1

Introduction

1.1 A Short Definition of Laboratory Automation

The term "automation" first appeared in 1936. Harder described automation as "the transfer of work tasks to machines in a production process without human intervention" [1]. In 1946, he while working as Vice President founded the Automation Department of Ford Motor Company. After World War II, two books by Diebold (1926–2005) appeared in 1952, describing automation as "automatic operation or a process for the automatic production of material goods." Diebold defined two main meanings of automation. On the one hand, he defined automation as an automatic control through feedback. On the other hand, automation for him was also the integration of a different number of machines [2]. The Diepold concept was further developed by Bright, who described the various stages of mechanization and automation [3], and Drucker, who recognized automation as "a conceptual system beyond technology." These three theories form the basis for understanding the concept and importance of automation [4]. "Automation" can be seen as an abbreviation for "automation technology" or "automatic operation." Alternatively, automation is also a combination of the Greek "automotos" (means "to move yourself") and the Latin "-ion" (means "a state"). "Mechanization" is the replacement of physical labor with machines; however, machine operation is controlled by human operators. "Automation" also replaces these control measures with machines, i.e. it replaces the physical and mental activities of humans with machines.

Laboratory automation is part of automation technology and aims to develop and optimize technologies for the automation of classic laboratories. This includes a wide variety of laboratories in the fields of medical diagnostics, environmental analysis, or quality control, for example, in the pharmaceutical industry, food monitoring, or industrial production. Laboratory automation is a strongly multidisciplinary field. The main goal of automating laboratory processes has not changed since the first steps in this area and consists of increasing the number of processed samples (and thus productivity), reducing the processing times required per sample, and improving the quality of those obtained experimental data or the creation of opportunities for examinations that would not be possible without suitable laboratory automation.

Laboratory automation can today be defined as a highly complex integration of robotics, liquid handling systems, sample processing, and analyzing devices and computers for process control. The most important part of laboratory automation is laboratory robotics, which

Devices and Systems for Laboratory Automation, First Edition. Kerstin Thurow and Steffen Junginger.

develops robots and robotic solutions adapted to the specificity of laboratory processes. Since the robots in laboratory automation systems generally only take on transport tasks, the development of suitable devices and components for the automatic execution of laboratory processes (e.g. dosing, shaking, incubating, etc.) is of immense importance. Suitable software algorithms are required to control the individual systems and to evaluate the data collected.

1.2 Short History of Laboratory Automation

The main drivers of the development of automation solutions are often the development of special branches of industry as well as new and more complex requirements for specific analytical processes. Very often, the impulses for the development of new solutions result from the end-users who are confronted with several problems and inadequacies in their everyday laboratory work. For a long time, the requirements of industrial process control drove the development of automated systems.

1.2.1 Early Developments in Laboratory Automation

The first reports on the use of automated devices can be traced back to 1875 [5]. The first steps that have been made accessible to automation seem very simple from today's perspective: washing filtration residues on filter paper or liquid extractions. In 1875, Stevens described a device that made it possible to wash filter residues with water at a controlled flow rate. The wash solution was in a closed reservoir, through which air was passed through an opening. The flow rate could be controlled by the size of the opening [6]. This concept was further developed by Mitchel [7] and Lathrop [8]. In the analysis of fertilizers, the samples were washed successively with 10 ml water each until a total volume of 2500 ml was reached in order to wash out the soluble components. For this purpose, Horne developed a device for the automatic washing of the samples [9]. The first automatic burette for laboratories with recurring titrations was described by Squibb in 1894 [10]. In the same year, Greiner presented an automatic pipette, which was used for the Babcock milk test [11]. The previous developments were not suitable for slow extractions over several hours; therefore, Hibbard developed a suitable system with which flow rates of approximately 40 drops/minute were possible. A further reduction in the dripping speed could be achieved by installing a splitter [12]. The first liquid–liquid extractors were used for botanical studies. By spraying the extraction solvent into the aqueous phase, the efficiency of the extraction could be increased considerably by increasing the surface area [13]. The first devices were developed by different scientists, who were faced with different problems in the laboratory. They were very fragile systems that could be easily broken and very difficult to clean; therefore, the solutions were proprietary and did not find widespread use.

The better understanding of combustion processes and the steadily growing production of electrical energy at the end of the nineteenth century revolutionized power generation. The development of automation was therefore decisively driven by the coal and power generation industry since at the beginning of the twentieth century, there was an increasing need for more precise knowledge of the quality of coal (calorific value). The first

commercial laboratory automation device was therefore a device for grinding coal samples. The Sturtevant Automatic Coal Crasher was operated by an external motor, and it made it possible to provide representative samples [14]. Another important parameter in industrial production was the determination of carbon dioxide in flue gases for the optimization of combustion processes. A commercial system was introduced to the market by Simmance and Abady. The system could be operated unattended for longer periods of time, but only provided intermittent values. A continuous variant was proposed by Stache et al. with the development of the autolyser [15]. Taylor and Hugh developed a system for the automated determination of carbon monoxide, which was based on a change in conductivity of a solution when the gas was passed through [16]. Conductivity measurements have also been reported for the control of sulfuric acid content in papermaking. Edelmann developed a device that enabled the automatic supply of sulfuric acid based on the measured values. This had previously been done manually and therefore represented an enormous source of errors [17].

The first commercial automated laboratory devices were developed during the First World War due to an increased need for rapid gas analysis. Such systems could now be used for the detection of chemical warfare agents in armed conflicts. The first systems were based on the measurement of changes in the conductivity of a heating wire. Since there was no chromatographic separation of the components prior to the measurement, clear identification of substances was not possible. Commercial variants were sold by the Cambridge Instrument Company and others [18].

