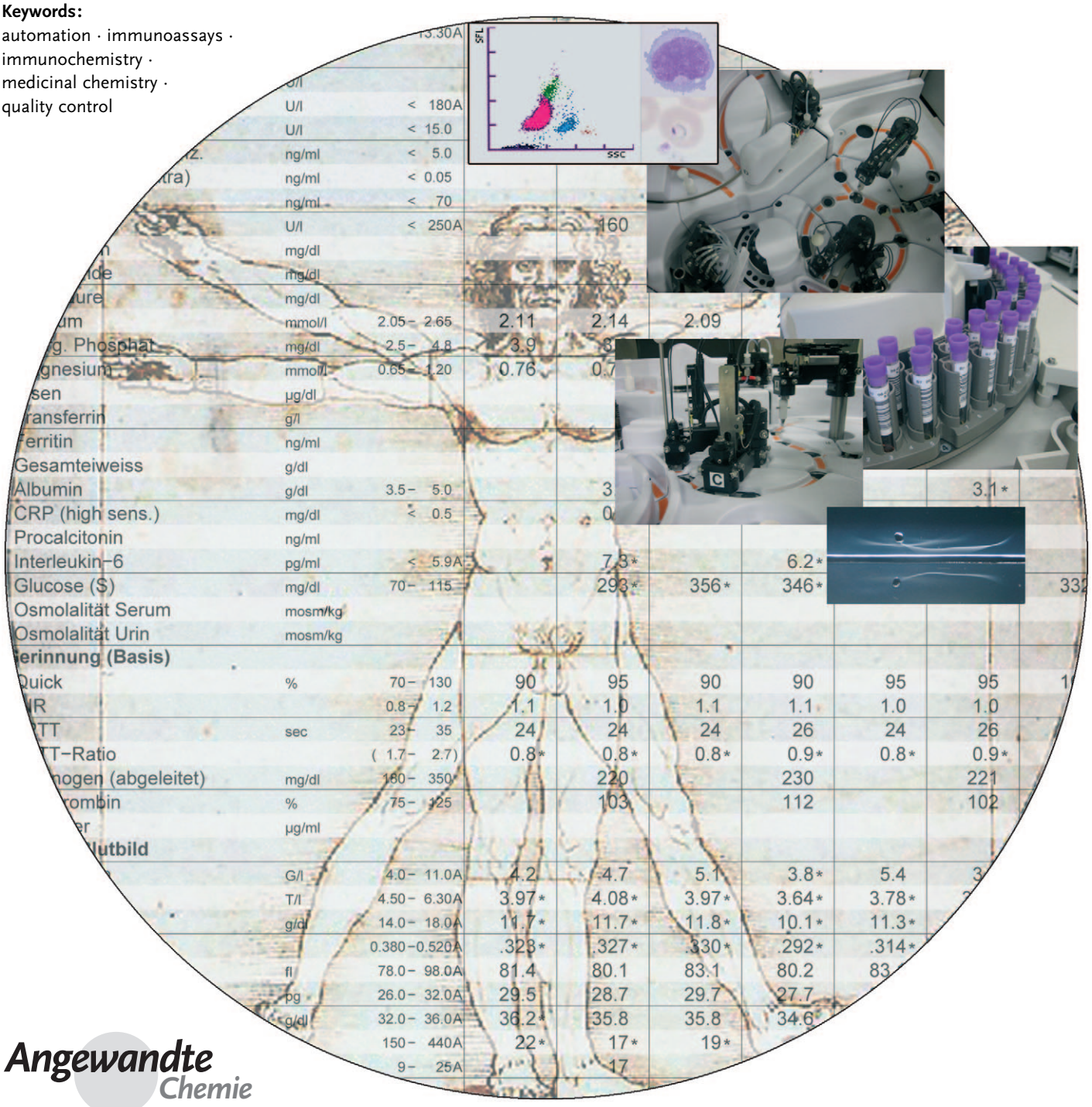
Clinical Chemistry

DOI: 10.1002/anie.200903363

Clinical Chemistry: Challenges for Analytical Chemistry and the Nanosciences from Medicine

*Jürgen Durner**

Keywords:
automation · immunoassays · immunochemistry · medicinal chemistry · quality control



Clinical chemistry and laboratory medicine can look back over more than 150 years of eventful history. The subject encompasses all the medicinal disciplines as well as the remaining natural sciences. Clinical chemistry demonstrates how new insights from basic research in biochemical, biological, analytical chemical, engineering, and information technology can be transferred into the daily routine of medicine to improve diagnosis, therapeutic monitoring, and prevention. This Review begins with a presentation of the development of clinical chemistry. Individual steps between the drawing of blood and interpretation of laboratory data are then illustrated; here not only are pitfalls described, but so are quality control systems. The introduction of new methods and trends into medicinal analysis is explored, along with opportunities and problems associated with personalized medicine.

From the Contents

> "*I feel it strongly and I am convinced that the efforts of chemistry will change the face of medicine.*[1]"
> Antoine François de Fourcroy

1. Introduction

1.1. Current Status of Clinical Chemistry in Medicine

There has been a fundamental transformation in medicine over the course of the last 20 years: Originally, laboratory parameters were determined only selectively, and usually during the course of therapy; laboratory analysis was intended to confirm a tentative diagnosis based on clinical or radiological information. Today, on the other hand, thanks to a broadening of the spectrum of analysis, increased sensitivity of detection methods, and a much higher informative value of the individual parameters, these determinations are made much earlier. In many cases, laboratory studies serve to provide diagnoses or to detect increased risks of disease. Moreover, for physicians with clinical responsibilities, the determination of laboratory parameters has become indispensable for monitoring the progress of therapy (decrease in signs of infection or decline in tumor markers) and early detection of side effects and complications (monitoring of liver and kidney parameters during chemotherapy). In this way, clinical chemistry definitively influences the targeted course of treatment as well as the quality of medical achievement—and this to an increasing extent on an individual basis, namely, tailored to the specific patient.

With respect to the most prevalent illnesses in society today—coronary and circulatory disease, cancer, and metabolic problems (above all, diabetes mellitus)—important roles are played not only by treatment but also by early diagnosis and the establishment of heightened risk (preventative medicine). Here laboratory medicine provides a support that is gaining in importance, since disease can be counteracted even at a preclinical stage (that is, before the first appearance of symptoms). Early diagnosis is clearly decisive not only for those affected and their future lives, but also—with a view toward the costs resulting from health care—for society at large. Both an illness itself and other diseases that result can run up enormous costs.

Modern diagnostic methods can often shorten or even eliminate hospital stays. Requested laboratory parameters may, for example, in patients with elevated temperatures and/or breathing difficulties, help distinguish a coronary insufficiency from a pulmonary embolism or pneumonia, and perhaps rule out one or the other. On the basis of physical measurements it may be possible to decide whether follow-up studies should be initiated—which may entail costly imaging or invasive procedures—or if hospitalization or even intensive care is required. Furthermore, decisions may be made about the extent to which medication (for example, antibiotics) should be administered. Certain laboratory parameters have shaped the characterization and management of disease, as with troponine in the case of myocardial infarction.

1.2. The Functions of Clinical Chemistry

According to the Sanz and Lous definition for the International Federation of Clinical Chemistry (IFCC), clinical chemistry is responsible for clinical-chemical analysis in the qualitative and quantitative description of a (patho)-physiological state. It is an integral part of diagnosis, therapy, and monitoring, but also the prevention of disease as well. To this comes also the introduction of new laboratory methods.[2] This definition was revised and extended by the IFCC in 1995. Accordingly, clinical chemistry is responsible for the application of chemical, molecular, and cellular strategies (concepts) and techniques for the better understanding and assessment of human health and disease. It encompasses the fields of hematology and hemostaseology, but is distinct from microbiology, virology, serology, histology, and cytology.

[*] Dr. Dr. J. Durner
Universitätsklinikum München
Institut für Klinische Chemie
Ludwig-Maximilians-Universität München
Marchioninistrasse 15, 81377 München
Fax: (+49) 89-7095-3240
E-mail: juergen.durner@med.uni-muenchen.de

1.3. Beginnings of Clinical Chemistry

The history of scientific medicine has been shaped by progress in physiological and organic chemistry. Hippocrates of Cos (ca. 460–370 BC) studied the appearance and the odor of feces. The first systematic medicinal laboratory diagnosis has been attributed to the twin brothers Saints Cosmas and Damian who, according to legend, succeeded in replacing a gangrenous leg with the leg of an Ethiopian who had recently died.[3] These two physicians were sentenced to death, in part as a result of their activities, but also for their conversion in the year 289 to Christianity.

Physicians attempted to derive information about the presence of disease through "laboratory chemistry", with experiments on blood (blood letting, hemoscopy) and especially urine (uroscopy). Hemoscopy was already being practiced by Hippocrates. Hemoscopic blood was collected in vessels to observe the possible formation of a "Crusta phlogistica" ("buffy coat"), which was regarded as signifying an inauspicious prognosis. Uroscopy reached its high point in the Middle Ages. An attempt was made to draw conclusions about the site of a disease within an organism on the basis of where the urine displayed turbidity in a urine bottle. Up until the middle of the 19th century, medicine was shaped by natural philosophy, which resulted in a host of different—and to some extent mutually contradictory—doctrines, such as the irritability school of Glisson (1597–1677) and Haller (1708–1777), which was diametrically opposed to the vitalism of Hufeland (1762–1836). Parallel to the latter, although differing in medications, was the homeopathic law of similarities championed by Hahnemann (1755–1843).[4]

The notion that organs were the site of diseases, and that it was essential to study the anatomy of organs, had its origin in the work of Morgagni (1682–1771).[5] The chemist and physician de Fourcroy (1755–1809) strove to integrate chemistry into medicine.[6] A new interpretation of the idea of "disease" appeared toward the end of the 18th century: no longer were individual symptoms in the foreground, instead these were replaced by the characteristics of a disease (clinical symptoms—signs that could be objectified, such as changes in the skin). A new concept was introduced to facilitate the study of these characteristics: large clinics were established, in which a great many patients with the same disease could be observed and studied, including by laboratory means.[7] The basis of this advance was recent headway in laboratory analysis, founded on such groundbreaking work as the elemental analysis of Gay-Lussac (1778–1850), Thenard (1777–1857), von Berzelius (1779–1848), and von Liebig (1803–1873).[8,9] By 1840, approximately 1400 organic compounds were known, and the period around 1840 is regarded as the point of origin of the discipline "clinical chemistry", since, especially in the German-speaking world, it was then that the first textbooks, handbooks, and journals appeared, scientific societies were organized, and independent university facilities were erected (for example, in Berlin, Würzburg, and Vienna). Clinical chemistry was practiced somewhat earlier in England and France, but there was no institutionalization of the subject at that time. It also became clearer at an earlier date in the German-speaking world that the subject owed its roots to chemistry, for which reason it was occasionally referred to as "pathological chemistry". In England and France, by contrast, the roots were perceived to be in medicine, thus leading to the alternative term "chemical pathology". The euphoria associated with the new discipline lasted only about a decade, however, since the chemical tools available (Table 1) for analysis and diagnosis were at first overrated, and also it became apparent how little was known about the physiobiochemical contexts ("a pathological chemistry with no physiological basis").[10] Moreover, chemists were dedicating themselves increasingly to organic synthesis. Many clinical laboratories served mainly for research purposes, with chemists leaving clinical work to the physicians. Bacteriology and parisitology developed toward the end of the 19th century, from which it was anticipated that results would be achieved more rapidly with respect to diagnosis and therapy of diseases.[4]

The discipline became established in the USA around the end of the 19th century, as biochemical analytical techniques were incorporated into the academic training of physicians.[11] The possibility to quantify analytes, as illustrated by the development of glucose determinations in urine (Table 2), as well as further advances in biochemistry and the construction

Table 1: Analytical instruments in early clinical laboratories. Modified from Ref. [13].

Analytical instrument	Examples
balance	apothecary's balance, microscales (< 1 mg)
volumetric instruments	graduated cylinder, volumetric flask, pipettes, burettes (as per Gay-Lussac and Mohr)
instruments for density measurement	hydrometer, "urine sampler"
instruments for gas analysis	gas pipettes, gas absorption tube
microscopy with analysis equipment	ocular micrometer, ocular goniometer
polarimeter	Biot/Soleil and Mitscherlicht
spectroscope	Kirchhoff and Bunsen
colorimeter	Duboscq
photometer	Vierordt, Hüfner, and Pulfrich

Jürgen Durner studied chemistry at the Munich Technical University and medicine at the Ludwig-Maximilians-Universität in Munich (LMU). He received his PhD in chemistry with Prof. Wolfgang A. Herrmann in 2000 after a stay at the TNO Prins Maurits Laboratorium Rijswijk (Netherlands), and his medical degree in 2005 with Prof. Ekkehard D. Albert. Since 2005 he has worked at the Institute for Clinical Chemistry of the LMU in Grosshadern. Besides routine diagnostics, he is concerned with the evaluation of analytical apparatus, disorders in lipid metabolism, and directs a lipid apheresis outpatient clinic.

Table 2: Glucose determination in urine: development of assay techniques.[a] Modified from Ref. [13].

Discoverer	Year	Nature of the assay	Characteristics	Results
Willis	1674	taste test	sensory sensation	sweet taste
Bobson	1776	extraction	chemical detection	quantitative: detectable or not detectable
Home	1780	fermentation		
Trommer	1841	chemical test		
Vogel	1858	chemical test	semiquantitative chemical test	reactivity: not detectable, +, ++, +++
Bouchardat	1841	polarimetry	quantitative analysis	concentration report
Heintz	1843	polarimetry		
Fehling	1848	titration		
Einhorn	1885	fermentation		

[a] "Diabetes" means "allow to pass through", and "Mellitus" means "honey-sweet"; Diabetes mellitus therefore means essentially "honey-sweet discharge".

of new laboratories during the First World War added new impulses to the discipline.[5] After the Second World War the internationally uniform designation "clinical chemistry" was introduced by the American Association of Clinical Chemists (AACC) and the IFCC.[12]

1.4. Automation of Analysis through Enzymatic Colorimetry

In the second half of the 20th century, enormous advances in the fields of biochemistry, technology (mechanics, optics, hydraulics, electronics, data processing), and methodology, as well as greater sensitivity and more specific analyses, transformed clinical chemistry into an important and indeed indispensable component of medical diagnostics.[14,15]

The need for automation in the field of clinical chemistry is illustrated by the following example: the clinic of the University of Munich (LMU) operates at two sites, in which about 83000 inpatients and 371000 outpatients are treated annually. The 44 specialty clinics, institutes, and divisions involved provide a total of more than 2400 beds. At the Grosshadern facility alone, there are between 15000 and 18000 individual analytical values to be determined every weekday. Apart from the need for standardized sample logistics, this presents a challenge from the standpoint of the analytical instrumentation. Rapid mastery of this workload was achieved in large measure through advances in enzymatic colorimetry, which is the basis for many routine detection methods.

Purely colorimetric procedures for analyte quantification are often nonspecific, since other substances can influence a color reaction, and thereby falsify the result. Starting in 1928, Warburg (1883–1970) developed photometric methods for use in his enzyme research. The first devices for routine analysis that took advantage of enzymatic colorimetry based on Warburg's efforts started to be marketed in 1955. Data derived from an enzymatic reaction were plotted on paper calibrated with a millimeter grid, and the slope of the best-fit line was established with a protractor. From the resulting angle and a conversion table, the enzyme activity could be established. The procedure was time-consuming and costly in terms of personnel. Serial analyses were especially arduous. Further automation presupposed an automatic pipetting system, a mechanical drive arrangement for the cuvettes, and an analogue/digital converter for the output signal. An American by the name of Skeggs was heavily involved in the development of an automated analyzer.[14] The first completely automatic determinations of urea, glucose, and calcium were demonstrated at the International Congress on Clinical Chemistry in 1956.[15] The most demanding challenge in producing a fully automatic analytical system was the photometer: it was necessary to obtain data at about ten different wavelengths to cover the complete spectrum of clinical-chemical parameters. For this reason, batch analyzers were initially employed, that is, a single parameter was first measured on up to 100 samples, after which the system was adjusted for the next parameter. A major breakthrough occurred in the early 1980s with the introduction of a new photometric principle: the diode-array spectrophotometer. Light from a high-pressure xenon lamp was passed first through a sample cuvette and then with the aid of a polychromator (grating monochromator, prism) split horizontally into its component wavelengths and spread out. Photodiodes for the various wavelengths of interest were distributed along this expanded light sector. Multiple wavelengths from a sample could, therefore, be analyzed simultaneously, thereby making it possible to take measurements at as many wavelengths as desired from a fixed spectrum, with work cycles of less than ten seconds.

Another milestone in routine analysis was the introduction of the immunoassay. Groundbreaking efforts from Yalow and Berson on the determination of peptide hormones by a radioimmunoassay technique go back to the 1950s, work for which Yalow received the Nobel Prize in 1977.[16] Further improvements and the introduction of a number of variants on the immunoassay technique have led to dramatic increases in its significance in recent years. The use of monoclonal antibodies has extended its effective range into the pmol L^{-1} region. In this way, immunoassays have made possible the determination, for example, of hormones, tumor markers, and medication levels.

2. Medicinal Laboratory Diagnostics and Quality Control

Medicinal laboratory diagnostics encompasses the fields:

- clinical chemistry (incl. hematology, hemostaseology),
- immunology (incl. allergology, immunogenetics, immunohematology),
- microbiology (incl. bacteriology, mycology, parasitology, infectious disease serology, molecular biology),
- virology (incl. infectious disease serology, molecular biology),

- human genetics (molecular human genetics, cytogenetics, tumor cytogenetics),
- pathology (incl. neuropathology, histology, cytology),
- transfusion medicine (incl. blood-group serology, transfusion serology),
- hospital hygiene,
- functional studies on patients (for example, determination of bleeding time, ^{13}C-breath tests, H_2-lactose breath tests, oral glucose-tolerance test, pancreolauryl test, xylose test).

In the following, only clinical chemistry (general clinical chemistry, serology immunochemistry, protein chemistry, endocrinology, metabolic chemistry, and drug monitoring), including the fields of hematology and hemostaseology, will be considered.

