146). If the haematocrit is above 0.55 a correction is necessary even if the ratio of anticoagulant to blood is 1+9 (1:10). This is because the plasma compartment is reduced, resulting in an excess of citrate in the specimen container which in turn complexes with the calcium ions added during measurement of the PT and APTT. Thus the clotting time is prolonged.

Specimen processing

After carefully inverting the specimen container several times to exclude clot formation and checking that the ratio of anticoagulant to blood is correct, the closed specimen tube is centrifuged at 2000 *g* for 10 minutes (155). Specimens with invisible clotting or haemolysis should not be used as activation of clotting factors may have taken place. In addition, it is very important to look out for lipaemic or icteric specimens as these can cause interference in the case of photo-optical measurements.

Unopened specimens for the performance of coagulation screening tests (PT and APTT) should be transported to the laboratory at room temperature (22–24°C) but not on ice.

The table in the Annex gives details on specimen stability for the different coagulation tests.

The following general principles can serve as a guide.

- If the test is to be performed immediately the specimen is kept at room temperature. The plasma can be left standing on top of the packed cells after centrifugation.
- The collection vessel should be closed to avoid changes in pH due to evaporation of volatile carbonic acids.
- Exposure to high temperatures (including direct sunlight) must be avoided at all costs.
- Specimens should not be refrigerated (+2 to 8°C) as cold activation of factor VII, and also of factors XI and XII, can lead to shortened clotting times in the respective screening tests.
- For tests to be performed at a later date platelet-free (< 5000/µL) citrated plasma is aliquoted and frozen in closed tubes.
- Frozen specimens should be thawed quickly in a water bath at +37°C and mixed thoroughly, making sure that any cryoprecipitates are completely dissolved. Repeated freezing and thawing is not recommendable.

Specimen storage

The plasma should be left standing on the sedimented cells and used within 2 to 6 hours (for details see Annex). If the storage period is to exceed 4 hours the plasma specimens can be kept in the refrigerator for 4 weeks at –20°C, and with rapid freezing to –70°C the storage time can be extended to 6 months (83, 155).

Factors V and VIII are unstable. The factor VIII activity (VIII.C) of the samples frozen at –20°C should be measured within two weeks. In specimens frozen at –70°C the factor VIII activity is stable for up to one year.

For single factor assays, particularly of factor VIII, the blood should be cryocentrifuged (+4°C) as soon as possible after collection and the plasma frozen at –20°C to –70°C.

Evaluation of fibrinolysis and monitoring of fibrinolytic therapy

After a freezing and thawing process, identical results are only obtained if the plasma contained no fibrin monomers, fibrin degradation products or heparin before freezing.

In plasmas which contain fibrin monomers gelling processes after thawing can result in negative fibrin monomer detection, prolongation of the APTT and prolonged thrombin and reptilase times. In plasmas with considerably prolonged thrombin times (heparin, FDP) instability after thawing can lead to shortening or even normalisation of the thrombin time and APTT after thawing (216).

The measurements of the PT and APTT are least influenced if the plasma is analysed at room temperature within 2 hours.

Specimens for monitoring fibrinolytic therapy with streptokinase or urokinase, for example, should be collected into tubes containing a mixture of EDTA and aprotinin as this combination immediately inhibits the streptokinase or urokinase induced plasmin activation (146). For this, aprotinin in a concentration of 150 kIU/mL blood should be combined with trisodium citrate (10 mmol/L) or EDTA (4.2 mmol/L).

Unlike FDP measurement, which requires a tube containing 10 units of thrombin and 1835 units of soybean trypsin inhibitor/mL of blood detection, D-dimer, which reflects the fibrinolytic activity, can be performed in citrated plasma.

The activity of rt-PA (recombinant tissue plasminogen activator) in the blood can be effectively inhibited with a mixture of 5 mmol/L D-phenylalanine-proline-arginine-chloromethylketone and 10 mmol/L citrate or 4.2 mmol/L EDTA (138, 142).

Optimum anticoagulant

The International Council for Standardization in Haematology (ICSH) has recommended dipotassium EDTA (K_2-EDTA) (ethylenediaminetetraacetic acid) as the anticoagulant of choice for the collection of blood specimens intended for blood cell counting and sizing (170).

K_2-EDTA was selected in preference to Na_2-EDTA because of the greater solubility of the potassium salt compared to the sodium salt.

With all EDTA salts, the cells shrink, thus affecting the centrifuged but not the calculated haematocrit. Because of the lower pH of Na_2- and K_2-EDTA compared to K_3-EDTA, the cells swell, thus compensating for osmotically induced cell shrinkage. Calibration of electronic blood cell counters for mean corpuscular volume (MCV) using the microhaematocrit value obtained from blood specimens collected in either Na_2- or K_2-EDTA have been reported to give acceptable results, in contrast to the unacceptable results obtained when microhaematocrit values obtained from blood specimens collected in K_3-EDTA were used to assay commercially available control material (170, 188).

With a variety of techniques now incorporated in the new automated instruments for counting, sizing and providing a 5-part white blood cell differential count, the question has been asked whether any salt of EDTA is the adequate anticoagulant for haematology (169).

Platelets change from their discoid to a spherical shape upon blood collection in EDTA, thus introducing an error in the mean platelet volume (MPV) determination.
The influence of EDTA on the stability of white blood cell populations, lymphocytes being relatively more stable and neutrophils and monocytes more likely to be influenced when stored in EDTA, introduces a variable that is compounded by the variation in the cluster analysis software packages used by the different analyzers.

Ratio of anticoagulant to blood

The volume of blood drawn should ensure maintenance of the recommended concentration range for the EDTA salt of 1.2–2.0 mg/mL (180).

Since the concentration of EDTA has an effect on neutrophil morphology, the quality of the peripheral blood smear can be compromised if attention is not paid to the anticoagulant to blood ratio and the time elapsed between blood collection and preparation of the smear.

At concentrations of EDTA of up to 1.50 g/L of blood in blood specimens not over 1 hour old, minor changes appear in neutrophil morphology. As the concentration of EDTA increases, more serious changes such as loss of bridges between lobules, loss of cytoplasmic boundary and early cross-over occurs within the first hour.

The effect of increase in the concentration of EDTA over the nominal value decreases the centrifuged microhaematocrit value, the decrease being more pronounced with K_3-EDTA than with K_2-EDTA.

However, with automated instruments, the mean corpuscular volume was not influenced at K_3-EDTA concentrations up to 10 times the nominal value; and results obtained with K_2-EDTA were shown to be dependent on the instrument used (63).

Using the recommended concentration of EDTA salts (K_2- and K_3-EDTA) and performing analysis between 1 and 4 hours after blood collection, no significant difference was seen in results obtained with blood collected in either of the 2 anticoagulants (63).

Specimen collection and handling

Thorough mixing of the blood specimen with the anticoagulant by inverting the tube several times is a prerequisite. The type of mixer (rocking versus rotary) used may affect the extent of mixing, especially if the tube is overfilled, thus yielding inaccurate results (166).

Transport, storage and stability of analytes

Because of the variations between newer automated instruments, including reagents, it has been recommended that the EDTA anticoagulated blood be analyzed within 6 hours of collection (150, 170). In some cases, however, this time is already too long to ensure constant results. Only haemoglobin and platelet number are stable over this time. Likewise, stability at refrigerator temperature is analyzer-dependent (for details see Annex). For the reasons given above, the blood smear should be prepared within 1–2 hours of blood collection. Extended storage for up to 24 hours is not recommended.

Pseudothrombocytopenia

Platelet clumping or agglutination, and platelets adhering to neutrophils (platelet satellism (Fig. 23-1)) have sometimes been observed with EDTA anticoagulated blood, such changes becoming progressive with the time elapsed after collection. This phenomenon elevates the white blood cell count while depressing the platelet count. The problem can be recognized by examination of the peripheral blood smear, and also by being alerted by the platelet flags or alarms in the instrument (39).

Accurate platelet counts on subjects showing EDTA-induced platelet satellism can be obtained by diluting blood from a finger prick or by collecting blood with citrate as the anticoagulant.

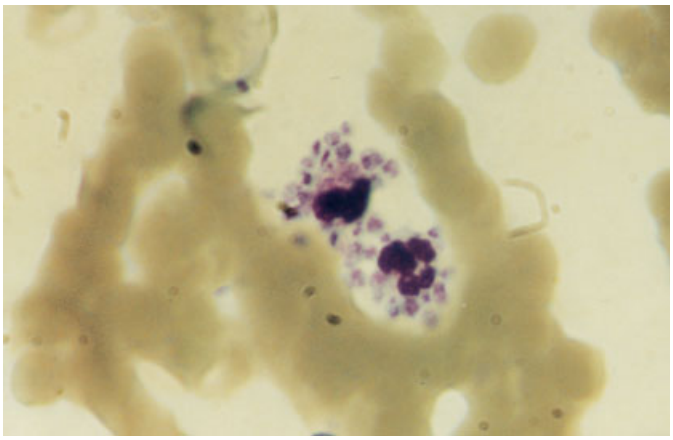


Fig. 23-1
Satellism of platelets on granulocytes (neutrophils)

If a blood collection tube is drawn to one-half of its nominal volume, the effective concentration of EDTA would be unsuitable for preparation of a peripheral blood smear intended for white blood cell differential count (180).

Blood collection tubes must have an air space representing at least 20% of the volume of the tube to facilitate mixing (170).

Special considerations for platelet content measurements

To assess in-vivo activation of platelets by measurement of constituents in the platelet α-granules such as platelet factor IV (PF4), β-thromboglobulin, fibronectin and platelet-derived growth factor, activation of platelets after blood collection should be minimized. This can be accomplished either by preventing the formation of thromboxane-A_2 or by maintaining high levels of cyclic AMP within the platelets. An additive mixture used for this purpose consists of 0.11 mol/L citric acid, 15 mmol/L theophylline, 3.7 mmol/L adenosine and 0.198 mmol/L dipyridamole with the final pH being adjusted to 5.0 (38, 138). PF4 levels in blood collected in this additive mixture called CTAD is almost 10 times less than that obtained in a conventional citrate tube (223).

In clinical chemistry, a number of reagent- and analyzer-specific problems have to be considered. Thus many interference factors act in a reagent-specific way. In addition there are differences between analyzers and analytical principles (i.e. "dry" and "wet" chemistry, direct and indirect potentiometry). In this chapter, a few examples only are demonstrated (66, 213, 239).

Special aspects when using so-called "dry chemistry" (99, 202)

When a carrier-bound reagent analyzer for capillary blood is used, the correct sampling of capillary blood samples is of decisive importance for the reliability of the results. The producer of the Reflotron® System (Roche Diagnostics, Germany) recommends:

"When a large free-hanging droplet has formed, it should be applied to the sample application field of the test carrier without directly touching the carrier with the finger. For an additional measurement it is necessary to prick the finger at a different site."

Results in a whole blood analyzer may depend on sample haematocrit, because the amount of plasma available to the reactive zone of the test strip varies at different packed cell volumes.

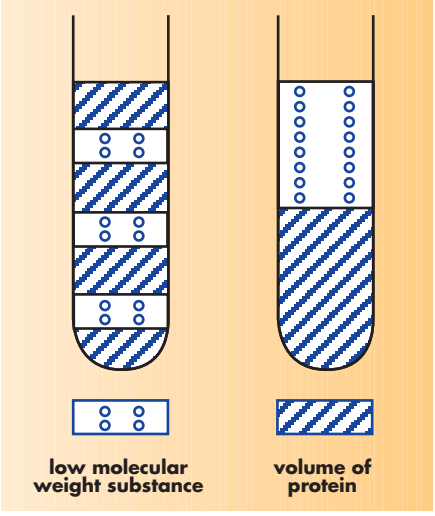


Fig. 24-1
Change of concentration of low molecular weight substances after deproteinization (from (25))

Dry chemistry methodologies on the one hand offer the possibility of separating the analyte from many disturbing components in the matrix. On the other hand the matrix is more diluted in wet chemistry procedures, resulting in lower analyte and possible interfering concentrations.

Different results using methods with and without deproteinization?

Protein molecules in serum-/plasma occupy a defined volume, dependent on the concentration and the size of the protein. As a result, the concentration of low molecular solutes (e.g. glucose, electrolytes etc.) in protein-free filtrates are found to be approximately 5% higher than in untreated serum-/plasma samples without deproteinization. The influence of deproteinization on the determination of low molecular weight substances which are dissolved in serum-/plasma water, is shown in Fig. 24-1 (25). The volume displacement effect of proteins is especially important for the determination of electrolytes using direct potentiometric in comparison to indirect measuring procedures (88) as well as for determination in deproteinised whole blood (e.g. glucose).

The volume displacement effect of lipids

A similar displacement effect is observed with triglycerides in blood. At 5000 mg/dL (57 mmol/L) triglyceride concentration, direct potentiometry gives about 5% higher (and more true) results of electrolytes compared to flame photometry or indirect (after dilution) potentiometry (110).

Whole blood versus plasma glucose

When comparing glucose in whole blood with that in plasma, similar, but larger differences are observed. Since blood cells have a higher protein and lipid content and glucose is not equally distributed between the intracellular and the extracellular space, results obtained in plasma are approximately 15% higher compared to whole blood, when related to the same volume. The WHO (3) and the ADA criteria (6) for the diagnosis of diabetes mellitus are therefore different for plasma and whole blood.

Electrolytes (Na^+, K^+, Cl^-, HCO_3^-)

If during transport and storage of whole blood the glucose concentration falls below a critical concentration, the cells lose their intracellular potassium and take up sodium in its place. If CO_2 escapes from a blood sample, the cells lose bicarbonate and replace it with chloride from the surrounding plasma (chloride shift) (Fig.24-2). This limits the stability of whole blood for the plasma/-serum analysis of electrolytes. Interestingly, the increase in plasma potassium is higher in refrigerated blood samples compared to samples stored at room temperature (84). This is caused by an inhibition of the cellular Na^+,K^+-ATPase activity brought about by cold.

Trace elements

Contamination plays a significant role in trace element analysis. Blood should therefore be obtained in a suitable sampling system that has been declared trace element-free by the laboratory.

Lipids

Storage alters triglyceride concentration due to the action of endogenous lipases.

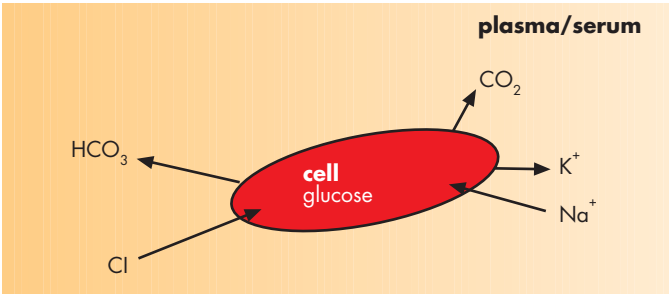


Fig. 24-2
Electrolyte fluxes between blood cells and plasma/serum during storage of whole blood

The triglyceride concentration in the sample falls while that of free glycerol rises. The extent of this effect varies from person to person and does not correlate with the initial triglyceride concentration. In addition, the composition of plasma lipids determines their behavior during centrifugation. Chylomicrons and their remnants tend to float to the top layer of plasma, whereas other lipoproteins remain equally distributed. This has to be considered when primary tubes are used in analyzers (131).

Creatinine

The concentration of non-creatinine chromogens increases at room temperature, this effect being more pronounced in whole blood than in serum/plasma (the higher the temperature, the greater the effect). This increase – which varies from person to person and is independent of the initial creatinine concentration – occurs when creatinine is determined by the Jaffé method (145).

Recommendation:

In clinical chemical analysis, method- and analyzer-specific prenalytical influences and interferences have to be taken into consideration. Results obtained with one system are not transferable to others without experimental proof.

Detailed information may be obtained from reagent and analyzer producers.

Sampling, storage and transport for analysis by immunochemical methods

Sensitive immunochemical methods lend themselves to the measurement of trace quantities of labile hormones, proteins and other analytes in blood. Because of the wide range of analytes measured by immunoassays, this chapter will attempt to focus on a few representative analytes (12).

Posture and timing

Variables such as posture during blood sampling and diurnal changes have to be taken into consideration.

Postural variations have a significant impact on renin, elevation of enzyme activity being observed when moving from the recumbent to the erect position.

Cortisol has a peak value between 4 a.m. and 6 a.m (see Fig. 5-2).

Hormones such as growth hormone, lutropin (LH) and follitropin (FSH) are released in bursts, and as such, several blood specimens taken within closely timed intervals are needed to establish a median value.

Refrigeration and freezing

Some hormones such as insulin, proinsulin and C-peptide can be stabilized by merely placing blood specimen containers on ice immediately after collection. Such specimens should be promptly centrifuged, preferably in a refrigerated centrifuge at 4°C, and the serum kept frozen until assayed. Of course, the frozen specimen should be completely thawed prior to assay. It is also important that haemolysis be avoided, since this will decrease both insulin and proinsulin values.

Processing of blood promptly upon clotting and freezing of serum at –70°C provides long-term stability for analytes such as gastrin, pepsinogen-1, and insulin.

Collection of blood in an appropriate anticoagulant

Most analytes determined by immunochemical methods can be measured in serum and/or heparinized plasma.

Collection of blood in EDTA and promptly freezing the plasma has been found to be adequate for preserving labile polypeptide hormones such as endorphin, vasoactive intestinal peptide, substance P and pancreatic peptide.

By its inhibitory effect on metalloproteinases EDTA plasma is also recommended for ACTH, parathyroid hormone and glucagon.

Collection of blood with proteolytic enzyme inhibitor

A proteinase inhibitor called aprotinin (also known by its trade name Trasylol) added to an anticoagulant such as EDTA or heparin has found application in the stabilization of labile polypeptide hormones and enzymes (137). Since aprotinin inhibits kallikrein, its potency is expressed in terms of kallikrein inhibitory units (KIU). The concentration of aprotinin used for the preservation of labile hormones and enzymes ranges from 500 to 2000 KIU/mL. Thus, a mixture of EDTA aprotinin has been used to stabilize glucagon, ACTH, renin and certain gastrointestinal hormones such as β-endorphin, secretin, neurotensin, gut glucagon, so-

matostatin and vasoactive intestinal peptide (137).

In one study it was demonstrated that glucagon levels measured by RIA in EDTA plasma were approximately 26% higher than in plasma obtained from blood collected in a mixture of 1.5 mg EDTA and 2000 KIU of aprotinin per mL of blood (Tab. 25-1). The high glucagon level in samples collected without aprotinin was due to the fragments of hormone produced by proteolytic enzyme degradation being apparently recognized as an intact molecule by the antibody used in the assay. In addition, some of the radio-labeled hormone underwent proteolytic enzyme degradation, thus limiting the amount of radio-label available to compete with the hormone in plasma for binding sites on the antibody (48).

Tab. 25-1 Effect of aprotinin on glucagon measurements in EDTA plasma

	EDTA + Aprotinin	EDTA
n	21	21
Mean pg/mL	386	518
% Difference	–25.5	

A mixture of lithium heparin and aprotinin has also been used to stabilize immunoreactive somatostatin, secretin, glucagon, C-peptide and cholecystokinin-pancreozymin.

Collection of blood in an anticoagulant-additive mixture

There are instances where EDTA alone is not sufficient for stabilizing an analyte. Thus, even when blood is collected in EDTA and the plasma is separated promptly and stored under ideal conditions, there is activation of complement. However, when a synthetic protease inhibitor such as nafamostat mesylate is added to EDTA, the stability of complement components (C_{3a}, C_{4a} and C_{5a}) is significantly improved. Furthermore, while the activity of complement components doubles with each freeze-thaw cycle when blood is collected with EDTA alone, no such discrepancy is seen in samples collected in EDTA supplemented with nafamostat mesylate (227).

Traditional anticoagulants such as EDTA and heparin have been ineffective in stabilizing a labile constituent such as parathyroid hormone related protein (PTH-RP). This tumor marker is so unstable that less than 10% of the original activity remains after 16 hours of storage at room temperature in blood specimens collected in either heparin or EDTA (164). The optimum mixture for blood collection is EDTA (1.5 mg/mL of blood), aprotinin (500 KIU/mL), leupeptin (2.5 mg) and pepstatin (2.5 mg). With this additive mixture, PTH-RP is stable for up to 1 hour at room temperature and for 24 hours if maintained refrigerated at 4°C (164).

For the measurement of catecholamines in plasma, blood should be collected in an anticoagulant mixture such as EGTA (90mg/mL) supplemented with sodium metabisulfite or glutathione (60 mg/mL). Even with this additive mixture, the blood has to be kept cold in an ice bath and centrifuged in a refrigerated centrifuge. The plasma should then be transferred to a plastic vial, capped and stored in a freezer at a temperature below –20°C until ready for analysis. Frozen plasma prepared under the above conditions preserves catecholamines for at least 3 months (19).

Blood cells can provide important information

A false negative result in HLA typing will have serious consequences when a patient or organ transplant donor is being typed for HLA compatibility.

Separation of cells from peripheral blood for cellular analysis.

For the collection and transport of patient blood samples for cell analysis propylen tubes are required, because cells are adsorbed on glass and polyethylen (178).
Separation of a pure population of cells such as lymphocytes from peripheral blood are required for tests such as human leukocyte antigen (HLA) typing studies. If the cell preparation is significantly contaminated with granulocytes and platelets, they may bind some of the HLA-antibody thus yielding a false negative result.

How to purify lymphocytes

Lymphocytes can be purified using the FICOLL-HYPAQUE centrifugation procedure.
A typical FICOLL-HYPAQUE mixture consists of 10 parts of 33.9% Hypaque with a density of 1.2 kg/L and 24 parts of 9% aqueous solution of Ficoll. Both Hypaque and Ficoll solutions should be mixed at room temperature. The final Ficoll concentration in the mixture should be 6.4% and the density of the mixture 1.077 kg/L (23).

In the procedure, diluted whole blood is layered over the FICOLL-HYPAQUE mixture and centrifuged at room temperature (20 °C) for 40 min. The centrifugal force attained at the interface is 400 *g*. Since the FICOLL-HYPAQUE medium is less dense than red blood cells and granulocytes but more dense than mononuclear cells (lymphocytes and monocytes) and platelets, the mononuclear cells will remain at the plasma-Ficoll-Hypaque interface. Subsequent washes eliminate platelets from the lymphocyte pellet.
A recent study described the use of either heparin or buffered citrate as an anticoagulant, polyester gel barrier and liquid density gradient medium in an evacuated tube for blood collection. A 20 min centrifugation step at 1,500 *g* at ambient temperature resulted in isolation of peripheral blood mononuclear cells (PBMC) over the gel barrier and separation from erythrocytes and granulocytes trapped underneath the gel (132, Fig. 26-1).