In the 1920s, new requirements came from the sugar and paper industries, where there was an increasing need for pH determinations in different production steps. An essential step is the liming of sugar cane juice to remove non-sugars, for which an automated system was first developed in 1928 [19]. This system marked the beginning of the era of the development of electrodes for pH control. The electrodes available at the time required too long equilibration times, were too complicated for use in an industrial environment and were too susceptible to poisoning from sulfur dioxide, which was used in the process. Balch and Kane used tungsten-calomel electrodes for their developments, but it turned out that these exhibited variabilities in calibration, did not last long, and were also susceptible to poisoning [20].

In 1929, the first automated titration systems were introduced, which used a photocell to detect the color change in the solution. After the color change was detected, a valve was automatically closed so that no further titrant was dosed. The authors reported that "the device was 165 times more sensitive than the human eye" [21]. Hickman and Sanford developed a much more sophisticated titration device at Eastman Kodak. The device had an option to empty the previous sample to avoid contamination. In addition, the indicator was automatically supplied [22].

With the beginning of World War II, there was a further boost in the development of automation solutions in process control. This resulted from increased demands on the production of war-relevant goods and a lack of qualified workers. Automated devices were also used to enable unskilled workers to perform complex tasks [23]. Particular attention was paid to the development of semi-automated distillation equipment; Ferguson developed a corresponding system for petroleum fractionation [24]. The automatic mercaptan titrator (Shell Oil Company) for the analysis of gasoline was also a typical example of an automation

solution that arose due to the existing shortage of skilled workers. Because the system was used in a refinery, the device was locked in an explosion-proof housing. To ensure overpressure in the system and to prevent the penetration of explosive gases, compressed air was fed into the housing. A potentiometric method developed in 1941 was automated in 1943. The device could easily be operated by unskilled workers [25]. In contrast to manual titration, in which the rate of addition is adjusted around the end point, the titrant was kept constant.

At the end of World War II, the use of automated systems in the chemical industry had already become routine; thus, there was an increasing need for appropriately trained specialists. New devices were developed for fraction collectors for chromatography or distillations. Electronic components were increasingly used to control valves, for example, for an automated system for paper chromatography [26]. The development of automated titrators was advanced. In 1948, a device was created that used a motor-driven syringe to add the titrant. The motor speed could be adapted for the respective titration applications and the titration curve could be printed [27]. The automated Karl Fischer titration was introduced in 1952 by the Merck company. Since this method works without water, it was not possible to use classic potentiometric methods to determine the end point. Instead, a polarization process with depolarization of the platinum electrodes used at the end point was chosen [28]. The automated coulometric Karl Fischer titration, which made it possible to recover the Karl Fischer reagent [29], represents a significant development. A summary of automated titration techniques and systems using photometric, amperometric, conductometric, thermal, and potentiometric methods can be found in Ewing [30]. The first reviews of automation technologies appeared in the 1950s [31]. From 1952, the "Instrument Engineer" journal was devoted to special automation topics.

Computers related to automation were first described in 1948. The "office-size electronic computer" presented by Reeves Instrument Corporation gave researchers an opportunity to simulate their processes for the first time [32]. The first use of digital computers was described as a system for the mass spectrometric determination of hydrocarbon mixtures (Atlantic Refining Company) [33]. In the following period, computers quickly found diverse uses in laboratory automation. Cerda and Ramis described, among other things, the automation of potentiometric titrations with a Commodore VIC-20 microcomputer and with an IBM PC. In some cases, separate computers were used for data handling due to the limited storage capacity. The latter system has been described for the titration of studies on chemical equilibria as well as for titrations to determine equivalence points. A system consisting of two burettes, an autosampler, a potentiometer, and an Acer 710 to control the entire system enabled the automatic determination of boron in industrial samples. A system for ion-selective potentiometry has also been described. The authors also described automatic systems for conductometric, photometric, spectrophotometric-potentiometric, fluorometric, and thermometric titrations [34].

In addition to the development of computers, the introduction of transistors also revolutionized laboratory automation.

Innovative technologies in the dosing of liquids were essential for further development of laboratory automation [35]. In 1957, Schnitger developed a new type of pipette that already had all the features of modern piston-operated pipettes today. It had a spring-loaded piston, a second coaxial spring for blowing out liquid residues, and replaceable plastic pipette tips. An air buffer separated the liquid from the reciprocating piston. The Eppendorf company

(Hamburg, Germany) secured exclusive production and marketing rights and introduced the first industrially manufactured piston-operated pipette into the market in 1961 [36]. Today's mechanically adjustable micropipettes are based on a model developed by Gilson, which he patented in 1974 [37].

The technical advances in the development of small motors and valves led to the introduction of semi-automated syringe-based pipetting systems in the 1970s. In 1971, the Digital Dilutor (Hamilton, Reno, NV) was introduced, which used two calibrated syringes as pipetting plungers. The establishment of microprocessor technology made it possible to create program sequences for controlling the motors and valves and this led to the first fully automatic pipetting systems. The first automated liquid handling systems emerged in the 1980s as a result of further electromechanical developments. The development of these systems has been driven by clinical radioimmunoassays. Hamilton (Reno, NV) and Tecan (Männedorf, Switzerland) cooperated in the late 1970s in the joint development of the Hamilton AMICA system, which was the basis for the later pipetting systems Hamilton 2000 Series and Tecan Sampler 500/RSP 5000 Series Workstation. Both systems were based on Cartesian robotic platforms and enabled single-channel pipetting. A short time later, systems with two separate Cartesian arms and a second pipetting channel were also available. With the Zymark Z510 Master Laboratory Station, Zymark (Hopkinton, MA) developed its own pipetting system for integration into more complex Zymark robot systems.