Over 6000 experimental procedures with subspecifications are available for analytical purposes. A selection of important methods has been assembled in Table 3.[17–23] The material for study in clinical-chemical laboratories is very diverse, depending upon its origin and nature. Quantification may need to be conducted starting with, for example:

- bodily fluids, such as blood, saliva, cerebrospinal fluids, gastric juices, bile, duodenal juice,
- excretions, such as sweat, feces, sputum, urine, pus,
- tissue samples, such as bone marrow.

Table 3: Choice of analytical procedure for the routine determination of analytes.

Spectrometry:

- absorption spectroscopy/photometry
- UV/Vis/NIR/IR spectroscopy
- atomic absorptions spectroscopy (AAS)
- atomic fluorescence spectroscopy (AFS)
- NMR spectroscopy
- luminescence spectroscopy: bioluminescence measurement, chemiluminescence, fluorescence, time-resolved fluorescence, fluorescence polarization and phosphorescence spectroscopy
- nephelometry/immunonephelometry
- turbidimetry/immunoturbidimetry
- atomic emission spectroscopy (AES)
- flame emission spektroscopy
- ICP mass spectrometry (ICP-MS), MALDI-TOF-MS

Blood cell counts:

- impedance measurement
- flow cytometric cell count determination with cytometric or cytochemical-cytometric cell classification
particle property determination with automated processing (particle counting and particle size determination of blood cells)
- immunophenotyping of haematopeoetic cells (flow cytometry)

Ligand assays:

- enzyme immunoassay
- fluorescence immunoassay
- immunoblot (Western blot)
- luminescence and electrochemiluminescence immunoassay (CLIA/ECLIA)
- fluorescence polarization enzyme immunoassay
- radioimmunoassay
- receptor assay

Electrochemical studies:

- amperometry: O_2 partial pressure (Clark electrode)
- potentiometry: pH value, CO_2 partial pressure, ion-selective electrodes
- coulometry
- voltammetry

Chromatography:

- thin-layer chromatography (TLC)
- liquid chromatography (LC)
- gas chromatography (GC) and GC-MS
- high-performance liquid chromatography (HPLC) and HPLC-MS

Molecular biological methods:

- (real-time) polymerase chain reaction
- Southern blot
- fluorescence in situ hybridization (FISH)
- evidence for single-nucleotide polymorphisms (SNPs) with, e.g. restriction fragment length polymorphisms (RFLPs), FRET probes (FRET: fluorescence resonance energy transfer), density gradient gel electrophoresis (DGGE), denaturing HPLC (DHPLC)

Electrophoresis:

- zone electrophoresis-cellulose acetate; electrophoresis
- immunoelectrophoresis/immunofixation
- isoelectric focusing
- pulse-field gel electrophoresis
- counterimmune electrophoresis (countercurrent electrophoresis)
- isotachophoresis
- capillary electrophoresis
- rocket electrophoresis

Other procedures:

- aggregometry (thrombocyte function tests: impedance aggregometry: PFA 100, ROTEM)
- filtration (adsorption filtration, membrane filtration, ultrafiltration)
- immunohistochemistry
- microscopy (light and dark field, fluorescence, and phase-contrast microscopy)
- qualitative studies with visual evaluation (e.g. osmotic erythrocyte resistance)
- reflectometry/carrier-bonded methods of analysis
- rheology, viscosimetry
- areometry
- X-ray diffraction (concretion analysis)
- coagulometry
- osmometry: cryoscopy, vapor-pressure osmometry
- sedimentation studies (erythrocyte sedimentation rate)
- radioactivity measurement
- centrifugation: analytical ultracentrifugation, density-gradient centrifugation

Analytical results from the sample types listed above make an important contribution toward diagnosis and therapeutic determination. High quality and reliability with respect to the findings is thus indispensable. To standardize the process and also provide transparency, as well as to exclude sources of error and to ensure that laboratory findings are correct and reliable, some system of quality control and management is indispensable.

The history of quality assurance in medicine had its origin in 1835 with the estimation of measurement errors through multiple determinations on the same sample (precision), and is closely associated with the name Quételet (1796–1874).[17,18] Medical laboratories and manufacturers of medical products can become accredited today if they fulfill certain legally and normatively defined principles. Such a foundation leads to a well-functioning system of quality control. This can be achieved in general and for all disciplines if the requirements of Norm DIN EN ISO/IEC 17025 are fulfilled. For laboratories devoted to human medicine, there is the further opportunity to meet the demands of Norm DIN EN ISO 15189 "Medizinische Laboratorien—Spezielle Anforderungen an Qualität und Kompetenz" ("Medical Laboratories—Particular Requirements for Quality and Competence"), an extension of DIN EN ISO/IEC 17025 for medical needs. There exist further norms as well, some of which are national in nature.[19] The goals are to achieve an accrediting procedure that is uniform and harmonized worldwide, to raise the level of quality, and to assure international comparability of laboratories.[27–29] The cited norms are intended to regulate in particular:[30,31]

- the disciplinary competence of laboratories (laboratory leadership, staff),
- structured and scheduled instruction, continuing education, and advanced training of staff,
- requirements with respect to documentation,
- internal and external quality-control systems,
- standards and measures for continuous in-house quality optimization (preemptive and corrective measures, causal analyses),
- internal and external auditing procedures,
- requirements with respect to outfitting of laboratories (apparatus, reagents, materials),
- unambiguous and intelligible instructions for pre-analysis, analysis, and postanalysis,
- standards regarding the data-processing systems utilized,
- improved orientation for patients and submitters.

Accreditation, once granted, is valid for five years. During this five-year period, monitoring is, as a rule, carried out annually. The purpose of such monitoring is to verify that the management system has in fact been implemented, and to determine whether the conditions for accreditation still apply, particularly disciplinary competence. If so desired by the laboratory, approximately six months before expiration of the accreditation period, a review will be conducted for the purpose of accreditation renewal.

3. The Phases in Analysis

The isolation and transport of the material of interest, analysis, and medical interpretation, followed by conveying the results to the submitter represents a complex process (Figure 1). Three phases can be distinguished prior to the generation of final results: [20]

1. pre-analysis,
2. analysis,
3. postanalysis.

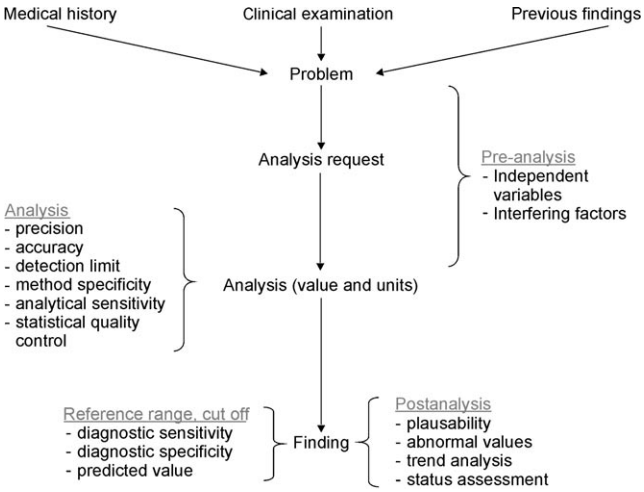


Figure 1. Pathway leading from the posing of a question to a clinical-chemical discovery.

The evaluation of an internal quality study of the medical laboratory of the University of Padua revealed that the introduction of a quality-control regimen resulted in the number of errors being reduced from 4667 to 3093 per million individual determinations over the course of a period of ten years, with an annual analytical load of 8000000 discrete values.[21] In this case, 61.9% of the errors originated in the pre-analysis phase, 15.0% in the analysis itself, and 23.1% in the postanalysis.

Unequivocal assignment of a sample and the corresponding measured data to a specific patient must be assured during the analytical phase. A common error in pre-analysis is a so-called mixing up of patients, namely patient data (patient name, date of birth, patient identification number) on the sample vessel and the sample itself do not correspond to the same patient.

3.1. Pre-analytical Phase

The term "pre-analysis" or "pre-analytical phase" encompasses all the administrative and functional factors and processes that occur prior to laboratory analysis, namely preparation, isolation, work-up (for example, separation of corpuscular components through centrifugation), storage, and transport of materials for clinical laboratory investigation.[22] In addition, it is helpful if information regarding the patient is

Table 4: Classification of independent variables, with examples; modified from Ref. [24,25].

Collection factors	Individual factors		
	permanent factors	long-term operative factors	short-term operative factors
body position		*endogenous factors:*	
length of the delay	sex	age	biorhythm
localization of the removal	ethnic idiosyncrasies	weight	pregnancy
time of day	interindividual variation	diseases	lactation
	genetic factors		
		exogenous factors:	
	geographic idiosyncrasies	drugs	Diet
		lifestyle habits	physical stress
		muscle mass	stimulants
		socioeconomic status	pharmaceuticals (or drugs)
		climate	diagnostic measures
		special exposure	operations

passed along to the laboratory so that analytical results, especially in areas bordering on medicine, will be properly interpreted (pregnancy, kidney deficiency, conditions following organ transplant).

Variability and sources of error in the pre-analytical phase are independent variables and interfering factors. All in vivo changes to the analytes in question are subsumed under "independent variables". A distinction is made between endogenous and exogenous independent variables. Endogenous independent variables are unalterable characteristics of an individual or unalterable biological circumstances. Exogenous independent variables encompass all alterable quantities that apply to an individual. In both cases, factors with permanent effects and those with long-term or short-term applicability are differentiated (Table 4). Interfering factors refer to in vitro changes, such as effects on the analytical process through, for example, interference in the analytical procedure as a result of drugs, icteric discoloration of the sample (transfer of gall coloring matter such as bilirubin into blood), hemolytic samples (destruction of erythrocytes and release thereby of hemoglobin), or lipamic samples (microscopically small lipoprotein particles in serum; Figure 2).

Most errors arise in the area of pre-analysis.[23] For this reason, it is important that patients receive precise instructions regarding what should be observed prior to an impending blood draw (for example, no physical exertion before a creatine kinase determination, no cycling or horseback or motorcycle riding before a determination of prostate-specific antigen). Equally important is advising the submitter (physician's practice, hospital ward) regarding patient preparation, proper sample removal and the right type of sample, as well as storage and transport (cooled transport, influence of light).[21]

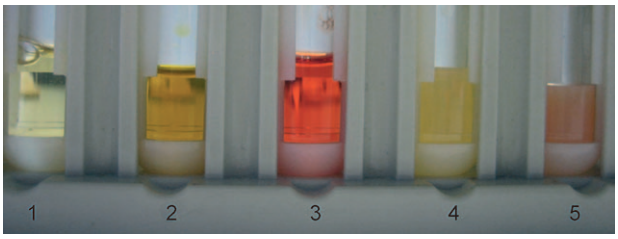


Figure 2. Various sera: 1) Normal coloration, 2) coloration with icteric metabolic state (collection of bile pigments), 3) serum in the presence of hemolysis, 4) serum with lipemia, 5) serum with hemolysis and lipemia.

3.2. Analytical Phase

3.2.1. Basic Considerations and General Remarks

The analytical phase covers taking aliquots and the general preparation of an analytical sample, the analysis itself, and acquisition of the appropriate data value(s). Quality control in the realm of analysis is also covered under DIN EN ISO 15189. Further regulations are to be found in ISO 15196 and 15198. Implementation occurs according to national guidelines. In Germany this is dealt with legally by the Medicinal Products Law (Medizinproduktegesetz), the Ordinance on Medicinal Products Distributors (Medizinproduktebetreiberverordnung), and guidelines of the German Medical Association Panel for Quality Control in Medical Laboratory Investigations (Bundesärztekammer zur Qualitätssicherung laboratoriumsmedizinischer Untersuchungen: RiLi-BÄK). These define quality-assurance measures (internal and external quality controls) specified for quantitative analysis of 67 measured parameters for serum/plasma/whole blood, 7 for cerebrospinal fluid, and 10 for urine. Compliance with the guidelines is monitored regularly by the relevant control agency.

Standard operating procedures (SOPs) are an integral part of quality management. These are procedural instructions for routine sample processing as well as for the operation of instruments. SOPs must be available in laboratories in written form, and should include factors that have the potential to influence analyses, together with information on error recognition and handling. Depending upon their purpose, a distinction is made between general SOPs, apparatus SOPs, and test SOPs. General SOPs govern, for example, responsibilities in the laboratory. Apparatus SOPs cover steps specific to pieces of apparatus, such as operation, calibration, and maintenance. Test SOPs specify details of carrying out analytical tests from the point of sampling through to the reporting of results. These may refer to self-

developed methods or standardized and/or published procedures.

One basic problem associated with the study of human samples is the extraordinarily complex and variable constitution of the samples. They may contain proteins (albumin, rheumatoidal factors, enzymes, lysozyme, complement systems, autoantibodies, endogenous hormones, and their binding proteins, as well as abnormal forms of the latter), carbohydrates, lipids, cellular components, electrolytes, and pharmaceuticals and their metabolites. The effects that these different components may have on the analytical system are referred to as matrix effects. These are defined as the sum of all the effects of the components of a system, apart from the analytes.[26] Also included are all the reagents utilized in sample preparation and in the analytical procedure. The results of a measurement can be seriously affected by these.[27]

The determination of an analyte may be either qualitative or quantitative. Results may be expressed according to scales characterized as nominal, ordinal, or differential/relational. A qualitative analysis is limited strictly to the identification of a substance, for example in a question of pregnancy prior to radiological investigation, or drug intoxication in the case of a somnolent patient brought to an emergency room. Test strips are typically available for qualitative tests (for example, urine test strips with test fields for the pH value, glucose, proteins, ketones, nitrite, erythrocytes, leucocytes, etc., on a carrier film) and immunoanalytical lateral flow assays (LFAs; Figure 3), the development of which has been accelerated through the EU-sponsored project "Allergen Test".[28] LFAs are extentions of the homogeneous latex agglutination test introduced in 1956.[40–42] These are based on the same principle as immunological assays, namely an antigen–antibody reaction. They have, in addition, chromatographic characteristics. The analyte is introduced through an opening onto a sample pad, upon which is present marked primary antibodies (conjugates) against the analyte. The antibodies are fixed on coated latex particles or colloidal gold (GLORIA technique: Gold-Labeled Optical-read Rapid Immuno Assay), or less often on selenium, carbon, or liposomes.[29] The antigen–antibody complex, along with excess conjugate, flows through the membrane system as a consequence of capillary action. As soon as the antigen–antibody complex reaches the test zone, it reacts with a secondary antibody and becomes fixed. Unbound conjugates travel to a control zone, and are bound there by so-called antispecies antibodies. The colloidal gold causes both zones to appear reddish in color. If no analyte is present, only the control line should be reddish. Excess liquid together with particles not bound on the test or control lines flows further through the membrane system until it is absorbed by filter paper, which prevents backflow. An LFA can be evaluated either visually (qualitative) or with a measuring device (semiquantitative). Nitrocellulose is often employed as the membrane material. LFAs are also of interest from a military standpoint, since they can be rapidly deployed outside of a laboratory environment, and can provide a first overview of possible contamination with bacterial toxins or other biological agents.[30]

Quantitative analysis takes advantage of routine methods (to provide adequate reliability and practicality), reference

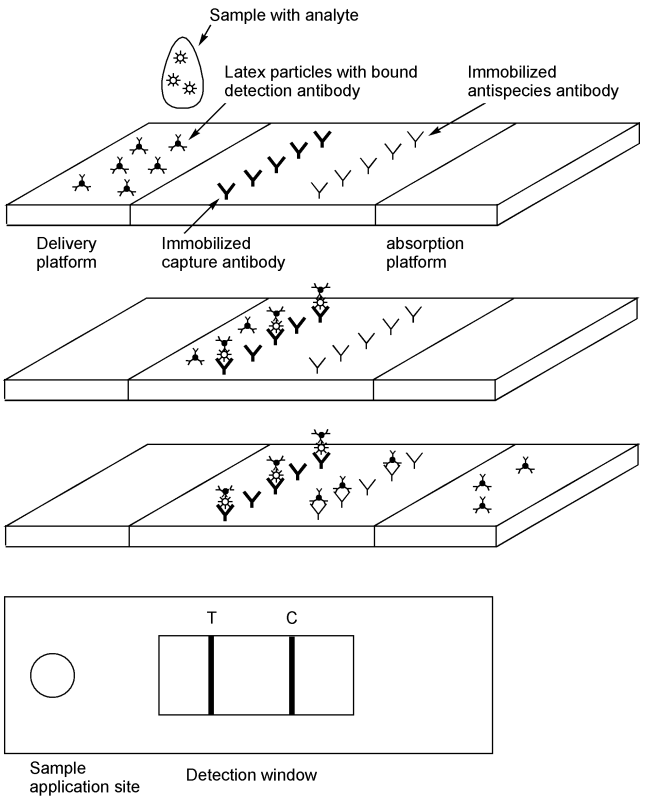


Figure 3. Mode of functioning of the lateral-flow rapid test. C: Control line, signal development with the test proceeding correctly; T: test line, signal development with a positive result.

methods (useful for the validation of other methods), and definitive methods (specific and accurate). This so-called methodological hierarchy permits inaccuracies of 0.1–1 % for definitive methods, 3–5 % for reference methods, and 5–10 % for routine methods.[31]

Analytical sensitivity expresses the ability of a particular method to differentiate between concentration-dependent signals, whereas analytical specificity describes an ability to capture exclusively the substance of interest, with no influence from other components of the matrix. Statistical quality control is the generic term for statistical procedures related to planning for random sampling and experimentation in general, for the purpose of assuring and maintaining the quality of a product, and in this way keeping a production process under control. Applying this to parameters for quantitative laboratory medicine involves controls both internal (precision and accuracy) and external (interlaboratory tests) in nature.

Participation in external quality-assurance measures (interlaboratory tests) ensures objective monitoring of the accuracy of results from quantitative laboratory investigations.