Effect of anticoagulants on the recovery of granulocytes

Granulocytes can be recovered from FICOLL-HYPAQUE centrifugation by re-

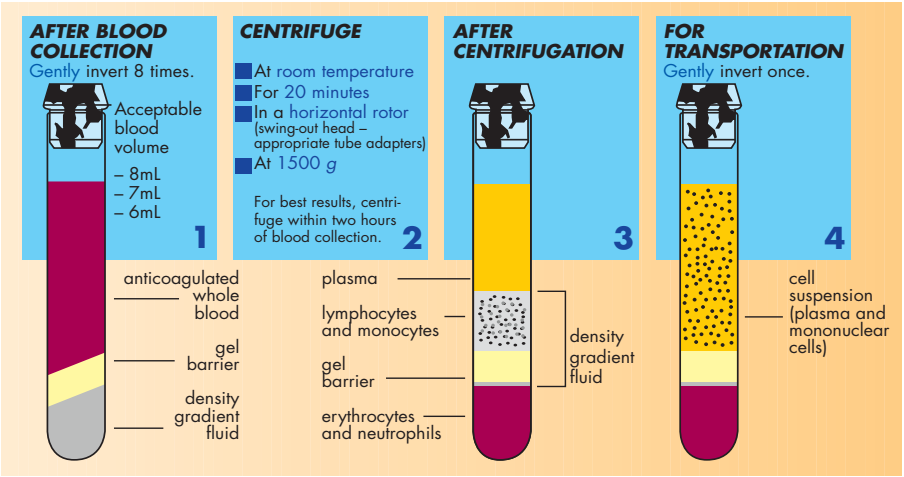


Fig. 26-1
Processing steps with the cell separation tube

moving the fraction above the FICOLL-HYPAQUE layer. 0.4 mL of 4.5% dextran and 1 mL of heparinized plasma are then added to this fraction. The residual red blood cells are allowed to sediment at 4°C for 40 min. Dextran promotes red blood cell rouleaux formation and favors sedimentation. The supernatant is thus enriched with granulocytes. Boyum found that EDTA gave better granulocyte yields (average 59%, range 43–71%) than heparin (average 49% range 38–61%) (23).

Relative merits of anticoagulants and nutrients for cell separation and stability

ACD (acid citrate dextrose), heparin and EDTA have all been used for the separation of mononuclear cells. However, regardless of whether EDTA, ACD or heparin was used as anticoagulant, it has been reported that the lymphocyte fraction was contaminated with granulocytes if the blood sample was over 14 hours old. Red cell contamination is a problem with 2-day-old EDTA blood (161).

From other authors it is reported that Ficoll gradient preparations of mononuclear leukocytes from EDTA-blood samples are contaminated by neurophile leukocytes and erythrocytes (178).

Nutrient dilution media would appear to influence the quality of FICOLL-HYPAQUE lymphocyte separations. Thus, blood collected in heparin and supplemented with glutamine and gentamicin gave good FICOLL-HYPAQUE separations with blood stored at room temperature for up to 3 days (135).

Whole blood lysis for flow cytometry applications

Whole blood lysis techniques for flow cytometric immunophenotyping applications have, because of their rapidity, supplanted the time-consuming FICOLL-HYPAQUE cell separation procedure (102). Even the lysis techniques have progressed from the original hypotonic lysis techniques to the currently commercially available non-hypotonic methods. In a study performed to evaluate the effects of various anticoagulants on both lysis methods and the FICOLL-HYPAQUE procedure, it was demonstrated that if analysis is performed on the same day, EDTA, ACD or heparin gave equivalent results. However, beyond 24 hours there is a significant decrease in granulocyte viability in EDTA. Furthermore, K_3EDTA has been reported to be associated with a loss of function in lymphocyte mitogen stimulation assays. Granulocyte viability was best for heparin. Lymphocytes were preserved equally in heparin and ACD. Although ACD could be used as an alternative to heparin, there is a tendency for platelets to aggregate in ACD, especially if specimens cannot be analyzed promptly (34).

If a flow cytometric test of the leukocytes is not performed during six hours after sample collection, the cell number should be determined immediately after sampling the EDTA blood. That is also necessary, if the immunophenotyping is performed with heparin blood (50 IE heparin/mL sample) (178). On the other hand, EDTA blood has the advantage that the loss of mature cells of the myeloid lineage by adsorption on the tube wall and platelet aggregation is diminished (178). For the platelet function analysis by flow cytometry technique the use of citrate blood is recommended despite some disadvantages. EDTA and heparin should be avoided because of their effects on glycoprotein structure and artifactual platelet activation (185).

Restriction fragment length polymorphism (RFLP)

To help understand some of the preanalytical problems, let us visualize a person whose DNA is being examined for RFLP to detect some genetic defect or gene rearrangements. When DNA is cleaved using restriction enzymes, fragments of varying sizes result depending on whether or not the polymorphism is within the restriction enzyme cleavage site (140).

It has been reported that aberrant or unexpected restriction fragments were obtained on DNA being examined for T and B cell gene rearrangements using blood collected in heparin (88). Such aberrant fragments were not found upon restriction enzyme digestion of DNA obtained from blood collected in either EDTA or ACD. Since these aberrant fragments found using heparinized blood may be confused with gene rearrangements, the choice of anticoagulant for blood collection intended for certain molecular biology procedures becomes critical (214).

Polymerase chain reaction (PCR)

Heparin at a concentration as low as 0.05 U/reaction mixture has been reported to retard or even completely inhibit the amplification of DNA during the polymerase chain reaction (PCR) (87). However, treatment of heparinized blood with heparinase to cleave heparin or the separation of leukocytes by centrifugation followed by at least two washings with a saline buffer is reported to overcome the effect of heparin (16).

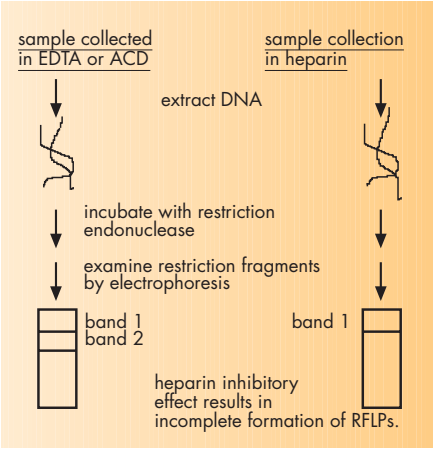


Fig. 27-1
Effect of anticoagulants on RFLPs

Actually, other anticoagulants such as EDTA and ACD also inhibit restriction enzymes. However, standard ethanol DNA precipitation techniques remove both EDTA and ACD while heparin is not removed (36).

Heparin should not be used as anticoagulant in the molecular biological analysis of blood.
If heparin is present in sample to be analysed, it should be eliminated before application of the sensitive rtPCR. This can be done by application of heparinase or precipitation of mRNA with LiCl (94, 95).

Erythrocytes can likewise inhibit taq DNA polymerase by formation of haematin (140).

Red blood cells can be removed by selective lysis with a buffer mixture consisting of 155 mmol/L ammonium chloride, 10 mmol/L potassium bicarbonate and 0.1 mmol/L EDTA adjusted to pH 4.

Alternatively, the cytoplasmic membrane of all cells can be dissolved with a buffer mixture containing the non-ionic detergent Triton-X100 leaving behind the nuclei of white blood cells from which DNA can be extracted. However, this technique will result in the loss of extranuclear DNA and RNA to the supernatant; hence, mitochondrial DNA will not be able to be extracted (27).

Considerations for the isolation of DNA and RNA

Classical techniques are based on lysing cells with lysozyme, alkali or detergents. The removal of proteins and other contaminants is effected by incubation with protease and/or extraction with phenol or chloroform.

The extract should be concentrated by precipitation with ethanol in the presence of sodium or ammonium acetate. If necessary, RNA can be removed using DNase-free RNAase.

The advantage of using proteinase K is that, in addition to releasing DNA from chromatin, it also destroys nucleases which would otherwise reduce the average molecular weight of DNA (140). However, proteinase K has to be removed before the isolated DNA can be subjected to restriction enzyme treatment. Also, Taq polymerase can be degraded by proteases.

Proteinase K can also be inactivated by heating the cell lysate or purified DNA to 95°C for 10 min.

Residual phenol can inhibit Taq polymerase. Hence, a final extraction with chloroform isoamylalcohol (49:1v/v) should be performed after phenolization to remove any trace quantities of phenol remaining in the aqueous phase.

Salts used to subsequently precipitate DNA should be removed by washing the pelleted DNA with 80% ethanol.

The type of detergent used for cell lysis may influence DNA amplification by PCR.

Generally, non-ionic detergents such as Tween 20 and Triton X-100 do not inhibit Taq polymerase in concentrations of less than 5% (v/v).

However, ionic detergents such as sodium dodecylsulfate (SDS) which are generally used in concentrations up to as high as 2.0% (w/v) can be inhibitory to Taq polymerase, since a concentration greater than 0.01% (w/v) has been found to be inhibitory.

Other ionic detergents such as sarkosyl and sodium desoxycholate have been shown to inhibit Taq polymerase at concentrations greater than 0.02% (w/v) and 0.06% (w/v) respectively. Hence, it is important that ionic detergents be efficiently removed by phenol/chloroform extraction and by ethanol precipitation and subsequent washing of the DNA pellet.

Even with non-ionic detergents such as nonidet P40 (NP40), where 1% (v/v) has no effect on reverse transcriptase enzyme, 0.1% (v/v) can inhibit Taq polymerase.

Hence, it is important to perform preliminary experiments to establish the effective concentrations of detergents and other known inhibitory reagents that may affect DNA amplification by PCR.

Chaotropic agents such as guanidinium isothiocyanate have been frequently used for the extraction of DNA or RNA.

The advantage of using 5 mol/L guanidinium isothiocyanate for RNA isolation is that it is able not only to remove proteins from RNA but also to denature ribonucleases which would otherwise degrade RNA (140).

Stabilizing RNA during transport, storage and sample preparation

A number of factors influence RNA stability. This rapid decrease in reverse transscriptase PCR sensitivity has been shown to be due to RNAses present in the sample, limiting the time of processing of native samples to 2 hours (140). By using 5 mol/L guanidinium isothiocyanate, the sample is stabilized by denaturation of RNAses for approximately one week at room temperature.

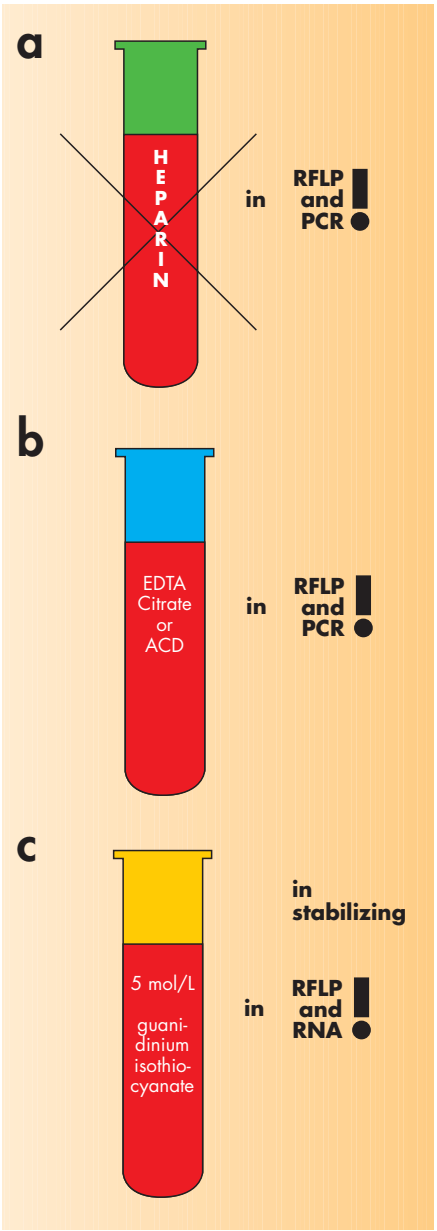


Fig. 27-2
Use of anticoagulants and stabilizers in sampling of blood for gene analysis

In terms of isolation of undegraded RNA, one must eliminate ribonuclease (RNase) contamination. These enzymes are so stable over a wide range of pH and resistant even to boiling that glassware, reagents and even the investigator's fingers are a source of potential contamination. Glassware should be treated with a 1% solution of diethylpyrocarbonate (DEPC) which is known to inhibit RNases. Residual DEPC, however, should be thoroughly removed by autoclaving the glassware in order to convert DEPC to carbon dioxide and water and subsequent heat-treatment of the glassware at 250°C for 4 hours.

Even sterile disposable plastic ware is not always free of RNAse! In case of contamination the materials like pipette tips and tubes should be autoclaved in hot air before use.

Contamination control

DNA from exogenous sources such as a person's hair or skin, door knobs, laboratory bench, dust, reagents, thermocycler and pipette tips are common sources of contamination.
Ideally, a laminar air flow bench with filtered air provides a clean dust-free environment.

Sample preparation should be carried out in a separate room or area.

Recently, Neumaier et al. have published recommendations on quality assessment of molecular biology diagnostics (160) desribing preanalytical aspects:

Specimens: PCR analysis can be applied to EDTA and citrate blood, dried blood (filter-paper cards), bone, marrow, buffy coat, sputum, mouthwash, bronchial lavage, cerebrospinal fluid, urine, stool, biopsy material, cell cultures, fixed or embedded tissue etc.). Depending on the test material, a pretreatment of the sample may be necessary prior to stabilization, e.g., liquefaction of sputum.

Sampling: sampling is best done in closed disposable sampling systems as are customary with other clinical test material. New disposable plasticware is considered to be free of nucleases. When using nonclosed sampling systems, at least disposable gloves are to be worn.

Sample stabilization: stabilization of test material is essential as nucleic acids degrade rapidly. This is especially important where RNA is to be analyzed. The fast inactivation of DNases and RNases is achieved reliably by chaotropic substances (here especially guanidinium-isothiocyanate, GITC). Organic solvents, e.g., phenol, may be added in parallel. Extraction systems with those additives are commercially available, e.g., RNazol, Trizol. The limited stability of reducing substances (here: β-mercaptoethanol) and their effect on sample stability is to be considered. The user has to be aware that batches of ready-to-use extraction solutions have limited shelf life due to instability of single components, e.g., β-mercaptoethanol. Enriched cells or native specimens of single components are lyzed by addition of GITC. The final concentration of GITC in the stabilized sample should not fall lower than 4 mol/L. Material stabilized in this manner needs not to be cooled. At temperatures lower than room temperature, GITC may crystallize. EDTA blood for the extraction of DNA from leukocytes requires no special stabilization.

Sample dispatch: samples stabilized appropriately may be dispatched by post at room temperature. This applies also to EDTA whole blood for DNA preparation and GITC stabilized samples for RNA recovery. Cooling is not necessary, but depending on the application the prolonged storage at room temperature will result in a critical loss in sensitivity. Samples are to be dispatched in breakproof containers. Non-stabilized samples must be shockfrozen and than be dispatched in dry ice. The cooling chain must not be interrupted.

Sample storage: specimen for DNA analysis are to be stored in 10 mM TRIS, 1 mM EDTA, pH 7.5–8.0 at 4°C. Specimens for RNA analysis should be kept in buffered solution preferably at –20°C. GITC stabilized RNA samples may be stored for approx. seven days at room temperature.

In conclusion, understanding the preanalytical pitfalls in molecular biology applications and minimizing or eliminating them is a prerequisite to the successful utilization of such techniques (95, 119, 143, 160, 190).

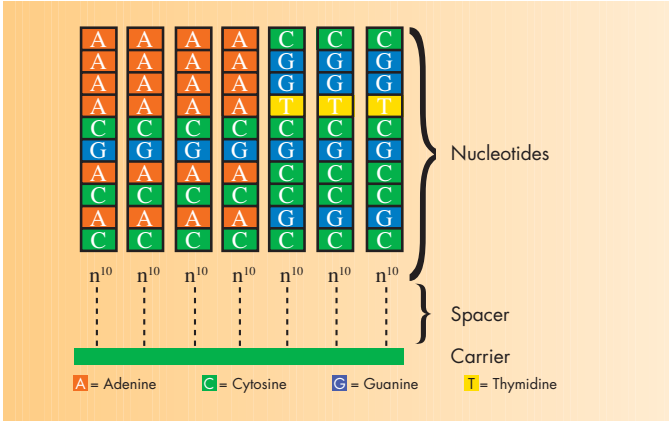


Fig. 27-3
Microchip PCR as diagnostic tool

Preanalytical variables in blood gas, acid-base and electrolyte measurement have recently been addressed in IFCC recommendations (30).

Anticoagulant

Heparin is the recommended anticoagulant for blood gas and electrolyte determinations in whole blood. Dry sodium heparinate may increase sodium, decrease pH, bicarbonate and base excess. In addition, ionized calcium is decreased if binding sites on heparin are not saturated. The recommended final heparin concentration in blood differs for glass and plastic tubes (195).

Recommendations on the use of heparin in blood gas and electrolyte analysis

Heparin solution may be added at a final concentration of 8–12 IU/mL in glass and 4–6 IU/mL in plastic tubes. The final concentration ranges for buffered heparin solutions should be, in mmol/L:

Sodium: 120–150
Potassium: 3.5–4.5
Ionized calcium: 1.2–1.4
Chloride: 100–130.

The pH of the heparin solution should be between 6 and 8 (30).

Specimen collection

There is a difference in blood gases and acid-base status between arterial and venous blood due to metabolism in the respective limb or tissue. In general, oxygen is consumed, CO_2 is increased and pH is decreased due to respiratory and metabolic components along the arteriovenous blood flow. Arterial and capillary sampling sites should therefore be preferred to obtain systemic acid-base and blood gas results. If indwelling catheters or cannulae are used for sampling, it has to be ensured that fluid or flush solutions are removed completely from the system by withdrawing a volume equal to three times the volume of the catheter system prior to blood collection (151). Blood should be collected anaerobically to prevent gas exchange with the surrounding air. Venous occlusion by tourniquet should be restricted to a maximum of 2 minutes. Formation of bubbles should be prevented. The sites recommended for sampling blood are those described in the chapters on arterial and capillary sampling (see p. 20–23).

Recommendation on capillary sampling

When sampling from skin, the first drop should be removed and blood allowed to flow into the heparinized capillary without any squeezing. The tip of the capillary should be placed deeply into the drop and filled completely without any pressure while holding in a horizontal or slightly downward position. The capillary in the drop should be closed off immediately with a plastic cap followed by insertion of a metal mixing bar ("flea"). The opposite end of the capillary should then be sealed. Blood and heparin are mixed by moving the bar with the aid of a magnet from end to end five times.

Storage and transport

Specimens for blood gas and electrolyte measurements may be affected during storage by the following processes (133):

● *Metabolism of blood cells:* glycolysis, mainly in red cells, causes formation of lactic acid and shifts in pH, bicarbonate and base excess towards the range of metabolic acidosis. Oxygen consumption in leukocytes and platelets decreases pO_2 and increases pCO_2. The fall in pO_2 is accelerated if the original sample pO_2 is elevated. The metabolic processes can be reduced by cooling the sample. Cooling is necessary if the sample cannot be analyzed within 15 min of sampling.

● *Ion release from blood cells:* prolonged storage, vibration during sample transport and severe thrombocytosis are factors which may contribute to an increase in potassium and a decrease in ionized calcium in the plasma. During the first hour of storage in ice water, the average increase in plasma potassium concentration is 0.1 mmol/L (133).

Ionised calcium can be stabilised up to 8 hours after separating plasma by centrifugation (96).

● *Gas exchange:* As mentioned above, plastic materials are not absolutely gas-tight. It has been observed that refrigerated plastic tubes leak more gases than those kept at room temperature. A compromise has therefore been suggested that storage in ice water should not exceed 30 min when stored in plastic. Glass capillaries and glass syringes remain gas-tight for several hours (133) (Fig. 28-1).

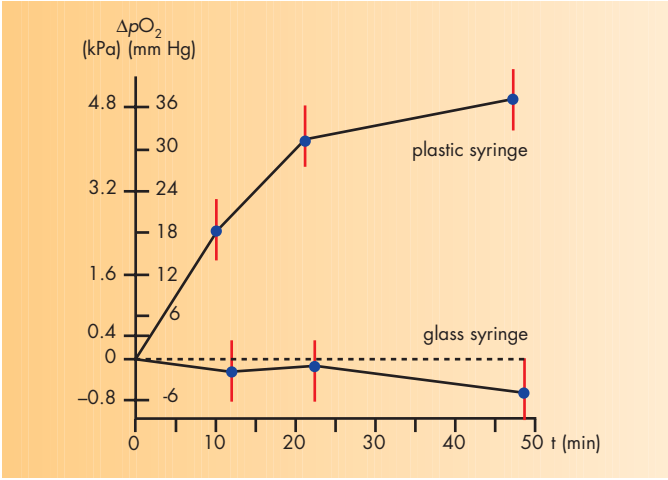


Fig. 28-1
Alteration of pO_2 in whole blood (pO_2 = 85 mm Hg ≙ 11.3 kPa) stored in a plastic or glass syringe for 45 min at room temperature (mean and SD of 15 measurements in each type of syringe) (133)

Sample preparation

Upon arrival of samples for blood gas and acid-base measurement, careful remixing of the samples prior to analysis is necessary.

Specimens in glass capillaries should be remixed by moving a metal wire from end to end for 5 to 10 sec. Glass or plastic syringes should be inverted 10 times and then rolled horizontally for 10 sec (30). No bubbles or dead space should be formed during mixing. Mixing the sample is of special importance if haemoglobin is measured simultaneously (134).