1.2.2 Advances in the Automation of Clinical Laboratories

Medical and clinical applications and requirements largely drove the development of laboratory automation. The first real automated systems with automated loading of samples into the system and then fully automated measurement appeared in medical laboratories in the mid-1950s. The *AutoAnalyzer* (Technicon), presented in December 1956, was able to determine the concentrations of urea, sugar, and calcium in blood samples within 2.5 minutes [38]. The concentration was determined by color changes that were read out using photocells [39]. The *AutoAnalyzer I* used flow analysis technology to increase sample throughput. Later versions enabled the simultaneous determination of 20 analytes, with a throughput of 150 samples per hour. The *AutoAnalyzer* started a long development in clinical automation. Devices such as the *Sequential Multiple Analyzer* (SMA, 1969) and *Sequential Multiple Analyzer with Computer* (1974) increased the throughput further [40]. The *AutoAnalyzer* was the first batch analyzer in clinical laboratories and led to numerous other batch analyzers, which could usually examine up to 100 samples continuously for individual analytes. In the early 1980s, the introduction of the photodiode for spectrometers with grating monochromators led to the development of systems that enabled simultaneous determination of different analytes in a sample using different specific wavelengths [41].

Another approach was followed by the Research Specialites Co., Richmond, CA, which presented the *Robot Chemist* in 1959 [42]. Although the *Robot Chemist* was able to take over all manual steps in sample preparation and enabled analysis with conventional cuvettes, it was not successful in the long term due to its excessive mechanical complexity; production stopped in 1969. The principle of batch sample processing has increasingly been replaced by discrete systems that work with positive displacement pipettes. The solutions were appropriately mixed by the dispensing steps themselves or by means of magnetic or

mechanical stirrers. Temperature monitoring was implemented, as well as washing steps between the individual sub-steps. Permanent (glass) or disposable (plastic) cuvettes were used. Depending on the application, different analyzers with different lamps, including tungsten, quartz halogen, mercury, xenon, or laser, were used. The monochromators used interference filters, prisms, or diffraction gratings. The signal detection was usually carried out with photodiodes since a wide range of wavelengths can be covered in this way [43].

In addition to the development of automated analysis systems, another important step was the introduction of ready-made kits for carrying out analytical determinations, which contained all the necessary solvents and reagents as well as the corresponding work instructions. Sigma Chemical Company introduced the first kit of its kind in the 1950s. This eliminated the need for the manual production of reagents in the laboratory, which, in addition to reducing the workload, also led to considerable improvements in the quality of the analytical tests.

With the beginning of the 1970s, the introduction of robots into clinical laboratories and with it the era of total automation began. A revolution in this area occurred in the 1980s when Sasaki opened the first fully automated laboratory [44, 45]. As professor and director of the Department of the Clinical Laboratory at Kochi Medical School (Kochi, Japan), he and his team built conveyor belts, robots for loading and unloading analyzers and developed the first process control software [46]. The automation efforts at this time resulted from extensive savings in technical personnel for the implementation of clinical-chemical investigations [47]. Through close cooperation with industrial partners, his ideas led to commercial products that were used in numerous clinical laboratories across Japan. Further, 72% of all university hospitals in Japan installed and used such systems [47]. In the 1990s, there were several commercial suppliers of fully automated systems for clinical laboratories [48]. Regardless of the success of these first laboratory automation systems, they remained stand-alone solutions that could not be used for smaller laboratories and institutions, particularly due to the high costs. In addition, different interfaces of devices from different manufacturers limited the general use, since communication between different devices was not possible in this way. Sasaki et al., therefore, recommended the introduction of binding standards and sizes of racks as well as the use of more flexible robotic technologies in order to achieve plug-and-play functionality in automation systems [47]. Some laboratories developed in-house solutions, but these were very proprietary systems and required a lot of maintenance.

Dr. Rod Markin (University of Nebraska Medical Center) developed one of the first clinical laboratory automation management systems. His system later enabled the "plug-and-play" integration of automation systems and clinical analyzers for managing and testing patient samples. His idea was to develop an automated transport system with which various test processes with commercially available test systems are possible. He paid particular attention to the management of the test processes, which resulted in greater efficiency, improved reporting, and lower laboratory costs.

1.2.3 Developments in Pharmaceutical Research

In addition to the requirements of clinical laboratories, the development of high-throughput screening (HTS) methods in the pharmaceutical industry has been of particular importance

for the development of laboratory automation since the 1980s [49, 50]. Due to the lack of drugs for numerous diseases (especially cancer and viral diseases), the increasing resistance of microorganisms to known antibiotics and the expiry of important patents, there was great pressure for faster development and testing of new potential active ingredients. In addition to the synthesis of new active ingredients, their testing with regard to biological activity, carcinogenicity, mutagenicity, and metabolism behavior is the focus of interest. The early identification of toxic properties of the potential drug candidates contributes significantly to reducing the costs of drug development and increasing safety. The main goal of HTS is to increase the number of samples processed per unit of time. The number of samples to be examined has increased dramatically. While in the 1980s, a sample volume of around 10 000 compounds was processed per year, at the beginning of the 1990s it was already 10 000 samples per month. Only five years later, there was a requirement to process the same number of samples within a week [51]. Today, HTS can include the processing of several thousand samples per day. In the area of ultra-HTS, up to 100 000 samples have to be processed per day [52, 53]. Since processing numerous of samples is associated with considerable costs for reagents, solvents, and consumables, there is great interest in minimizing these costs by miniaturizing the experimental approaches [53]. In the period from 1998 to 2006, Novartis (Basel, Switzerland) succeeded in significantly increasing the number of compounds examined while at the same time drastically reducing the cost per substance.