3.2.2. Selected Analytical Methods

3.2.2.1. Immunoassay

The term "immunoassay" is understood to refer to one of a series of methods that take advantage of the antigen–antibody reaction for detecting an analyte (for example, a

hormone, a protein, or a drug).[32,33] Haptens can also be employed in place of complete antigens. The principle of immunoassays is based on a competitive and cooperative interaction between the analyte to be determined and a labeled ligand (L*), which is thus measurable, and an unlabeled ligand (L), both of which occupy the same binding site on a protein (P). The labeling can be achieved with a fluorescent dye, a luminogen, a fluorophor, an enzyme, or a radioactive substance (it is incidentally also possible to measure the turbidity resulting from formation of an immune complex (see Section 3.2.2.2)). A multivalent interaction between the antigen and antibody leads to Equations (1) and (2):[34]

$$x\,\mathrm{L} \quad y\,\mathrm{P} \quad \mathrm{L}_x\mathrm{P}_y \qquad 1$$

$$x\,\mathrm{L}^* \quad y\,\mathrm{P} \quad \mathrm{L}^*_x\mathrm{P}_y \qquad 2$$

The concentration of complex $\mathrm{L}_x\mathrm{P}_y$ [$\mathrm{L}_x\mathrm{P}_y$] is obtained as a function of the concentration of ligand [L], according to the law of mass action, in the form of Equation (3):

$$\mathrm{L}_x\mathrm{P}_y \quad \frac{\mathrm{L}_x\mathrm{P}_{y\,\max}\ \mathrm{L}^{\,b}}{K \quad \mathrm{L}^{\,b}} \qquad 3$$

This relationship corresponds to a case of cooperative enzyme kinetics, and is expressed by the Hill Equation. In analogy to enzymological applications, the ligand in this case corresponds to the substrate and the protein to the enzyme, whereby *b* (the Hill exponent) is absent from the corresponding equation.

Immunoassays are distinguished by a high specificity and affinity to the antibody. A great many analytes can be determined in this way, commensurate with the large number of commercially available antibodies. Automation also makes a high throughput of samples possible. Sample manipulation is simple, and in most cases there is no need for an elaborate processing of samples. One problem is cross-reactivity with additional matrix components, such as metabolites or structurally related materials. Apart from clinical diagnostics, immunoassays are also utilized in environmental and foodstuffs analysis.

Depending upon their implementation, various immunoassay variants can be distinguished.[35] The classification of immunoassays can also be made as a function of the wide range of variants. What follows is a classification common in clinical chemistry, together with a presentation of certain important immunoassay types. Depending on realization, a homogeneous or heterogeneous immunoassay can be distinguished. In contrast to homogeneous immunoassays, the unbound reactants are separated prior to measurement in heterogeneous immunoassays.

In the case of heterogeneous immunoassays, two additional types are distinguished: competitive immunoassays and immunometric assays, also known as "two-site" or sandwich immunoassays.

In a competitive immunoassay, an added labeled analyte (tracer) competes with the analyte to be determined from the sample for an insufficient amount of antibody (catcher), which is fixed to the wall of the test vessel. During incubation, there is competition between the labeled antigen and the antigen to be determined for binding sites on the antibodies attached to the vessel. After work-up, the ratio of sample antigen to tracer occupying the binding sites serves to permit the analyte concentration in the sample to be calculated with the aid of calibration curves.

Two additional important forms can be distinguished in "sandwich" immunoassays: the antigen-capture assay and the antibody-capture assay.

In an antigen-capture assay, the antibody is fixed in excess relative to the analyte to the wall of the reaction vessel or a suspended latex or magnetic particle. The sample and the labeled antibody are subsequently added simultaneously (single-step assay); alternatively, the sample is prepared first and, after incubation and cleansing, the labeled antibody is added (two-step assay). For this test, the antigen must exist in at least two different epitopes.

With an antibody-capture assay, specific antibodies in the sample are determined. For this purpose either the antigen can be immobilized in excess on a solid phase, or a class-specific antihuman antibody can be employed (usually one against IgM). After a washing step, the labeled secondary antibody is introduced. This binds to a constant Fc fraction of the bound primary antibody. Quantification occurs following further washing steps.

A widespread form of labeling is the coupling of an enzyme (horse radish peroxidase, alkaline phosphatase, β-galactosidase) to the antibody. In a subsequent reaction, the enzyme converts a substrate into a chromogenic reaction product, the concentration of which is determined through absorption spectroscopy according to the Lambert–Beer law. This is the underlying principle behind what is known as the ELISA test (ELISA: enzyme-linked immunosorbent assay), which permits rapid investigation and quantification of an antigen (for example, protein, virus, hormone, toxin, pesticide) in a sample (blood serum, milk, urine).

A homogeneous immunoassay is less sensitive than the heterogeneous version, but involves a simpler work-up. One popular approach is the "enzyme-multiplied immunoassay test" (EMIT). The principle underlying this test corresponds to a competitive immunoassay, but without immobilization of the antibody. The added enzyme-labeled antigen, which is incubated with the antigen to be determined, competes for a binding site on the added antibody, which leads either to enzyme inactivation or enzyme activation. Measurement requires no additional separation of the bound analytes from free analytes.

Enzymes commonly employed for labeling include the glucose-6-phosphate dehydrogenases and the malate dehydrogenases. The formation of an antigen–antibody complex reduces the enzyme activity by labeling with glucose-6-phosphate dehydrogenases, or increases it on labeling with malate dehydrogenase, that is a high analyte concentration in the sample leads in the first case to high enzyme activities, and in the second case to low enzyme activities. A disadvantage is that the antibody is not able to precisely turn the enzyme activity on or off, but only to increase or diminish it, which results in a significant activity value for a blank sample.

3.2.2.2. ***Immunonephelometry***

Immunonephelometry belongs to the optical analytical methods that take advantage of the Tyndall effect for quantification of an analyte. The formation of antigen–antibody complexes leads to formation of dispersed particles in fliuds, suspensions, or aerosols that scatter light.[36,37] The intensity of the scattered light depends among other things on the size and number of particles. Immunonephelometry can be subdivided into immunoturbidimetry (measurement of turbidity) and immunotydallometry (measurement of scattering). From a methodological perspective, immunoturbidimetry and immunotydallometry correspond to absorption and fluorescence photometry, respectively, with the distinction that instead of measuring the absorption and emission of light at different wavelengths, scattered light is measured at the same wavelength.[46,47] In the general literature, and also in ordinary laboratory usage, immunotydallometry is usually referred to as immunonephelometry (although this is actually a broader term), in which the laterally scattered light is measured. In the case of immunoturbidimetry, the decrease in intensity of the incident light is measured. The latter extinction is not due to an absorption process, but rather to scattering.

Since with both absorption photometry and immunoturbidimetry, a difference is determined between two strong signals, these procedures are less sensitive at low analyte concentrations than fluorescence measurements or immunotyndallometry, which at low analyte concentrations involve differences between weak signals.[38] Multiple methods exist for quantification of an analyte from a reaction on the basis of light-scattering dynamics. With the endpoint method, the maximum value of the scattered-light is utilized. With the two-point (fixed-time) method, the intensity of scattered light is measured at defined periods of time at two points, and the antigen concentration is established on the basis of the difference between the measured values. In kinetic immunonephelometry (rate method), advantage is taken of the maximum reaction rate for the formation of the immunocomplex. For this purpose, a difference is obtained between successive scattered light intensity curves, and a point of inflection is established.[39,40]

One variant of immunonephelometry that offers increased sensitivity is the particle-amplified nephelometric test. In this case, latex particles are coated with immunoglobulin (Ig) to create larger particles during the course of the immunological reaction, whereby the intensity of the scattered light is increased and the detection limit can be reduced 1000-fold.[41] This in turn makes it possible to determine very low analyte concentrations, for example, IgA and IgM in cerebrospinal fluids.

The precipitation reaction in solution requires 90–240 minutes. The reaction time can be reduced to a few minutes by the addition of 4% polyethylene glycol 6000 (immunotyndallometry) or to 10–15 seconds (immunoturbidimetry). In clinical chemistry, calibration curves are used to convert the measured results into SI units prior to distribution. This is one of the ways clinical chemistry differs from other disciplines, such as water processing, where experimental results are calibrated against a standard suspension—specific to a country or a field—for conversion into nephelometric turbidity units (NTU) or formazin turbidity units (FTUs).

Special attention must be given to low measured values. Heidelberger and Kendall studied the dependence of immunoprecipitation on the antigen/antibody concentration ratio.[42,43] In the graphic presentation that bears their name (Heidelberger–Kendall curve; Figure 4), three regions are

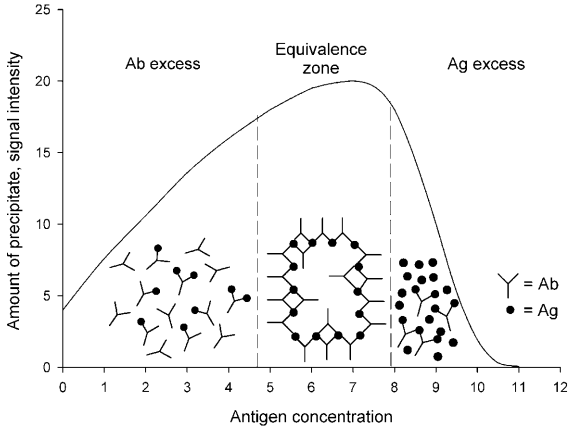


Figure 4. Curve according to Heidelberger and Kendal representing the dependence of agglutination on the antigen/antibody concentration ratio and an antigen excess (high-dose Hook effect). The light scattering intensity of the complex formed between the antigen (Ag) and the antibody (Ab) is plotted as a function of the Ag concentration at a constant Ab level. At maximum precipitation, the supernatant after sedimentation contains neither antigen nor antibody, that is both the antibody and antigen are completely linked within the precipitate. The region with maximal precipitation is designated as the equivalence zone, namely equivalence of epitope and paratope. To the right and left of the equivalence zone, the solution after sedimentation contains either antibody (zone of antibody excess; linear relationship between amount of precipitate and antigen concentration) or soluble antigen (zone of antigen excess, dissolution of the crystal lattice, and decrease in the amount of precipitate as a consequence; false-low analytical result).

distinguished, which result from the fact that both the antigen and antibody possess multiple binding sites. For this reason, depending upon the concentration relationship, antigen/antibody complexes of various sizes, and thus various immunoprecipitates, can develop. In the first region (ascending branch, zone of excess antibody; linear region), the antibody is present in excess, and the result is a nearly linear relationship between the amount of precipitate and the antigen concentration. In the second region (equivalence zone, maximum formation of precipitate), the antibody concentration continues to decrease until all the antibody is bound by the antigen, and a space lattice develops. In the third region (descending branch, zone of antigen excess), there exists a high antigen concentration. This leads to dispersal of the space lattice, and a corresponding decrease in the amount of precipitate. For this reason, quantitative analyses must take place within the first region of the Heidelberger–Kendall curve. Evaluation software attempts to recognize this source

of error, known as the Hook Effect. A repeat of the measurement is initiated through automatic dilution to carry out the evaluation in the region of antibody excess.

The Hook Effect (or high-dose Hook Effect) refers to a false-low determination for analytes present in samples in very high concentration. As soon as the analyte concentration is too high, all the antibody binding sites may be occupied by the analyte, and additional analyte molecules will no longer be determined within the range of the binding curve. This leads to false-low results. Parallel measurements at various sample dilutions can lead to recognition of a Hook Effect and allow the determination to be corrected accordingly. Clinical parameters known to be the source of Hook Effects include, for example, calcitonin, hCG, CRP, AFP, CA 125, PSA, ferritin, prolactin, and TSH.

3.2.2.3. **Blood Cell Counts**

3.2.2.3.1. Impedance Measurement

Performing cell counts and differentiations with blood samples is both personnel and time intensive, which is why attempts have long been made to automate the differentiation process. In the 1940s, Coulter invented electronic counting and size determination of microscopic particles on the basis of impedance measurements.[44] The principle is widely disseminated: Blood cells are suspended in an electrolyte solution, and this suspension is drawn through the opening in a capillary. Two electrodes are situated aound the capillary (one inside, one outside). The passage of a blood cell alters the electrical resistance and conductivity in proportion to the volume of the displaced electrolyte solution and thus the cell volume. Threshold values have been established that allow the various cell types to be distinguished.

3.2.2.3.2. Flow Cytometry

Flow cytometry is suited to physical and molecular identification, counting, and sorting of cells or particles in suspensions. It is often referred to as flow photometry or FACS (fluorescence-activated cell sorting); the latter technique is a trademark of the Becton Dickinson Company.

The automatic determination of cells in a flow system was first demonstrated by Moldavan in 1934.[45] One problem proved to be cell aggregation in the thin measuring capillaries. This problem was circumvented in the early 1950s by surrounding the stream of cells with a laminar streaming liquid sheath (Figure 5).[46]

The quantification and analysis of cells is based on the emission of scattered light during passage of a laser beam as a function of the relative size, granularity, or complexity of the cells. Here the cells in a solution are drawn into a capillary and passed, in a sensor module, individually through a laser beam. The cells scatter a portion of the light, which is then detected by photomultipliers. The extent of light scattering correlates with the size of the cells as well as their granularity or complexity. Thus, segmented granulocytes, which involve numerous cytoplasmatic granula, scatter considerably more light than the very smooth lymphocytes. Forward scatter (FSC) is a measure of low-angle diffraction, and depends upon the cell volume. Sideward scatter (SSC) is a measure of the perpendicular refraction of light, which depends on the granularity of the cells, the size and structure of the cell nucleus, and the number of intracellular components (Figure 6). With these two parameters it is possible to distinguish very effectively, for example, the cell types of blood. Essentially nothing has changed in regard to this principle, which was introduced by Hulett, Bonner et al. in the early 1970s, with the exception of the number of detection channels used for simultaneous detection of multiple fluorescent dyes. The number of laser excitation sources has also increased.[47–49]

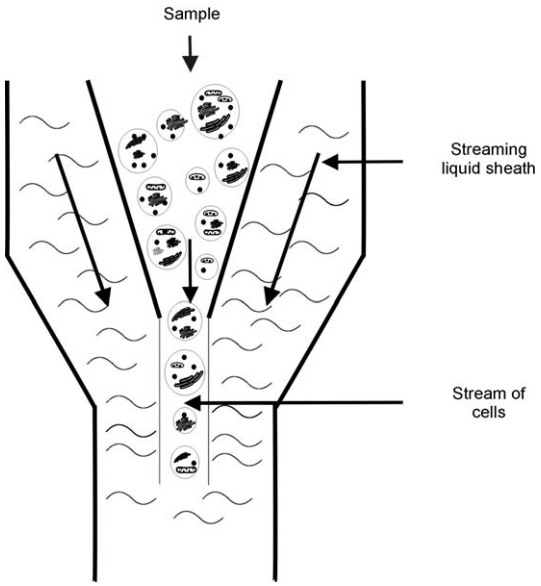


Figure 5. Principle of hydrodynamic focusing: In flow cytometry, cell suspensions are examined at the single-cell level. For this purpose the cells are brought into a single-cell suspension with hydrodynamic focusing. The cell suspension from the sample is drawn out of the sample reservoir. A sheath flow with a higher flow velocity than the sample flow surrounds the sample flow and constricts the latter to a diameter of a few micrometers.

In parallel with this, it is possible, depending on the features of the device utilized, to determine fluorescence-labeled cells. Thus, for the diagnosis and differentiation of various types of leukemia, the leukocytes are incubated with fluorescence-labeled antibodies against leukemia-specific surface proteins. It is possible in subsequent determinations to show whether binding of the labeled antibody has occurred, and thus whether this particular surface protein is expressed on the leukocyte. It is also possible to sort cells according to their labeling. The use of lasers operating at different wavelengths in combination with various filters allows the number of applicable dyes to be increased, and thus the corresponding information density to be increased (Figure 6).

Flow cytometry is a routine procedure in immunology and hematology. It is used in daily routine analysis to provide an automatic differential blood count. Figure 7 shows the distribution pattern (scatter diagram, dot plot) for an unexceptional differential blood count. The cell populations of the white blood cells (WBCs; = leucocytes) take the form

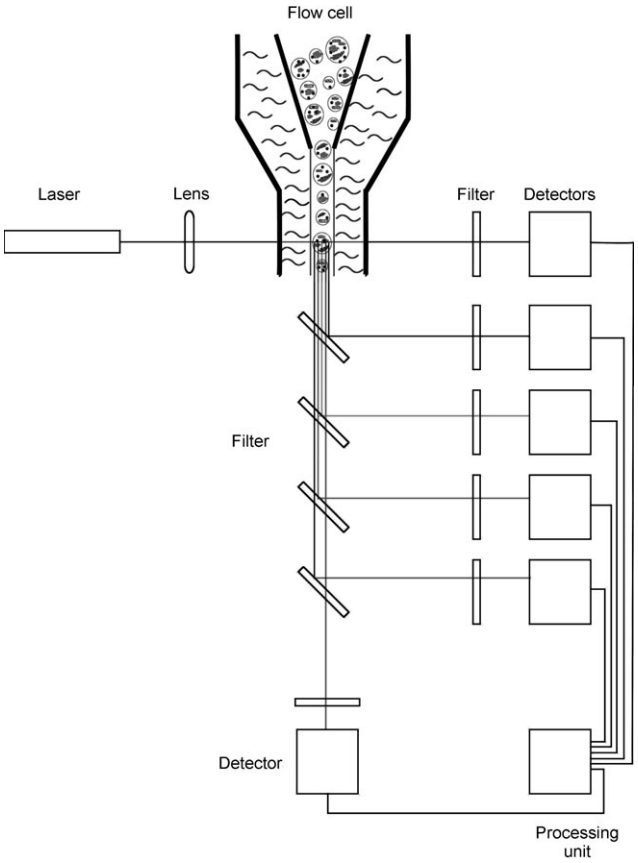


Figure 6. Principle of flow cytometry. A particle (perhaps fluorescence labeled) or a cell (again, perhaps fluorescence labeled) travels in a liquid stream through a laser beam. In the process, a determination is made for each cell or particle of how much light is absorbed (forward scatter; indicates the relative size), how much light is reflected (sidewards scatter; provides the relative optical density), as well as, if fluorescence marking is being conducted, what fluorescence is emitted (detection dependent upon the detectors, what fluorescent dye is employed, and the wavelength of the excitation source). Filters and mirrors adjust the light so that it falls on the proper detector.