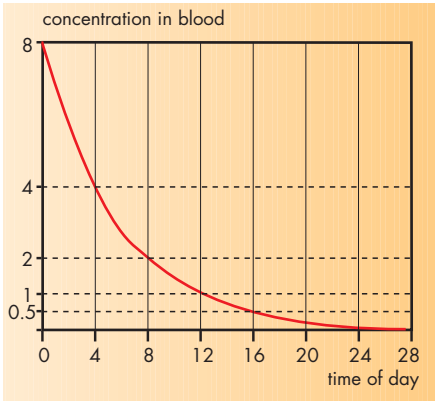


Fig. 29-1
The elimination of most pharmaceuticals takes place in accordance with a first-order reaction, i.e. a certain percentage of the total quantity in the body is eliminated per unit of time irrespective of the administered dose and the concentration of the pharmaceutical agent in the sample (167)

Which sample for TDM? (49, 124, 136)

The most commonly sampled body fluids are blood, plasma and serum because a good correlation between drug concentration and the therapeutic effect can usually be found. In addition, TDM in plasma/serum is useful in preventing toxic side effects of the drug by overdosage or decreased elimination (metabolism).

In special cases, urine is also useful as a sample material; saliva and CSF are analyzed less frequently, although they may better represent the free drug concentration in some cases.

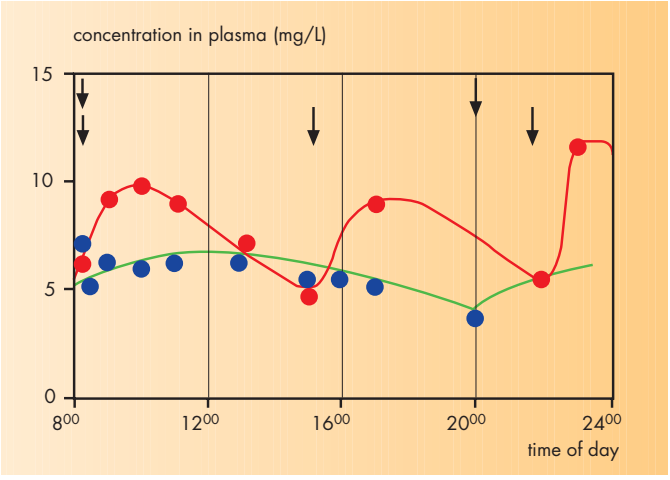


Fig. 29-2
Concentration of theophylline in plasma after oral administration of pills either as immediate-release formulation (red points) or as sustained-release formulation (blue points). The immediate-release formulation was administered 3 times a day (at 6.00, 15.00 and 22.00 h), the sustained-release formulation 2 times a day at 8.00 and at 20.00 h (53)

Sampling – a matter of timing

The optimal sampling time varies with the drug and the dose schedule used (see Tab. 29-2 and Fig. 29-2).

When making an estimate of a meaningful interval to select between two determinations, it should be remembered that approximately five half-lives are necessary for equilibrium to be reached in the body between intake and elimination, i.e. before adapting the dosage one should wait until at least this interval has elapsed before the next measurement of the drug concentration. Maxima are given in Tab. 29-2, minimum concentrations should be obtained shortly before the next dose is applied (Tab. 29-2).

Tab. 29-1 Rules for blood sampling in TDM

Time at which blood sample should be taken	
Long-term therapy	Basically always in the steady-state (after approx. 5 half-lives)
Intravenous administration	One must wait until the distribution phase is completed (approx. 1–2 hours after completion of the infusion)
	Exception:
	Digoxin 6 – 8 hours
	Digitoxin 6 – 8 hours

For further details, see the NCCLS-document on therapeutic drug monitoring (148).

Which specific aspects of sampling are important for TDM?

Blood should not be taken from the arm into which drugs or transfusion fluids are being infused.

Heparin, EDTA, or potassium oxalate can be used as anticoagulants. However, heparin leads to a change in

protein binding in some drugs (236). EDTA is believed to be the optimal anticoagulant and should be the agent of choice for measuring levels of tricyclic antidepressants since, by chelating divalent cations, EDTA might contribute to the stability of these drugs by protecting them from oxidation (147).

Haemolyzed blood samples are preferred to serum or plasma for cyclosporine determination, because numerous factors (temperature, haematocrit, concentration of lipoproteins) affect the blood/plasma relationship of ciclosporine (238). EDTA is also recommended as an anticoagulant.

Some immunoassays for drugs can be disturbed by unspecific cross-reactions of endogenous interference factors, e.g. digoxin-like immunoreactive substances (steroids, lipids) may interfere with digoxin (falsely elevated results).

Sampling of urine should be carried out over at least 7–10 biological half-lives (see Annex and (52)*), which covers more than 99% of the excretion phase.*

Sampling times for saliva should be similar to those for blood. The mouth should be thoroughly cleansed with water before sampling. The saliva must be completely free of food particles and any drug retained in the mouth following oral administration.

What about storage and transport?

Disposable containers should be used to collect the specimens in order to reduce the possibility of contamination. Some plasticizers released from plastic syringes or from the rubber closures of glass containers may affect assay results (191).

To ensure their integrity, the samples should be sent to the laboratory without delay. To avoid haemolysis and decomposition, only plasma, serum, urine or CSF should be sent. Nitrazepam, chlorazepam and cocaine, for example, have been found to decompose during storage of blood samples at 4°C.

For ciclosporine A (CsA) which partitions into the red blood cell rapidly when blood cools from body temperature (37°C) to room temperature (20°C) after collection, whole blood is the specimen of choice. The preferred anticoagulant for monitoring CsA is EDTA (192).

When plasma is separated at room temperature, the plasma to blood ratio for CsG is 0.8, whereas for CsA it is 0.6 (147).

How stable are samples at different temperatures?

Stability of samples for TDM is summarized in the "List of Analytes" (see annex). Strict observation of the kit producer's instructions is recommended.

The freezing of blood for the determination of ciclosporins should be strictly avoided. In addition, samples for fluorouracil need to be collected on ice (unstable at room temperature). Frozen samples should preferably be thawed at room temperature. Too rapid thawing by warming the sample may cause overheating and decomposition. Thorough mixing is very important in order to secure homogeneity prior to analysis.

Tab. 29-2 Therapeutic Drug Monitoring: Pharmacokinetic properties with recommendations for sample collection. Data for adults (52, 74, 187).

Substance	Time to reach maximum concentration	Time to reach steady-state	Elimination half-life	Protein binding	Recommendation on sample collection
ANTI-ARRHYTHMIC AGENTS					
Amiodaron	3–7 h (oral) 15 min (i.v.)		4 h – 25 d (single dose i.v.) 7 h – 80 h (oral) at the time of steady state: 20 – 100 d	> 90%	
Quinidine	1–3 h	2 d	6–7 h	80–90 %	Maximum about 8 h after administration of sustained-release preparations
Disopyramide	2–3 h	1–2 d	4–9 h	10–65 %	Protein binding is concentration-dependent
Lidocaine	End of the initial dose	30–90 min	70–200 min	60–70 %	During the infusion; protein binding is concentration-dependent. Formation of active metabolite
Procainamide/ N-acetyl-procainamide (NAPA)	1–4 h (oral) 15–30 min (i.v.)	15–25 h	3–5 h 6–10 h	approx. 15 %	Maximum immediately after the last orally administered dose; oral absorption is very variable
ANTIBIOTICS					
Gentamicin* Tobramycin* Netilmicin Amikacin	1 h after i.m. administration; 30 min after i.v. administration	< 30 y of age 2.5–15 h > 30 y of age 7.5–75 h	1.5–6 h 0.5–3.0 h 2–3 h 1.5–15 h	≤ 10 %	Sampling time 1 h after administration Trough level just before subsequent injection
Vancomycin	30 min	20–30 h	4–10 h	30–55 %	Peak 30 min after infusion of 1 h
Streptomycin	1–2 h	10–15 h	2–3 h	≤ 30 %	Peak 1–2 h after i.m. infusion
ANTICONVULSANTS					
Carbamazepine	6–18 h	2–6 d	10–25 h	65–80 %	During the dosage interval; actual sampling time unimportant
Clonazepan	1–2 h		20–60 h	83–87 %	
Ethosuximide	2–4 h	7–14 d	10–60 h	0 %	
Phenobarbital	6–18 h	10–25 d	50–120 h	50 %	Actual sampling time unimportant
Phenytoin Sustained-release preparations	15–30 h 3–9 h	8–50 d	25–200 h	92 %	During the dosage interval; actual sampling time unimportant
Primidone Phenobarbital	0.5–7 h	2–4 d 10–25 d	6–8 h 48–120 h	35 %	
Valproic acid	2–8 h	2–3 d	8–15 h	90 %	▶▶

Substance	Time to reach maximum concentration	Time to reach steady-state	Elimination half-life	Protein binding	Recommendation on sample collection
BRONCHOSPASMOLYTIC AGENTS					
Theophylline	1–4 h	2 d	3–12 h	55–65 %	Maximum approx. 4 h for sustained-release preparations
CARDIAC GLYCOSIDES					
Digitoxin	3–6 h	30 d	6–8 d	90–97 %	8 to 24 h after ingestion
Digoxin	60–90 min	5–7 d	40 h	20–40 %	8 to 24 h after ingestion
IMMUNOSUPPRESSANTS					
Ciclosporine A	2–6 h	approx. 2 d	10–27 h	90 %	Immediately before the next dose
Tacrolimus	1–2 h	3 d	6–21 h (liver transplantation) 4–57 h (kidney transplantation)	99 %	Use EDTA whole blood
PSYCHOPHARMACEUTIC AGENTS					
Amitriptiline	2–6 h	3–8 d	17–40 h	90 %	Non-critical. In equilibrium, just before next dose is taken
Desipramine	2–6 h	2–11 d	12–54 h	75–90 %	
Imipramine	1–6 h	2–5 d	9–24 h	63–95 %	
Nortriptiline	2–6 h	4–20 d	18–56 h	87–93 %	
Lithium	1–3 h	3–7 d	14–33 h	0 %	12 h after the last administration
CYTOSTATIC AGENTS					
Methotrexate	1–2 h	12–24 h	2–4 h	50–60 %	Varies from person to person

* In newborns the biological elimination half-life is about 8 h (187)

The diagnostic value of a microbiological test is influenced by a number of pre-analytical factors (129, 184). Attention must be given to the following:

- Unambiguous diagnostic question
- Precise details of the collection site
- Correct method and time of collection
- Suitable transport systems
- Shortest possible transport times
- Correct storage of the specimens before processing

Various containers for the collection and transport of specimens for microbiological testing and the requirements for such containers are summarised in Fig. 30-1 and Tab. 30-1.

Even the most sophisticated transport system can never be a substitute for short transport times and immediate specimen processing.

Tab. 30-1: Requirements for specimen containers for the transportation of microbiological specimens

1. Sterile
2. Non-corrosive
3. Sufficiently large
4. Secure closure
5. Unbreakable
6. Transport media
without growth enhancers or
with culture media for certain organisms

Bacteria

When collecting specimens for bacteriological testing particular care is to be taken to avoid contamination. Before aspiration the skin must always be meticulously disinfected. Purulent lesions should be aspirated through the skin, if possible, as this is easier to disinfect than the mucous membranes. Liquid material is more suitable for microbiological testing than swab specimens. Aspirates are delivered to the laboratory in the syringe after the needle has been removed and the syringe securely capped. In the case of open wounds, the superficial secretions should be wiped off to remove interfering secondary organisms and a swab specimen then collected from the margins of the wound. Swab specimens must be protected from drying out during transport. This can be done by placing the swab either in a liquid broth or in a transport medium. In the case of low bacterial counts the volume of the specimen should be as large as possible. Specimens for blood cultures if possible should be collected while the fever is rising. If infective endocarditis is suspected up to ten blood cultures must be collected.

Short transport times and variable transport or storage temperatures are important for several reasons. Refrigeration and pH changes of the specimen or exposure to oxygen reduce the survival times of a number of organisms such as meningococci, gonococci, Haemophilus, pneumococci, Bordetella, Salmonella, Shigella, cholera vibrios, Helicobacter pylori and anaerobic organisms. While the viability of these environmentally sensitive organism can decrease rapidly during the transport, others multiply if the transport times are too long. This makes the quantitative evaluation of a culture difficult (urine). However, the sought organism can also become overgrown with other organisms. All specimens for bacteriological testing should therefore be delivered to the laboratory within two hours after collection. The transport and storage requirements for bacteriological testing are summarised in Tab. 30-2. If these conditions cannot be met, inoculation of a culture bottle is recommended or, for urine samples for example, the use of dipslides.

Tab. 30-2: Transport and storage conditions for various specimens for bacteriological testing (29, 129)

Specimen	Transport	Storage temperature
Blood	Blood culture bottle	Room temperature or 37 °C
Abscess material Cerebrospinal fluid Pleural, pericardial, peritoneal, synovial fluid Paranasal sinus secretions	Short transport times: leave specimen in (capped) syringe under anaerobic conditions. Delayed transport: use transport medium	Room temperature, do not incubate, protect from cooling
Bronchoalveolar lavage (BAL) fluid Sputum, other secretions Stool	Prompt transport (2–3 h)	Cool
Urine	Dipslide	Room temperature or 37 °C
Swab specimens from Eyes Ears Mouth Throat Nose Urethra Cervix Rectum Wounds	Swab in transport medium (> 4 h transport time)	Room temperature, do not incubate
Biopsy material	Prompt transport in sterile isotonic saline	Cool

Fungi (130)

To obtain skin specimens for mycological testing, scrapings from the active areas of the lesions, i.e. the edges, are collected with a scalpel after thoroughly disinfecting the skin area. Skin, hair (collected from the edges with epilation pipettes or excision off in the case of deposits on the hair) and nail clippings should be sent to the laboratory dry in sterile containers. Scrapings from the underside of the nail are used for culture. For detection of yeasts in urine, a random urine specimen should be sent to the laboratory promptly in a sterile container. The same applies to the detection of yeasts or moulds in sputum specimens with morning sputum specimens being preferred. Tissue specimens for mycological, as for bacteriological, testing should be placed in isotonic saline and sent to the laboratory as rapidly as possible. For mycological testing of the vagina, the upper respiratory tract or the stool, submission of two swab specimens in sterile containers is recommended. Specimen transport at room temperature is usually uncritical for mycological cultures if the

Tab. 30-3: Specimen collection, processing and transport for parasitological tests (I) = Immediate examination (92)

Specimen material	Specimen type and transport	Parasites (direct and indirect detection)
Parasite itself or components of parasites	Isoton. NaCl (endoparasites) 70% alcohol (ectoparasites)	e.g. Ascaris, proglottides e.g. fleas, lice
Stool for transport	Stool tube For Lawless-stain fix in alcohol sublimate (alc./$HgCl_2$)	Eggs or larvae of intestinal nematodes, cestodes, intestinal trematodes, liver trematodes, lung trematodes. Cysts of protozoa: amoebae, flagellates, ciliates, coccidia, microsporidia. Vegetative forms of protozoa (particularly amoebae, Giardia)
Stool for immediate examination	At room temperature for direct examination (I)	Vegetative forms of protozoa (particularly amoebae, Giardia)
Duodenal fluid	At room temp. for direct examination (I)	Vegetative forms of Giardia
Urine	24-hour urine	Schistosoma haematobium
Blood	Thin film, thick film, heparinised blood	Plasmodia, trypanosomes, microfilariae
Bone marrow	Smear, sterile bone marrow	Leishmania
Sputum	Sputum tube	Paragonimus eggs, larvae of intestinal nematodes, in some cases Echinococcus hooklets
Skin	Skin snip in isoton. NaCl (I) Sterile skin biopsy	Onchocerca (microfilariae) Leishmania
Recovery of eggs or adults from perianal skin	Cellulose tape technique	Pinworms

transport times are short. In the case of long transport distances refrigeration of the specimens (not necessary for swab specimens) is recommended in order to prevent overgrowth of the slowly growing fungi with bacteria. If a phycomycete infection is suspected (e.g. Mucor) rapid specimen transport without refrigeration is necessary.

Parasites (92)

Specimens examined for the diagnosis of parasitic infections are blood (plasmodia, trypanosomes, leishmaniae, microfilariae, Loa loa), stool (Giardia, ciliates, helminths, cestodes), tissue specimens of the affected organs (Trichinella spiralis larvae, Echinococcus) or the parasites themselves (arthropods: ticks, mites, insects). In the case of stool specimens it should be noted that the vegetative stages are unstable. These stages can only be detected by examination of fresh, body-warm stool. Cysts are stable. MIF (merthiolate-iodine-formalin) and SAF solution (sodium acetate – formalin) have become routine for parasite concentration and preservation in stool specimens. For most specimens transport is uncritical and special transport conditions need not be observed.

Refrigeration of the specimens is rarely ever necessary. Arthropods are sent to the laboratory in 70% alcohol. Tab. 30-3 gives a brief overview of preanalytical factors important for parasitological testing.

A fundamental prerequisite for a parasitological examination is a travel history (place, time and duration of visit) and information on onset of symptoms, treatment and whether or not the patient is immunosuppressed. A request stating "test for parasites" is inadequate.

Viruses

The time of specimen collection for virus isolation and identification is critical. Usually the material should be collected immediately after the onset of symptoms (if possible within the first three days). As a general rule, specimens should be delivered to the laboratory rapidly at 4°C in an insulated container. Viruses usually remain stable for 2 to 3 days under these conditions (130). Swab specimens (nose, throat, eyes), pharyngeal washings, vesicular fluid from skin lesions, stool, urine and CSF are used for analysis.

Specimens for microbiological studies which do not meet certain standards (see Tab. 30-4) should either be rejected or only analysed after consultation with the requester.

Tab. 30-4: Cases in which the specimen should only be processed after consultation with the requester (129)

1.	No label/identification
2.	Abnormally long transport time
3.	Unsuitable or leaking containers
4.	Unsuitable specimen
5.	Submission of the same specimen material with the same question or request within 24 hours (except blood samples)

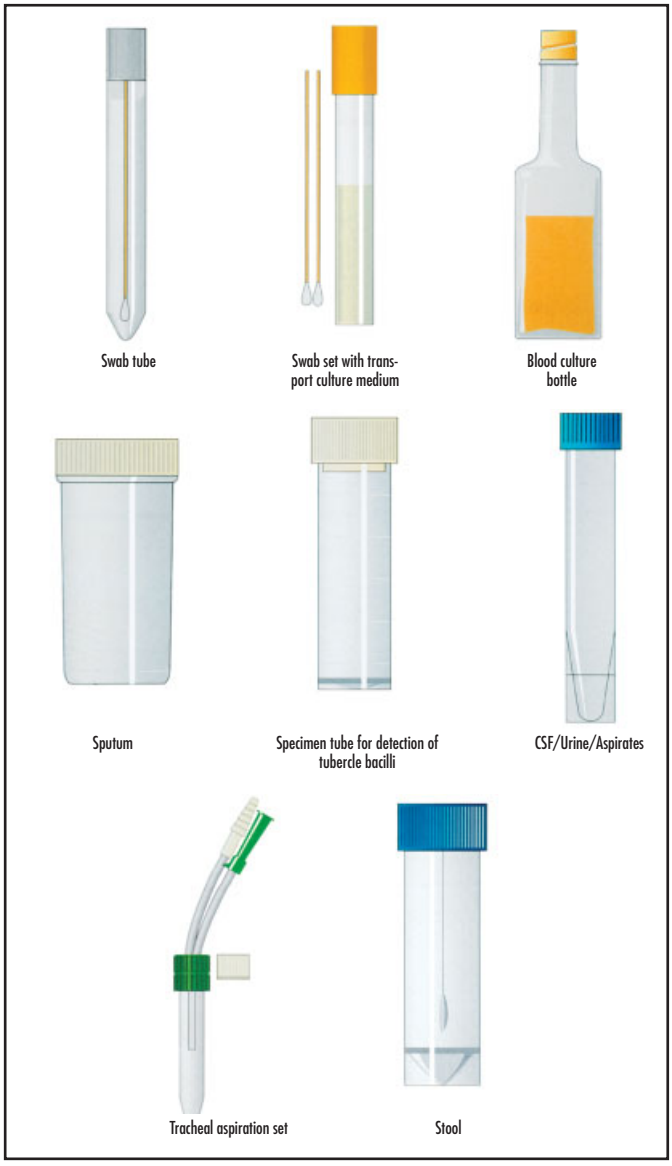


Fig. 30-1
Containers for transport of specimens for microbiological studies

Can turbid samples be used?

The lipemic sample

Plasma and serum samples are sometimes turbid to varying degrees due to an increased lipoprotein content (Fig. 31-1). In nearly all cases, turbidity is caused by an increased triglyceride concentration. The turbidity may be slight, often called opaque, translucent, turbid or milky. The degree of turbidity depends not only on the amount of triglycerides but also more markedly on the presence of macromolecular species of lipoproteins. Turbid samples are therefore called lipemic.

Fig. 31-1
Plasma samples with different degrees of turbidity

The diagnostic importance of turbidity

Because normal samples do not exhibit any turbidity except after a fatty meal, the turbidity of a sample is always of clinical relevance and should be assessed, documented and reported by the laboratory (73 and Annex). It may indicate hypertriglyceridemia due to an increase in chylomicrons, very low density lipoproteins (VLDL) or both. As described in textbooks, these forms can be differentiated by observing the floating of lipoproteins during centrifugation and storage (31, 66, 213). A distinct creamy layer floating over a clean layer after centrifugation on storage over at least 12 hours in the refrigerator indicates the presence of chylomicrons. In contrast, a more homogeneous turbidity is in most cases caused by the presence of increased concentrations of VLDL.

The concentration of triglycerides leading to turbidity depends on the composition of lipoproteins. Chylomicrons, due to their size, deflect light at detectable rates even at triglyceride concentrations below 300 mg/dL (3.4 mmol/L). However, the intermediate and low density lipoproteins can be invisible even at triglyceride concentration of 800 mg/dL, or higher. Varying degrees of turbidity are observed with VLDL, depending on their size and composition (10, 60).

Relevance of turbidity as an interference factor

Whereas the degree of hyperlipidemia is of diagnostic relevance, the interference of lipoproteins with the determination of lipids and other blood constituents should be regarded as disturbing interference factors which should be avoided as far as possible.

Mechanisms of interference

The following mechanisms have been found to cause either falsely low or high laboratory results:

- *Inhomogeneity:* Triglyceride-rich lipoproteins cause them to float during centrifugation and storage of serum/plasma samples. When analyzed after such treatment (centrifugation) without careful mixing, triglycerides and other constituents may be inhomogeneously distributed in the sample. This may cause a disproportionately high concentration of lipids in the upper layer and cause interference in other methods like total protein. On the other hand, lipids may displace water in the upper phase of a

ISBN: 978-3-527-30981-8

sample thereby leading to a lower apparent concentration of water-soluble components like electrolytes and metabolites.