Parallel sample processing was increasingly used in the automation of bioscreening. The development of a uniform standardized format, the microtiter plate, played an important role. Depending on the format used (see Chapter 3), up to 384 or more samples can be processed in parallel today. This required the development of parallel working systems for the dosing of liquids, but also for the technical determination of the parameters by means of adsorption or fluorescence methods.

Microtiter plate-based test methods were presented for the first time in 1986 at the *Fourth International Symposium on Laboratory Robotics* [54]. The systems used an early version of Zymark's microplate management system and, thanks to interchangeable hands, were able to carry out various laboratory processes such as pipetting, washing plates or adding reagents. The systems were referred to as "one-armed chemists" [55] and were initially used for enzyme-linked immunosorbent assays (ELISAs) investigations [56]. However, their throughput and unattended operation were severely limited.

The use of articulated robots (see Chapter 8) represented a very cost-intensive variant of the automation of such processes and was therefore not generally applicable. Numerous companies, therefore, developed specialized liquid handling systems based on a Cartesian robot structure. The *Cetus Propette*, a 12-channel pipetting system for the transfer of liquids in microtiter format, was introduced in 1996. The device originally developed for the automation of interleukin-2 assays was later used extensively in polymerase chain reaction (PCR) analysis [57]. The *Biomek 1000* (Beckman Coulter), originally a development by Infinitek, was launched in 1984. It enabled the single or parallel multi-channel pipetting of several samples. The interchangeable pipetting heads were a special feature. Another Cartesian liquid handling platform, the *Star 700*, was introduced by Kemble (U.K.) in 1985. The *MikrolabAT* (Hamilton Company) was launched in 1987 for the batch screening of blood samples for HIV and hepatitis viruses. The *system* had 12 channels with variable span and used disposable pipette tips. The first 96-channel pipetting system was the

Quadra96, developed by TomTec in 1990 [58], later followed by a variant with a 384 pipetting head. In contrast to solutions with liquid-handling hands-on articulated robot arms, Cartesian systems enable a significantly faster and better quality liquid transfer. The liquid handling workstations available today represent all further developments of these early pipetting systems. Workstation technology quickly found its way into molecular biology and genomics, as both areas of science were characterized by low throughputs and numerous labor-intensive liquid handling steps.

In order to avoid bottlenecks in the metrological determination of the samples, it was also necessary to develop parallel reading systems based on absorption or fluorescence methods. One of the first automated plate readers, the *EL310*, was introduced by BioTek in 1984 [59]. Today's plate readers enable the parallel reading of up to 1536 samples in microtiter plate format.

Various automated systems have been described for biological studies. One of the best-known applications is the Tox21 Initiative, which was started in 2008 with the aim of determining the toxicity of environmentally relevant compounds. The *Tox21 Screening System* has been used to screen more than 10 000 compounds. To determine the reproducibility of the results, the substances were examined on three days each with three replicates in different well positions. Various devices such as incubators, contactless dispensers for liquid dosing in the nano range and fluorescence or luminescence-based plate readers were positioned around a central robot [60]. Approximately 40 different assays were used for the biological testing, the parallel testing of the samples was performed in the 1536 format. All results have been made accessible in public databases and are thus available to scientists worldwide for further data evaluation, the formation of new hypotheses, and the establishment of reliable QSAR models.

The earliest automated systems in pharmaceutical screening were developed for finding biologically active compounds in natural products. The majority of these systems, if not all, were tailor-made in-house developments that were usually not published for reasons of competition. Therefore, no general formats and technologies could be derived and developed from these developments. One of the few published studies comes from Eli Lilly and Company (Indianapolis, IN). They used a *PUMA 560* robot for inoculation of microbial colonies in sample vessels combined with a subsequent test of the antibiotic effect of the fermentation extracts [61].

Pfizer (Groton, CT) has also been using HTS methods since 1986 for the screening of natural products by replacing fermentation broths with dimethyl sulfoxide solutions of synthetic compounds using 96-well plates and reduced assay volumes of 50–100 µl. After initially 800 compounds per week examined, a volume of 7200 compounds per week was already achieved in 1989. Autoradiography and image analysis were introduced for ^{125}I receptor-ligand screens. The coupling of reverse transcriptase (RT), quantitative PCR, and multiplexing enabled multiple targets to be addressed in a single assay. By 1992, around 40% of the hits were produced using HTS as starting materials for the discovery portfolio. In 1995 the HTS methodology was expanded to include ADMET (absorption, distribution, metabolism, excretion, toxicity) targets. ADMET examinations require the unique identification of every single compound, which leads to the development of an automated high-throughput liquid chromatography-mass spectrometry (LC-MS). In 1996, the testing of approximately 90 compounds per week in microsomal, protein binding, and

serum stability assays was possible. Until 1999, the HTS for ADME examinations was completely integrated into the drug discovery process.