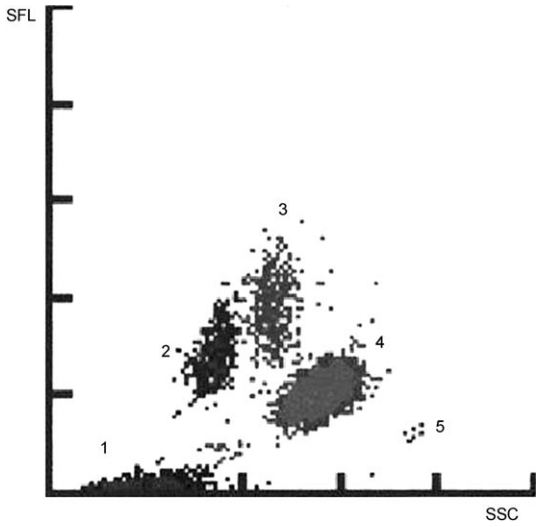


Figure 7. Distribution pattern (scatter diagram) of the blood cells in flow cytometry (differential blood count). The individual cell populations of the white blood count appear as a scatter plot. Evaluation as a normal blood count. 1 = Debris, arising, for example, from lysing of erythrocytes; 2 = lymphocytes; 3 = monocytes; 4 = neutrophilic and basophilic granulocytes; 5 = eosinophilic granulocytes. SSC = sidewards-scattered light; SFL = fluorescence intensity = side fluorescence.

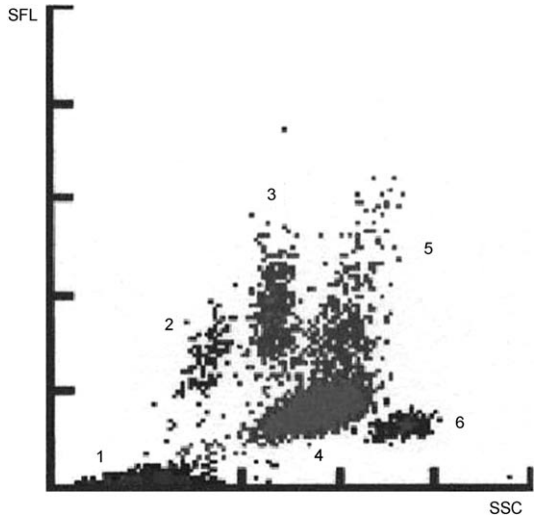


Figure 8. Distribution pattern (scatter diagram) of the blood cells in flow cytometry (differential blood count). Cell population 4 appears not as a scatter plot, but rather as a second, inhomgeneous cell population (5). This involves precursor cells of granulocytopoese (immature form) in the context of a "left shift", which occurs, for example, with infections, inflammations, and neoplasia. 1 = Debris, arising, for example, from lysing of erythrocytes; 2 = lymphocytes; 3 = monocytes; 4 = neutrophilic and basophilic granulocytes; 5 = precursor cells of granulocytopoese; 6 = eosinophilic granulocytes.

of a cloud of points. If the point cloud no longer displays boundaries, this may be an indication of a change in the white blood cells. Figure 8 shows a so-called "left shift", namely the appearance of young, immature granulocytes. This can arise, for example, in connection with infections, inflammations, and neoplasias. In cases with unclear boundaries for the cell populations in a scatter diagram, a smear preparation and differentiation of the white blood cells by a physician are obligatory.

Flow cytometry is also employed in biological research and environmental analysis. Investigation of the expression of surface molecules or intracellular molecules at the single-cell level is often of interest. The cells are tagged with fluorescence-labeled monoclonal antibodies or with the aid of fluorescing fusion proteins. Other applications include experiments related to cell-cycle analysis, apoptosis assays involving RNA/DNA dyes, or the analysis of intracellular pH values or of ion flow at membranes with the aid of appropriate fluorescent dyes.

3.2.2.4. ***Functional Coagulation Diagnostics***

Apart from the quantification of analytes, a determination of their functional capability from a physiological standpoint also plays an important role. Thus, in the management of pre- and postoperative cases, not only are the number of throm-

bocytes relevant, but so is their functional capability with respect to cellular coagulation.[50,51]

The first attempt at a uniform description of coagulation, that of cellular and plasmatic coagulation, was developed in 1878 by Vierordt (1818–1884).[52] He drew a white horse hair through a 5 cm long glass capillary filled with blood, and measured the time required until the first appearance of a fibrin strand. The introduction of thromboelastography (TEG), a procedure for determining functional coagulation (global test of the coagulation system), by Hartert in 1948 made possible the determination not only of individual or a few coagulation parameters, but rather observation of the dynamics of coagulation, as well as fibrinolysis.[53–55] TEG provides a viscoelastic measure of the formation and disappearance of a clot, with the possibility of simultaneously determining clot status, thrombocyte/fibrinogen interaction, thrombocyte function, and fibrinolysis. The method was improved with respect to routine measurement time and sensitivity by Calatzis et al. in 1996 with the development of rotational thromboelastometry (ROTEM).[56] This technique is based on a change in the oscillation of a pin dipped in a cuvette filled with blood. The pin, stabilized by passage through a ball-bearing system, oscillates around an angle of 4.75°, driven by a spring (Figure 9). With the onset of clotting and formation of fibrin fibers between the pin and the wall of the cuvette, oscillation begins to be damped, and this is detected by an optical system (light source—mirror—CCD linear imaging sensor) and transmitted to a computer. As measurement proceeds, fibrinolysis begins, causing oscillation of the pin to increase again. Changes in the pin oscillation are recorded, which results in a typical TEG curve (Figure 10). Coagulation disturbances can be diagnosed and differentiated on the basis of the shape of the curve as a function of time and the steepness of the curve, together with a comparison with various standardized test batches.

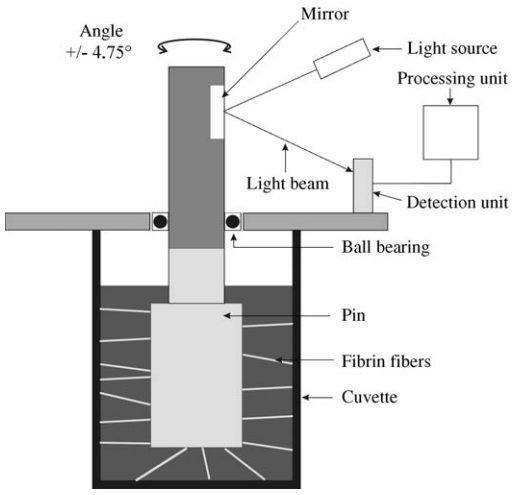


Figure 9. ROTEM principle.[57] Prior to starting the measurement, the pin is fixed in its holder on the measuring apparaturs, and the cuvette is pressed into a metal block. After introduction of the blood sample and the initiating reagent, specific reagents for the test in question are added, and measurement is begun. Motion of the pin is mechanooptically passed along to the computer system. The reduced motion of the pin occasioned by formation of fibrin fibers is converted into thromboelastographic (TEG) amplitude, which thus provides a progressive measure of the strength of the fibrin fibers. The activators developed for the ROTEM accelerate the onset of clotting and thus lead to results in significantly less time (10 min instead of 45—60 min with classical apparatus).

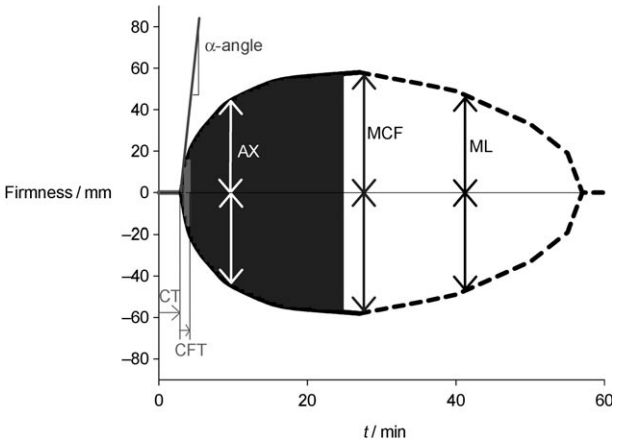


Figure 10. Time course and parameters in the thromboelastogram (ROTEM analysis) of a healthy person, modified from Ref. [56] (for historic reasons the curve is presented with two branches, and the ordinate is calibrated in mm). The coagulation time (clotting time, CT [s]) is the time from the start of the measurement until the onset of clotting (activation of the clotting cascade, thrombin fomation, onset of clot formation). The clot formation time (CFT [s]) is defined as the time from the start of clotting until a clot firmness of 20 mm is achieved (strengthening of the clot through a combination of fibrin polymerization, thrombocyte aggregation, and the activity of fibrin stabilizing factor XIII; the CFT and α-angle are a measure of the elasticity increase of the clot. The maximum clot firmness (MCF [mm]) is the maximum mechanical form of the clot (increasing firmness of the clot through the combination of fibrin polymerization and cross-linking thrombocyte aggregation, and activity of fibrin stabilizing factor XIII). The maximum lysis (ML [%]) is the stability of the clot relative to fibrolytic activity (clot lysis). A prolongation in the CT is observed when there is a deficiency of procoagulatory factors, thrombopenia, or the action of an anticoagulant such as heparin. AX [mm] is the amplitude at time X; this enters into an evaluation of the MCF. Clot firmness is a measure of the strength and ability to withstand the stress of a clot (clot quality), and depends, for example, on the thrombocyte count, the fibrinogen concentration, and the factor-XIII activity. Lysis sets in physiologically within 60 min.

Whole blood impedance thrombocyte aggregometry was introduced in the early 1980s by Cardinal and Flower.[58] A further development of this method is the Multiplate, developed with significant help from Calatzis et al.[59,60] In this method, two parallel electrodes are immersed in a blood sample (Figure 11). During initial contact with whole blood, the electrodes become partially coated with thrombocytes. In the absence of reagents encouraging aggregation, the conductivity remains constant at a given current flow. After the addition of aggregation stimulants (for example, adenosine diphosphate (ADP), arachidonic acid, prostaglandins, ristocetin, thrombin), the impedance (alternating current resistance) increases as a result of aggregation of thrombocytes at the electrodes. The change in impedance is proportional to

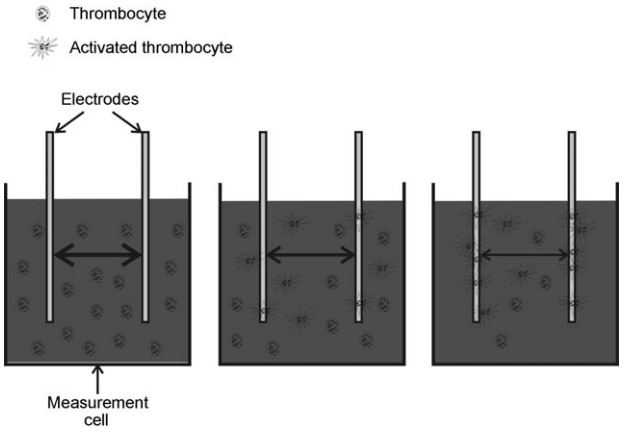


Figure 11. Principle of the impedance thrombocyte aggregation test with Multiplate. Two electrodes, across which there is an alternating voltage, are immersed in a cuvette filled with whole blood. The increase in impedance (alternating current resistance) after activation of the thrombocytes with various activators is determined as the area under the aggregation curve within 6 min.

thrombocyte aggregation, and is displayed as the area under the aggregation curve.

Thrombocyte function can be further assayed with the aid of the Platelet Function Analyzer (PFA 100) by determining the capillary closure time.[61–63] Whole blood is loaded into a chamber, which is then introduced into the apparatus. With the aid of a vacuum, the device draws the blood sample through a thin tube, the inside of which is coated with collagen and adrenalin or ADP. These substances induce the passing thrombocytes to aggregate and adhere. A thrombus thus forms on the interior of the tube, which causes further blood flow to be halted. The time between onset of the test and cessation of blood flow is measured and reported as the closure time.

3.2.3. ***Point-of-Care Testing (POCT)***

POCT means essentially "on-site diagnosis", since even with the best possible organization of all pre-analytical (in particular, material transport) and analytical procedures, particularly because of the multitude of investigatory requests, there will be a delay before laboratory results become available, and thus before further assurance of the correctness of a diagnosis. To keep this "turnaround time" (TAT) as short as possible, more and more emergency rooms, first-aid workers, surgical suites, and hospital-based ambulances, as well as in-house physicians, are turning increasingly to decentralized and "near-patient" testing for important analyses by taking advantage of POCT equipment.[64] Characteristics of POCT include quantitative or semiquantitative sample processing near the patient, absence of sample preparation (usually consisting of whole blood), lack of steps involving pipetting, ready-to-use reagents, and analytical devices that are easy to operate and maintain.[65] Another distinctive feature is the availability of "unit-use" reagents, that is, all the substances required for an examination are present on a test strip. The underlying technique is based on biosensor technology. The analytical system itself relies on dry chemical, immunochromatographic, and electrochemical methods. Measurement times are in the range of minutes. Especially with acute emergency cases and in intensive-care monitoring, blood-gas analysis (BGA)—an application of POCT—has achieved a high status, since it provides the actual status of the individual patient in terms of the oxygen level/carbon dioxide level, pH value, blood-glucose level, and electrolyte and acid/base budgets. This makes possible a very personal therapy for the patient in the context of artificial respiration or dialysis for, example.[66]

Also widespread today is the determination of enzymes (alkaline phosphatase (AP), glutamate oxaloacetate transaminase (GOT = aspartate aminotransferase = ASAT = AST), glutamate pyruvate transaminase (GPT = alanine aminotransferase = ALAT = ALT), γ-glutamyl transferase (γ-GT), α-amylase, creatine kinase (CK)), and electrolytes (Na^+, K^+, Ca^{2+}, Cl , Mg^{2+}), as well as a wide range of metabolite values (bilirubin, total, HDL (high-density lipoprotein) and LDL (low-density lipoprotein) cholesterol, triglycerides, glucose, uric acid, creatinine, urea, and lactate). Attempts are also being made to use POCT to establish additional parameters important in emergency cases, such as cardiac markers (troponine T or I) or the D dimer in the context of excluding a pulmonary embolism.[67] Generally speaking, the analytical quality that is obtained is adequate for clinical purposes: acceptable correlations have been shown between results from POCT analyses and those with established laboratory methods.[68,69] It is important to note, however, that accuracy and precision with POCT measurements often fail to equal those of laboratory diagnostics for methodological reasons (susceptibility to interference, the elimination of sample preparation, and the experimental principles themselves), for which reason the validity of POCT results in clinical emergency situations must be checked particularly carefully, for example the analytical levels of patients with elevated blood glucose or hematocrit values.[70,71]

3.2.4. ***"Laboratory Streets"***

The term "laboratory streets" or "laboratory assembly lines" refers to facilities optimized from a procedural standpoint, which assume such time-consuming tasks as the sorting of samples, centrifugation, and removing caps from sample vessels. With the aid of bar codes, a decision is made as to which parameters are to be determined for a given sample. Subsequently, sample vessels are then directed—completely automatically—to the appropriate analytical station. After the analytes have been determined, the sample vessels are sorted into repositories at the end of the laboratory assembly line. The preparation of aliquots is also possible, and takes place entirely automatically in the event that parameters are to be determined at multiple stations. If the analysis of a parameter must be repeated, the appropriate sample vessel is automatically withdrawn from the proper repository and once more analyzed. With parameters for special analyses, such as flow cytometry, samples are automatically sorted out and collected in the release section (Figure 12).

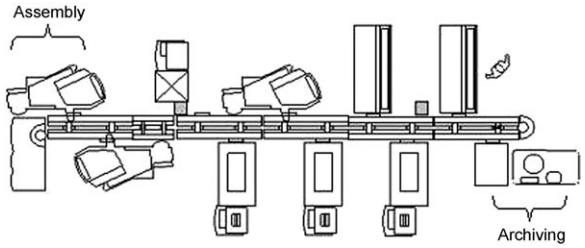


Figure 12. A laboratory "street" or "assembly line". Sample tubes are automatically introduced into the system and perhaps centrifuged; caps on the tubes are removed automatically (at the left in the picture). On the basis of the parameters requested (coded into the bar-code label), sample tubes are directed to the individual analysis atomats and subsequently to the collecting point for archiving (at the right in the picture).

3.2.5. Research and Recent Developments

3.2.5.1. Biosensing

Biosensing refers to a process wherein specific information regarding the biochemical constitution of an analytical sample can be obtained in real time. A biosensor is a miniaturized analytical system that can determine biospecific interactions in a flow system in the absence of additional markers. Biological recognition elements able to enter into biospecific interactions with particular analytes are immobilized on the sensor surface.[72] Such an interaction is translated into a physically measurable optical, electrochemical, thermal, mechanical, electrical, or magnetic signal, which is expressed either quantitatively or qualitatively.[73]

The principle of enzyme electrodes (in which enzymes are captured on a sensing element) was patented in the 1960s by Clark, Jr. Clark and Lyons described an enzyme electrode for the determination of blood glucose, in which the enzyme glucose oxidase is fixed in front of an oxygen electrode by a semipermeable membrane.[74] Since that time, numerous other enzymes have been employed. Moreover, other biocomponents such as organelles, intact cells, antibodies, and receptors have been utilized as recognition elements. This list of traditionally employed recognition systems has more recently been extended through the use of nucleic acids as well as synthetic and semisynthetic (biomimetic) recognition substances. Particularly worth mentioning here are DNA, RNA, peptide nucleic acids (PNAs), synthetic enzymes (synzymes), and molecular imprinting (molecularly imprinted polymers which detect specific target structures (cf. antibody-similar affinity and selectivity)). The use of protein engineering has enabled specifically altered proteins, antibodies, and enzymes to be obtained. The preparation of optimal binding molecules on the basis of amino acids and nucleic acids has been realized through a combination of the synthesis of large combinatorial libraries of peptides and RNAs, selection on the basis of binding strength to analytes, and enzymatic amplification.

Examples include immuno and nucleic acid sensors, enzymatic and microbial sensors, as well as receptor-based sensors. Detection in the field of biosensors is achieved primarily by enzymatic conversion and the piezoelectric quartz microbalance, as well as by various optical procedures such as time-resolved fluorescence spectroscopy and surface plasmon resonance (SPR) spectroscopy (detection of changes in mass in an evanescent field). This has led to a series of biochemical, molecular biological, and medicinal applications.[75–77] A good example in the laboratory medicine sector is the development of a glucose sensor which, placed in tissue, permits a continuous real-time blood-glucose reading, and—through a feedback mechanism—direct and self-acting administration of insulin (artificial pancreas). Biosensors also find application in the field of POCT determinations (blood gases, electrolytes, metabolic intermediates). Other areas of future application include the recognition of very small tumors in the context of cancer detection, allergy diagnosis, study of the human genome, and microbiology. The detection of bacteria depends, for example, on electrochemical measurements with carbon nanotubes, which are coated with aptamers (namely, artificial DNA or RNA fragments) to serve as bacteria-specific binding sites. A change in the electrical voltage can be detected if bacteria bind to the aptamers.[78]

3.2.5.2. Personalized Medicine

This category includes targeted treatment for a patient after previous establishment of a genetic profile, since the latter may influence the extent and duration of treatment as well as the effectiveness and side effects of certain medications. Personalized medicine permits drug therapy to be increasingly adapted to individual patient groups as a function of genetic profile.