● *Water displacement* is also responsible for the higher concentration of sodium and potassium observed in direct ion-sensitive electrode measurement compared to flame photometry (110). In exceptional cases, lipids can displace up to 10 % of the water content of a serum/plasma sample.

● *Interference by turbidity:* Photometric procedures are sensitive to turbidity at nearly all wavelengths (65). This leads to various degrees of absorption (Fig. 31-2).

● *Interference by physicochemical mechanisms:* Lipoproteins in the sample may incorporate lipophilic constituents, thereby decreasing their accessibility to antibodies. Likewise, electrophoretic and chromatographic procedures may be disturbed by lipoproteins.

"Diagnosing" and "treatment" of interference due to turbidity

Relevant turbidity can easily be detected with the naked eye. Alternatively, the turbidity of each sample can be measured by automatic analyzers using a specific wavelength (660–700 nm) (65). The degree of interference of each method can be quantified by adding different amounts of sample from a hyperlipemic patient to a clear sample subsequent to analyzing the concentration of both samples separately. When a test is known to be disturbed by any of the mechanisms mentioned, triglycerides may either be removed from the sample by ultracentrifugation (14) or precipitation (114)

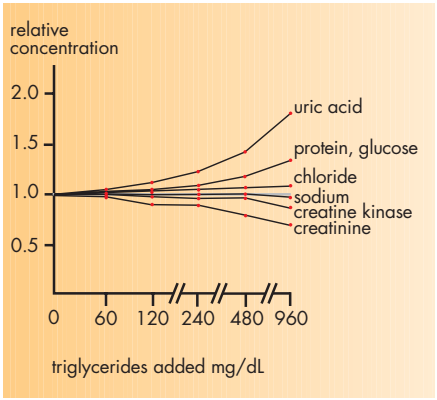


Fig. 31-2
Method – dependent inferenence of various analyte determinatins by increased triglycerides (65)

and the analysis repeated with the clarified sample.

Care has to be taken regarding the clarification procedure itself as a possible cause of interference.

In some cases a change in methodology may be helpful in eliminating interference due to lipids. Thus, a second wavelength may compensate for turbidity. Alternatively, a blank sample may be run without the relevant reactant but under otherwise identical conditions. In each case the degree and type of turbidity should be documented and reported and an aliquot of the untreated sample stored for later testing for verification purposes.

Procedures for treating lipemic samples should be documented in the quality assurance manuals of each laboratory. Producers of test kits should indicate that interference by lipemic samples has been tested for and provide respective information in the product leaflet (73 and Annex).

Cold agglutinins

Antibodies as interference factors in clinical chemistry are often neglected since detection of this factor is difficult under day to day routine conditions. Analytical procedures in clinical chemistry, haematology and immunohaematology can be affected by antibodies (107). Antibodies may affect the cell count of erythrocytes, leukocytes and platelets (234). High titers of cold agglutinins directed against erythrocytes lead to agglutination. Such agglutination alters the electronic cell count in the following way: erythrocyte count is low at normal haemoglobin concentrations; MCV is grossly enhanced (Fig. 32-1); calculated haematocrit values are low, resulting in high MCH and MCHC values. The leukocyte and thrombocyte counts are falsely elevated, because the agglutinates depending on their size are either counted in the leukocyte or thrombocyte channel. The blood smear shows agglutination of erythrocytes. Both the determination of blood group and crossmatching procedures can be affected by cold agglutination in two ways. First, panagglutination introduced by antibodies can influence the correct assignment of blood group antigens and the crossmatching procedure. Second, the cold agglutination antibodies may mask other types of antibodies which may affect analytical procedures in a different manner.

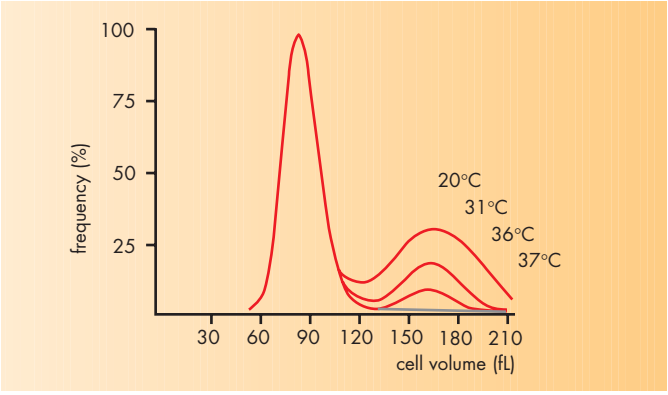


Fig. 32-1
"MCV"-determination of blood in cold agglutinin disease at different temperatures (15)

Cryoglobulins

Cryoglobulins crystallize in samples kept at room temperature. The resulting particles are of varying shape and may mimic leukocytes, resulting in a falsely elevated leukocyte count (Fig. 32-2). Moreover, high concentrations of cryoglobulins can affect erythrocyte count, haemoglobin determination (flocculation phenomenon) and platelet count (pseudothrombocytosis) (Fig. 32-2). The blood smear shows flocculated dark blue crystals without elevated leukocyte numbers. The phenomenon of pseudoleukocytosis depends on the length of contact, temperature, concentration of cryoglobulins and the interaction of cryoglobulins with other plasma proteins (1). This is valid for all cell number determinations by particle counting.

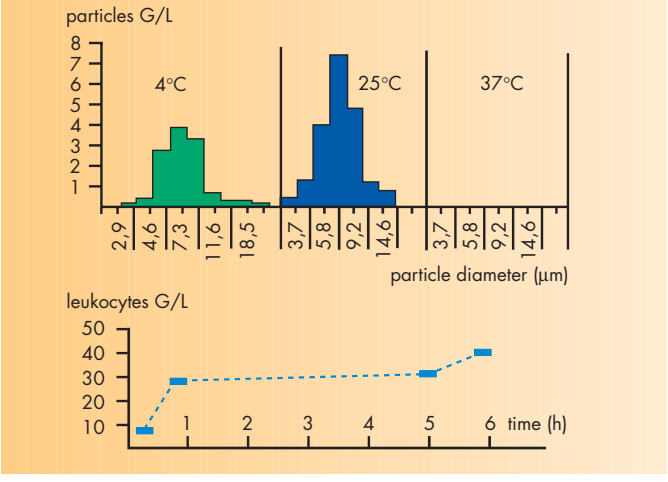


Fig. 32-2
Distribution of cryoglobulin particles at different temperatures and the corresponding increase in leukocyte count at different storage times at room temperature (1)

EDTA-dependent antibodies

Antibody-induced falsely decreased platelet counts (without haemorrhagic diathesis) can be due to cold agglutinins or antibodies active in presence

of EDTA. In both cases, agglutination takes some time. Thus, a prolonged delay between obtaining the sample and platelet counting results in a more pronounced pseudothrombocytopenia. Platelets of patients with thrombasthenia, which lack the membrane glycoproteins IIb and IIIA, do not react with EDTA-dependent antibodies. This observation suggests that these glycoproteins are actively involved in binding the antibody. Depending on their shape and volume, thrombocyte aggregates may be counted as leukocytes. In addition to pseudothrombocytopenia elevated cell counts for leukocytes may be observed. Detection of particles of the size of lymphocytes in the white cell histogram is evidence of a spurious count of leukocytes. Staining of peripheral blood allows detection of aggregates of platelets. Other causes of falsely decreased thrombocyte counts are platelets adhering to leukocytes (platelet satellism), giant platelets, or analysis of partly coagulated blood samples caused by a wrong sampling technique.

"Macroenzymes"

The possibility of complexes with immunoglobulins (macroenzymes) has been demonstrated for all diagnostically relevant enzymes. A consequence of such phenomena is an increased biological half-life of such enzymes. The increased half-life may in turn result in enhanced enzyme activity which can provoke further diagnostic measures. The phenomenon of macroenzymes is primarily observed in elderly patients with chronic diseases. Well-described examples are macro creatine kinase (CK) type I and type II. Macro CK type I is an immunoglobulin CK-BB complex. Type II represents polymers of mitochondrial CK, which can be detected by electrophoresis. Both types of macro CK may affect accurate quantification of CK-MB by means of CK-M-inhibiting antibodies resulting in falsely elevated CK-MB activities. Another example is macroamylase which is characterized by enhanced activity in serum while urinary amylase excretion is unchanged (209).

Autoantibodies

Immunoassays can be affected by autoantibodies or heterophilic antibodies (21). Well-described examples are autoantibodies directed against triiodothyronine and thyroxine. Thyroid hormone concentrations are apparently enhanced since the tracer is bound not only to the receptor antibody added to the sample but also to the autoantibody. Antiphospholipid antibodies in plasma results in increased APTT values because the antibody binds phospholipids used as reagent in the assay.

Heterophilic antibodies

Heterophilic antibodies are detected in some human serum samples, the mechanism underlying generation of these antibodies being unknown.
In some cases, interference by heterophilic antibodies can be of diagnostic significance. If antibodies have anti-mouse specificity and assays employing immunoantibodies from mice are used (murine monoclonal antibodies), interference of these assays is possible. There are several reports in the literature describing wrong therapeutic measures as a consequence of such antibody induced analytical errors (21). In this case monoclonal antibodies caused the interference. However, antibodies have been described, which are unspecific and interfere as well. Only the latter are classified as heterophilic antibodies in the narrow sense (116).

The serum sample looks reddish

Blood consists of cells and plasma. Many constituents measured in plasma have relatively high concentrations in blood cells (233). Therefore, haemolysis should be avoided to obtain reliable results.

What is haemolysis?

The absence of red colour does not, however, exclude interference by haemolysis, because haemoglobin is seen with the naked eye only at a level approximating 300 mg/L and higher.

Haemolysis has been defined as the "release of blood cell constituents into plasma/serum". It is usually recognized by a more or less reddish appearance of the plasma/serum after centrifugation (Fig. 33-1), caused by haemoglobin released from the erythrocytes. As such, interference can occur even at lower concentrations of haemoglobin otherwise invisible to the naked eye.

Fig. 33-1
Plasma samples with various degrees of haemolysis

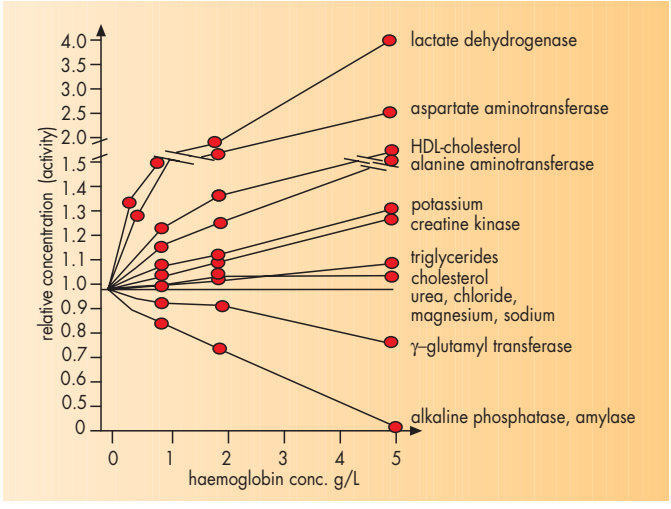


Fig. 33-2
Changes in various analytes with increasing haemolysis in a dual wavelength routine analyzer

Haemolysis is not always accompanied by the release of haemoglobin (for instance, if blood is stored at low, but non-freezing temperature). Interferents may also arise from both platelet lysis and granulocyte lysis.

Mechanisms of interference (73 and Annex)

The effects of haemolysis may be classified according to the mechanisms involved:

● *Increase of intracellular constituents in the extra-cellular fluid.* The efflux of intracellular constituents may occur in-vivo, during sampling and at all stages of the preanalytical phase. Accordingly, haemolysis may be a diagnostically relevant observation, defined as an in-vitro influence factor when occurring during sampling or other steps of the preanalytical phase as it leads to alteration of the sample composition. Fig. 33-2 shows the effect of increasing haemolysis on various serum analytes.

● *Optical interference* may be due to the colour of the haemoglobin, which may change during sample storage due to haemoglobin formation. The direction and degree of interference differs not only with the wavelength(s) but also with the type of blank and reagent used. Recently therapeutically applied artificial oxygen carriers based on haemoglobin structure (HbOC) have been introduced (32). This when applied in concentrations up to 50 g haemoglobin/L create optical interferences nearly indistinguishable from those caused by natural haemoglobin.

● *Interference by intracellular constituents with the reaction mechanism of the assay* (chemical, biochemical and immunological interference). In this

case, a method-dependent interference is observed which is not due to optical interference by haemoglobin. Thus, adenylate kinase released from blood cells interferes with most standard methods for the measurement of creatine kinase activity, the interference being dependent on the concentration of the inhibitors of adenylate kinase added to the reagent mixture (210). Fig. 33-3 illustrates the method-dependence of haemoglobin interference with various diazo methods for bilirubin, caused by the peroxidative effect of the haeme (224).

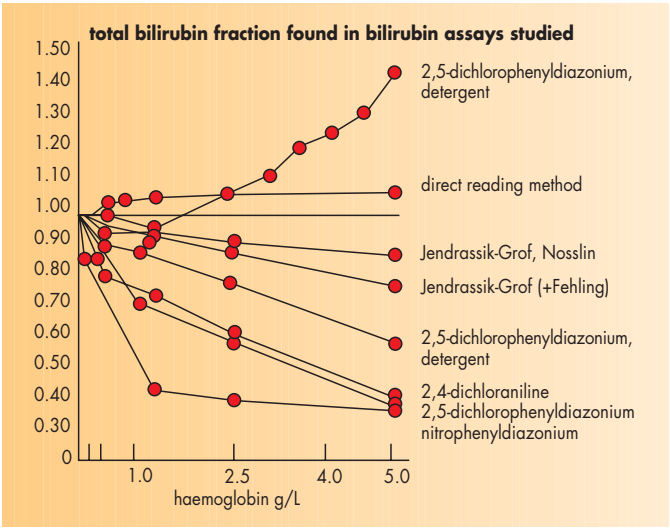


Fig. 33-3
Interference of haemoglobin with various diazo methods to measure bilirubin (from (224))

How to "diagnose", prevent and "treat" interference brought about by haemolysis (73 and Annex)

Overt haemolysis is easily detected when the sample is visually controlled before being analyzed. In-vivo and in-vitro haemolysis may be differentiated by comparing various samples of the same patient and by analysis of sensitive markers of in-vivo haemolysis such as haptoglobin and consideration of clinical information. Consulting the clinician is advisable for any suspected in-vivo haemolysis. In addition any unexpected increase in "sensitive" analytes should be regarded as arising from in-vitro haemolysis unless this can be excluded. Free haemoglobin, lactate dehydrogenase (LDH) activity and potassium should increase in parallel in this case.

Once diagnosed, results obtained from a haemolytic sample should be withheld (or sample not measured) if interference is to be expected. If a new sample cannot be obtained, the clinician should receive information about the possible degree of interference along with the result. A correction formula as suggested by Caraway (33) can be applied only if in-vitro haemolysis with parallel release of all constituents can be ascertained. Evaluation of the possible cause of haemolysis will certainly help to prevent interference.

In-vitro haemolysis can be prevented by standardizing the preanalytical phase. Use of standardized needles, closed tubes and calibrated centrifuges is of great help in reducing haemolysis. The use of plasma instead of serum can also minimize haemolysis, especially by avoiding the release of cellular constituents from platelets (121). Fig. 13-2 (see p. 32) shows how differences between serum and plasma potassium concentrations depend on the number of platelets.

The laboratory should be aware of the effects of haemolysis on specific tests (201). The user should expect new reagents and kits to be tested for the effect of haemolysis by the manufacturer and respective information given in the product application manual.

Every laboratory should document how haemolyzed samples are to be handled in their quality assurance manual. The responsibility of the laboratory for diagnostically reliable results can only be fulfilled by taking rigorous measures to prevent misinterpretation of results caused by haemolysis (73 and Annex).

Mechanisms of drug interference

Interference with laboratory tests by drugs is so widespread due to the multiplicity of drugs and laboratory procedures that a computerized directory is the best source of information available on the subject. In the main, however, drug interference on laboratory tests can be broadly categorized as being either biological or chemical. A pharmacological effect arises as a consequence of the drug being metabolized in the body and the metabolite subsequently interfering with the laboratory test. Thus, while the parent drug propranolol does not interfere with the bilirubin methods of both Jendrassik-Grof and Evelyn Malloy, the metabolite 4-hydroxy propranolol interferes with the bilirubin measurement by both the above methods (208).

Another pharmacological effect relates to the ability of the drug to increase the level of binding proteins. This can result in an increase in the level of analytes bound to such proteins. For instance, oral contraceptives increase the plasma concentration of thyroxine-binding globulin, ceruloplasmin, transferrin and transcortin, thus increasing the level of analytes bound (thyroxine, copper, iron and cortisol) (241).

Drug interference that is classified as technical, on the other hand, relates to in-vitro interference which could be either chemical or physical, such as

Tab. 34-1 Drug effects and interference; mechanisms and analyte changes

Category	Mechanism	Example of drug	Analytes	Change in plasma
Biological influence in vivo	Enzyme induction	phenytoin	γ-glutamyl transferase	↗
	Enzyme inhibition in liver	allopurinol	uric acid	↘
	Enzyme inhibition in plasma	cyclophosphamid	cholinesterase	↘
	Increased binding protein	oral contraceptives	copper (ceruloplasmin)	↗
	Competing with endogeneous component for glucuronidation	novobiocin	bilirubin, unconjugated	↗
	Antivitamin effect	warfarin, phenprocoumon	protein C prothrombin	↘ ↘
	Cytotoxicity liver	biguanides	lactate,	↗
	kidney	gentamicin	alanine aminotransferase	↗
		cis-platinum	creatinine	↗
Chemical and physical interference in vitro	Cross-reactivity in immunoassays	spironolactone	digoxin	apparrent ↗
	Chemical reaction with Jaffe' reagent	cephalotin	creatinine	↗
	Production of atypical haemoglobins	salicylates	haemoglobin A_1	↗

haemolyzed or icteric samples etc. (141). Tab. 34-1 lists some of the other types of interference by the more common drugs on laboratory tests (98, 123, 141, 145, 241).

Binding of drugs to protein

The binding of drugs to proteins can be altered due either to the presence of other drugs competing for the same binding site on protein or due to an increased level of fatty acids (141). In general, drugs that are weakly bound tend to be displaced from their protein binding sites by a competing drug or fatty acid. Thus, free drug levels can be increased under such circumstances. Furthermore, unless the displaced drug is rapidly metabolized, the patient would become toxic at the therapeutic level of dosage.

Low albumin levels found in patients with liver and kidney disease affect protein binding. In such a case when multiple drugs are being administered, competition for binding sites on albumin can be significant. For instance, when valproic acid is co-administered with phenytoin, the competition for protein binding sites can lead to the displacement of phenytoin by valproic acid leading to decreased total phenytoin levels, since the displaced phenytoin is rapidly transformed into an inactive metabolite (136).

The protein binding capacity of a particular drug can be dramatically altered in conditions such as in uremia where for instance the protein binding of phenytoin can vary from 70% to near 0%.

The broad spectrum of drug interference with laboratory tests has been compiled in several reviews and books (123, 179, 218, 241). When using these lists, the method-dependence of many of the effects described has to be kept in mind. As with other types of interference, comparison of results obtained using two independent methods may indicate the mechanism of interference. Consulting with a clinician is advised in order to prevent misinterpretation due to drug interference.

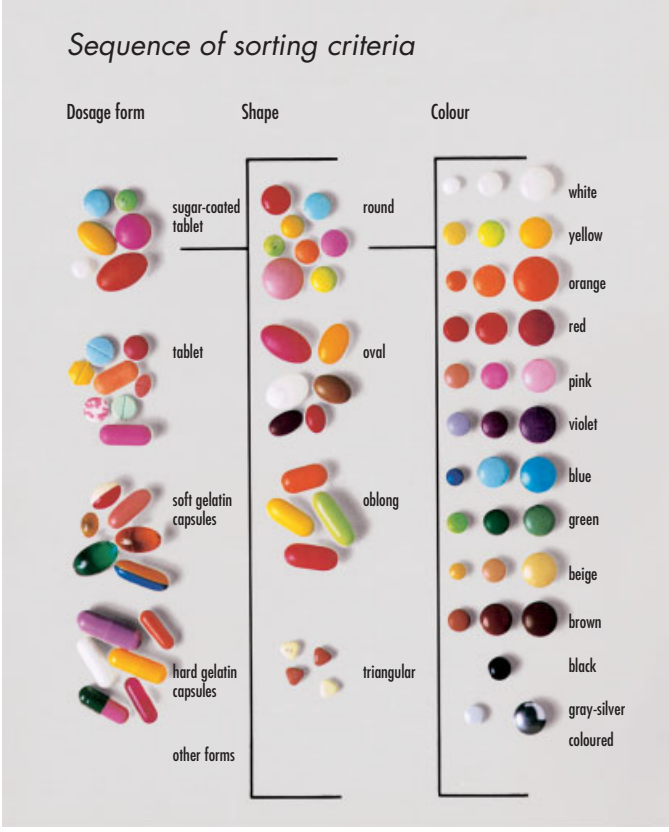


Fig. 34-1
Colour chart of drugs used to characterize and identify drugs found

Usually, the quality of a laboratory test result is assessed by defining the imprecision and accuracy in comparison to quality standards. These standards are either obtained from experts, formulated through one's own experiences or defined by external quality control agencies (46, 221). No such standards exist for defining the quality of the preanalytical phase. From present knowledge however, several criteria may be derived which may be taken as quality criteria in each individual laboratory.

Defining quality

The aim of analyzing samples taken from patients is to obtain a result which describes the condition of the patient as represented by the analyte concentration in blood or other body fluids. This result helps the doctor to arrive at a diagnosis, providing it reaches him before he has to make a decision which may well be influenced by the result. Thus, the adequateness of the request, the type of sample and the timing has to be defined in relation to the individual needs in any given clinical situation. In contrast to the analytical result, which is often defined as a "product", the preanalytical phase may be defined as a mixture of processes and materials. Tab. 35-1 summarizes numerous examples of materials and procedures (processes) used during the preanalytical phase.

Who defines quality?