Automated screening systems have also been used at the Genomic Institute of the Novartis Research Foundation (GNF). A system developed for genome screening was used for almost 200 genome screens from 60 000 to 100 000 wells. The system not only carried out the transports, but also enabled the plates to be transported between the liquid handlers, incubators, and plate readers. The actual measurement was carried out on an integrated *ViewLux* plate reader (Perkin Elmer) or, for fluorescence-based assays, on a confocal *Opera 384* well system, on which the cells can be displayed directly. In order to optimally use all genomic information generated for structural biology, an automated system was developed that enables the automatic expression and purification of bacterial cells, baculoviruses and mammalian cells. Bacterial proteins were expressed using a parallel fermentation system consisting of 96 arranged 100 ml culture tubes, which enabled high-density cell growth and yields of 2–4 g cell pellet for each culture with minimal variation. Protein purification was performed using GNF's automated protein purification system, which included a 96-tube centrifuge, sonication probes, and liquid handling and affinity purification functions. As a result, 10 mg of purified protein could be obtained per tube; the overall process took 96 hours [62].

1.3 Laboratory Applications and Requirements

1.3.1 Bioscreening and Pharmaceutical Testing

As described above, the development of laboratory automation has been largely influenced by the needs of the pharmaceutical industry since the 1980s. The need to find new potential drugs and reliable early screening for biological activity remain critical. The essential processes in this area include enzyme and cell-based assays, ELISAs, DNA/RNA extraction, purification and quantification, PCR and qPCR, gene expression experiments and next generation sequencing (NGS).

1.3.1.1 Enzymatic Assays

Enzymatic assays use the determination of enzyme activity and are used to determine substances that inhibit or activate certain enzymes as well as the enzyme kinetics. Usually, a blank value and a measured value of the sample are measured after 5–10 minutes of exposure and the extinction difference is calculated, from which quantitative statements can be derived.

Enzymatic reactions use optical measurement methods. As early as 1935, Warburg described an optical-enzymatic test for measuring the enzyme activities of NAD^+ reducing enzymes. A photometric measurement of the change in color intensity during the reduction from NAD^+ to NADH was carried out [63]. This test was used to measure the activities of lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and glutamate dehydrogenase (GLDH) [64]. The biochemical detection of enzyme activities is also possible using composite enzymatic tests. In this case, enzyme activity is measured for which no colored substrate is available. The combination of the reaction of the enzyme

to be determined (indicator reaction) with a further enzymatic reaction (measurement reaction) with a change in color intensity enables the extension of the method. The second reaction partially uses the products of the first reaction. This indirectly determines the enzyme activity and quantifies it in comparison to a standard series. Examples of composite enzymatic tests are the glucose oxidase (GOD)-horseradish peroxidase (HRP) test and the GPT-LDH test. The measurement of cell metabolic activity, cytotoxicity, or cytostatic activity is of great importance in the process of drug development. The detection of cell vitality by means of the MTT test uses the reduction of the yellow, water-soluble dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) into a blue-violet, water-insoluble formazan. The conversion takes place by NAD(P) H-dependent cellular oxidoreductase, which is present in viable cells. This non-radioactive, colorimetric assay system using MTT was first described by Mosmann T and improved in subsequent years by several other investigators [65–67].

Enzymatic assays can be carried out continuously or discontinuously. The timed (discontinuous) assay measures the enzyme concentration in fixed periods of time. A common timed test method is to use a microplate reader to read multiple concentrations of the solution. Multiple dilution series are examined, which contain dilution series for the substrate, the enzyme, and for the substrate and enzyme together. After the start of the reactions, the solutions are incubated for a specified period of time. A stop solution is then added to prevent a further enzyme reaction. Continuous assays measure the formation of a product or the conversion of a substrate in real-time. The disadvantage of a continuous assay is that only one reaction can be measured at a time. The advantage, however, is the convenience of easily measurable reaction rates. Enzymatic reactions are widely used in drug development for early testing of potential drug candidates [68].

1.3.1.2 Cell-Based Assays

A higher level of information about the biological relevance of active ingredients can be achieved through cellular assays. Investigations can take place either in the cell network or at the level of an individual cell. Cell-based assays are therefore used extensively in drug development, where they make up more than half of all tests for target validation and ADMET [69]. Classically, proliferation, migration, invasion, apoptosis, etc. are examined. Cell-based assays are analytical tools that can be used to study a mechanism or process of cell function. They typically include intact or fixed cells. The following important types of cell-based assays can be defined [70]:

- Intracellular signal transmission: It is an important mechanism by which cells can react to their environment and extracellular signals. Cells can perceive their environment and modify gene expression, mRNA splicing, protein expression, and protein modifications to respond to these extracellular influences.
- Cell viability assays: These tests determine the ratio of living and dead cells. Cell viability tests are used to determine the cellular response of drug candidates as well as for the optimization of cell culture conditions.
- Proliferation Assays: Cell proliferation describes the biological process in which the number of cells increases over time due to cell division. They thus monitor the growth rates of cell populations. Cell proliferation is important in the regular homeostasis of tissues and cells to ensure an optimized growth, development, and maintenance of the organism.

- Cytotoxicity assays: These assays determine the number of living and dead cells in a population after treatment with a drug candidate or pharmacological agent.
- Cell senescence assays: Assays for assessing cell health include, e.g. assays for determining the senescence of cells. One example is the detection of senescence markers associated with the activity of β-galactosidase which reflects the integrity of the cell membrane.
- Cell death assays:

Apoptosis (programmed cell death type 1): Apoptosis investigations are essential for the development, homeostasis, and pathogenesis of various diseases including cancer. Apoptotic cells appear in response to extrinsic or intrinsic signals. Typical signs of apoptotic cell death include the exposure of phosphatidylserine on the extracellular side of the plasma membrane, the activation of caspases, the disruption of the mitochondrial membrane potential, or the shrinkage of the cells. Other markers are DNA fragmentation and condensation.

Autophagy (programmed cell death type 2): Autophagy is defined as the selective degradation of intracellular targets that serve as an important homeostatic function. This process enables the destruction of misfolded proteins by ubiquitination followed by a breakdown via the lysosomal route.