Personalized medicine has already come into play in isolated cases. The American Food and Drug Administration (FDA) in 2005 recommended for the first time a genetic test prior to the administration of warfarin-containing products.[79] Warfarin, a vitamin K antagonist in the coumarin family, is used to inhibit blood clotting as a way, for example, to lower the risk of thrombosis or strokes in at-risk patients. As a result of specific genetic variations, approximately 30% of patients metabolize warfarin more slowly than normal, and thus—at standard dosages—may lead to the warfarin level in the body being too high, which in turn results in heavy bleeding. On the other hand, with too low a dosage, the desired reduction in the risk of a thrombosis is not achieved. This differing metabolism of warfarin is due to variants in cytochrome P450 (*cyp*) *2c9*- and *vkorcl*-genes.[80] Further examples are the genes *cyp2d6* and *cyp2c19*, which code for enzymes that transform prodrugs into the active metabolites (*cyp2dx*: tamoxifen, a therapeutic agent for estrogen-receptor-positive breast cancer cases, in the active agent endoxifen; *cyp2c19*: clopidogrel, a drug to protect against blood clots (by thrombocyte aggregation inhibition), e.g. in peripheral vascular desease, and mainly to prevent thrombosis after placement of an intracoronary stent, in the pharmacologically active thiol derivative).[81,82] A genetic profile is helpful in adjusting the dosage of these active agents for particular patients to ensure the desired activity, and above all to avoid undesirable side effects.

Human epidermal growth factor receptor 2 (HER2 = HER2/neu = c-erbB-2) plays an important role in normal

growth and in the maturation of somatic cells. Compared to normal somatic cells, the number of receptors in cancer cells can increase to 10- or 100-fold of the norm. One refers in this case to an HER2 overexpression, which is usually related to increased gene amplification, that is an increase in the number of HER2 gene copies in the cell nucleus. Detection of HER2 proteins in breast cancer through specific tests makes it possible to identify patients likely to respond to therapy with the recombinant monoclonal IgG_1 antibody trastuzumab (herceptin), a drug that acts specifically on this growth-factor receptor.[83]

Panitumumab (Vectibix) is a human recombinant anti-EGFR-antibody (IgG_2; antiepidermal growth-factor receptor) against epidermal growth factor receptor used in therapy for metastasized colorectal carcinoma. Therapy costs in this case can exceed 100000 Euros per patient. According to current data, therapy with anti-EGFR antibodies is advisable only with patients whose tumors demonstrate no mutation in the K-ras gene (ca. 60% of all patients).[84] Determination of the K-ras mutation status is a predictive biomarker for the effectiveness of therapy with panitumumab, whereby the patient may be spared serious side effects, and major costs can be avoided for the insurer.

Moreover, a gene chip is available for establishing the individual endowment of a particular patient with respect to genes for liver enzymes that break down specific medications, since these are of great significance in regards to the choice of the needed and suitable dosage of certain drugs. Further examples include azathioprin and mercaptopurin, which are used against autoimmune diseases and leukemia, respectively.[85]

In general, three basic aspects must be investigated and safeguarded in establishing parameters for personalized medicine:

1. Both the accuracy and sensitivity of a measurement must be valid for the marker in question.
2. The test must be able to determine or predict a disease reliably.
3. The test must be clinically relevant; that is, the significance of a finding must be clear for the individual patient, including possible preventive or therapeutic starting points.

3.2.5.3. ***Control, Calibration, and Reference Materials***

In the context of internal quality control, a control journal must be maintained according to established guidelines for every analyte. Furthermore, for every analyte and for each series of analyses, control studies must be performed at the start of measurements and during the procedure, in various concentration ranges. In particular, this means that analytical values of the controls must not lie exclusively in the middle of the analytical range, but tests must instead be run at the upper and lower limits of the test range as well, and results must be documented in the control journal. Deviations from the concentration range must also be documented there. If it proves impossible to maintain the prescribed concentration range, then a calibration of the analytical device must be undertaken. Through evaluation of the control journal, it should be possible to determine both the resulting precision (extent of agreement of independent data values acquired under differing conditions, for example, time, examining person) and the accuracy (extent of agreement within a particular control cycle, for example, within a month, between an observed mean value and the target value).

It is recommended that these minimum requirements be voluntarily extended through additional controls, so that errors in analytical determinations (for example, from instrument contamination) may be discovered promptly. Moreover, so-called pool results are determined in many laboratories. These may be prepared in the laboratory itself by mixing human sera, so that they present a more complex matrix than the controls alone, which are often produced synthetically. Pools are also often prepared for various concentration ranges.

Control and calibration materials are generally offered by apparatus manufacturers. A problem arises here in that a set of commercial reagents for analyte determination may indeed give good results with the synthetic control and calibration materials supplied (since they are from the same manufacturer), but cross-reactivity or other interferences can develop with seriously matrix-encumbered patient samples.[86,87] For this reason, a measurement with another test system is indispensable for some studies of human material, or else extensive sample preparation is required. There is thus an interest in improving the calibration and reference materials as well as their matrices to make them more comparable to biological and human samples.[88–90] Fundamental guidelines for reference materials are specified by ISO and its committee for reference materials (ISO-REMCO). According to ISO/IEC Guide 99:2007, International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM), reference materials or substances are to be of sufficient homogeneity such that one or more characteristic values are defined so precisely that they can be used for the calibration of measuring instruments, for evaluation of measuring procedures, or for the assignment of material constants (for example, the content of a particular ion or the electrical conductivity of a substance). Reference materials are normally supplied with a certificate, that is, the reference values are cited along with a corresponding measuring inaccuracy (confidence interval). Moreover, the material constants cited by the manufacturer are confirmed by an independent analytical procedure. Laboratories that are authorized to supply such a certificate are themselves subject to continuous quality control. The certificate also indicates the homogeneity of the reference material, and it states the minimum initial quantity of sample for which the certified value for a material constant is valid. Reference materials are provided for various fields (for example, pharmaceuticals or the foodstuffs industry). Apart from the calibration of measuring instruments, reference materials are also used for comparative analyses between various laboratories (ring analyses) and for training in analytical chemistry. They are offered by both private producers and suppliers as well as by government institutes.

3.2.5.4. Nanodiagnostics

Nanodiagnostics refers to the use of nanomaterials for the detection of diseases.[91,92] A review published by Jain in 2005 provides an overview of the present and possible future utilization of nanotechnology in medicinal analysis.[93] Important fields for the employment of nanodiagnostics include immunohistochemistry, genotyping, and the detection of biomarkers and infectious microorganisms. The simultaneous determination of various analyte molecules (multiplexing) is possible.[94] The detection principle is in every case similar: surface characteristics are altered as a result of the binding of an analyte to an antibody that has been fixed upon a carrier surface.[95] These changes are in turn detected with appropriate technology.

Of particular interest in clinical chemical analysis are bifunctionalized nanoparticles, for example, of gold and its alloys, quantum dots (QDs), magnetic nanoparticles, and nanomechanical cantilever arrays.

3.2.5.4.1. Gold Nanoparticles

Spherical gold nanoparticles, in some cases alloyed with silver or copper, are suited to the simultaneous detection of various proteins or DNA molecules (multiplexing, multianalyst procedures), either in solution or at a solid phase through the use of a modified form of vibrational Raman spectroscopy.[96] Here the gold nanoparticles are coated with a self-assembled monolayer (SAM) of a Raman-active dye. This combination leads to a sensitivity increase above that required for bioanalysis (surface-enhanced Raman scattering, SERS).[97,98] An antibody is also conjugated to the Raman-active dye. The result is a bifunctionalized nanoparticle (gold nanoparticle + Raman-active dye + antibody), which in this particular case (with reference to the form of labeling) is also designated a SERS-labeled antibody (cf. also radioactively or fluorochrome-labeled antibodies).[99] Immunocomplex formation occurs in the presence of the corresponding antigen, for example on a histologically prepared section, and thus fixation of the bifunctionalized gold nanoparticle occurs. Analysis can be carried out after treatment is complete. The principle underlying this method is similar to the labeling technique in fluorescence spectroscopy. Signals from the marker molecules are less numerous, however, and up to 100-times narrower than those from fluorophores. Thus, there will be no spectral overlap with the appropriate combination of Raman-active dyes, thereby allowing several analyses to be conducted in parallel.[93]

3.2.5.4.2. Quantum Dots

Quantum dots are semiconductor crystals only a few nanometers in size.[100,101] As a consequence of their size they behave neither like individual molecules nor as voluminous materials. Their constitution (for example, CdSe/ZnS) and size give the QDs their properties. The absorption and emission wavelengths can be adjusted by varying the size and composition.[102] In contrast to other fluorescent inorganic and organic compounds, QDs are distinguished by their brightness, symmetric emission spectrum, color stability, and the numerous resolvable colors that can be excited simultaneously by a single wavelength. Another unique photophysical characteristic of these semiconductor particles is the fact that, because of the quantum confinement effect, modification of their size makes it possible to adjust the absorption and also emission wavelengths to any desired value.[103]

3.2.5.4.3. Magnetic Nanoparticles

Magnetic nanoparticles are coated with antibodies and incubated with a sample.[104] Analyte detection can take place in a homogeneous liquid phase or in the solid phase, for example in a histological organ section in which tumor cells are being sought. The formation of an antigen–antibody complex occurs in the presence of the target analyte, whereby the change in an externally applied magnetic field can be measured. Alternatively, magnetic relaxation studies can be conducted. Apart from in vitro diagnoses, magnetic nanoparticles are also utilized in in vivo investigations and therapy schemes.[105,106]

3.2.5.4.4. Nanomechanical Cantilever Sensors

The surface of a cantilever is coated with DNA or with antibodies that are capable of binding the target analyte. The elastically flexible cantilever is fastened onto a chip that signals every displacement. If the target analyte is present in the sample, a displacement will occur that is proportional to the concentration. Parallel arrangements of multiple analyte-specific cantilevers makes it possible to quantify several different analytes simultaneously.[107]

3.2.5.5. Systemic Biology

A human being has 30000–40000 genes, each of which codes for a single protein. This number is very large, but the number of biochemical reactions catalyzed by these proteins is virtually incalculable. Systemic biology examines the behavior and interactions of all the elements in a specific biological system.[108] In this interdisciplinary field, which encompasses the natural sciences, mathematics, and information science, it is not the investigation of individual genes or proteins that occupies the foreground, but rather the behavior and the interaction of all the components of a system. From data concerning biological systems or from human physiology (derived, for example, from genomics, transcriptomics, lipomics, proteomics, metabolomics, or glycomics (the "omics" technologies), as well as other studies), mathematical models are developed, Hypotheses regarding systemic properties and behaviors are then devised with the aid of such models.[109] This may transpire at a computer ("in silico") and in vitro or in vivo.

In addition, results (biomarkers) from "omics" technologies and genome association studies related to widespread diseases are being discussed for their value in disease prevention and increasing the efficiency of laboratory diagnostics. Biomarkers found in the context of these investigations find application in four clinical areas:

- screening for preclinical diseases in asymptomatic people,
- differential diagnostic significance in patients with clinical symptoms,
- risk stratification for patients with clinically manifested diseases,
- support in the choice and management of therapy.

From the points listed, it can be deduced that few biomarkers actually live up to clinical standards and find a place among the routinely used techniques.[110]

3.2.6. *Research into Established Methods and Implementation of Measurement Methods Derived from Chemistry*

Current areas of investigation include the development of quantitative analysis methods that are insensitive to interference, as well as the testing (and possible implementation) of chemical analysis methods for application in clinical chemistry.

The robustness of immunoassays can be improved by the use of new antibodies and by altering the matrix of the test system.[111–117] Chromatographic columns can be used during the sample preparation for removal of interfering substances. The cell analysis with flow cytometry was extended by the introduction of new markers in leukemia diagnostics, the study of stem cells and precursor cells, as well as the detection of pathological microorganisms in blood.[118–120]

3.2.6.1. *LC-MS/MS*

Well-established, although relatively expensive, test systems such as immunoassays are being carried over to new platforms, such as LC-MS/MS.[121–123] For example, in the field of drug analysis, laboratory cost savings of several 100000 Euro per year are being achieved at the Munich University Clinic alone. However, for the establishment of a new platform it is first necessary that the measurement conditions are explored and, in a second phase, the steps in the sample preparation defined. Ultimately, the method must prove suitable for routine, continuous service. Many chromatographic methods fail this test because the measurement time exceeds 15 minutes, since it is then no longer possible to process sufficient samples in a reasonable time period. Moreover, it is not easy to incorporate a new analytical principle into routine diagnostics, with parameters that must be accessible over a 24 h period, since the personnel must learn an additional method, with its associated susceptibility to errors. Manual sample preparation has also proved to be problematic. On the one hand it is personnel-intensive, but on the other hand the risk of inadvertent sample interchange rises.[124,125] Some form of automation is thus desirable. One possible approach could involve the use of functionalized ferromagnetic microparticles as well as automated plasma protein precipitation.[126–128]

3.2.6.2. *NMR Spectroscopy*

Routine application of NMR spectroscopy is not yet universal.[129] In the context of lipid analysis, it is expected to result not only in differentiation of the cholesterol fractions HDL, LDL, and VLDL cholesterol, but also in further subclassification of LDL cholesterol, which should have considerable potential in atherogenesis.[130] The goal is to reduce the effort otherwise expended in ultracentrifugation. Another possible field of application is early clarification of metabolic disease and study of the steps in metabolism.[131–135]

3.2.7. *New Analytical Parameters and Principles of Cell Physiology*

Main focus of research are the pursuit of clinical studies or clarification of physiological and pathological mechanisms at the molecular level. As a consequence of the diversity of the fields involved, only a few isolated cases can be delineated here.

MALDI-TOF-MS finds application in the context of protein and lipid analysis (proteome analysis),[136–138] post-translational glycolysis of proteins,[139] and the search for new biomarkers.[140–146] MALDI-TOF-MS is increasingly being utilized in microbiology for the identification of bacteria, viruses, and fungi. The procedure is distinctive for its minimal sample requirements, as well as rapid and easy sample preparation. In contrast to biochemical differentiation, no preliminary classification is required (for example, Gram positive/Gram negative; enterobacteriaceae/non-enterobacteriaceae). Incubation of germs with substrates, as in the case of biochemical differentiation, is unnecessary, so the time entailed in a single differentiation amounts to at most two minutes. Species identification on the basis of the resulting mass spectrum is accomplished through spectra databases.[147,148]

Nucleosomes, for example consisting of a histone octamer and DNA, which in turn is wrapped around the histone octamer in about two coils, function as markers for cell destruction.[149] Cell destruction can have a host of causes, including a traumatic event, sepsis, apoplexy, and various tumors (such as small-cell lung carcinoma), as well as therapy monitoring during chemotherapy.[150] The extent of DNA methylation as a prognostic biomarker in various tumor conditions is also a topic of current investigation.[151–154] Methyltransferases methylate cytosine at specific locations in the genome, with formation of methylcytosine. This methylation has consequences for the regulation of gene expression. Methylated cytosine bases in the promoter region of a gene leads to its inactivation. It has been shown that aberrant DNA methylation in various promoter regions is a characteristic of human tumors, and that this methylation is associated with the disease process.[155–157]

Structure, function, molecular mechanisms and markers of signal transduction, cell proliferation, and cell differentiation, as well as cell migration and cell adhesion are all being studied from a standpoint of cell physiology.[158] Key areas in this context include cytokines as colony-stimulating factors, interferones, interleukins, tumor necrosis factors, chemokines, cadherins, catenins, integrins, selectins, proteoglycans, mucins, and the immunoglobulin supergene family (such as cellular adhesion molecules, CAMs).[159–165]

Special clinical relevance is attached to clarification of molecular mechanisms and markers of sepsis, specific and nonspecific defensive reactions, and inflammatory cascades, and, in this context, the role of cytokines.[166–170]

Within the framework of molecular study of diseases associated with affluence, the development of diabetes and atherosclerosis are under investigation (lipid metabolism, role of lipoprotein(a)).[171–173] Similarly, bone metabolism is being studied at the molecular level, particularly against the background of osteoporosis.[174–176]

The influence of genetic components on the development and extent of disease is a focus of research, as is the response to specific therapies. Simultaneous quantification[177] of many of the most diverse genes should become possible with the help of DNA chips or microarrays.[178–183]

3.3. Further Development of Medical Laboratory Methodology

Prognoses of the future are to some extent little more than guesswork and opinion. Thus, in the 1980s and early 1990s a great future was prophesied for molecular diagnostics,[184] but their significance and volume in present day diagnostics remain of secondary importance. The high point in automation is considered already to have been reached with the introduction of "laboratory assembly lines", which provides a maximal increase in efficiency with simultaneous reduction in personnel engagement. This results indirectly in a centralization of laboratory effort, wherein transport times are increased and there is no longer the assurance of an "on-site presence". With acute disease patterns, it often takes too long before laboratory results are received. The modular concept of a handy readout device and an interchangeable multisensor cassette permits accommodation of various analyte portfolios, such as cardiac markers, drugs, allergens, or hormones. Against this background one should anticipate an increased prevalence of POCT devices.

From a technical standpoint, hopes are pinned on the trend toward miniaturization (lab-on-a-chip), parallelization (multiplex techniques: biochips, MS, pyrosequencing), cellular analytics (FACS and FISH), as well as mass data analysis (bioinformatics). Further developments in medical analytics will, therefore, be influenced by microtechnology.

Thanks to advances in microfluidics, chip technology, and electronic detection methods, it is now possible to make measurements on a sample containing only a few microliters of DNA or RNA. The structures for these systems are only a few micrometers in size. Immunoassays and electrophoretic analyses for proteins can also be carried out with micro total analysis systems (μ-TASs). The expression μ-TAS was coined in 1990 by scientists at Ciba-Geigy AG.[185] It refers to measuring devices in which all the necessary steps for the chemical analysis of a substance are carried out automatically (for example, separations, filtrations, dilutions, and evaluations).