Complaints, suggestions and proposals from persons involved are the major sources of information upon which a quality assurance program for the preanalytical phase can be initiated. The quality of the products and processes have to be defined in relation to the medical needs. This can be done by a group of experts in a "quality circle" or by an external audit. Local, national or international standards and recommendations may also be used. The results of these activities are published in the preliminary quality manual, which, after evaluation, can be used as the basis for future quality assessment schemes. The

Tab. 35-1 Preanalytical processes and materials that can be subjected to quality assurance

Step	Process	Materials
Patient preparation	Information on diet, posture and sampling procedures	Urine containers
Preparation of sampling	Defining request, entering request, labeling tube	Request form, request program, patient and sample identification system
Sampling	Identification of patient, timing, tourniquet, cleaning site of sampling, selection of vein, artery or capillary sampling site, positioning of needle, changing tubes	Needles, tubes, disinfectant
Transport	Collecting samples, transporting samples	Sample containers, pneumatic tube system, cooling systems.
Sample treatment	Registration, centrifugation, distribution, mixing, identification, extraction	Identification and registration program
Storage	Timing of storage, selection of site and temperature, finishing storage, remixing after storage	Storage device, freezing device, temperature control

results of this evaluation and the objectives of the quality assurance program should be defined in a quality manual.

Quality of the sample

The adequateness of a sample should be addressed from the standpoint of patient, doctor and the laboratory.

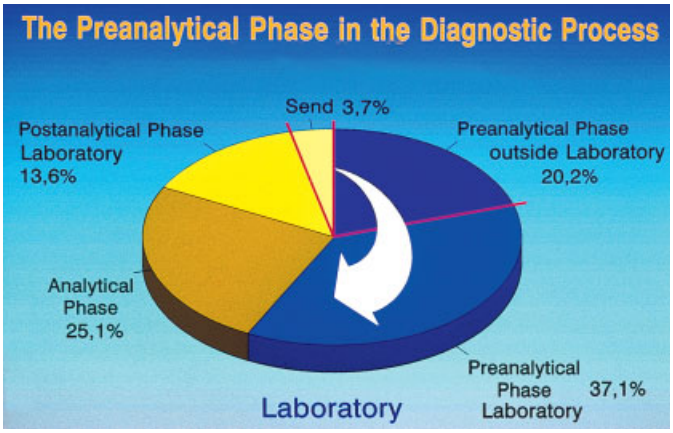
Patient's criteria: Painless sampling is preferable to painful sampling. Less blood is better than excessive blood collection. Recently a formula was recommended defining the optimal volume of sample considering technical and biological variables. If these rules are followed, the minimal amount of sample can be assured for the analysis ordered (see (71) and Annex).
A rapid procedure of sampling is better than a drawn out procedure (i.e. spot or random urine sample versus 24 h urine except where it is absolutely necessary).

Doctor's criteria: More information from one sample is preferable. Rapid sampling is preferred to time-consuming sampling. A rapid test is preferable to a slow one. The ideal sampling procedure is one presenting a low degree of risk to both patient and phlebotomist.

Laboratory's criteria: It is better to have more sample than is needed rather than an insufficient amount. An adequate amount has to be defined. A normal sample is preferred to a sample with potentially interfering (haemolytic, lipemic) or risky (infectious) factors. Standard sample size and anticoagulant to blood ratio is preferred to non-standardized samples.

Quality of timing

The preanalytical phase takes more time than the analytical phase. Therefore, it's timing is of critical importance for the entire diagnostic procedure.



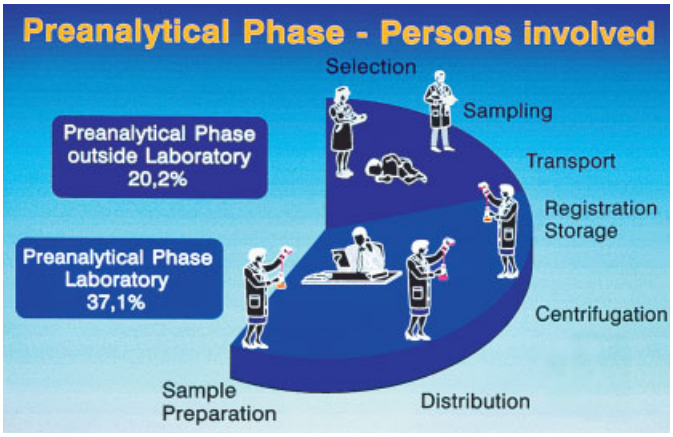


Fig. 35-1a ▲▲ ***Relative contribution of the preanalytical phase to the total turn-around time of a diagnostic test***

Fig. 35-1b ▲ ***Persons involved in the preanalytical phase***

Fig. 35-1a gives an example of the prelaboratory and intralaboratory preanalytical times analyzed (62, 70). Fig. 35-1b shows that many different persons are involved; these have to be considered when quality is to be improved.

How can timing be documented and controlled?

In order to clearly document the timing of laboratory testing, three essential times need to be monitored in assessing quality:
1. Time of sampling

2. Time of arrival of sample in the laboratory
3. Time of printing of result

The difference between time 1 and 2 gives the prelaboratory preanalytical time; the time interval between 2 and 3 provides the intralaboratory preanalytical plus analytical and postanalytical time. Further documentation of the various phases of the preanalytical time is possible if analytical time is subtracted. By doing this, preanalytical phase was shown to be responsible for more than 50% of the total turn-around time in most laboratories. Fig. 35-2 gives an example of the documentation of preanalytical times in a laboratory information system.

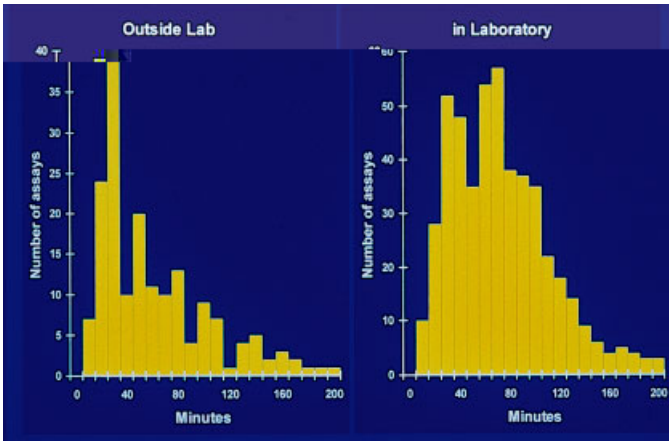


Fig. 35-2
Documentation of preanalytical times during a usual working day

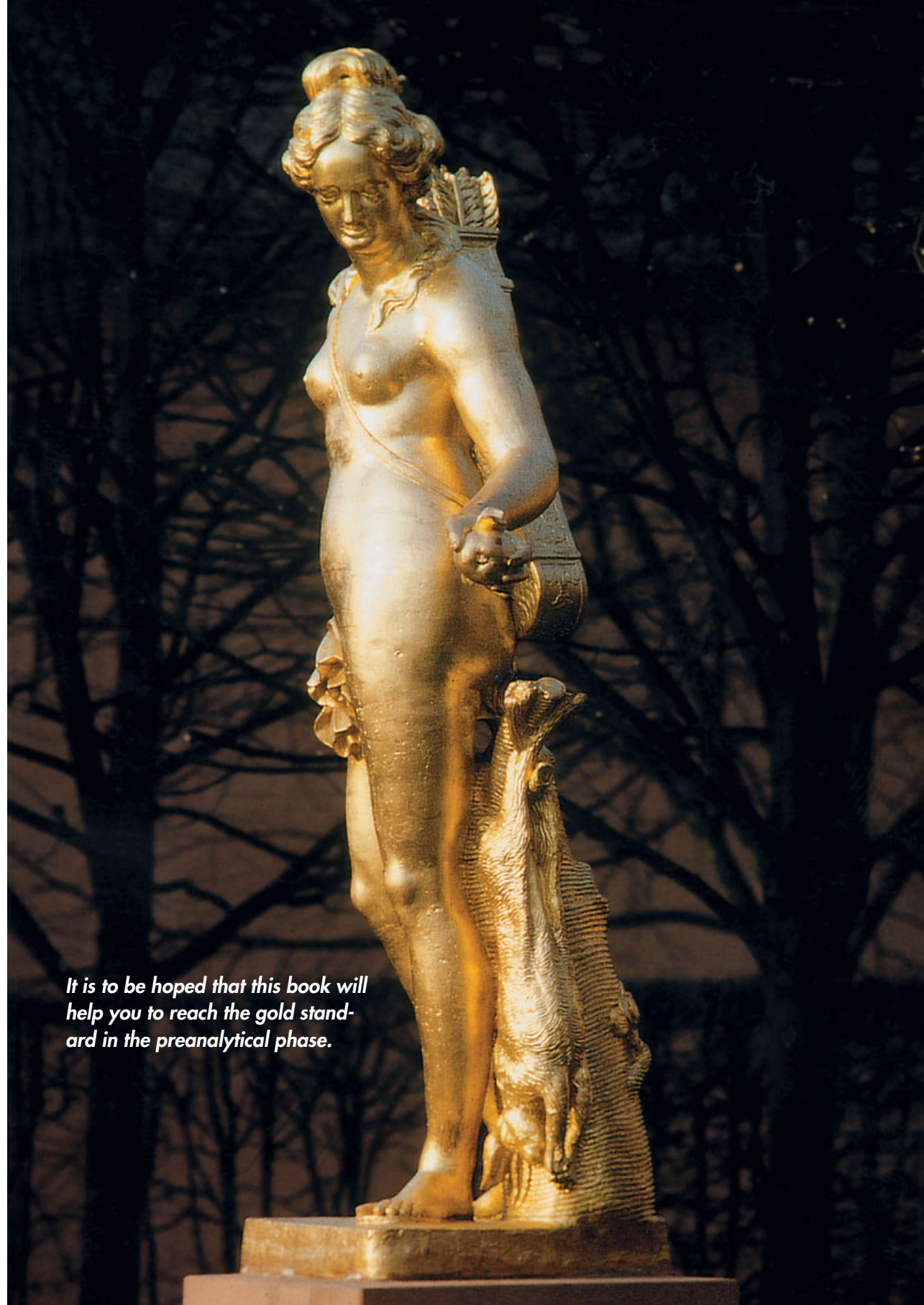
The quality journal of the preanalytical phase

Procedures and standards used in the individual laboratory should be documented in a quality manual accessible to all employees as well as visitors and external quality managers. Tab. 35-2 gives an example of the possible table of contents of such a quality manual. According to the ISO standard on quality management of medical laboratories the quality manual has to contain a detailed description on responsibilities, procedures and aims of several preanalytical aspects of the laboratory, including consequences in cases of non compliance. This list also includes procedures on requests, sampling and handling details as well as regulations for transport to other laboratories (90).

A Working Group on Preanalytical Quality has published recommendations which can serve as a basis to define quality standards (71, 72, Annex).

Tab. 35-2 Contents of the quality manual for the preanalytical phase

1. Request procedure
a. Forms
b. Patient identification
2. Sampling
Materials
a. Needles
b. Tubes
c. Containers for non-blood samples
Procedures
a. Venous blood
b. Capillary blood
c. Arterial blood
d. Timed urine
e. Spot urine
f. Cerebrospinal fluid
g. Sputum
h. Ascites and pleural fluid
i. Other samples
3. Transport
4. Registration
5. Centrifugation
6. Sample identification
7. Storage
8. Handling of interference
a. Haemolysis
b. Lipemia
c. Icterus
d. Drugs and containments
9. Disposal of samples
10. Timing of the preanalytical phase
11. Documentation
12. Responsibilities



It is to be hoped that this book will help you to reach the gold standard in the preanalytical phase.

References

1. Abela M, Mc Ardle B, Qureshi M. Pseudoleucocytosis due to cryoglobulinaemia. J Clin Pathol 1980; 33: 796.
2. Adcock DM, Kressin DC, Marlar RA. Effect of 3.2 % vs 3.8 % sodium citrate concentration on routine coagulation testing. Am J Clin Pathol 1997; 107: 105-10.
3. Albert KGMM, Zimmet BZ for the WHO Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med 1998; 15: 539-53.
4. Alexander S. Physiologic and biochemical effects of exercise. Clin Biochem 1984; 17: 126 – 31.
5. American Association of Blood Banks. Pretransfusion testing. In: Walker RH, editor. Technical Manual of the American Association of Blood Banks. Arlington: 1990: 269 – 92.
6. American Diabetes Association. The expert committee on the diagnosis and classification of diabetes mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997; 20: 1183-97.
7. Anonymus, Results of the project "Painreduced blood sampling" and development of the softclix® device, 1994. Boehringer Mannheim GmbH.
8. Assmann G. Lipidstoffwechsel und Arteriosklerose. Stuttgart: Schattauer Verlag, 1982.
9. Astles R, Williams Ch P, Sedor F. Stability of plasma lactate in vitro in the presence of antiglycolytic agents. Clin Chem 1994; 40: 1327 – 30.
10. Aufenanger J, Zawta B. Pre-analytical aspects of lipoprotein measurement. Clin Lab 1999; 45: 535 – 46.
11. Bain B, Seed M, Godsland I. Normal values of peripheral blood white cell counts in women of four different ethnic groups. J Clin Pathol 1984; 37: 188 – 93.
12. Banfi G. Preanalytical variables in immunoassays in "4th expert symposium biological and technical aspects of the preanalytical phase in clinical chemistry and laboratory medicine". Karlovy Vary, Czech Republic, 1998, Becton Dickinson VACUTAINER® Systems.
13. Berg B, Estborn B, Tryding N. Stability of serum and blood constituents during mail transport. Scand J Clin Lab 1981; 41: 425 – 30.
14. Bermes EW, Young DS. Specimen collection and processing; sources of biological variation. In: Tietz NW. Fundamentals of Clinical Chemistry, Philadelphia: WB Saunders Company, 4th ed. 1996: 33 – 52.
15. Bessman JD, Banks D. Spurious macrocytosis, a common clue to erythrocyte cold agglutinins. Am J Clin Pathol 1980; 74: 797 – 800.
16. Beutler E, Gelbart T, Kuhl W. Interference of heparin with the polymerase chain reaction. Bio Techniques 1990; 9: 166.
17. Bode JC. Die internistischen Folgeerkrankungen des Alkoholismus. In: Kisker KP, Lauter H, Meyer J-E, Müller C, Strömgren E, Hrsg. Psychiatrie der Gegenwart. Abhängigkeit und Sucht. Berlin. Heidelberg. New York: Springer, 3rd ed. 1987: 206 – 41.
18. Bonini PA, Alpert N, Luzzana M, Rubin R. Guidelines for the identification and distribution of patient samples in the medical laboratory. J Autom Chem 1994; 16: 25 – 32.
19. Boomsma F, Alberts G, van Eijk L, Man in't Feld AJ, Schalekamp ADH. Optimal collection and storage conditions for catecholamine measurement in human plasma and urine. Clin Chem 1993; 39: 2503 – 8.
20. Boone DJ, Steindel StD, Herron R, Howanitz PJ, Bachner P, Meier F et al. Transfusion medicine monitoring practices. Arch Pathol Lab Med 1995; 119: 999–1006.
21. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988; 34: 27 – 33.
22. Bowman HS. Red cell preservation in citrate-phosphate-dextrose and in acid-citrate dextrose. Transfusion 1963; 3: 364 – 7.
23. Boyum A. Separation of lymphocytes from blood and bone marrow. Scand J Clin Lab Invest 1968; 21 Suppl 97: 77 – 106.
24. Braune HJ, Huffmann G. A prospective double-blind clinical trial, comparing the sharp Quincke needle (22 G) with an "atraumatic" needle (22 G) in the induction of post-lumbar puncture headache. Acta Neurol Scand 1992; 86: 50 – 4.
25. Bürgi W, Richterich R, Mittelholzer ML. Der Einfluss der Enteiweißung auf die Resultate von Serum- und Plasma-Analysen. Klin Wschr 1967; 45: 83 – 6.

ISBN: 978-3-527-30981-8

26. Büttner J. Unspecificity and interference in analytical systems: concepts and theoretical aspects. Klin Chem Mitt 1991; 22: 3 – 12.
27. Buffone GJ, Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. Clin Chem 1985; 31: 164 – 5.
28. Bundschuh G, Schneeweiss B, Bräuer H. Lexikon der Immunologie. München: Medical Service. 2. Auflage, 1992.
29. Burkhardt F, Schaal KP. Gewinnung und Verarbeitung von Untersuchungsmaterial – Bewertung von Befunden. In: Burkhardt F, Herausgeber. Mikrobiologische Diagnostik. Stuttgart, New York: Thieme 1992: 15– 49.
30. Burnett RW, Covington AK, Fogh-Andersen N, Külpmann WR, Maas AHJ, Müller-Plathe O, et al. Approved IFCC recommendations on whole blood sampling, transport and storage for simultaneous determination of pH, blood gases, and electrolytes. Eur J Clin Chem Clin Biochem 1995; 33: 247 – 53.
31. Burtis CA, Ashwood ER. Tietz Textbook of Clinical Chemistry. Philadelphia: Saunders, 1998, 3rd Edition.
32. Callas DD, Clark TL, Moreira PL, Lansden C, Gawryl MS, Kahn S, et al. In vitro effects of a novel hemoglobin-based oxygen carrier on routine chemistry, therapeutic drug, coagulation, hematology, and blood bank assays. Clin Chem 1997; 43: 1744– 8.
33. Caraway WT. Chemical and diagnostic specifity of laboratory tests. Am J Clin Path 1962; 37: 445 – 64.
34. Carter PH, Resto-Ruiz S, Washington GC, Ethridge S, Palini A, Vogt R, et al. Flow cytometric analysis of whole blood lysis, three anticoagulants, and five cell preparations. Cytometry 1992; 13: 68–74.
34a. Chan AYW, Swaminathan R, Cockram CS. Effectiveness of sodium fluoride as a preservative of glucose in blood. Clin Chem 1989; 35: 315 – 7.
35. Ciba Geigy AG. Wissenschaftliche Tabellen. Teilband Hämatologie und Humangenetik. Basel: Ciba Geigy AG, 8th ed. 1979.
36. Coad JE, Lander TA, Litz CE. Inhibition of restriction endonucleases by common clinical anticoagulants. Anal Biochem 1992; 205: 368 – 9.
37. Cohn JS, McNamara JR, Cohn SD, Ordovas JM, Schaefer EJ. Postprandial plasma lipoprotein change in human subjects of different ages. J Lipid Res 1988; 29: 469 – 79.
38. Contant G, Gouault-Heilmann M, Martinoli JL. Heparin inactivation during blood storage: Its prevention by blood collection in citric acid, theophylline, adenosine, dipyridamole – C.T.A.D. mixture. Thromb Res 1983; 31: 365 – 74.
39. Cornbleet J. Spurious results from automated haematology cell counters. Lab Med 1983; 14: 509 – 14.
40. Dale JC, Pruett SK. Phlebotomy – a minimalist approach. Mayo Clinic Proc 1993; 68: 249 – 55.
41. de Maat MPM, Kluft C, de Boer K, Knot EAR, Jie AFH. Acid treatment of plasma for the inactivation of plasminogen activator inhibitor-1 (PAI-1). Thromb Res 1988; 52: 425 – 30.
42. Deutsche Forschungsgemeinschaft. MAK- und BAT-Werte-Liste 1995: Maximale Arbeitsplatzkonzentrationen und Biologische Arbeitsstofftoleranzwerte. Weinheim: Verlag Chemie, 1995.
43. Deutsche Post AG. Regelungen über den Postversand von medizinischem und biologischem Untersuchungsgut. Mitteilungsblatt 1996; 34: 545.
44. Ditschuneit H, Ditschuneit H, Wechsler J. Probleme der ambulanten Nulldiätbehandlung. Dtsch Ärztebl 1979; 76: 871 – 80.
45. Don BR, Sebastian A, Cheitlin M, Christiansen M, Schambelan M. Pseudohyperkalemia caused by fist clenching during phlebotomy. N Engl J Med 1990; 322: 1290-2.
46. Dybkaer R. Quality assurance, accreditation and certification: needs and possibilities. Clin Chem 1994; 40: 1416 – 20.
47. Dybkaer R. Vocabulary for use in measurement procedures and description of reference materials in laboratory medicine. Eur J Clin Chem Clin Biochem 1997; 35: 141-73.
48. Echemendia E, Van Heertum RL, Guzman L, Narayanan S, Ince G. The stabilizing effect of aprotinin on selected hormone assays. Clin Chem 1984; 30: 1051. (Abstract)
49. Einer G, Zawta B. Präanalytikfibel. Heidelberg. Leipzig: Johann Abrosius Barth, 2nd ed. 1991.
50. EN 829. In vitro diagnostic systems. Transport packages for medical and biological specimens. Requirements, tests. Brussels: European Committee for Standardization (CEN), 1996.
51. Evans K, Laker MF. Intraindividual factors affecting lipid, lipoprotein and apolipoprotein measurement: a review. Ann Clin Biochem 1995; 32: 261– 80.