Necrosis (programmed cell death type 3): Cell swelling and destruction of the plasma membrane and subcellular organelles are typical signs of necrosis. Necrotic cell death is a heterogeneous phenomenon including both, programmed and accidental cell death.

- Antibody-dependent cell-mediated cytotoxicity (ADCC): ADCC is an immunological mechanism in which an effector cell of the immune system destroys an antibody-loaded target cell. NK cells, but also macrophages, dendritic cells, neutrophils, and eosinophils primarily take over the role of the effector cell. The ADCC thus represents a connection between the innate and the adaptive immune system.
- Complement depending cytotoxicity: Complement-dependent cytotoxicity (CDC) is an effector function of IgG and IgM antibodies. If they are bound to surface antigen on the target cell (e.g. bacterially or virally infected cell), the classic complement pathway is triggered by binding of the protein C1q to these antibodies. This leads to the formation of a Membrane Attack Complex (MAC) and lysis of the target cell. The complement system is efficiently activated by human IgG1, IgG3, and IgM antibodies, weakly by IgG2 antibodies and not by IgG4 antibodies [71]. It is a mechanism of action through which therapeutic antibodies [72] or antibody fragments [73] can achieve an antitumor effect [74].
- Antibody-dependent cell phagocytosis (ADCP): ADCP is the mechanism by which antibody opsonized target cells activate the FcγRs on the surface of macrophages to induce phagocytosis, resulting in internalization and degradation of the target cell through phagosomal acidification.

1.3.1.3 ELISAs

ELISAs are antibody-based detection methods that belong to the enzymatic immunosorbent methods and are based on an enzymatic color reaction. The antigen to be detected is adsorptively bound and enriched via a first antibody, an enzyme-coupled second antibody (detection antibody) leads to the reaction of a dye substrate. With the help of the ELISA, proteins (e.g. SARS-CoV-2 antibodies [75]) and viruses (e.g. Zika virus [76]), but also low

molecular weight compounds such as hormones [77], toxins [78], and pesticides [79] in a sample (blood serum, milk, urine, food, etc.) can be detected using the property of specific antibodies to bind to the substance to be detected (antigen). An antibody is previously marked with an enzyme. The reaction catalyzed by the reporter enzyme serves as proof of the presence of the antigen. The reporter enzymes often used are HRP, alkaline phosphatase (AP), or, less often, GOD. In the case of the alkaline phosphatase a dye substrate (synonym: chromogen), for example, *p*-nitrophenyl phosphate (pNPP), is added, while for peroxidase *o*-phenylenediamine (oPD) is mostly used. The alkaline phosphatase splits off the phosphate residue from the colorless nitrophenyl phosphate and *p*-nitrophenol is formed, which is pale yellow. The change in concentration of the dye produced by the enzymatic reaction can be followed with a photometer according to Lambert–Beer's law. The color intensity changes with the concentration of the nitrophenol formed and thus also the concentration of the antigen to be determined in the sample in comparison with a dilution series with known concentrations [80].

1.3.1.4 DNA/RNA Extraction, Purification, and Quantification

DNA extraction is one of the methods of DNA purification and involves the process of extracting DNA from cells. Usually, in the first step, the cells are concentrated by means of centrifugation, followed by cell disruption. Different procedures are required depending on the type of cells used. Plant, fungal, and bacterial cells usually require additional enzymatic or mechanical steps. Chemical cell disruption (alkaline lysis) is usually used for plasmid preparation from bacteria. The homogenate is clarified by filtration or centrifugation. DNA from mitochondria or chloroplasts is separated from the DNA of the cell nucleus by cell fractionation. Hirt extraction is used to isolate extrachromosomal DNA such as viral DNA [81]. An RNAse digestion can be performed to remove RNA. DNA extractions are usually based on two-phase extraction [82] or precipitation [83], the latter being carried out with additional selective adsorption onto a DNA-binding matrix. Some extraction processes are also combined with one another. Final ethanol precipitation usually follows [84], in some cases with the addition of ammonium acetate [85].

The quantification of DNA is possible with different methods [86]. The classic diphenylamine method uses colorimetric detection [87]. It has a detection limit of 3 µg but is very labor-intensive and time-consuming. Absorption-based methods typically use microvolume spectrophotometers and are simple and quick. Their low specificity and sensitivity to impurities are disadvantageous. The sensitivity is around 2 ng/µl. Fluorescence measurements have better detection limits (10–50 pg/µl depending on the kit used). They have high specificity but require very expensive reagents [88]. Sometimes a digital PCR is also used, which is very sensitive and specific [89].

1.3.1.5 PCR/RT-PCR/q-PCR

The PCR is a method to reproduce genetic material (DNA) *in vitro* [90]. PCR uses the enzyme DNA polymerase. The term chain reaction indicates that the products of previous cycles serve as starting materials for the next cycle and thus enable exponential replication. Kleppe et al. used first a process for the amplification of DNA sections in 1971 by Kleppe et al. [91]. The actual developer of the method is considered to be Mullis (1944–2019, Nobel Prize in Chemistry 1993). The reaction usually uses volumes of 10–200 µl in small reaction

vessels (200–500 µl) in a thermal cycler. Today, PCR is one of the most important methods of modern molecular biology and is used in biological and clinical-diagnostic laboratories for genetic fingerprints, parentage reports, the cloning of genes, or the detection of hereditary diseases [92] and viral infections (e.g. dengue virus) [93]. The PCR test is currently the gold standard among the SARS-CoV-2 test procedures [94, 95].