Even today, after nearly 20 years of development, complete μ-TASs are still rare. The reasons lie in part in the technical complexity of integrating various functionalities in a single automated, rapid, robust, and reliable system that still provides the necessary sensitivity, but also in a continued lack of acceptance on the part of users.[185]

An important aspect of the miniaturized laboratory is the meager amount of liquid involved at the nano- and picoliter level. Apart from a reduction in reagent use, there is also the potential time saving in temperature adjustment and rapid mixing, which in turn cuts the time required for an analysis. Thus, if DNA amplification through a polymerase chain reaction (PCR) requires minutes at the microliter scale, only seconds are involved in a chip laboratory.[186] An additional advance in DNA sequencing is pyrosequencing. Similar to Sanger sequencing, this utilizes DNA polymerase for synthesizing DNA, and entails enzymatic cleavage of pyrophospate from the deoxynucleoside triphosphates (dNTPs: dATP, dGTP, dCTP, dTTP). In a follow-up reaction involving luciferase, the released pyrophosphate is so transformed that a bioluminescent signal is emitted, which can in turn be detected and measured. Starting with a primer, DNA chain lengthening takes place, nucleotide by nucleotide, through controlled addition of the individual dNTPs. The addition of the proper nucleotide, namely one complimentary to the matrix, results in a luminescent signal, whereas there will be no signal with dNTPs that fail to fit. Excess dNTP is rapidly removed from the solution, so that no mixed signals arise. The simultaneous inclusion of several identical nucleotides generates a stronger light signal, one that is proportional to the number of nucleotides involved. The opportunity for quantitative evaluation of signals is one of the strengths of pyrosequencing. It is used in determining the frequency of single-nucleotide polymorphisms (SNPs), for example in the study of genetic diseases, and is thus incorporated into personalized medicine. Pyrosequencing is readily automated, and is well-suited to the parallel analysis of DNA.[187]

Apart from technical modifications in analytical apparatus, changes also occur in the spectrum of analytes, in other words in the medical realm, which adapt to the socioeconomic structure of the land and thus to those diseases that are widespread. In this way, great significance will be attached in classical analysis to predictive diagnosis and early recognition (especially in coronary and circulatory system diseases, diabetes, osteoporosis, dementia, and cancer), to individualized medicine (theragnostics), and to personalized medicine. Challenges here of course include the discovery of cancer at relevant early stages, recognition of diagnostically and/or therapeutically useful patterns, and development of clinically more meaningful, scientifically based indices for the recognition and specification of risk. At the same time, scientifically validated calculators enter the picture (risk factors derived from a patient's biographical data), on the basis of which relevant risks of disease can be computed (for example, the relative risk of developing a certain terminal disease within the next two years).

Parallel analysis of multiple parameters will acquire great significance, especially with respect to nonspecific symptoms (for example, tiredness, sense of having a cold, vomiting) in differential diagnosis (for example, a simple cold rather than some more malign illness). Thus, the American certification agency, the FDA, has approved a parallel test that can simultaneously verify 12 different viral pathogens responsible

for infections of the respiratory path. One vendor is offering a rapid immunological test for simultaneously distinguishing anthrax, staphylococcal enterotoxin B (SEB), ricin, Yersinia pestis, and botulinum toxin, the most important representatives of the "dirty dozen", which are of interest both in disaster management and the militarily. In this context it should be noted that this particular test meets NATO Standard Agreement 4571, which specifies, for example, that it must function reliably over a wide range of temperatures (environmental temperatures between 4 and 49 °C).[188,189]

An increase in the sensitivity of detection for individual analytes as well as parallel analysis of multiple parameters will also be facilitated by nanodiagnostics. As already noted, research and industrial development are concentrating on gold nanoparticles. This has arisen for several reasons: gold is well-suited to clinical chemical investigations because of its optical properties, its chemical robustness, and the possibility for functionalization with ligands via thiol compounds (DNA, proteins, antibodies). It has been shown that, in this way, DNA and proteins can be detected with exceptionally high sensitivity. Thus, DNA can be detected at a concentration of 23 pM.[190] Simultaneous measurement of multiple analytes is possible with the aid of various antibody surface coatings, thus facilitating screening for widely distributed or chronic diseases.[191,192] The fields of nanochips/lab-on-a-chip/microarrays, microfluidics, nanoparticles, as well as microelectomechanical systems (MEMS; for example, tablet-size cameras) are all subsumed under the term nanodiagnostics.

Short-term implantable in vivo diagnostic systems are playing an increasing role, particularly for immediate diagnosis of patients in intensive-care stations. These include sensors that can be implanted in a blood vessel for permanent monitoring of, for example, the pH value, blood-glucose levels, temperature, and oxygen partial pressure.[193,194] A further development is measuring systems that are implantable and provide long-term stability, and which can be recalibrated in vivo. One example makes possible permanent glucose determination for diabetics.[195] Another application of biosensors permits an in vivo dose determination in radiation therapy based on fiber-optic sensors that function without the need for an electrical supply. Such systems make possible the local monitoring of radiation dosage directly at a tumor, in surrounding tissue, or a neighboring organ.[196]

Changes can be anticipated not only in apparatus and diagnostic tools: there will also be a pooling of disciplinary competencies from laboratory medicine, microbiology/infectious epidemiology, transfusion medicine, human genetics, hygiene, and pathology. By using diagnostic expert systems, neuronal networks, knowledge-based diagnostic systems, and electronic differentiation tools it will become possible to judge individual findings differently, and to interpret them in individualized ways. Preventative laboratory diagnostics harbors great advantages for the individual if risks can be identified through biochemical markers before clinical or organic findings appear, thus allowing therapy to be initiated early.[197,198]

When will one be able to speak of a transition to a new generation of systems? The zeroth generation involved no automation, namely it had to do strictly with manual methods—the so-called "test-tube systems". An advance to the first generation began in Germany in 1950; this generation was driven by technology and characterized by a mechanization of apparatus and the packaging of reagents (standardization), together with parametric sample processing. The second generation arrived in 1975, and was application-driven, that is analysis systems came with prepackaged reagents along with software management. Selective sample processing became possible. The third generation appeared in 2000, and was driven by efficiency (integration of laboratory systems on a platform with "intelligent" sample processing in a laboratory assembly line, integration of pre- and postanalysis). The fourth generation will again be driven by technology, characterized by miniaturization and parallel arrangement of analytical techniques together with increasing IT networking. When the fourth generation of laboratory systems will arrive is unclear, however. Estimates suggest that its introduction will begin around 2025.[199]

Miniaturized products, the realization of which would be impossible without microtechnology, are already appearing on the market, are in clinical testing, or await market introduction in the near future. The deployment of microtechniques in medicine can be characterized as a success only if the new products contribute to a sustainable increase in the efficiency of patient care; in other words, clinical utilization will always be the decisive factor.

3.4. Postanalytical Phase

3.4.1. General Observations

The postanalytical phase, or postanalysis, covers the analytical and medical assessment of analytical results and the recorded set of definitive findings.[200] Such findings constitute a legally valid conveyance of analytically and medically approved measured data after technical and medical validation (longitudinal and transverse comparisons, plausibility controls). Technical validation occurs through the person working with the technical apparatus, or through a quality control program, whereas medical validation includes consideration of all clinically relevant data. An official finding must contain the date and time of sampling and sample receipt, as well as the date and time of issue of the finding. Also documented must be identification information (patient, submitter, laboratory, person responsible for issue), the nature of the material under investigation, description of the investigation, results including units, and the reference range for interpretation.

The interpretation, classification, and judgement of clinical relevance of analytical results with respect to an overall clinical picture presuppose, in addition to substantiated knowledge or skills from pathobiochemistry, analysis (analytical sensitivity and specificity, precision, and interfering factors related to the methods of analysis) as well as diagnostic characteristics (physiological scatter, influencing variables, diagnostic sensitivity and specificity, predictive value) of the measured value under investigation, together with the case history of the patient and the patient's

complaints.[201] Differential diagnostic judgements made by the laboratory should also be indicated. The attending physician can then make a determination in context, including, for example, radiological findings and results from functional diagnosis.[202,203]

Conveyance of the findings occurs in consultation with the submitter in the form of a printed report delivered by courier, post, fax, or electronically, as well as by HTML. Combinations are also possible. With appropriate advance notice on the investigation request, a telephone or fax report may also be transmitted. In the case of an investigation requiring a prolonged period of time, a partial report may be prepared, which is followed by a final report containing all the requested information. If necessary, a corrected report may be issued, including reference to the original report, should that be found necessary on the basis of internal control or a subsequent inquiry. Addenda, suitably labeled, are normally made a part of a final report.

Critical laboratory results, based on lists of extreme values (which may or may not be the subject of individual agreement with the submitter), can be communicated at once to the physician in question by telephone or by fax, and also in the absence of any special labeling, for example, significantly high or low potassium levels, hormone controls in pregnancy, or the monitoring of fertility therapy.

Depending upon agreements between the submitter and the analytical laboratory, results of various types can be distinguished. Some of the most important examples are described below:

Cumulative findings: These provide a summary of multiple requests, collected together on a single sheet. The measured data are arranged in such a way that values for a particular quantity derived from various samples/times appear in a single row. The basis for attribution and summary of requests on a single cumulative finding is the identification number from the patient management system, which remains valid for life. The cumulative finding is the obligatory finding. A variant of this is the XML-cumulative finding, which collects electronic individual results together and presents them in tabular form. The user can selectively adapt the time span and content to be included. There may also be a graphic depiction of data as a function of time.

Detailed finding/individual finding: The results of only a single request, often with a preliminary examination of a patient, presented in such a way that the descriptions of a measured quantity are written out in detail to make it easier for the submitter to interpret. Further individualization through appropriate interpretations of the results is also possible.

Label finding: A label finding provides results, together with appropriate units, on one or more labels. These make it possible to stick laboratory values into patient overviews, directly in the patient's ward.

HTML online finding: This would normally be available only to submitters at a clinic or hospital, and it ensures timely transmission of results for patient care. It consists of a set of electronic findings that can be called up by the submitter on a PC. Anyone authorized to access such information is required to submit to a privacy agreement supplied by the accredited privacy agent of the clinic or hospital.

A distinction is made between a laboratory finding and a laboratory report. A laboratory report consists of a set of measured values together with a physician's interpretation, whereas a finding is limited simply to raw data, perhaps together with a reference such as "hemolytic sample".

All the results of an investigation are documented electronically under both the patient's master data file and the laboratory assignment number. These records are maintained for a period of ten years, and can be called up at any time. The specified reference ranges, which are essential for interpreting the results, are consistent with the specifications of the creator and the current state of an investigation or the related literature.

The last step in sample processing is disposal according to appropriate legal provisions.

3.4.2. *Determination of Reference Ranges for Individual Parameters*

Reference ranges are an expression of interindividual variability.[204,205] They are influenced by the method of determination and pre-analytical factors such as age, sex, height, weight, circadian rhythm, and nutritional habits of the patient. According to DIN EN ISO 15189, reference ranges should be adapted to conform to the current state of knowledge.[206] It is always important to demarcate a physiological scattering of a parameter as would be observed with healthy patients, from the pathological values observed with the sick.[207] For this reason, parameters/biomarkers must be examined for their specificity with respect to an illness, for example by the introduction and interpretation of new tumor markers. This concerns proteins, peptides, or other substances formed concurrently with the emergence and growth of malignant tumors, and produced either by the abnormal cells themselves or from healthy tissue in reaction to the growth of the tumor. Tumor markers may also appear in healthy people. The purpose of the reference range is to separate a healthy from a diseased collective; thus, the diagnostic sensitivity and specificity should ideally approach 100% in every case (Figure 13).[208] Diagnostic sensitivity concerns the number of healthy individuals with a normal test result. The less false-negative results obtained, the more sensitive is the test method. Diagnostic specificity refers to the number of diseased persons with a pathological test result. The less false-positive results encountered, the more specific is the test method.

For determining a normal range, only a random sample of the "normal healthy" that can ever be examined, so the normal range is thus a range of values that will, with a specified probability, be observed among healthy persons. Often the normal collective is poorly defined. The information will be deficient:

- regarding the selection criteria,
- the sampling technique (preparation of the subjects),
- the method(s) of analysis (reliability criteria),
- the number of people examined,
- and/or the evaluation procedure.

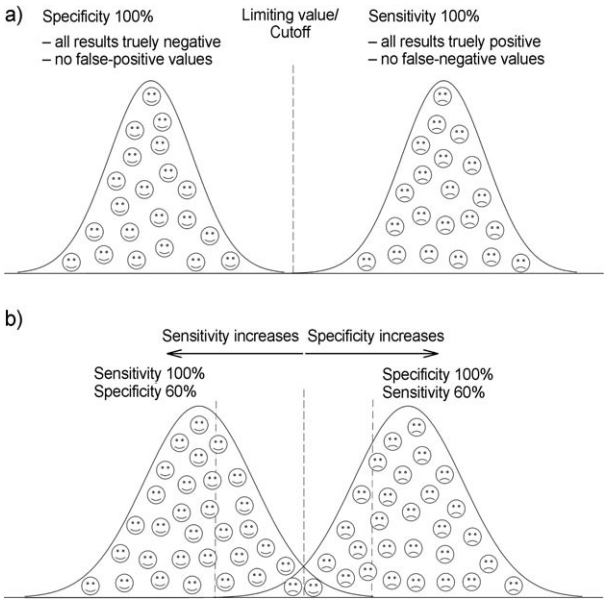


Figure 13. a) Idealized concept of a marker: 100% diagnostic specificity, that is, no false-positive results. This provides a categorical distinction between healthy and sick patients. b) More realistic test: it is impossible to provide a categorical distinction between healthy and sick patients.

This will be illustrated on the basis of two examples. Interpretation of prostate-specific antigen (PSA) data requires considerable care, since the result is influenced by many factors.[209,210] One of these factors is the test method itself.[211] Over 80 different methods for total PSA determination are available worldwide. It is therefore important that the same laboratory method is always used when monitoring changes in PSA over time.[212] Factors influencing how high the measured value is include, apart from age and ethnicity, intensive cycling and other vigorous physical activity (increases in total PSA values are demonstrable for up to a week after such exertion), ejaculation (total PSA enhancement up to 48 hours later), or acute urinary activity or prostate biopsy (total PSA increase up to a month later). Above all, these PSA increases must be distinguished from ones arising from diseases of the prostate (carcinoma, inflammation, or enlargement). Since this is often difficult based simply on a total PSA value, a free PSA (fPSA) value is often determined as well, and then a quotient is taken (fPSA/total PSA). Strict borderline values are replaced in such cases by a stepwise diagnostic process. When an elevated total PSA concentration is found, a subsequent fPSA determination may follow. Patients with prostate carcinoma often show a lower percentage of fPSA relative to patients with a benign prostate hyperplasia.[213] The variability of a biomarker such as PSA in the diagnosis of prostate carcinoma means that further biomarkers can be called upon to increase the diagnostic sensitivity, such as the prostate-specific membrane antigen, as well as prostate carcinoma-specific antibodies, whereby their significance must first be verified through clinical studies.[214]

Natriuretric peptides, such as the brain natriuretic peptide (BNP), are released as prohormones (namely, inactive precursors of a hormone) from cardiomyocytes when there is increased heart-wall tension as a consequence of volume and/or pressure stress. This is intended to encourage renal sodium elimination, and thus water expulsion. Every volume and/or pressure burden leads to the release of BNP, and qualitative and quantitative distinctions offer information about the cause.[215,216] It can be established through additional parameters, such as determination of troponin, whether the secretion of BNP has a cardial origin, or, with an elevated creatinin level, a renal basis.

In the establishment of a reference range of one parameter are, by definition, only 95% of the analytical results of this parameter from test subjects with a defined degree of health (for example from clinical or epidemological registers/studies as well as national or international reference data) included in the confidence interval. The confidence interval is selected in such a way that at both the upper and the lower end of the measured values a standard deviation of 2.5% is taken as the limit. This procedure implies that 2.5% of all healthy people will show an elevated value, and 2.5% a diminished value, which for them is nevertheless physiologically normal. Thus, 5% of healthy test subjects fall outside the normal range and would incorrectly be categorized as ill. For this reason the determination of additional parameters is indispensable, as is the presence of clinical indications, to achieve a correct appraisal and interpretation of the results.

4. Summary and Outlook

A reliable laboratory result will only be acquired if work is carried out correctly at all three stages. Errors in pre-analysis continue to be the most common cause of "false" findings. Economic pressures in the health-care system have meant that attempts have long been made to reconcile the elements of the tension triangle: "fast—good—cheap". So far, however, it has only been possible to satisfy only two of these elements. Based on data from the Society of the Diagnostics Industry, laboratory investigations play a decisive role in approximately 70% of all clinical diagnoses. Data from the USA attest to these numbers: in hospitals there, between 70 and 80% of medical decisions are made on the basis of laboratory analyses, which represent only 3–5% of the costs in the USA, and are thus relatively inexpensive.

An aging population, changes in lifestyle, and high expectations for a good quality of life offer a major challenge to health-care systems. The goal is thus to strengthen preventative diagnostics. For this reasons, tests are being developed, for example, for early recognition of widespread diseases such as coronary and circulatory problems, cancer, and metabolic disorders (above all, diabetes mellitus), as well as osteoporosis, before these manifest clinical symptoms. New diagnostic approaches such as nanotechnology and DNA microarrays offer very promising avenues for individualized systemic biology in this personalized medicine. Nobel prize winner Richard Phillips Feynman (1918–1988) predicted in 1959 that "*there is plenty of room at the bottom*",[217] where he was alluding to still undreamt of possibilities with respect to modification of material in its tiniest dimension. Theranostics,

a neologism from therapy and diagnostics, will acquire greater significance here.[92] However, not everything in this area that is analytically and technically possible will become routine. Thus, for example, Raman spectroscopy in the context of diagnosis with biofunctionalized gold nanoparticles, NMR spectroscopy, and DNA microarrays must first demonstrate their superiority over routine procedures in regard to analytical stability, sensitivity, and specificity, and a large number of parameters must be determinable with these methods before investment in the corresponding equipment and training of appropriate personnel become worthwhile. Furthermore, new parameters and biomarkers must be tested explicitly in terms of their diagnostic sensitivity and specificity. Many new analytical methods, approaches, and biomarkers for the improvement of patient care need still to prove themselves in everyday practice.