52. Evans WE, Oellerich M, Holt DW. Drug monitoring. Abbott Laboratories Diagnostic Division, 2nd ed.1994.
53. Fagerström PO, Mellstrand T, Svedmyr N. Absorption of theophylline from conventional and sustained-release tablets. Int J Clin Pharmacol Ther Toxicol 1981; 19:131 – 8.
54. Felder RA, Boyd JC, Margrey K, Holman W, Savory J. Robotics in the medical laboratory. Clin Chem 1990; 36: 1534 – 43.
55. Felding P, Tryding N, Hyltoft Petersen P, Hørder M. Effects of posture on concentrations of blood constituents in healthy adults: practical application of blood specimen collection procedure recommended by the Scandinavian Committee of reference values. Scand J Clin Lab Invest 1980; 40: 615 – 21.
56. da Fonseca-Wollheim F. Deamidation of glutamine by increased plasma gmma-glutamyltransferase is a source of rapid ammonia formation in blood and plasma specimens. Clin Chem 1990; 36: 1479 – 82.
57. Freer DE, Statland BE. Effects of ethanol (0.75 g/kg body weight) on the activities of selected enzymes in sera of healthy young adults : 2. Interindividual variations in response of γ-glutamyltransferase to repeated ethanol challenges. Clin Chem 1977; 23: 2099 – 102.
58. Friedel R. Mattenheimer H, Trautschold I, Forster G. Der vorgetäuschte Enzymaustritt. J Clin Chem Clin Biochem 1976; 14: 109 – 17.
59. Garza D. Urine collection and preservation. In: Ross DL, Neely AE, editors. Textbook of Urinalysis and Body Fluids. Norwalk (Conn): Appleton-Century-Crofts, 1983: 57 – 66.
60. Glick MR, Ryder KW, Glick SJ, Woods JR. Unreliable visual estimation of the incidence and amount of turbidity, haemolysis, and icterus in serum from hospitalized patients. Clin Chem 1989; 35: 837–9.
61. Godolphin W, Bodtker K, Uyeno D, Goh LO. Automated blood-sample handling in the clinical laboratory. Clin Chem 1990; 36: 1551–5.
62. Godolphin W, Bodtker K, Wilson L. Simulation modelling: a tool to help predict the impact of automation in clinical laboratories. Lab Robot Autom 1992; 4: 249–55.
63. Goossens W, Van Duppen V, Verwilghen RL. K_2- or K_3-EDTA: The anticoagulant of choice in routine haematology? Clin Lab Haemat 1991; 13: 291 – 5.
64. Gorodischer R, Burtin P, Hwang P, Levine M, Koren G. Saliva versus blood sampling for the therapeutic drug monitoring in children: patient and parental preferences and an economic analysis. Ther Drug Monit 1994; 16: 437 – 43.
65. Grafmeyer D, Bondon M, Manchon M, Levillain P. The influence of bilirubin, haemolysis and turbidity on 20 analytical tests performed on automatic analysers. Eur J Clin Chem Clin Biochem. 1995; 33: 31–52.
66. Greiling H, Gressner AM. Lehrbuch der Klinischen Chemie und Pathobiochemie. Stuttgart. New York: Schattauer, 3rd ed. 1995.
67. Grunst J, Dietze G, Wicklmayr M, Mehnert H, Hepp KD, Eisenburg J. Einfluß von Äthanol auf den Purinkatabolismus der menschlichen Leber. Verh D Gesellsch Innere Med 1973; 79: 914 – 7.
68. Guder WG. Einflussgrößen und Störfaktoren bei klinisch-chemischen Untersuchungen. Internist 1980; 21: 533 – 42.
69. Guder WG. Thrombocytes as interfering factors in Clinical Chemistry. Editorial Comment. J Clin Chem Clin Biochem 1990; 28: 445.
70. Guder WG. Preanalytical factors and their influence on analytical quality specifications. Scand J Clin Lab Invest 1999; 59: 545-9.
71. Guder WG, Ehret W, da Fonseca-Wollheim F, Heil W, Müller-Plathe O, Schmitt Y, et al. The quality of diagnostic samples. (in german) J Lab med 2002; 26: 267-83, and CD-ROM in english, german and spanish, Zug: Chronolab, Switzerland. 2003. www.diagnosticsample.com
72. Guder WG, Ehret W, da Fonseca-Wollheim F, Heil W, Müller-Plathe O, Töpfer G, et al. Serum, plasma or whole blood? Which anticoagulants to use? J Lab Med 1998; 22: 297-312 and WHO/DIL/LAB/99.1 Rev. 2 and http://www.who.int/bct/Main_areas_of_work/DIL/Lab_Tech/Documents.htm.
73. Guder WG, da Fonseca-Wollheim F, Heil W, Schmitt YM, Töpfer G, Wisser H, et al. The haemolytic, icteric and lipemic sample. Recommendations regarding their recognition and prevention of clinically relevant interferences. J Lab Med 2000; 24: 357-64.
74. Gummert JF, Ikonen T, Morris RE. Newer immunosuppressive drugs: a review. J Am Soc Nephrol 1999; 10: 1366–80.
75. Guyton JR, Dahlen GH, Patsch W, Kautz JA, Gotto AM jr. Relationship of plasma lipoprotein

Lp(a) levels to race and to apolipoprotein B. Arteriosclerosis 1985; 5: 265 – 72.
76. Haboubi NAA, Bignell AHC, Haboubi NY. Serum angiotensin converting enzyme activity in cigarette smokers. Clin Chim Acta 1986; 154: 69 – 72.
77. Haeckel R, Colic D. Verfahren der Speichelgewinnung. In: Haeckel R, editor. Speicheldiagnostik. Darmstadt: GIT VERLAG, 1988: 1 – 12.
78. Haeckel R, Hänecke P. Application of saliva for drug monitoring. An in vivo model for transmembrane transport. Eur J Clin Chem Clin Biochem 1996; 34: 171 – 91.
79. Hagemann P. Qualität im Arztlabor, Optimierung der Präanalytik, Heidelberg: Springer-Verlag 1994.
80. Hallbach J, Hoffmann GE, Guder WG. Overestimation of albumin in heparinized plasma. Clin Chem 1991; 37: 566 – 8.
81. Harris EK, Wong ET, Shaw ST jr. Statistical criteria for separate reference intervals: race and gender groups in creatine kinase. Clin Chem 1991; 37: 1580 – 2.
82. Harrop IS, Ashwell K, Hopton MR. Circannual and within-individual variation of thyroid function tests in normal subjects. Ann Clin Biochem 1985; 22: 371 – 5.
83. Heil W, Grunewald R, Amend M, Heins M. Influence of time and temperature on coagulation analytes in stored plasma. Clin Chem Lab Med 1998; 36: 459-62
84. Heins M, Heil W, Withold W. Storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes. Eur J Clin Chem Clin Biochem 1995; 33: 231 – 8.
85. Hoffmann GE. Integrierte Automationssysteme, Die dritte Generation der Laborsysteme. Videofilm, Trillium GmbH, Grafrath, Deutschland, 1998.
86. Hoffmann GE. Concepts for the 3rd Generation of Laboratory Systems. Clin Chim Acta 1998; 278: 203–16.
87. Holodniy M, Kim S, Katzenstein D, Konrad M, Groves E, Merigan TC. Inhibition of human immunodeficiency virus gene amplification by heparin. J Clin Microbiol 1991; 29: 676 – 9.
88. Housman DE. DNA on trial – The molecular basis of DNA finger printing. N Engl J Med 1995; 332 : 534 – 5.
89. ISO/DIS 6710.2 (prEN ISO 6710) Single-use containers for human venous blood specimen collection, draft 2002.
90. ISO/DIS 15189 (pe EN ISO 15189) Quality management in the medical laboratory. Genève: International Organization for Standardization. 1999.
91. Jahnen A, Claßen A, Hesse A. Erprobung von Methoden zur Sammlung und Konservierung von Urinproben für die Labordiagnostik bei Urolithiasis. Lab Med 1989; 13: 425 – 8.
92. Janitschke K, Kimmig P, Seitz HM, Frosch M, Groß U, Hlobil H et al. MiQ4: Parasitosen. Stuttgart, Jena, Lübeck, Ulm: Gustav Fischer, 1998: 72 – 80.
93. Junge B, Hoffmeister H, Feddersen HM, Röcker L. Standardisierung der Blutentnahme. Dtsch Med Wschr 1978; 103: 260 – 5.
94. Jung R, Lübcke C, Wagener C, Neumaier M. Reversal of RT–PCR inhibition observed in heparinized clinical specimens. Bio Techniques 1997; 23: 24 – 8.
95. Jung R, Soondrum K, Wimmer M, Neumaier M. Rolle der Präanalytik bei molekularbiologischen Untersuchungen von humanen Zellen. J Lab Med 1999; 23: 275 – 9.
96. Kallner A. Preanalytical procedures in the measurement of ionized calcium in serum and plasma. Eur J Clin Chem Clin Biochem 1996; 34: 53 – 8.
97. Karayalcin G, Rosner F, Sawitsky A. Pseudo-neutropenia in American negroes. Lancet 1972; 1: 387.
98. Kates RE. Therapeutic monitoring of antiarrhythmic drugs. Ther Drug Monit 1980; 2: 119 – 26.
99. Keller H. Klinisch-chemische Labordiagnostik für die Praxis. Analyse, Befund, Interpretation. Stuttgart. New York: Thieme, 2. Auflage, 1991.
100. Keller H, Guder WG, Hansert E, Stamm D. Biological influence factors and interference factors in clinical chemistry: General considerations. J Clin Chem Clin Biochem 1985; 23: 3 – 6.
101. Keshgegian AA, Bull GE. Evaluation of a soft handling computerized pneumatic tube specimen delivery system. Am J Clin Pathol 1992; 97: 535 – 40.
102. Kilpatrick DC. Simplified preparation from anticoagulated blood for HLA-DR genomic typing. Tissue antigens 1993; 41: 219 – 20.
103. Kleine TO, Hackler R, Lehmitz R, Meyer-Riemecker H. Liquordiagnostik: Klinisch-chemische Kenngrößen – eine kritische Bilanz. Klin Chem Mitt 1994; 25: 199 – 214.

104. Knoll E. Praeanalytische Faktoren bei der Harnanalytik. Klin Lab 1992; 38: 578 – 82.
105. Koepke JA, Rodgers J, Ollivier MJ. Pre-instrumental variables in coagulation testing. Am J Clin Pathol 1975; 64: 591 – 6.
106. Kohse KP, Wisser H. Problems of quantitative urine analysis. Ann Biol Clin 1987; 45: 630 – 41.
107. Kohse KP, Wisser H. Antibodies as a source of analytical errors. J Clin Chem Clin Biochem 1990; 28: 881 – 92.
108. Kollée LAA, Willems JL, de Kort AFM, Monnens LAH, Trijbels JMF. Blood sampling technique for lactate and pyruvate estimation in children. Ann Clin Biochem 1977; 14: 285 – 7.
109. Kouri T, Fogazzi G, Gant V, Hallander A, Hofmann W, Guder WG. European Urinalysis Guidelines, Scand J Clin Lab Invest 2000; 60 Suppl 231: 1 – 96.
110. Külpmann WR. Determination of electrolytes in serum and serum water. Wiener Klin Wschr 1992; 104 Suppl 192: 34 –8.
111. Kupke IR. Evaluation of the polyester gel barrier microtainer®. Clin Chem 1987; 33: 1468 – 9.
112. Laessig RH, Indriksons AA, Hassemer DJ, Paskey TA, Schwartz TH. Changes in serum chemical values as a result of prolonged contact with the clot. Am J Clin Pathol 1976; 66: 598 – 604.
113. Lamers KJB, Doesburg WH, Gabreëls FJM, Lemmens WAJG, Romson AC, Wevers RA, et al. The concentration of blood components related to fuel metabolism during prolonged fasting in children. Clin Chim Acta 1985; 152: 155 – 63.
114. Lamprecht J, Seidel D. Enzymdiagnostik bei Patienten mit Hyperlipoproteinämie: Beseitigung von Plasmatrübungen durch selektive Polyanionenpräzipitation von Plasma-Lipoproteinen. Z Klin Chem Klin Biochem 1974; 12: 154.
115. Leppaluoto J, Vuolteenaho O, Arjamaa O, Ruskoao H. Plasma immunoreactive atrial natriuretic peptide and vasopressin after ethanol intake in man. Acta Physiol Scand 1992; 144: 121 – 7.
116. Levinson StS. Antibody multispecificity in immunoassay interference. Clin Biochem 1992; 25: 77–87.
117. Liss E, Bechtel S. Improvement of glucose preservation in blood samples. J Clin Chem Clin Biochem 1990; 28: 689 – 90.
118. Lob WS. Robotic transportation. Clin Chem 1990; 36: 1544 – 50.
119. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions. Gene 1990; 93: 125 – 8.
120. Luft D, Götz R. Lactat im Liquor cerebrospinalis. Bedeutung für Differentialdiagnose, Therapiekontrolle und Prognose zerebraler und meningealer Erkrankungen. Lab Med 1983; 7:55 – 9.
121. Lutomski DM, Bower RH. The effect of thrombocytosis on serum potassium and phosphorus concentration. Am J Med Sci 1994; 307: 255 – 8.
122. Massarrat S, Herbert V. Haltbarkeit der Enzyme beim Versand. Dtsch Ärztebl 1977; 74: 1909 – 10.
123. McKenney JM, Goodman RP, Wright JT jr., Rifai N, Aycock DG, King ME. The effect of low-dose of hydrochlorothiazide on blood pressure, serum potassium and lipoproteins. Pharmacotherapy 1986; 6: 179 – 84.
124. Mehta AC. Review article: preanalytical considerations in drug assays in clinical pharmacokinetic studies. J Clin Pharm Ther 1989; 14: 285 – 95.
125. Meites S. Skin-puncture and blood-collecting technique for infants: update and problems. Clin Chem 1988; 34: 1890 – 4.
126. Meites S. Saniel-Banrey K. Preservation, distribution and assay of glucose in blood, with special reference to the newborn. Clin Chem 1979; 25: 531 – 4.
127. Middleton S, Mountain P, Kemp A. Laboratory automation: A model. Leadership in Health Services. 1993; 2:20 – 4.
128. Miet I, Haeckel R. Proposals for nomenclature in specimen identification. J Clin Chem Clin Biochem 1982; 20: 253 – 7.
129. Milatovic D, Braveny I. Infektionen: Praktische Hinweise zur antimikrobiellen Therapie und Diagnostik. München: MMV Medizin 1997: 293–301.
130. Miller JM, Holmes HT. Specimen collection transport and storage. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, editors. Manual of Clinical Microbiology. Washington DC: ASM Press, 6th ed. 1995: 19–32.
131. Miller M, Bachorik PS, Cloey TA. Normal variation of plasmalipoproteins: postural effects on plasma concentrations of lipids, lipoproteins and apolipoproteins. Clin Chem 1992; 38: 569 – 74.
132. Mole L, Margolis D, Carroll R, Todd D, Holodniy M. Stabilities of quantitative plasma culture for human immunodeficiency virus, RNA, and p24 antigen from samples collected in VACUTAINER® CPT and standard VACUTAINER® tubes. J Clin Microbiol 1994; 32: 2212 – 5.

133. Müller-Plathe O., Heyduck S. Stability of blood gases, electrolytes and haemoglobin in heparinized whole blood samples: influence of the type of syringe. Eur J Clin Chem Clin Biochem 1992, 30: 349 – 55.
134. Müller-Plathe O. Säure-Basen-Stoffwechsel und Blutgase. In: Greiling H, Gressner AM, Herausgeber. Lehrbuch der Klinischen Chemie und Pathobiochemie. Stuttgart, New York: Schattauer, 3. Auflage, 1995: 501–23.
135. Muirhead KA, Wallace PK, Schmitt TC, Frescatore RL, Franco JA, Horan PK. Methodological considerations for implementation of lymphocyte subset analysis in a clinical reference laboratory. Ann NY Acad Sci 1986; 468: 113 – 27.
136. Narayanan S. Considerations in therapeutic drug monitoring. Italian J Clin Patol 1984; 1: 73 – 5.
137. Narayanan S. Protection of peptidic substrates by protease inhibitors. Biochim Clin 1987; 11: 954 – 6.
138. Narayanan S. Inhibition of in vitro platelet aggregation and release and fibrinolysis. Ann Clin Lab Sci 1989; 19: 260 – 5.
139. Narayanan S. Approaches to laboratory automation. In: Principles and Applications of Laboratory Instrumentation. Chicago, Illinois: ASCP Press, 1989: 197 – 207.
140. Narayanan S. Overview of principles and current uses of DNA probes in clinical and laboratory medicine. Ann Clin Lab Sci 1992; 22: 353 – 76.
141. Narayanan S. Physiological variables in blood sampling. Klin Chem Mitt 1993; 24: 130 – 4.
142. Narayanan S. Preanalytical aspects of coagulation testing. Haematogica 1995; 80 Suppl. 2: 1 – 6.
143. Narayanan S. Concepts, principles and applications of selected molecular biology techniques in Clinical Biochemistry. Adv Clin Chem 1996; 32: 1 – 38.
144. Narayanan S. The preanalytical phase. An important component of laboratory medicine. Am J Clin Pathol 2000; 113: 429-52.
145. Narayanan S, Appleton HD. Creatinine: A review. Clin Chem 1980, 26: 1119 – 26.
146. Narayanan S, Hamasaki N. Current concepts of coagulation and fibrinolysis. Adv Clin Chem 1999; 33:133 – 68.
147. Narayanan S, Lin FC. Sampling technique. In: Wong SHY, editor. Therapeutic Drug Monitoring and Toxicology by Liquid Chromatography. New York: Dekker 1985; 79 – 88.
148. NCCLS. Development of requisition form for therapeutic drug monitoring. Wayne, PA: Document TDM 1A, 1985.
149. NCCLS. Interference testing in clinical chemistry. Proposed guideline. Wayne, PA: Document EP7-P, 1986.
150. NCCLS. Additives to blood collection devices: EDTA. Wayne, PA: Document H35-P, 1989.
151. NCCLS. Blood gas preanalytical considerations: Specimen collection, calibration, and controls. Approved guideline. Wayne, PA: Document C27-A, 1993.
152. NCCLS. Clinical laboratory waste management. Aproved guideline. Wayne, PA: Document GP5-A, 1993.
153. NCCLS. Routine urinalysis and collection, transportation and preservation of urine specimens; Approved guideline. Wayne, PA: Document GP 16-A, 1995.
154. NCCLS. Clinical laboratory safety. Approved guideline. Wayne, PA: Document GP17-A, 1996.
155. NCCLS. Collection, transport, and processing of blood specimens for coagulation testing and general performance of coagulation assays. Wayne, PA: Approved guideline, 3rd ed., 2000.
156. NCCLS. Procedure of the collection of diagnostic blood specimens by venipuncture. Approved Standard – 4th ed. Wayne, PA: Document H3-A4, 1998.
157. NCCLS. Procedure of the collection of diagnostic blood specimens by skin puncture. Approved Standard – 4th ed. Wayne, PA: Document H4-A4, 1999.
158. NCCLS. Procedures for the collection of arterial blood specimens. Approved Standard, 3rd ed. Wayne, PA: Document H11-A3, 1999.
159. NCCLS. Procedures for the handling and processing of blood specimens. Approved guideline, 2nd ed. Wayne, PA: Document H18-A2, 1999.
160. Neumaier M, Braun A, Wagener C. Fundamentals of quality assessment of molecular amplification methods in clinical diagnostics. Clin Chem 1998; 44: 12-26.
161. Nicholson JKA, Jones BM, Cross D, McDougal JS. Comparison of T and B cell analyses on fresh and aged blood. J Immunol Methods 1984; 73: 29 – 40.
162. Nordin BEC, Wilkinson R, Marshall DH, Galagher JC, Williams A, Peacock M. Calcium absorption in the elderly. Calcif Tiss Res 1976; 21: 442 – 51.

163. Ono T, Kitaguchi K, Takehara M, Shiiba M, Hayami K. Serum-constituents analyses: effect of duration and temperature of storage of clotted blood. Clin Chem 1981; 27: 35 – 8.
164. Pandian MR, Morgan CH, Carlton E, Segre GV. Modified immunoradiometric assay of parathyroid hormone – related protein: clinical application in the differential diagnosis of hypercalcemia. Clin Chem 1992; 38: 282 – 8.
165. Peheim E, Colombo JP. Die mikroskopische Urinuntersuchung. In: Colombo JP, editor. Klinisch-chemische Urindiagnostik. Rotkreuz: Labo Life Verlagsgemeinschaft, 1994: 103 – 31.
166. Pewarchuk W, Vander Boom J, Blajchman MA. Pseudopolycythemia, pseudothrombocytopenia, and pseudoleukopenia due to overfilling of blood collection vacuum tubes. Arch Pathol Lab Med 1992; 116: 90 – 2.
167. Picard-Maureau A, Vollmer C, Sparrer W. Therapeutisches Drug Monitoring. Extracta Diagnostica 1989; 3: 266 – 71. 1990; 4: 14 – 20.
168. Ratge D, Brugger G, Wehr M, Bode JCh, Wisser H. Catecholamines in plasma and urine of patients with alcoholic liver damage under resting and excercise conditions. J Clin Chem Clin Biochem 1985; 23: 447 – 52.
169. Reardon DM, Warner B, Trowbridge EA. EDTA, the traditional anticoagulant of haematology: with increased automation is it time for a review? Med Lab Sci 1991; 48: 72 – 5.
170. Recommendations of the International Council for Standardization in haematology for ethylene diamine tetraacetic acid anticoagulation of blood for blood cell counting and sizing. Am J Clin Pathol 1993; 100:371 – 2.
171. Rehak NN, Chiang BT. Storage of whole blood: effect of temperature on the measured concentration of analytes in serum. Clin Chem 1988; 34: 2111 – 4.
172. Renoe BW, Mc Donald JM, Ladenson JH. Influence of posture on free calcium and related compounds. Clin Chem 1979; 25: 1766 – 9.
173. Rico H. Alcohol and bone disease. Alcohol Alcoholism 1990; 25: 345 – 52.
174. Rifai N, Merill JR, Holly RG. Postprandial effect of a high fat meal on plasma lipid, lipoprotein cholesterol and apolipoprotein measurements. Ann Clin Biochem 1990; 27: 489 – 93.
175. Robertson D, Frölich JC, Carr RK, Watson JTh, Holliefield JW, Shand DG, et al. Effects of caffeine on plasma renin activity, catecholamines and blood pressure. N Engl J Med 1978; 298: 181 – 6.
176. Röcker L, Schmidt HM, Junge B, Hoffmeister H. Orthostasebedingte Fehler bei Laboratoriumsbefunden. Med Lab 1975; 28: 267 – 75.
177. Röcker L, Schmidt HM, Motz W. Der Einfluss körperlicher Leistungen auf Laboratoriumsbefunde im Blut. Ärztl Lab 1977; 23: 351 – 7.
178. Rothe G, Schmitz G for the Working Group on Flow Cytometry and Image Analysis. Consensus protocol for the flow cytometric immunophenotyping of haematopoietic malignancies. Leukemia 1996; 10: 877–95.
179. Routledge PA, Stargel WW, Wagner GS, Shand DG. Increased a_1-acid glycoprotein and disposition in myocardial infarction. Ann Int Med 1980; 93: 701 – 4.
180. Sacker IS. Specimen Collection. In: Lewis SM, Coster JF, editors. Quality Control in Haematology. Symposium of the International Committee for Standardization in Haematology. New York: Academic Press, 1975: 211 – 29.
181. Sasaki M. An innovative conveyor belt system for a clinical laboratory. In: Lin HJ, Swaminathan R, Robertshaw AM, editors. Proc. 4th Asian-Pacific Congr. Clin Biochem. Hongkong: Gardiner Caldwell Communications Ltd., 1988: 301 – 6.
182. Sasaki M, Ogura K. A fully robotic assay for human hormone analysis. Clin Chem 1990; 36: 1567 – 71.
183. Saxena S, Carmel R. Racial difference in vitamin B_{12} levels in the United States. Am J Clin Pathol 1987; 88: 95 – 7.
184. Schaal KP. Entnahme und Transport von Untersuchungsmaterial zur mikrobiologischen, parasitologischen und serologischen Diagnostik von Infektionskrankheiten. Der Krankenhausarzt 1976; 49: 1–71.
185. Schmitz G, Rothe G, Ruf A, Barlage S, Tschöpe D, Clemetson KJ, et al. European working group on clinical cell analysis: consensus protocol for the flow cytometric characterisation of platelet function. Thromb Haemost 1998; 79: 885–96.
186. Scholer A. Urinkonservierung. In: Colombo JP, editor. Klinisch chemische Urindiagnostik. Rotkreuz: Labo Life Verlagsgemeinschaft, 1994: 43 – 50.
187. Schulz M, Schmoldt A. Therapeutic and toxic blood concentration of more than 500 drugs. Pharmazie 1997; 52: 895–911.