Real-time quantitative PCR (qPCR or RTD-PCR) is an amplification method for nucleic acids based on the principle of ordinary PCR. In addition, it also enables the quantification of the DNA obtained. The quantification is carried out with the help of fluorescence measurements, which are recorded in real-time during a PCR cycle.

1.3.1.6 Gene Expression Analysis

The gene expression analysis examines the implementation of genetic information (gene expression) with molecular biological and biochemical methods. It enables qualitative and quantitative statements about the activity of genes and can be used for individual transcripts as well as the complete transcriptome. Typical qualitative questions are the general expression of a gene and the type of cells in which the expression takes place. In the case of quantitative analysis, the size of the difference in expression compared to a defined reference is determined. Applications can be found in cancer research [96] or the investigation of viral diseases such as Zika [97] or SARS-CoV-2 [98].

1.3.1.7 Next-Generation Sequencing

NGS is an improved technology for DNA sequencing. In contrast to classic enzymatic (Sanger sequencing) or chemical sequencing (Maxam-Gilbert method), this method allows higher speeds and thus enables the sequencing of a complete human genome within one day [99, 100]. The NGS processes are often automated; the results are obtained in parallel with the sequencing. In addition, the results can be compared with a human reference genome. In the first step, DNA fragments are generated with the help of enzymes or centrifugation. In the next step, specific adapter oligonucleotides are bound to the fragments and a DNA library is created. The DNA fragments are bound to solid reaction media (for example a chip) and amplified. Due to the division into clusters of identical DNA, in which the actual sequencing takes place, many sequencing processes can take place parallel in a very short time. The data obtained are stored in the form of a DNA chip and analyzed using bioinformatics methods [101]. For the sequencing of the human genome, Illumina sequencing [102, 103] and SOLiD sequencing [104, 105] are mainly used.

1.3.1.8 Cell Culturing

The cultivation of animal or plant cells in a nutrient medium outside the organism is another typical application in life science laboratories. A distinction can be made between adherent cells growing on surfaces (e.g. fibroblasts, endothelial or cartilage cells) and suspension cells floating freely in the nutrient medium (e.g. lymphocytes). Further differentiation is possible in 2D and 3D cell cultures. The culture conditions differ greatly depending on the cell lines to be cultivated, which concerns both the nutrient media, pH values and the necessary nutrients. Depending on the rate of division and density of the cells, they are distributed to new vessels at regular intervals (passage or splitting). The passage number indicates the frequency with which the cells have already been passaged.

In the case of adherent cells in continuous culture, the cells are regularly isolated in order to avoid confluence and the associated inhibition of cell contact. The process of cell cultivation includes numerous sub-processes of dosing nutrient medium, taking aliquots for cell counting and sowing the cells on microtiter plates. Classically, these are manual and therefore labor-intensive processes, which have been increasingly automated in recent years. Both the cultivation of 2D and 3D cells on an automation system have been described [106, 107].

Cell cultures are widely used in biological and medical research, development, and production. Cell cultivation is also of great importance for the manufacture of biotechnological products. In addition to numerous vaccines (e.g. influenza vaccines [108]), erythropoietin, a growth factor for the formation of red blood cells, is also produced in cell culture.

1.3.1.9 Requirements

Although the underlying biochemical reactions in enzymatic and cellular reactions are very complex, carrying out the corresponding assays is quite simple (see Figure 1.1). Essentially, it involves pipetting steps for dosing the components involved (enzyme solutions, substrates, possibly stop solutions and other solutions) as well as the analytical detection of the reactions, usually using optical methods. Mixing the solutions is an important point in order to achieve the most homogeneous distribution possible. All disturbances, such as

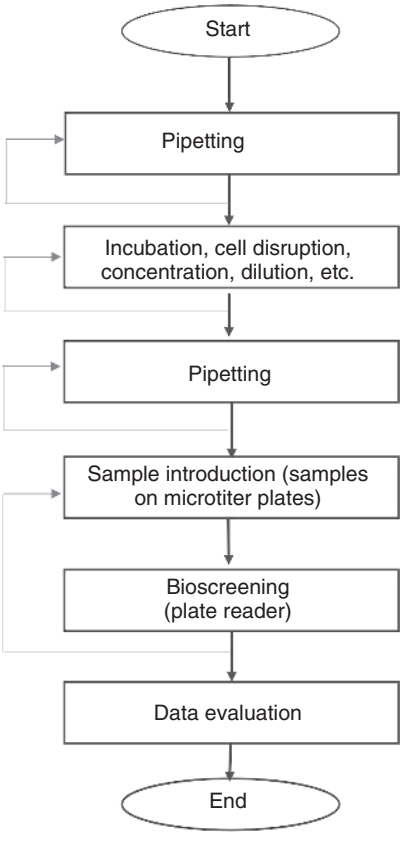

Figure 1.1 Classical process of enzymatic and cellular assays in drug discovery.

the introduction of air bubbles or dust particles into the solutions, must be avoided [109]. Biological assays run under mild ambient conditions, i.e. they make little demands on temperature, pressure, or the inertness of the ambient air. The assays are usually carried out at room temperature or an assay temperature of 37 °C. Aqueous, buffer-containing solutions are traditionally used, complex organic mixtures are not required. For cell-based assays, sterile conditions are also required, which can be implemented, for example, by means of high-efficiency particulate air (HEPA) filters or the use of UV lamps. Simple optical methods such as absorption spectroscopy or fluorescence spectroscopy, which do not require any preanalytical preparation of the samples, enable the detection. The sensitivity of the optical detection methods used enables working with very small volumes. In addition, the strong parallelization of the processes through the introduction of the microtiter plate (see Chapter 3) is a great advantage.