In addition, it must be demonstrated that values obtained and consequences drawn would increase the quality of life and overall lifetimes of the patients themselves. Current studies on early detection of prostate cancer, in part through annual PSA tests, are unable to demonstrate unambiguous benefits.[218,219] Also, there is no increased chance of recovery through urine screening in newborns for the presence of a neuroblastoma (malign neoformation of sympathetic nerve tissue),[220,221] whereas newborn screening of blood from a heel stick for possible metabolic disease (for example, phenylketonuria, hypothyroidism) is of definite benefit to the patient.[222,223] A subject of current discussion is expansion of the number of newborn screenings to 21 metabolic diseases with the aid of tandem MS.[224]

A further step, the "lab-on-a-chip", offers the possibility of real-time observation of a vast array of blood parameters or cellular processes (optimistic authors speak in terms of thousands).[225–227] The large number of parameters, rapid availability, and simple acquisition of results leads to new problems, which are especially important for the clinical physician. A single result is important, but how is it to be interpreted?[228] How variable is a single parameter? How specific is a change in that parameter? Is it necessary, in the context of personalized medicine, to acquire data on a regular basis, especially in a phase of good health, to properly interpret changes in the event of illness? Over all these questions stands, in the end, questions of financial feasibility.

Received: June 22, 2009
Published online: December 29, 2009

Translated by Prof. William Russey, Laconia

[1] A. F. Fourcroy, *Systeme des connaissances Chimiques Part 9*, Baudoin, Paris, **1801**.
[2] M. C. Sanz, P. Lous, *IFCC News Lett.* **1971**, *6*, 1.
[3] B. D. Kahan, *N. Engl. J. Med.* **1981**, *305*, 280–281.
[4] J. Buettner, *J. Clin. Chem. Clin. Biochem.* **1985**, *23*, 797–804.
[5] L. Rosenfeld, *Clin. Chem.* **2002**, *48*, 186–197.
[6] W. A. Smeaton, *Fourcroy, chemist and revolutionary*, W. Heffer & Sons Ltd, Cambridge, **1962**.
[7] J. Buettner, *Eur. J. Clin. Chem. Clin. Biochem.* **1992**, *30*, 585–593.
[8] J. J. Berzelius, *Ann. Philos.* **1814**, *4*, 401–409.
[9] J. L. Gay-Lussac, L. J. Thenard, Recherches physico-chimiques, faites sur la pile, sur la preparation chimique et les properietes du potassium et du sodium, sur la decomposition de L'acide boracique, sur les acides fluorique, muriatique et muriatique oxigene, sur l'action chimique de La Lumiere, sur l'analyse vegetale et animale, Vol. 1, 2, Deterville, Paris, **1811**.
[10] C. G. Lehmann, *Lehrbuch der physiologischen Chemie, Vol. 1*, 2nd ed., Engelmann, Leipzig, **1850**.
[11] A. M. Chesney, *The Johns Hopkins Hospital and the Johns Hopkins University School of Medicine. A chronicle, 1893–1905, Vol. 2*, The Johns Hopkins Press, Baltimore, **1958**.
[12] S. Natelson, *Clin. Chem.* **1970**, *16*, 258–259.
[13] J. Buettner, *Ber. Wissenschaftsgesch.* **1957**, *28*, 311–322.
[14] L. T. Skeggs, Jr., *Am. J. Clin. Phathol.* **1957**, *28*, 311–322.
[15] L. T. Skeggs, Jr., *Clin. Chem.* **1956**, *2*, 241, Abstract 028.
[16] R. S. Yalow, S. A. Berson, *J. Clin. Invest.* **1960**, *39*, 1157–1175.
[17] A. Quételet, Sur l'homme et le développement de ses facultés, ou Essai de physique sociale., Paris, **1835**.
[18] A. Quételet, Lettres à S.A.R. le duc Régnant de Saxe-Cobourg et Gotha sur la théorie des probabilités, appliquée aux sciences morales et politiques, Hayez, Bruxelles, **1846**.
[19] P. Pascal, F. Beyerle, *Pathol. Biol.* **2006**, *54*, 317–324.
[20] M. Elston Dirk, *Clin. Lab. Med.* **2008**, *28*, 173–177.
[21] P. Carraro, M. Plebani, *Clin. Chem.* **2007**, *53*, 1338–1342.
[22] M. J. Alsina, V. Alvarez, N. Barba, S. Bullich, M. Cortes, I. Escoda, C. Martinez-Bru, *Clin. Chem. Lab. Med.* **2008**, *46*, 849–854.
[23] G. Lippi, N. Blanckaert, P. Bonini, S. Green, S. Kitchen, V. Palicka, A. J. Vassault, C. Mattiuzzi, M. Plebani, *Clin. Chem. Lab. Med.* **2008**, *0*, 0.
[24] G. Halwachs-Baumann, *Labormedizin: Klinik—Praxis—Fallbeispiele*, Springer, Vienna, **2006**.
[25] M. G. Scott, A. M. Gronowski, C. S. Eby, *Tietz's Applied Laboratory Medicine*, 2nd ed., Wiley, Hoboken, **2007**.
[26] W. G. Wood, Scand. *J. Clin. Lab. Invest. Suppl.* **1991**, *205*, 105–112.
[27] C. Selby, *Ann. Clin. Biochem.* **1999**, *36(Pt6)*, 704–721.
[28] G. A. Posthuma-Trumpie, J. Korf, A. van Amerongen, *Anal. Bioanal. Chem.* **2009**, *393*, 569–582.
[29] R. J. Davies, S. S. Eapen, S. J. Carlisle, *Handb. Biosens. Biochips* **2007**, *2*, 1151–1165.
[30] G. A. Posthuma-Trumpie, J. Korf, A. van Amerongen, *Anal. Bioanal. Chem.* **2009**, *393*, 569–582.
[31] G. A. Uriano, J. P. Cali in *Validation of the measurement process* (Ed.: J. R. DeVoe), American Chemical Society, Washington, DC, **1977**, pp. 140–161.
[32] J. N. Miller, R. Niessner, D. Knopp, *Ullmann's Biotechnol. Biochem. Eng.* **2007**, *2*, 585–612.
[33] M. Seydack, *Springer Ser. Fluoresc.* **2008**, *6*, 401–428.
[34] J. F. Brady, *Immunoassay Other Bioanal. Tech*. **2007**, pp. 249–263.
[35] R. F. Taylor, *Kirk-Othmer Encycl. Chem. Technol., Vol. 14*, 5th ed., Wiley, Hoboken, **2005**, pp. 135–159.
[36] C. P. Price, D. J. Newman, *Methods Immunol. Anal.* **1993**, *1*, 134–158.
[37] D. J. Marmer, P. E. Hurtubise, *Immunoassay* **1996**, 363–387.
[38] I. Deverill, *Res. Monogr. Immunol.* **1984**, *6*, 27–49.
[39] S. Blirup-Jensen, *Clin. Chem. Lab. Med.* **2001**, *39*, 1098–1109.
[40] J. C. Sternberg, *Clin. Chem.* **1977**, *23*, 1456–1464.
[41] J. A. Molina-Bolivar, F. Galisteo-Gonzalez, *J. Macromol. Sci. Part C* **2005**, *45*, 59–98.
[42] M. Heidelberger, F. E. Kendall, *J. Exp. Med.* **1932**, *55*, 555–561.
[43] M. Heidelberger, F. E. Kendall, *J. Exp. Med.* **1935**, *62*, 697–720.
[44] "Device for the determination of particles in liquid suspensions": W. H. Coulter, DE 964810, **1957**.
[45] A. Moldavan, *Science* **1934**, *80*, 188–189.
[46] P. J. Crosland-Taylor, *Nature* **1953**, *171*, 37–38.

[47] H. R. Hulett, W. A. Bonner, J. Barrett, L. A. Herzenberg, *Science* **1969**, *166*, 747 – 749.
[48] H. R. Hulett, W. A. Bonner, R. G. Sweet, L. A. Herzenberg, *Clin. Chem.* **1973**, *19*, 813 – 816.
[49] W. A. Bonner, H. R. Hulett, R. G. Sweet, L. A. Herzenberg, *Rev. Sci. Instrum.* **1972**, *43*, 404 – 409.
[50] A. R. Hobson, R. A. Agarwala, R. A. Swallow, K. D. Dawkins, N. P. Curzen, *Platelets* **2006**, *17*, 509 – 518.
[51] R. J. Luddington, *Clin. Lab. Haematol.* **2005**, *27*, 81 – 90.
[52] K. Vierordt, *Arch. Heilkd.* **1878**, *19*, 193.
[53] H. Hartert, *Klin. Wochenschr.* **1948**, *26*, 577 – 583.
[54] H. Hartert, *Schweiz. Med. Wochenschr.* **1949**, *79*, 318 – 322.
[55] H. Hartert, *Z. Gesamte Exp. Med.* **1951**, *117*, 189 – 203.
[56] A. Calatzis, S. Haas, O. Gödje, A. Calatzis, R. Hipp, J. M. Walenga in *Management of bleeding in cardiovascular surgery* (Ed.: R. Pifarré), Hanley & Belfus, Inc., Philadelphia, **2000**, pp. 215 – 226.
[57] Nobis Labordiagnostica GmbH: Benutzerinformation für die roTEG Vollblutgerinnungsanalyse/Thrombelastographie.
[58] D. C. Cardinal, R. J. Flower, *J. Pharmacol. Methods* **1980**, *3*, 135 – 158.
[59] A. Calatzis, M. Wittwer, B. Krueger, *Platelets* **2004**, *15*, 485 – 486.
[60] A. Calatzis, B. Krueger, M. Wittwer, (Dynabyte Informationssysteme G.m.b.H.), WO, Application, **2005**, p. 50.
[61] M. A. Kratzer, S. Bellucci, J. P. Caen, *Haemostasis* **1985**, *15*, 363 – 370.
[62] M. A. Kratzer, G. V. Born, *Haemostasis* **1985**, *15*, 357 – 362.
[63] V. Von der Goltz, US Patent 5051239, **1991**.
[64] R. L. Sautter, E. H. Lipford, *N. C. Med. J.* **2007**, *68*, 132 – 135.
[65] S. S. Ehrmeyer, R. H. Laessig, *Clin. Chem. Lab. Med.* **2007**, *45*, 766 – 773.
[66] S. M. Caples, R. D. Hubmayr, *Curr. Opin. Crit. Care* **2003**, *9*, 230 – 235.
[67] G. J. Kost, N. K. Tran, *Cardiol. Clin.* **2005**, *23*, 467 – 490, vi.
[68] G. J. Kost, H. T. Vu, J. H. Lee, P. Bourgeois, F. L. Kiechle, C. Martin, S. S. Miller, A. O. Okorodudu, J. J. Podczasy, R. Webster, K. J. Whitlow, *Crit. Care Med.* **1998**, *26*, 581 – 590.
[69] Oral Anticoagulation Monitoring Study Group, *Am. J. Clin. Pathol.* **2001**, *115*, 288 – 296.
[70] Z. Tang, J. H. Lee, R. F. Louie, G. J. Kost, *Arch. Pathol. Lab. Med.* **2000**, *124*, 1135 – 1140.
[71] R. Gosselin, J. T. Owings, R. H. White, R. Hutchinson, J. Branch, K. Mahackian, M. Johnston, E. C. Larkin, *Thromb. Haemostasis* **2000**, *83*, 698 – 703.
[72] P. B. Luppa, L. J. Sokoll, D. W. Chan, *Clin. Chim. Acta* **2001**, *314*, 1 – 26.
[73] D. Wild, *The Immunoassay Handbook*, Nature Publishing Group, New York, **2001**.
[74] L. C. Clark, Jr., C. Lyons, *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29 – 45.
[75] E. Katz, L. Sheeney-Haj-Ichia, I. Willner, *Angew. Chem.* **2004**, *116*, 3354 – 3362; *Angew. Chem. Int. Ed.* **2004**, *43*, 3292 – 3300.
[76] E. Katz, I. Willner, *Angew. Chem.* **2004**, *116*, 6166 – 6235; *Angew. Chem. Int. Ed.* **2004**, *43*, 6042 – 6108.
[77] E. Katz, I. Willner, *Angew. Chem.* **2005**, *117*, 4869 – 4872; *Angew. Chem. Int. Ed.* **2005**, *44*, 4791 – 4794.
[78] G. A. Zelada-Guillen, J. Riu, A. Düzgün, F. X. Rius, *Angew. Chem.* **2009**, *121*, 7470 – 7473; *Angew. Chem. Int. Ed.* **2009**, *48*, 7334 – 7337.
[79] M. J. Kim, S. M. Huang, U. A. Meyer, A. Rahman, L. J. Lesko, *J. Clin. Pharmacol.* **2009**, *49*, 138 – 146.
[80] M. H. Eckman, J. Rosand, S. M. Greenberg, B. F. Gage, *Ann. Intern. Med.* **2009**, *150*, 73 – 83.
[81] S. Briest, V. Stearns, *Clin. Adv. Hematol. Oncol.* **2009**, *7*, 185 – 192.
[82] T. Simon, C. Verstuyft, M. Mary-Krause, L. Quteineh, E. Drouet, N. Meneveau, P. G. Steg, J. Ferrieres, N. Danchin, L. Becquemont, *N. Engl. J. Med.* **2009**, *360*, 363 – 375.
[83] R. Nanda, *Rev. Recent Clin. Trials* **2007**, *2*, 111 – 116.
[84] E. S. Schernhammer, E. Giovannucci, C. S. Fuchs, S. Ogino , *Cancer Epidemiol. Biomarkers Prev.* **2008**, *17*, 2895 – 2898.
[85] C. Csillag, R. Borup, J. Olsen, F. C. Nielsen, O. H. Nielsen, *Scand. J. Gastroenterol.* **2007**, *42*, 834 – 840.
[86] C. Franzini, F. Ceriotti, *Clin. Biochem.* **1998**, *31*, 449 – 457.
[87] A. Uldall, *Accredit. Qual. Assur.* **1996**, *1*, 218 – 222.
[88] WHO Expert Committee on Biological Standardization, *Tech. Rep. Ser.* **2005**, *932*(i–vi), 1–137.
[89] W. J. Philipp, P. Iwaarden, H. Schimmel, N. Meeus, N. Kollmorgen, *Accredit. Qual. Assur.* **2007**, *12*, 134 – 138.
[90] K. W. Phinney, *Am. J. Clin. Nutr.* **2008**, *88*, 511S – 512S.
[91] H. M. Azzazy, M. M. Mansour, S. C. Kazmierczak, *Clin. Chem.* **2006**, *52*, 1238 – 1246.
[92] K. Riehemann, S. W. Schneider, T. A. Luger, B. Godin, M. Ferrari, H. Fuchs, *Angew. Chem.* **2009**, *121*, 886 – 913; *Angew. Chem. Int. Ed.* **2009**, *48*, 872 – 897.
[93] K. K. Jain, *Clin. Chim. Acta* **2005**, *358*, 37 – 54.
[94] L. Wang, M. B. O'Donoghue, W. Tan, *Nanomedicine* **2006**, *1*, 413 – 426.
[95] G. Wu, R. H. Datar, K. M. Hansen, T. Thundat, R. J. Cote, A. Majumdar, *Nat. Biotechnol.* **2001**, *19*, 856 – 860.
[96] C. Schmuck, P. Wich, B. Kustner, W. Kiefer, S. Schlucker, *Angew. Chem.* **2007**, *119*, 4870 – 4873; *Angew. Chem. Int. Ed.* **2007**, *46*, 4786 – 4789.
[97] D. L. Jeanmaire, R. P. Van Duyne, *J. Electroanal. Chem. Interfacial Electrochem.* **1977**, *84*, 1 – 20.
[98] M. D. Porter, R. J. Lipert, L. M. Siperko, G. Wang, R. Narayanan, *Chem. Soc. Rev.* **2008**, *37*, 1001 – 1011.
[99] S. Schlücker, B. Kuestner, A. Punge, R. Bonfig, A. Marx, P. Stroebel, *J. Raman Spectrosc.* **2006**, *37*, 719 – 721.
[100] X. Gao, M. Yezhelyev, Y. Xing, R. M. O'Regan, S. Nie, *Immunoassay Other Bioanal. Tech.* **2007**, 465 – 476.
[101] K. E. Sapsford, L. Berti, I. L. Medintz, *Angew. Chem.* **2006**, *118*, 4676 – 4704; *Angew. Chem. Int. Ed.* **2006**, *45*, 4562 – 4588.
[102] V. Biju, T. Itoh, A. Anas, A. Sujith, M. Ishikawa, *Anal. Bioanal. Chem.* **2008**, *391*, 2469 – 2495.
[103] R. Gill, M. Zayats, I. Willner, *Angew. Chem.* **2008**, *120*, 7714 – 7736; *Angew. Chem. Int. Ed.* **2008**, *47*, 7602 – 7625.
[104] P. Tartaj, M. P. Morales, S. Veintemillas-Verdaguer, T. Gonzalez-Carreno, C. J. Serna, *Handb. Magn. Mater.* **2006**, *16*, 403 – 482.
[105] C. Alexiou, R. Jurgons, C. Seliger, H. Iro, J. *Nanosci. Nanotechnol.* **2006**, *6*, 2762 – 2768.
[106] E. Duguet, S. Vasseur, S. Mornet, J. M. Devoisselle, *Nanomedicine* **2006**, *1*, 157 – 168.
[107] R. McKendry, J. Zhang, Y. Arntz, T. Strunz, M. Hegner, H. P. Lang, M. K. Baller, U. Certa, E. Meyer, H. J. Guntherodt, C. Gerber, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 9783 – 9788.
[108] T. Ideker, T. Galitski, L. Hood, Annu. *Rev. Genomics Hum. Genet.* **2001**, *2*, 343 – 372.
[109] E. C. Butcher, E. L. Berg, E. J. Kunkel, *Nat. Biotechnol.* **2004**, *22*, 1253 – 1259.
[110] J. W. Lee, W. C. Smith, G. D. Nordblom, R. R. Bowsher, *Drugs Pharm. Sci.* **2003**, *132*, 119 – 148.
[111] A. Dorn-Beineke, C. E. Dempfle, T. Bertsch, H. Wisser, *Thromb. Res.* **2005**, *116*, 171 – 179.
[112] C. P. Chan, Y. C. Cheung, R. Renneberg, M. Seydack, *Adv. Biochem. Eng. Biotechnol.* **2008**, *109*, 123 – 154.
[113] Z. Fu, H. Liu, Z. Yang, H. Ju, *Curr. Trends Biotechnol. Pharm.* **2007**, *1*, 1 – 17.
[114] R. E. Gerszten, M. Sabatine, T. J. Wang, *Clin. Chem.* **2009**, *55*, 404 – 406.
[115] I. Hemmilä, *Springer Ser. Fluoresc.* **2008**, *6*, 429 – 447.