188. Sears D, Charache S, Perlstein M. Electronic Blood Cell Counters. Faulty calibration due to type and amount of anticoagulant in collection tubes. Arch Pathol Lab Med 1985; 109: 247 – 9.
189. Sepkovic DW, Haley NJ, Wynder EL. Thyroid activity in cigarette smokers. Arch Intern Med 1984; 144: 501 – 3.
190. Sestini R, Orlando C, Zentilin L, Gelmini S, Pinzani P, Bianchi S, et al. Measuring c-erb B-2 oncogene amplification in fresh and paraffin-embedded tumors by competitive polymerase chain reaction. Clin Chem 1994; 40 : 630 – 6.
191. Shang-Quiang JI, Evenson MA. Effect of contaminants in blood collection devices on measurement of therapeutic drugs. Clin Chem 1983; 29: 456 – 61.
192. Shaw LM, Bowers L, Demers L, Freeman D. Moyer T, Sanghvi A et al. Critical issues in cyclosporine monitoring: Report of the task force on cyclosporine monitoring. Clin Chem 1987; 33: 1269 – 88.
193. Sidebottom RA, Williams PR, Kanarek KS. Glucose determinations in plasma and serum: potential error related to increased haematocrit. Clin Chem 1982; 28: 190 – 2.
194. Siegel JE, Bernard DW, Swami VK, Sazama K. Monitoring heparin therapy: APTT results from partial vs. full-draw tubes. Am J Clin Pathol 1998; 110: 184-7.
195. Siggard-Andersen O. The acid base status of the blood. Copenhagen: Munksgaard, 4th ed. 1974.
196. Simonnet C. Quelque règles à propos du transport par route des échantillons biologiques. Ann Biol Clin 2000; 58: 111-7.
197. Skinner SL, Adelaide MB. A cause of erroneous potassium levels. Lancet 1 1961: 478 – 80.
198. Smoller BR, Kruskall MS. Phlebotomy for diagnostic laboratory tests in adults. Pattern of use and effect on transfusion requirements. N Engl J Med 1986; 314: 1233 – 5.
199. Solberg A. A guide to IFCC recommendations of reference values. IFCC J 1993; 5: 160 – 4.
200. Sommer R, Eitelberger F, Hohenauer L. Gentamicin-Dosierung beim Neugeborenen. Klin Pädiatr 1989; 201: 287-92.
201. Sonntag O. Haemolysis as an interference factor in clinical chemistry. J Clin Chem Clin Biochem 1986; 24: 127–39.
202. Sonntag O. Trockenchemie, Analytik mit trägergebundenen Reagenzien. Stuttgart, New York: Thieme, 1988.
203. Stansbie D, Begley JP. Biochemical consequence of exercise. IFCC J 1991; 3: 87 – 91.
204. Statland BE, Winkel P. Selected pre-analytical sources of variation. In: Gräsbeck R, Alström T, editors. Reference Values in Laboratory Medicine. Chichester: John Whiley & Sons Ltd, 1981: 127–37.
205. Statland BE, Winkel P. Effects of preanalytical factors on the intraindividual variation of analytes in the blood of healthy subjects: consideration of preparation of the subject and time of venipuncture. Crit Rev Clin Lab Sci 1977; 8: 105 – 44.
206. Steinmetz J, Panek, E, Sourieau F, Siest G. Influence of food intake on biological parameters. In: Siest G, editor. Reference Values in Human Chemistry. Basel: Karger, 1973: 195 – 200.
207. Stevens EJ, Hoffmann GE. Developments and economic factors in preanalytical automation. 3rd Symposium: Samples from patients to laboratories, Basel, Switzerland: Becton Dickinson VACUTAINER® Systems 1997: 21.
208. Stone WJ, MacKinney TD, Warnock LG. Spurious hyperbilirubinemia in uremic patients on propranolol therapy. Clin Chem 1979; 25: 1761 – 75.
209. Sturk A, Sanders GT. Macro enzymes: prevalence, composition, detection and clinical relevance. J Clin Chem Clin Biochem 1990; 28: 65 – 81.
210. Szasz G, Gerhardt W, Gruber W, Bernt E. Creatine kinase in serum: 2. Interference of adenylate kinase with the assay. Clin Chem 1976; 22:1806–11.
211. Tan AC, Kloppenborg PWC, Benraad TJ. Influence of age, posture and intra-individual variation on plasma levels of atrial natriuretic peptide. Ann Clin Biochem 1989; 26: 481 – 6.
212. The Hiteco Advanced Automation for the Health Care Sector. Milano: Hiteco, 1994.
213. Thomas L (Editor). Clinical Laboratory Diagnostics. Frankfurt/Main: TH Books 1st Edition 1998.
214. Todd DM, Buccini FJ. Apparent heparin interference with restriction enzyme digestion of genomic DNA. Clin Chem 1993; 39: 362 – 3.
215. Töpfer G, Funke U, Schulze M, Lutze G, Ziemer S, Siegert G. Determination of coagulation parameters in citrated venous blood, catheter blood, and capillary blood: preanalytical problems. J Lab Med 2000; 24: 514-20.
216. Töpfer G, Lutze G, Sauer K, Friedel G, Hornig F, Kühnert T et al. Influence of the sample tube's citrate concentration on coagulation analytes. J Lab Med 2001; 25: 162-8.

217. Touitou Y, Haus E, editors. Biologic Rhythms in Clinical and Laboratory Medicine. Berlin. Heidelberg. New York: Springer, 1992.
218. Tryding N, Tufvesson C, Sonntag O. Drug Effects in Clinical Chemistry. Clinically important analytical interferences and biological effects of drugs on biochemical and haematological laboratory investigations. Stockholm: AB Realtryck, 7th ed. 1996. Supplement Vol.: Tryding N, Tufvesson C. References to drug effects in Clinical Chemistry. Stockholm: AB Realtryck, 7th ed. 1996.
219. Tsianos EB, Jalali MT, Gowenlock AH, Braganza JM. Ethnic "hyperamylasaemia": clarification by isoamylase analysis. Clin Chim Acta 1982; 124: 13 – 21.
220. Uchida K, Matuse R, Toyoda E, Okuda S, Tomita S. A new method of inhibiting glycolysis in blood samples. Clin Chim Acta 1988; 172: 101 – 8.
221. US Dept of Health and Human Services. Medicare, Medicaid, and CLIA programs: regulations implementing the clinical laboratory improvemend amendments of 1988 (CLIA). Final rule. Fed Regist 1992; 57: 7002–186.
222. U.S. Environmental Protection Agency. Title 40, CFR, Parts 260 – 5.
223. Van den Besselaar AMH, Meeuwisse-Braun J, Jansen-Gruter R, Bertina RM. Monitoring heparin therapy by the activated partial thromboplastin time. The effect of preanalytical conditions. Thromb Haemost 1987: 58: 226 – 31.
224. Van der Woerd-de Lange JA, Guder WG, Schleicher E, Paetzke I, Schleithoff M, Wieland OH. Studies on the interference by haemoglobin in the determination of bilirubin. J Clin Chem Clin Biochem 1983; 21: 437–43.
225. Van Steirteghem AC, Young DS. Povidone-iodine ("Betadine") desinfectant as a source of error. Clin Chem 1977; 23: 1512. (Letter to the editor)
226. Voit R. Plasma-Serum-Unterschiede und Lagerungsstabilität. Klinisch-chemische Messgrößen bei Verwendung von Plasmatrennröhrchen. Dissertation, Ludwig-Maximilians-Universität München, 1993.
227. Watkins J, Wild G, Smith S. Nafamostat to stabilise plasma samples taken for complement measurements. Lancet. 1989; 1: 896 – 7.
228. Watson KR, O'Kell T, Joyce JT. Data regarding blood drawing sites in patients receiving intravenous fluids. Amer J Clin Pathol 1983; 79: 119 – 21.
229. WHO. The SI for the health professions. Prepared at the request of the 30th World Health Assembly, May 1977. Geneva: World Health Organization, 1977.
230. Wissenschaftlicher Beirat der Bundesärztekammer und Paul-Ehrlich-Institut. Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hämotherapie). Köln: Deutscher Ärzte-Verlag, 2000.
231. Wisser H. Praeanalytische Faktoren bei der Harnanalytik. In: Guder WG, Lang H, Hrsg. Pathobiochemie und Funktionsdiagnostik der Niere. Berlin. Heidelberg. New York: Springer Verlag, 1989: 171 – 82.
232. Wisser H. Einflussgrößen und Störgrößen. In: Greiling H, Gressner AM, Hrsg. Lehrbuch der Klinischen Chemie und Pathobiochemie. Stuttgart. New York: Schattauer, 3rd ed., 1995: 50 – 71.
233. Wisser H. Präanalytik. In: Greiling H, Gressner AM, Hrsg. Lehrbuch der Klinischen Chemie und Pathobiochemie. Stuttgart. New York: Schattauer, 3. Auflage, 1995: 37 – 49.
234. Wisser H. Störungen der Messgrößen des kleinen Blutbildes durch Antikörper. GIT LaborMedizin 1995; 18: 83 – 5.
235. Wisser H, Knoll E. Tageszeitliche Änderungen klinisch-chemischer Messgrößen. Ärztl Lab 1982; 28: 99 – 108.
236. Wood M, Shand DG, Wood AJJ. Altered drug binding due to the use of indwelling heparinized cannulas (heparin lock) for sampling. Clin Pharm Ther 1979; 25: 103 – 7.
237. Wurster U, Stark E, Engelhardt P. Cerebrospinal fluid cytology after combined centrifugation and cytocentrifugation compared to sedimentation and membrane filtration. Ärztl Lab 1984; 30: 184 – 8.
238. Yatscoff RW, Honcharik N, Lukowski M, Thliveris J, Chackowsky P, Faraci C. Distribution of cyclosporin G (NVa2 cyclosporin) in blood and plasma. Clin Chem 1993; 39: 213 – 7.
239. Young DS. Effects of Preanalytical Variables on Clinical Laboratory Tests. Washington: AACC Press, 2nd ed, 1997.
240. Young DS. Biological variability. In: Brown STS. Mitchell FL, Young DS, editors. Chemical Diagnosis of Disease. Amsterdam. New York. Oxford: Elsevier, North-Holland, Biomedical Press, 1979: 1 – 113.
241. Young D. Effects of Drugs on Clinical Laboratory Tests. Washington: ACC Press, 2000, 5th ed.
242. Zawta B. Infusionstherapie und Labordiagnostik. GIT LaborMedizin, 1994; 17: 171 – 3.

The definition of terms used in this volume follows the "Vocabulary of Reference Method Procedures and Materials in Laboratory Medicine", prepared by R. Dybkaer (47). Other sources are given in brackets (26, 68, 90, 100, 149, 156, 229).

accession:
All steps necessary to ensure that a specific blood specimen and the accompanying forms are unmistak-ably identified as referring to a specific person.

accuracy:
Closeness of the agreement between the result of a measurement and the true value of the measurand.

additive:
Substance, other than surface treatment designed to be irremovable, that is placed inside the receptacle to facilitate the preservation of the specimen, or is intended to react with the specimen, in order to allow the intended analysis to be performed (89).

analyte:
Component of a sample indicated in the name of a measurable quantity.

analytical interference:
Systematic error of measurement caused by an analytical interferent (90, 149).

analytical portion:
The portion of material taken from the analytical sample and on which the measurement of the appropriate measurable quantity is actually carried out.

analytical sample:
A sample prepared from the laboratory sample and from which analytical portions may be taken.

analytical sensitivity:
The slope of the analytical calibration function. The term "analytical sensitivity" is not a synonym for detection limit.

analytical specificity:
The ability of a measurement procedure to determine solely the measurable quantity it purports to measure.

bias of measurement:
Bias is the difference between the expectation of the results of measurement and the true value of the measurand.

biological influence quantity:
See influence factor.

biological reference value; reference value:
Value of a measurement in an individual belonging to a defined reference sample group of individuals.

The term "biological reference value" is not a synonym of the ambiguous term "normal value" as the individuals of the reference sample group may suffer from a defined state of disease.

component:
Definable part of a system.
In analytical chemistry the components of a system are sometimes divided into "analyte", "concomitants", and "solvent"; the latter two are often called "matrix".

detection limit; limit of detection:
The minimum detectable value. Result of a measurement by given measurement procedure for which the probability of an analytically false negative result is b, given the probability a of an analytically false positive result (IUPAC recommends default values for a and b equal to 0.05.)

Samples: From the Patient to the Laboratory. W. G. Guder, S. Narayanan, H. Wisser, and B. Zawata

ISBN: 978-3-527-30981-8

diagnostic sensitivity:
See nosographic sensitivity.

diagnostic specificity:
See nosographic specificity.

endogeneous:
Any factor or mechanism acting or derived from the system from which the analytical sample is taken. See also interference factor.

error of measurement; error:
Result of a measurement minus a true value of the measurand.

exogeneous:
Any factor or mechanism added to the sample either in vivo (i.e. drug) or to the sample in vitro (i.e. contaminant).

good laboratory practice; GLP:
Organization process and the conditions under which laboratory studies are planned, performed, monitored, recorded, and reported.

imprecision of measurements; imprecision:
Dispersion of independent results of measurements obtained under specified conditions.

Imprecision of measurements, when applied to sets of results of measurements, depends solely on the dispersion of random error of measurement and does not relate to a true value of the measurable quantity. Imprecision is usually expressed numerically as the repeatability standard deviation, an intermediate precision standard deviation, or a reproducibility standard deviation of results of measurements.

inaccuracy of measurement; inaccuracy:
Discrepancy between the result of measurement and the true value of a measurement. Inaccuracy of a measurement describes a combination of systematic effects and random effects that contribute individual components of error of measurement.

influence, influence factor:
Biological (in vivo and in vitro) influence on the concentration of a measurand in a system (i.e. venous blood).

interference:
Systematic error of measurement caused by a sample component which does not by itself produce a signal in the measuring system (CEN). The effect of a substance upon any step in the determination of the concentration or catalytic activity of the analyte.

interference factor:
Substance or component of the matrix of a sample which differs from the analyte and interfering with the analytical procedure to give a false measuring signal.

Interference factor is called influence quantity by Dybkaer (47), defined as the measurable quantity that is not the measurand, but that effects the result of the measurement.

inter-individual variation:
Distribution of the values within individuals of a given set.

internal quality control:
Operational techniques and activities within a production site that are used to fulfil requirements for quality.

international system of units; SI:
Coherent system of units of measurement adopted and recommended by the General Conference on Weights and Measures (CGPM). The SI is presently based on seven base units of measurements: metre (m), kilogram

(kg), second (s), ampere (A), kelvin (K), mole (mol), and candela (cd) (229).

intra-individual variation:
Distribution of the values in a given individual. It is usually assumed that the variation occurs with time as an independent variable.

matrix:
All components of the material system except the analyte.

matrix effect:
Influence of a sample property, other than the measurand, on the measurement and thereby on the value of the measurand.

measurable quantity; quantity:
Attribute of a phenomenon, body, or substance that may be distinguished qualitatively and determined quantitatively.

measurand:
measurable quantity subject to measurement.

measurement:
Set of operations having the object of determining a value of a measurable quantity.

measurement procedure:
Set of operations described specifically, used in the performance of measurements according to a given method of measurement.

metrology:
Science of measurement.

nosographic sensitivity;
not diagnostic sensitivity:
Number of persons correctly classified by the results of measurement being in a defined state divided by the number of all persons in that state.

Clinically true positive classifications divided by the sum of clinically true positive class plus clinically false negative classifications.

nosographic specificity;
not diagnostic specificity:
Number of persons correctly classified by the results of measurement as not being in a defined state divided by the number of all persons not in the defined state.

Clinically true negative classifications divided by the sum of clinically true negative class plus clinically false positive classifications.

patient sample (specimen):
An aliquot of a specimen that has been appropriately collected, transported and processed in the laboratory to provide material for a specific laboratory test (153 – 159).

preanalytical phase:
synonymous with **pre-metrological phase** and **pre-examination phase**, starting from the request, including the examination requisition, preparation of the patient, collection of the primary sample, transportation to and within the laboratory, and ending when the analytical examination procedure starts. Procedures included herein are called pre-examination procedures (89).

precision of measurements;
precision:
Closeness of agreement between independent results of measurements obtained under stipulated conditions (ISO 3534-1).

quality assurance:
All the planned and systematic activities implemented within the quality system, and demonstrated as needed, to

provide adequate confidence that an entity will fulfil requirements for quality (ISO 8402-3.5). In the clinical laboratory sciences, it is customary to consider internal quality control and external quality assessment as complementary but not complete parts of quality assurance. The term "external quality assurance" is also being used to comprise the actions ensuring the transferability of results of measurement.

quality system:
Organisational structure, procedures, processes, and resources needed to implement quality management (ISO 8402-3.6).

quantity:
see measurable quantity.

random error of measurement; random error:
Result of a measurement minus the mean that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions.

reagent:
Substance employed to produce a chemical reaction in order to measure quantities, pertaining to other substances or convert one substance into another.

receptacle:
Vessel, wether evacuated or not, intended to contain a specimen, together with any receptacle accessory and additive, with closure in place.
Receptacle accessory is a component inside the receptacle which is intended to assist in the collection, or mixing, or separation, of the specimen (89).

reference interval, reference values:
Do not use normal values because of the inherent ambiguity of the word "normal". The reference interval defines the 95% range of reference values obtained from a reference population (199).

reference limit:
Upper or lower limit of reference interval, not identical with clinical decision limit.

reference material:
Material or substance, one or more of whose property values are sufficiently homogenous and well established to be used for the calibration of a measuring system, the assessment of a measurement procedure, or for assigning values to materials (ISO-Guide 30-2.1).

reference population, reference individual:
Reference individuals are persons selected by inclusion and exclusion criteria from a healthy population to form the reference populations from which reference values are obtained for comparison with an individual having a specific disease. The reference population should be as similar as possible to the persons tested except for the disease being investigated.

repeatability of results of measurements; repeatability:
Closeness of agreement between the results of successive measurements of the same measurand carried out under repeatability conditions.

sample:
One or more parts taken from a system and intended to provide information on the system, often to serve as a basis for a decision on the system or its production.

A portion taken from a system is sometimes called a "specimen".
It may be useful to distinguish between "primary sample" (taken from the original system), "laboratory sample" (as received by the laboratory), and "analytical sample" from which the "analytical portion" is taken.
See also analytical sample, patient sample (specimen).

sampling:
Process of drawing or constituting samples, usually qualified by a description of the sampling procedure (ISO 3534-2).

sampling procedure:
Operational requirements and/or instructions relating to the use of particular sampling plan, that is the planned procedure of selection, withdrawal, and preparation of one or more samples from an inspection lot to yield knowledge of the characteristics of the lot (ISO 3534-2).
Note: In laboratory medicine, the inspection lot usually is a person.

specimen:
Biological material which is obtained in order to detect properties or to measure one or more quantities (89).

stability:
Ability of a system, when kept under specificed conditions, to maintain a stated property value within specified limits for a specified period of time.

sterility:
Sterility is the absence of living organisms.

systematic error of measurement; systematic error:
Means that would result from an infinite number of measurements of the same measurand carried out under repeatability conditions minus a true value of the measurand.

traceability:
Ability to trace the history, application or location of that which is under consideration (CEN-ISO 9000: 2000).
Property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties (VIM; 1993, 6.10).

turn-around-time:
Time interval between collection of primary sample (patient sample) and report to the ordering health service, or Time interval between receive of request and report to the requesting health service (90).

unspecificity:
Effects of sample components other than the analyte that by themselves produce a signal of the measuring system.

unit of measurement; unit:
Particular measurable quantity, defined and adopted by convention, with which other measurable quantities of the same kind are compared in order to express their magnitudes relative to that quantity.

value of a measurable quantity; value:
Magnitude of a measurable quantity generally expressed as a unit of measurement multiplied by a number.

venipuncture:
All steps involved in obtaining an appropriately identified blood specimen from a person's vein.

Index

A

acetate 13
acetoacetate 8
N-acetyl-procainamide (NAPA) 70
acid citrate dextrose (ACD) **35, 61, 62**
acid phosphatase 7, 32, 42
ACTH (see corticotropin)
activated partial thromboplastin time (APTT) 17, 52–53
acute phase proteins 7
addictive drugs **13**
additives **34–35**
adenylate kinase **81**
ADH (see vasopressin)
adrenaline (see epinephrine)
age **6**
air temperature 10
alanine aminotransferase (ALT) 8–9, 13, 19, 36–37, 80, 82
albumin 7–9, 17, 18–19, 27, 30, 32, 37, 83
alcohol (ethanol) **13**,
aldosterone 12–13, 14–15, 17, 18
alkaline phosphatase 6–7, 9, 18–19, 32, 37, 76
allopurinol **82**
altitude **11**
amikacin 70
amino acids 7
amiodaron 70
amitriptiline 71
ammonia 7, 9, 32–33, 36
ammonia excretion 29
amphetamine 13
amylase 6, 13, 16, 79, 80
analyte absorption to the vessel wall **28**
analytical time **85 – 86**
angiotensin 17
angiotensin converting enzyme 12
antibodies **37, 78 – 79**
anticoagulant 21, **32, Annex**
antiphospholipid antibodies 79
apolipoprotein AI 7, 18, 41
apolipoprotein B 8, 18, 41
aprotinin **53, 59**
arterial puncture **20–21**
arterialization of the puncture site **23**
aspartate aminotransferase (AST) 7–9, 13, 32–33, 18–19, 37, 80
"atraumatic" pencil shaped needle **26**
atrial natriuretic peptide (ANP) 13, 18
autoantibodies **79**

B

bacteria **72**
bacteriological testing **72 – 75**
bar-code **24–25**
bed rest **18**
bicarbonate 8, 13, 57
biguanides **82**
bilirubin 6–9, 13, 16, 19, 32, 37, 40, 81, 82
biohazard **38**
biological halflife **69**
biological influences 5, 13, **82**
biopsies 16
blank **80**
blood cell lysis **62**
blood cells **54–55, 60, 62–65**
blood collection **60–61**
blood culture 21
blood gas analysis 21, 23
blood gases 21, **66–67**
blood grouping 16, **50–51**
blood smear 37, 55
blood transfusion **50–51**
body temperature **15**
borate serine EDTA 36
bronchoalveolar lavage (BAL) 73

C

cadmium 12
calcium (free or total) 8–9, 18–19, 28–30, 37, 40
cannabis 13
capillary sampling **22–23, 66, 84**
carbamazepine 70
carbohydrate deficient transferins 13
carbondioxide (pCO_2) 13, 66–67
carboxyhemoglobin (CO-Hb) 12
carcino embrionic antigen (CEA) 12
β-carotinoids 12
cartesian robot **45**
casts 28
catecholamines 17, 30, 59
cation interference **33**
cellular analysis **61**
cell separation tube **60–61**
centrifugation **33, 42–43, 76, 84, Annex**
cephalotin **82**
cerebrospinal fluid (CSF) **26–27, 69**
cerebrospinal fluid (CSF) cytology 27
ceruloplasmin 7, 82
chemical waste **48–49**
chlorazepam 69
chloride 19, 29, 57, 66, 77, 80
cholecalciferol (25-OH) 14
cholecystokinin-pancreozymin 59
cholesterol 7–8, 12–13, 16–17, 18, 80
cholinesterase 7, 9, 32, 82
chronobiological influence **14–15**
chylomicronemia 15
chylomicrons **57, 76**
cigarettes **12**
circadian rhythm **14–15**
cis-platinum **82**
citrate **16,** 30, **34, 52–53, 55**
citrate buffered **52, 60**
citric acid/theophilline/adenosine/dipyridanole (CTAD) **55**
clinical chemistry **56–57**
clonazepan 70
clot activators 20
clot formation **32**
clotting factors 7, **32**
caffeine 12
coagulation **52–53**
cocaine 69
coffee 12
cold agglutination **78**
color codes **34–35**
competition for binding sites 83
complement activation 50, 59
complement components 59
condensation **40**
contamination 16–17, **69**
contamination control in molecular biology **64–65**
contamination with the anticoagulant 21
conveyer belt system **45**
copper 7, 12, 78
corrosive chemicals **48–49**
corticotropin (ACTH) 9, 15, **58**
cortisol **9**, 13, 14–15, 17, 58, 82
cotinine 12
CO_2 57, 66
C-peptide 58, 59
C-reactive protein (CRP) 11, 16
creatine kinase 6–10, 19, 32, 40, 77, 80
creatinine 2–3, 7–9, 16, 19, 28, 29–30, 37, 57, 77, 80

Samples: From the Patient to the Laboratory. W. G. Guder, S. Narayanan, H. Wisser, and B. Zawata

ISBN: 978-3-527-30981-8

creatinine clearance *7*, 11
cristals in urine 30
cryoglobulins **78**
cyclic AMP 12
cyclophospamid **82**
ciclosporine A (CsA) 69, *71*
cylindrical robot **45**
cytotoxicity **82**

D

date of birth 24
D-dimer 53
deproteinization **56**
desipramine *71*
detergents **28, 63**
dextran **16**
diabetes mellitus **2**
diagnostic and therapeutic intervention **16**
diagnostic and therapeutic procedures **14–15,** 16–17
diet **8, 84**
diethylpyrocarbonate (DEPC) 64
digitoxin 68, *71*
digoxin 68, *71*, 82
digoxin-like immunoreactive substances 69
disopyramide *70*
direct bilirubin 32
direct potentiometry 56
disposal **46–49**
disposal of chemicals **48–49**
disposal of needles **46, 48**
disposal of specim **47–48**
disposal of sharp objects **46–47**
distribution of sample **42–43, 84**
diurnal changes **58**
diurnal variation **14–15**
DNA (desoxyribonuclein acid) **62–65**
documentation **85 – 86**
dopamine 32
drinking 8
drug colour chart **83**
drug effects and interferences **82–83**
drug monitoring 15, 31, **68–71**
drugs **68–71, 82 – 83**
"dry chemistry" **56**
duration of storage **36**

E

edema formation **18**
EDTA (ethylenediaminetetraacetic-acid) 2, **34,** 50, **54–55, 58–59, 61, 62, 68–69**
EDTA-aprotinin mixture **53**
EDTA dependent antibodies **78**
EDTA plasma 58
EGTA **59**
effect of light **40**
electrolytes 56, *57*, 66
elimination half-life of drugs **70–71**
endorphin 58
β-endorphin 58
enzyme induction **82**
eosinophils 15
epinephrine 9, 12–13, 15, 18
ergometry 16
European standard on sample transportation 39
erythocyte sedimetation rate *7*
erythocytes *7*, 18–19, *37*, *78*
estriol 11
estradiol 13
ethanol **13**
ethosuximide *70*
evaporation **40**
exercise **9–10,** 14, 16, **19**
explosive chemicals and waste **48–49**
extraction **84**

F

factor VII *53*
factor VIII *53*
factor XI *53*
factor XII *53*
femoral artery 21
ferritin *7*
fibrin degradation products (FDP) *53*
fibrin monomers *17*, 41
fibrinogen 12, 16–17, **32–33,** *53*
fibrinolysis **53**
FICOLL-HYPAQUE **60–61**
finger puncture 22–23
first morning urine 28
flammable waste **48–49**
flow cytometry applications **61**
fluoride 26, **34–35**
folate 13
folic acid 40
follitropin (FSH) 58
food depriviation **8**
food intake **8**
free fatty acids 8, 13
free hemoglobin 81
freezing of blood **69**
fructose **16**
fungi **73 – 74**

G

gas diffusion **40**
gas exchange **67**
gastrin 58
gastro-inestinal tract surgery 16
gel barrier 60–61
gender **7**
gene rearrangements 62
genes **62–65**
gentamicin *70*, **82**
γ-globulin **16,** 33
glas capillaries **67**
glas syringes **67, 69**
glomerular filtration rate *7*
glucagon 8–9, *59*
glucose 2–3, *7*–9, 12–13, **16–17,** 19, 21, 30, 36, 40, *57*, *77*
glucose tolerance 8, 14
glutamate dehydrogenase 13
glutamine hydrolysis **32**
γ-glutamyltransferase *7*, 9, 13, 19, 32, *37*, 80, 82
glutathione **59**
glycerol 8, 12
glycolysis **40, 67**
glycolytic inhibitors 21, **34–35**
glycoproteins IIIA, IIb *79*
gold standard **5, 87**
granulocyte lysis **80**
granulocytes 6, 61
granulocyte viablity 61
growth hormone 58
guanidinium isothiocyanate **63**
gut glucagon 58

H

haematin **62**
haematocrit 9, 11, 12, 18, *37*, **52, 56**
haematology **54–55**
haemoglobin 6–*7*, 11, 15, *17*, 18–19, *37*, **80 – 81**
haemoglobin A1c 3–4, 82
haemolysis **33,** 50, **80 – 81, Annex**
haemostasiology **52–53**
hazardous waste **48–49**
HDL-cholesterol 6–*7*, 12, 18, 80
heavy metals 12
heparin **34, 58–59, 60–61,** 62, **66,** 68
heparin interference **33, 62**
heroin 13
heterophilic antibodies **79**
high fat diet 8

human leucocyte antigen (HLA typing) 60
hydrochloric acid (HCL) **30**
5-hydroxy indolacetic acid 30
hydroxyproline in urine *7*
hydroxybutyrate 8
hydrolysis of glutamine **32**
hyperlipidemia **76 – 77**
hypotonic lysis **61**
hypoxia 10

I

icteric sample **Annex**
identification of patient **20, 50, 84**
identity of samples **24–25, 41**
imipramine *71*
immune hematology **50–51**
immunoassays **59**
immunocytology *27*
immunoglobulin A 18
immunoglobulin G (IgG) *7*, 18, *27*, 41
immunoglobulin M 18
indirect sample identification **25**
infectious substance label **38**
influence of temperature on serum analytes **36**
information flow **24**
infusion site *17*
infusions **16–17**
injections **16**
inorganic phosphate 8–9, 16, 19, 29–30, 32, 36, 42
insulin 8–9, 13, 16–17, *58*
interference **33, 76–77, 80 – 81**
interference factors **5**
iodoacetate **35**
ionising radiation **16**
ionized calcium 66–*67*
ion release from blood cells **67**
iron *7*, 15, 32, 82
isometric exercise 9
isopropanol **22**
isotonic exercise 9

J

jointed robot **45**

K

ketone bodies 8–10

L

labeling **80**
laboratory computer 24–25
lactate 8, 10, 13, *17*, 19, 21, *27*, 32, 40, **67,** 82
lactate dehydrogenase (LDH) 19, 32–33, *37*, 42, 43, 80
LDL-cholesterol 6–*7*, 12, 18, 41
lead 12
leucocytes 18–19, *27*, *37*, *78 – 79*
leupeptin **59**
lidocaine *70*
light effects **40**
lipase 13
lipemia **76 – 77, Annex**
lipemic sample **76 – 77, Annex**
lipids *57*
lipoprotein(a) 12
lipoprotein electrophoresis 41
lipoprotein X 41
lithium 32, *71*
long term starvation **8**
lutropin (LH) *58*
lymphocyte separation 60–61
lymphocytes 6, 12, 60, *78*
lysis of red blood cells **62**
lysis of the cells **32**

M

macroamylase *79*
macro creatinine kinase (CK) *79*
macroenzymes **79**
magnesium 29–30
magnesium phosphate 41
mailing of blood **36–37, 38–39**
malnutrition **8**
marathon race **9**
material safety data sheets (MSDS) **49**
Mean cellular (corpuscalar) volume (MCV) 12–13, *37*, *54*, *78*
meal 8, **15**
menstrual cycle **14**
mental stress **16–17**
metabisulfite **59**
metabolic acidosis 13
metabolism of blood cells **67**
method-dependent interference **33, 81**
methotrexate *71*
microbiology testing **72 – 75**
microbiology in CSF 26–*27*, *73*
micro blood collection sets (disposal) **47–48**
microcollection technique **23**
microcollection tubes **42–43**
mid-stream urine **29**
mitochondrial DNA 62
mixing *55*, *67*, **84**
mixing bar ("flea") 66
mixing of deep-frozen samples **41**
mixing of specimen **20, 67**
molecular biology **62–65**
mononuclear cells 61
monocytes 6, 12, 60–61
morphine 13
murine monoclonal antibodies **79**
muscle mass *7*, **10**
mycological testing **73 – 74**
mycobacteria 29
myoglobin *7*

N

Na^+, K^+-ATPase **57**
nafamostate mesylate **59**
needle disposal **46–48**
needle reshielding **47**
netilmicin *70*
neuron specific enolase 32
neurotensin 13, *58*
neutrophils 12
neutrophil morphology *54*
nicotine **12**
nitrazepam *69*
non-ionic detergents **63**
norepinephrine 9, 13, 15, 18
nortryptiline *71*
novobiocin **82**

O

oligoclonal protein bands *27*
operations **16**
optical interference **80**
oral contraceptives **82**
oral glucose tolerance test 16, 22
order of collection **21**
osmolality 18, 30
osteocalcin 13
overfilling tubes **20**
ovulation 14
oxalate 30, **68**
oxygen (pO_2) *67*

P

pancreatic peptide *58*
pancreatic polypeptide 13
parasites **74 – 75**
parasitological testing *74 – 75*
parathyroid hormon related protein (PTH-RP) *59*
patient preparation **84**
patient's criteria of quality **85**
pepsinogen-1 *58*

pepstatin **59**
pH 8, 21, 40, *67*
pharmacological effect **82**
phenobarbitol *70*
phenol **63**
phenprocoumon **82**
D-phenylalanine-proline-arginine-chlormethylketone **53**
phenytoin *70*, 82 – 83
pH value in blood 16, 21, 66–*67*
phlebotomy **20–21**
phosphate, phosphorus 8–9, 15, 16, 22, 30, 32
phosphodiesterase 12
plantar surface of the heel 22
plasma 16, **32–33, 57**
plasma-serum differences **32–33**
plasma water *56*
plastic syringes **67, 69**
plastic tubes **66**
plasticizers **69**
platelet contamination **43**
platelet count 32, **55**
platelet derived growth factor *55*
platelet factor IV (PF4) *55*
platelet free plasma **33**
platelet lysis **80**
platelet poor plasma **33**
platelet rich plasma **33**
platelet satellism **55**
platelets **32–33, 43, 54–55,** 60, 61, *78*
pneumatic tube delivery system **36**
polyagglutination **50–51**
polymerase chain reaction (PCR) **62,** *65*
porpyrins 30, 40
position of patient **20**
post-centrifugal coagulation **33**
posture **18, 58, 84**
potassium 2–4, 8–9, 13, 15, 16–*17*, 19, 22, 29–30, 32–33, 36–*37*, 42, *57*, 66–*67*, 80 – 81
pO_2 *67*
preanalytical times **85 – 86**
pre-ovulatory increase **15**
pregnancy **7**
preservation of cells **35**
primidone *70*
procainamide *70*
proinsulin *58*
prolactin *7*, 12–13, 15, *17*
protease inhibitor **59**
protein: see total protein
protein binding of proteins **70–71, 83**
protein C *78*
protein electrophoresis 33
proteolytic enzyme inhibitor **58**
prothrombin time (PT) *17*, *52-53*, 82
pseudoagglutination 16
pseudoleucocytosis **78**
pseudothrombocytopenia **55, 79**
pseudothrombocytosis **78**
proteolytic enzyme inhibitor **58–59**
pyridoxal phosphate 12
pyruvate 8, 19
pyruvate kinase 9

Q

quality assurance **84 – 87**
quality managers **86**
quality manual **86**
quality of the sample **85, Annex**
quality of timing **85**
quinidine *70*

R

race *6–7*
radial artery 21
ratio of anticoagulant to blood **52, 54**
red cell mass 12
refrigeration **58**
renin 11, 13, 15, *17*, 18, *58*
reptilase time 16–*17*
request **84**
request form 24, **84**
reshielding of needles **47**
restriction fragment lenght polymorphism (RFLP) 62–64
reticulocytes *7*
retinol binding protein 8
rethawing effects **40–41**
ribonuclease (RNAses) contamination **64**
rimixing after storage **41, 84**
RNA (ribonucleic acid) **62–64**
robotics **44–45**
rubber closures **69**
running 9–10

S

safety aspects **46–49**
safety flow lancet 22
safety labels **49**
salicylates **78**
saliva collection **31, 69**
sample handling **43, 55**
sample identification **24–25**
sample inhomogeneity **40, 72**
sample preparation **64, 67**
sample sorter **44**
sample treatment **84**
sampling **14–15, 26–27, 52, 58, 67, 68–71**
sampling from catheters **17, 21**
satellism of platelets **55**
scalp artery 21
scheduling infusions and blood sampling **17**
seasonal influences **14**
second morning urine **28**
secretin 58–*59*
selection of vein **84**
selenium 12
serotonin 32
serum 16, **32–33**
serum osmolality 11
serum-plasma differences **32–33**
site for sampling blood 20, **22–23**
skin puncture 20, **22–23**
sleep **14**
smoking **12**
sodium 8–9, 13, 15, 19, 30, 32, *37*, *57*, 66, *77*, 80
sodium azide **30**
sodiumborate serine EDTA **36**
sodium carbonate **30**
sodium dodecylsulfate (SDS) **63**
somatotropin (growth hormone) 9, 15, *17*
somatostatin *58–59*
soybean trypsin inhibitor **53**
specimen collection **28–31, 52, 55, 66**
specimen collection bags **28**
specimen processing **42–43**
spironolactone **82**
sputum tube **74 – 75**
stability of the constituents in the sample 30, **40–41, 55, 69**
stabilising RNA **64**
standard meal **8**
starvation **8,** 15, **29**
steady-state of drugs **70–71**
stool tube **73 – 75**
storage of samples **27, 30, 40–41, 55, 57–58, 64, 67, 69, 84, Annex**
storage temperature **30, 36, 51**
streptokinase *53*
streptomycin *70*
subsamples 24
substance P *58*
supine position **18**
surgical procedures 9, **16**
sustained-release preparations *70*
swap specimen and tube **73 – 75**
swimming 9

T

tacrolimus 71
Taq DNA polymerase 62
temperature (body) 15
temperature control **84**
temperature during transport **36–37**
testosterone 15
thawing samples **40–41**
theophylline 68, 71
therapeutic drug monitoring (TDM) **68–71**
thiocyanate 12
thrombin 53
thrombin time 16–17
β-thromboglobulin 55
thrombocytosis **67**
thromboxane A_2 55
thymol **30**
thyroid-stimulating hormone (TSH) 13, 15, 17, 32
thyroxine 7, 9, 13, 15, 18, 79, 82
thyroxine-binding globulin 82
time after drug **14–15**
time after last meal **14–15**
time after last sample **14–15**
time dependent changes 14
time of sampling **14–15,** 24
time of the day **14–15**
time saving **33**
timing **14–15,** 24, **58, 68, 84 – 85**
timing of storage **84, Annex**
tobacco smoke **12**
tobramycin 70
total bilirubin 32
total protein 7, 16, 18–19, 30, 32, 77, 82
tourniquet **19, 20, 66, 84**
tourniquet application time **19**
toxic chemicals **48–49**
trace elements 57
training status **9–10,** 11
transcortin 78
transferrin 32
transfusions **16–17**
transport of diagnostic specimen **27, 36–37, 38–39, 53, 55, 58, 64, 67, 69, 84**
transport of analytes **55**
tricyclic antidepressants 69
triglycerides 7–8, 13, 18–19, 32, **56,** 57, **76 – 77,** 80
triiodothyronine (T3) 8, 32, 79
thrombocytolysis **32–33**
thrombocytosis **67**
tube and sample disposal **47**
turbidity 15, **76 – 77**
turbid samples **76 – 77**
turn around time (TAT) 44, 85 – 86
two-dimensional bar code **25**
two-dimensional dotcode **25**

U

ultracentrifugation **77**
umbilical artery 21
upright position **18**
urea 7–9, 16, 19, 29–30
uric acid 7–11, 13, 16, 19, 22, 30, 41, 73, 78
urinary creatinine 10–11
urinary excretion of calcium 18, 28
urine **28–30, 69**
urine containers **28, 84**
urine pH 30
urine preservation **30**
urine sediment 41
urine volume 7, 15
urobilinogen 30
urokinase 53

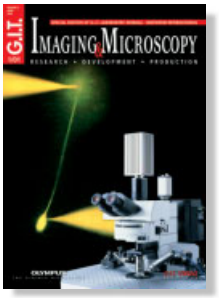
V

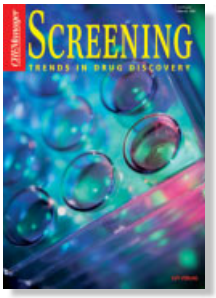
valproic acid 70, 83
vanillylmandelic acid (VMA) 13
vancomycin 70
vasoactive intestinal peptide 58
vasopressin 13, 17
venipuncture **20–21**
very low density lipoproteins (VLDL) 9, 76
viruses **75**
volume displacement effect **56**
volume errors **33**
volume of blood collected **21**
volume shift 10, **18–19**
von Willebrand factor 16

W

warfarin **82**
water displacement **77**
white blood cell differential count 54
whole blood **57, 67**
whole blood lysis **61**
workflow **44, 45**

Competence in laboratory procedures





G.I.T. "Imaging and Microscopy" offers a qualified international forum for the presentation of products, services and company. This international publication is published in English and is distributed to decision-makers and opinion leaders in industry, research and at universities in Europe.

"Imaging & Microscopy" provides up-to-the-minute coverage of applications and products in the areas of image processing, microscopy and other modern imaging processes. The methods and instruments involved, such as sample preparation and sample handling, are presented in a practical, easy-to-understand manner in an attractive layout.

Screening – Trends in Drug Discovery focuses on the latest trends and developments in the field of active substance research and trends in drug discovery. It covers all aspects of research as well as strategies of pharmaceutical companies and start-up companies. This editorial mix means that this publication is relevant reading for all those concerned with drug discovery. Internet and/or E-mail offer readers the possibility of direct contact without delay. Screening – Trends in Drug Discovery is published in English.

Management & Krankenhaus is a source of information for decisionmakers in hospitals and is also aimed at representatives of health insurance organisations, professional associations and industry, amongst others. "Networking" is the motto for the future and the constructive collaboration of all professional groups in the hospital setting is becoming more and more important. For this reason, in addition to its regular columns, each issue of M & K focus on special interdisciplinary topics, thereby offering a platform for dialogue, highlighting trends, and making visions known. The special "Pain" and "Modern Cancer Therapy" and the magazine "MedAmbiente" round of this series of journals. Published in German.

The well-established, pan-European bi-monthly journal, EUROPEAN HOSPITAL, is aimed at decisionmakers in healthcare organisations throughout the EU and those in greater Europe. Highly varied editorial coverage includes news of clinical & technological advances, administration and healthcare politics, financial strategies, exciting innovations, plus interviews with scientists working in R & D, as well as people on the cutting edge of healthcare, manufacturing, and policy making. EUROPEAN HOSPITAL is published in English.

GIT VERLAG **Roesslerstrasse 90 · 64293 Darmstadt · Germany · Tel. (+49) 61 51/80 90-0 · Fax (+49) 61 51/80 90-146**
www.gitverlag.com

Samples: From the Patient to the Laboratory. W. G. Guder, S. Narayanan, H. Wisser, and B. Zawata

ISBN: 978-3-527-30981-8