[116] H. Kuramitz, *Anal. Bioanal. Chem.* **2009**, *394*, 61 – 69.
[117] C. Mastichiadis, A. E. Niotis, P. S. Petrou, S. E. Kakabakos, K. Misiakos, *TrAC Trends Anal. Chem.* **2008**, *27*, 771 – 784.
[118] M. S. Rodeheffer, K. Birsoy, J. M. Friedman, *Cell* **2008**, *135*, 240 – 249.
[119] W. A. Woodward, E. P. Sulman, *Cancer Metastasis Rev.* **2008**, *27*, 459 – 470.
[120] R. M. O'Hara, Jr., S. E. Benoit, C. J. Groves, M. Collins, *Drug Discovery Today* **2006**, *11*, 342 – 347.
[121] M. Vogeser, C. Seger, *Clin. Biochem.* **2008**, *41*, 649 – 662.
[122] K. Gempel, K.-D. Gerbitz, B. Casetta, M. F. Bauer, *Clin. Chem.* **2000**, *46*, 122 – 123.
[123] Z. Yang, S. Wang, *J. Immunol. Methods* **2008**, *336*, 98 – 103.
[124] H. H. Maurer, *Anal. Bioanal. Chem.* **2007**, *388*, 1315 – 1325.
[125] U. Ceglarek, A. Leichtle, M. Brugel, L. Kortz, R. Brauer, K. Bresler, J. Thiery, G. M. Fiedler, *Mol. Cell. Endocrinol.* **2009**, *301*, 266 – 271.
[126] M. Vogeser, A. Geiger, R. Herrmann, U. Kobold, *Clin. Biochem.* **2008**, *41*, 1417 – 1419.
[127] U. Kobold, A. Geiger, R. Herrmann, M. Vogeser, (Roche Diagnostics GmbH; F.Hoffmann-La Roche AG), Application EP 200810569, **2008**, pp. 35.
[128] J. Ma, J. Shi, H. Le, R. Cho, J. C.-J. Huang, S. Miao, B. K. Wong, *J. Chromatogr. B* **2008**, *862*, 219 – 226.
[129] D. Leibfritz, *Anticancer Res.* **1996**, *16*, 1317 – 1324.
[130] S. Mora, J. D. Otvos, N. Rifai, R. S. Rosenson, J. E. Buring, P. M. Ridker, *Circulation* **2009**, *119*, 931 – 939.
[131] J. Willmann, D. Leibfritz, H. Thiele, *J. Biomol. Tech.* **2008**, *19*, 211 – 216.
[132] W. Dreher, D. Leibfritz, *Magn. Reson. Med.* **2005**, *54*, 190 – 195.
[133] V.-P. Makinen, P. Soininen, C. Forsblom, M. Parkkonen, P. Ingman, K. Kaski, P.-H. Groop, M. Ala-Korpela, *Magn. Reson. Mater. Phys. Biol. Med.* **2006**, *19*, 281 – 296.
[134] M. E. Bollard, E. G. Stanley, J. C. Lindon, J. K. Nicholson, E. Holmes, *NMR Biomed.* **2005**, *18*, 143 – 162.
[135] M. Coen, E. Holmes, J. C. Lindon, J. K. Nicholson, *Chem. Res. Toxicol.* **2008**, *21*, 9 – 27.
[136] B. Fuchs, J. Schiller, *Subcell. Biochem.* **2008**, *49*, 541 – 565.
[137] K. Sparbier, T. Wenzel, H. Dihazi, S. Blaschke, G.-A. Mueller, A. Deelder, T. Flad, M. Kostrzewa, *Proteomics* **2009**, *9*, 1442 – 1450.
[138] Z. Meng, T. D. Veenstra, *Proteomics Clin. Appl.* **2007**, *1*, 747 – 757.
[139] W. Morelle, V. Faid, F. Chirat, J. C. Michalski, *Methods Mol. Biol.* **2009**, *534*, 1 – 19.
[140] T. Alexandrov, J. Decker, B. Mertens, A. M. Deelder, R. A. Tollenaar, P. Maass, H. Thiele, *Bioinformatics* **2009**, *25*, 643 – 649.
[141] C. Pan, S. Xu, H. Zhou, Y. Fu, M. Ye, H. Zou, *Anal. Bioanal. Chem.* **2007**, *387*, 193 – 204.
[142] F. Vivanco, L. R. Padial, V. M. Darde, F. de La Cuesta, G. Alvarez-Llamas, N. Diaz-Prieto, M. G. Barderas, *Biomarker Insights* **2008**, *3*, 101 – 113.
[143] J. V. Tricoli, Transl. *Med. Ser.* **2007**, *1*, 39 – 57.
[144] A. Lonneborg, *Mol. Diagn. Ther.* **2008**, *12*, 307 – 320.
[145] O. F. Laterza, V. R. Modur, J. H. Ladenson, *Biomarkers Med.* **2008**, *2*, 81 – 92.
[146] D. C. Gaze, *Curr. Opin. Invest. Drugs (Thomson Sci.)* **2007**, *8*, 711 – 717.
[147] P. Seng, M. Drancourt, F. Gouriet, B. La Scola, P. E. Fournier, J. M. Rolain, D. Raoult, *Clin. Infect. Dis.* **2009**, *49*, 543 – 551.
[148] G. Marklein, M. Josten, U. Klanke, E. Müller, R. Horré, T. Maier, T. Wenzel, M. Kostrzewa, G. Bierbaum, A. Hoerauf, H. G. Sahl, *J. Clin. Microbiol.* **2009**, *47*, 2912 – 2917.
[149] S. Holdenrieder, P. Stieber, *Crit. Rev. Clin. Lab. Sci.* **2009**, *46*, 1 – 24.
[150] S. Holdenrieder, J. von Pawel, E. Dankelmann, T. Duell, B. Faderl, A. Markus, M. Siakavara, H. Wagner, K. Feldmann, H. Hoffmann, H. Raith, D. Nagel, P. Stieber, *Clin. Cancer Res.* **2008**, *14*, 7813 – 7821.
[151] A. Sulewska, W. Niklinska, M. Kozlowski, L. Minarowski, W. Naumnik, J. Niklinski, K. Dabrowska, L. Chyczewski, *Folia Histochem. Cytobiol.* **2007**, *45*, 315 – 324.
[152] M. Esteller, *DNA Methylation 2005*, 1 – 9.
[153] E. Prokhortchouk, P.-A. Defossez, *Biochim. Biophys. Acta Mol. Cell Res.* **2008**, *1783*, 2167 – 2173.
[154] S. Thiagalingam, P. Papageorgis, *Cancer Epigenet.* **2009**, 333 – 346.
[155] R. Lesche, F. Eckhardt, *Curr. Opin. Mol. Ther.* **2007**, *9*, 222 – 230.
[156] A. Dobrovic, L. S. Kristensen, *Int. J. Biochem. Cell Biol.* **2009**, *41*, 34 – 39.
[157] S. Gopalakrishnan, B. O. Van Emburgh, K. D. Robertson, *Mutat. Res. Fundam. Mol. Mech. Mutagen.* **2008**, *647*, 30 – 38.
[158] E. A. Klein, Y. Yung, P. Castagnino, D. Kothapalli, R. K. Assoian, *Methods Enzymol.* **2007**, *426*, 155 – 175.
[159] J. P. Thiery, *Curr. Opin. Genet. Dev.* **2003**, *13*, 365 – 371.
[160] M. K. Chelbi-Alix, J. Wietzerbin, *Biochimie* **2007**, *89*, 713 – 718.
[161] E. A. Evans, D. A. Calderwood, *Science* **2007**, *316*, 1148 – 1153.
[162] J. Monnier, M. Samson, *FEBS J.* **2008**, *275*, 4014 – 4021.
[163] S. A. Mousa, *Mol. Biotechnol.* **2008**, *38*, 33 – 40.
[164] K. R. Pang, J. J. Wu, D. B. Huang, S. K. Tyring, S. Baron, *Methods Mol. Med.* **2005**, *116*, 1 – 23.
[165] F. W. Quelle, *Immunol. Res.* **2007**, *39*, 173 – 184.
[166] K. Ley, J. Reutershan, *Handb. Exp. Pharmacol.* **2006**, *176*, 97 – 133.
[167] I. Cinel, S. M. Opal, *Crit. Care Med.* **2009**, *37*, 291 – 304.
[168] I. Herzum, H. Renz, *Curr. Med. Chem.* **2008**, *15*, 581 – 587.
[169] M. Bajenoff, R. N. Germain, *Eur. J. Immunol.* **2007**, *37*, S18 – S33.
[170] N. Sachdeva, D. Asthana, *Front. Biosci.* **2007**, *12*, 4682 – 4695.
[171] M. Mahmoudi, N. Curzen, P. J. Gallagher, *Histopathology* **2007**, *50*, 535 – 546.
[172] D. Aronson, *Adv. Cardiol.* **2008**, *45*, 1 – 16.
[173] M. Cnop, *Biochem. Soc. Trans.* **2008**, *36*, 348 – 352.
[174] B. Akduman, E. D. Crawford, *Curr. Urol. Rep.* **2007**, *8*, 233 – 238.
[175] H. K. Datta, W. F. Ng, J. A. Walker, S. P. Tuck, S. S. Varanasi, *J. Clin. Pathol.* **2008**, *61*, 577 – 587.
[176] H. Jüppner, *Ther. Apheresis Dial.* **2007**, 11 Suppl 1, S3 – S22.
[177] M. Wiltgen, G. P. Tilz, *Hematology* **2007**, *12*, 271 – 287.
[178] S. F. Grant, H. Hakonarson, *Clin. Chem.* **2008**, *54*, 1116 – 1124.
[179] K. Ogawa, S. Murayama, M. Mori, *Oncol. Rep.* **2007**, *18*, 1243 – 1248.
[180] G. P. Page, S. O. Zakharkin, K. Kim, T. Mehta, L. Chen, K. Zhang, *Methods Mol. Biol.* **2007**, *404*, 409 – 430.
[181] A. T. Weeraratna, D. D. Taub, *Methods Mol. Biol.* **2007**, *377*, 1 – 16.
[182] K. Sato, T. Kitamori, Handb. *Capillary Microchip Electrophor. Assoc. Microtech.*, 3rd ed., CRC, Boca Raton, **2008**, pp. 1013 – 1019.
[183] M. Seidel, R. Niessner, *Anal. Bioanal. Chem.* **2008**, *391*, 1521 – 1544.
[184] A. R. Newman, *Anal. Chem.* **1990**, *62*, 1063A – 1065A.
[185] S. J. Lee, S. Y. Lee, *Appl. Microbiol. Biotechnol.* **2004**, *64*, 289 – 299.
[186] C. Zhang, D. Xing, *Nucleic Acids Res.* **2007**, *35*, 4223 – 4237.
[187] A. Ahmadian, M. Ehn, S. Hober, *Clin. Chim. Acta* **2006**, *363*, 83 – 94.
[188] B. Huelseweh, R. Ehricht, H. J. Marschall, *Proteomics* **2006**, *6*, 2972 – 2981.
[189] J. Weile, C. Knabbe, *Anal. Bioanal. Chem.* **2009**, *394*, 731 – 742.

[190] P. Baptista, E. Pereira, P. Eaton, G. Doria, A. Miranda, I. Gomes, P. Quaresma, R. Franco, *Anal. Bioanal. Chem.* **2008**, *391*, 943 – 950.
[191] C. C. You, O. R. Miranda, B. Gider, P. S. Ghosh, I. B. Kim, B. Erdogan, S. A. Krovi, U. H. Bunz, V. M. Rotello, *Nat. Nanotechnol.* **2007**, *2*, 318 – 323.
[192] K. K. Jain, *Expert Rev. Mol. Diagn.* **2003**, *3*, 153 – 161.
[193] W. Wang, P. Vadgama, *J. R. Soc. Interface* **2004**, *1*, 109 – 117.
[194] W. L. Wright, *J. Neurol. Sci.* **2007**, *261*, 10 – 15.
[195] J. Metzger, P. B. Luppa, D. M. Good, H. Mischak, *Crit. Rev. Clin. Lab. Sci.* **2009**, *46*, 129 – 152.
[196] R. Gaza, S. W. McKeever, *Radiat. Prot. Dosim.* **2006**, *120*, 14 – 19.
[197] V. Van Hoof, A. Wormek, S. Schleutermann, T. Schumacher, O. Lothaire, C. Trendelenburg, *Stud. Health Technol. Inform.* **2004**, *107*, 89 – 93.
[198] A. Kratz, M. Laposata, *Clin. Chim. Acta* **2002**, *319*, 117 – 125.
[199] G. E. Hoffmann, *Clin. Chim. Acta* **1998**, *278*, 203 – 216.
[200] D. M. Elston, *Clin. Lab. Med.* **2008**, *28*, 173 – 177.
[201] M. J. Goldschmidt Henk, *Clin. Chem. Lab. Med.* **2004**, *42*, 868 – 873.
[202] Y. A. Park, M. B. Marques, *Clin. Lab. Med*. **2007**, *27*, 411 – 424, abstract ix.
[203] M. J. O'Kane, P. L. Lynch, N. McGowan, *Ann. Clin. Biochem.* **2008**, *45*, 129 – 134.
[204] R. Gräsbeck, *Clin. Chem. Lab. Med.* **2004**, *42*, 692 – 697.
[205] C. Ricós, V. Domenech Maria, C. Perich, *Clin. Chem. Lab. Med.* **2004**, *42*, 858 – 862.
[206] F. Ceriotti, R. Hinzmann, M. Panteghini, *Ann. Clin. Biochem.* **2009**, *46*, 8 – 17.
[207] B. N. Swanson, *Dis. Markers* **2002**, *18*, 47 – 56.
[208] J. Feldschuh, S. Katz, *Am. J. Med. Sci.* **2007**, *334*, 41 – 46.
[209] S. F. Shariat, P. T. Scardino, H. Lilja, *Can. J. Urol.* **2008**, *15*, 4363 – 4374.
[210] D. J. Parekh, D. P. Ankerst, D. Troyer, S. Srivastava, I. M. Thompson, *J. Urol.* **2007**, *178*, 2252 – 2259.
[211] D. L. Meany, D. W. Chan, *Clin. Chem. Lab. Med.* **2008**, *46*, 575 – 576.
[212] C. M. Sturgeon, A. R. Ellis, *Clin. Chim. Acta* **2007**, *381*, 85 – 92.
[213] D. Ilic, D. O'Connor, S. Green, T. Wilt, *Cochrane Database Syst. Rev.* **2006**, *3*, CD004720.
[214] K. Bensalah, Y. Lotan, J. A. Karam, S. F. Shariat, *Prostate Cancer Prostatic Dis.* **2008**, *11*, 112 – 120.
[215] M. A. Burke, W. G. Cotts, *Heart Failure Rev.* **2007**, *12*, 23 – 36.
[216] V. K. Munagala, J. C. Burnett, Jr., M. M. Redfield, *Curr. Probl. Cardiol.* **2004**, *29*, 707 – 769.
[217] R. Feynman, There is plenty of room at the bottom. Lecture on 29.12.1959 at the "Annual meeting of the American Physical Society" at the California Institute of Technology, Pasadena, CA, 1959.
[218] G. L. Andriole, E. D. Crawford, R. L. Grubb III, S. S. Buys, D. Chia, T. R. Church, M. N. Fouad, E. P. Gelmann, P. A. Kvale, D. J. Reding, J. L. Weissfeld, L. A. Yokochi, B. O'Brien, J. D. Clapp, J. M. Rathmell, T. L. Riley, R. B. Hayes, B. S. Kramer, G. Izmirlian, A. B. Miller, P. F. Pinsky, P. C. Prorok, J. K. Gohagan, C. D. Berg, *N. Engl. J. Med.* **2009**, *360*, 1310 – 1319.
[219] F. H. Schroder, J. Hugosson, M. J. Roobol, T. L. Tammela, S. Ciatto, V. Nelen, M. Kwiatkowski, M. Lujan, H. Lilja, M. Zappa, L. J. Denis, F. Recker, A. Berenguer, L. Maattanen, C. H. Bangma, G. Aus, A. Villers, X. Rebillard, T. van der Kwast, B. G. Blijenberg, S. M. Moss, H. J. de Koning, A. Auvinen, *N. Engl. J. Med.* **2009**, *360*, 1320 – 1328.
[220] W. G. Woods, R. N. Gao, J. J. Shuster, L. L. Robison, M. Bernstein, S. Weitzman, G. Bunin, I. Levy, J. Brossard, G. Dougherty, M. Tuchman, B. Lemieux, *N. Engl. J. Med.* **2002**, *346*, 1041 – 1046.
[221] F. H. Schilling, C. Spix, F. Berthold, R. Erttmann, N. Fehse, B. Hero, G. Klein, J. Sander, K. Schwarz, J. Treuner, U. Zorn, J. Michaelis, *N. Engl. J. Med.* **2002**, *346*, 1047 – 1053.
[222] C. I. Kaye, F. Accurso, S. La Franchi, P. A. Lane, H. Northrup, S. Pang, G. B. Schaefer, *Pediatrics* **2006**, *118*, 1304 – 1312.
[223] B. Wilcken, V. Wiley, *Pathology* **2008**, *40*, 104 – 115.
[224] L. E. Cipriano, C. A. Rupar, G. S. Zaric, *Value Health* **2007**, *10*, 83 – 97.
[225] A. M. Dupuy, S. Lehmann, J. P. Cristol, *Clin. Chem. Lab. Med.* **2005**, *43*, 1291 – 1302.
[226] T. A. Franke, A. Wixforth, *ChemPhysChem* **2008**, *9*, 2140 – 2156.
[227] D. Kohlheyer, J. C. Eijkel, A. van den Berg, R. B. Schasfoort, *Electrophoresis* **2008**, *29*, 977 – 993.
[228] A. A. Conti, A. Conti, G. F. Gensini, *Panminerva Med.* **2006**, *48*, 203 – 205.