1.3.2 Clinical Applications

For a long time, clinical applications were a key driver in the development of laboratory automation. While simpler parameters were initially of interest, new requirements increasingly include the determination of a wide variety of organic compounds. Table 1.1 gives an overview of important clinical parameters.

1.3.2.1 Determination of Classical Parameter

Clinical-chemical analyses generally refer to the determination of enzymes, substrates, and metabolic products. A wide range of analytics offered in clinical-chemical laboratories is also available for near-patient diagnostics. This includes, e.g. the determination of enzymes (AP, GOT, GPT, γ-GT, amylase, CK), electrolytes (Na^+, K^+, Ca^{2+}, Cl^-, Mg^{2+}) and numerous metabolic variables (total bilirubin, HDL and LDL cholesterol, triglycerides, glucose, uric acid, creatinine, urea, and lactate) [110].

One of the most important clinical parameters to be determined is the glucose content. For this purpose, enzymatic measuring methods using the enzymes GOD and glucose dehydrogenase are almost exclusively used today. The enzyme GOD oxidizes the glucose to gluconic acid in the presence of water and oxygen. The co-factor flavin-adenine-dinucleotide (FAD) serves as the first electron acceptor, which is reduced to FADH. After that, FADH is re-oxidized by molecular oxygen (O_2), the final electron acceptor. This creates hydrogen peroxide (H_2O_2). The oxygen consumption or the resulting H_2O_2 can then be detected using electrochemical or chromogenic methods. As chromogens, e.g. *o*-dianisidine, *p*-aminophenazone/phenol, and iodide/molybdate can be used. The chromogen is oxidized by the resulting H_2O_2 and measured reflectometrically (e.g. *GlucoTouch* from LifeScan (Malvern, PA). The measurement reaction is highly specific, but the indicator reaction can be affected to varying degrees by reducing substances such as ascorbic acid or acetaminophen. A special analytical problem is the sometimes considerable dependence of the measurement results on the oxygen content of the sample. Here, too, the individual variants of the GOD methods must be carefully considered. Those methods that use oxygen as the last electron acceptor (*Blood Gas Devices*, YSI, Yellow Springs, OH or *GlucoTouch*) are insensitive to changing oxygen concentrations as long as there is enough oxygen in the sample. The opposite is the case for methods

Table 1.1 Clinically relevant parameters and clinical areas of application.

Area of application	Parameter
Acid–base balance, blood gases	pH, pCO_2, pO_2
Electrolytes	Na^+, K^+, Cl^-, ionized Ca^{2+}, ionized Mg^{2+}
Metabolites	Cholesterol, HDL cholesterol, triglycerides, creatinine, urea, uric acid, bilirubin, lactate, ammonia
Enzymes	Amylase, alkaline phosphatase, CK, AST, ALT, y-GT
Hemostaseology	Activated whole blood clotting time (ACT), partial thromboplastin time (PPT, aPPT), thromboplastin time (Quick-Test, INR), D-dimer, platelet function tests, bleeding time
Hematology	Hemoglobin, hematocrit, erythrocytes, leukocytes, platelets
Hemoglobin fractions	CO oximetry
Cardiac markers	Troponin T, troponin I, myoglobin, CK-MB, natriuretic peptides (BNP/NT-pro-BNP)
Diabetes mellitus	Glucose, HbA1c, minimally invasive continuous glucose measurement, ketones
Acute-phase proteins	CRP
Allergy diagnostics	Allergen specific IgE
Drug levels and drug screening	Medicines, alcohol, amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates
Infectiology	HIV, infectious mononucleosis, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Plasmodium falciparum*, *Plasmodium vivax*, Influenza A, Influenza B, *Streptococci* A and B
Fertility	HCG, LH, and FSH in urine
Urine diagnostics	Test strips (pH, protein, glucose, ketones, bilirubin, urobilinogen, nitrite, leukocytes, blood, specific gravity), microalbumin, bacteria

Source: Based on Junker and Gässler [110].

that use ferrocene (*Precision PCx*, Abbott, Chicago, IL) or hexacyanoferrate (*Ascensia Elite*, Bayer. Leverkusen, Germany) instead of oxygen as the last electron acceptor. Here, oxygen, as a possible electron acceptor, competes with the mediators, so that the glucose is determined to be incorrectly low in the case of increased oxygen values in the sample. Further interferences are to be expected from the body's own metabolites and drugs with reducing properties (vitamin C, acetaminophen, dopamine, etc.). These are mostly measurement methods that use GOD-based peroxide reactions as detection methods. Processes that use glucose dehydrogenase as an enzyme are much more stable toward such substances. Whole blood, hemolysate, serum/plasma (with and without deproteinization), urine, liquor and interstitial fluid or the dialysate obtained from them can generally be used as sample materials.

Hematological examinations using point of care tests (POCT) range from the measurement of the hematocrit (HC) and hemoglobin (Hb) to the complete determination of the blood count. The term blood count summarizes the count of red blood cells, leukocytes

(including the distribution of granulocytes, lymphocytes, and monocytes), and platelets. In addition, the determination of HK and Hb, as well as cell properties (e.g. erythrocyte indices, mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC)) and other information (e.g. size distribution curves and stages of maturation of individual cell rows), are further parameters of interest. In hematology, in addition to fully-fledged machines for determining blood counts, which have a corresponding range of measurement technology, devices for determining individual hematological parameters are also used. Depending on the device system used, the required sample volume ranges from a few microliters to 200 µl; the measurement time is usually a few seconds.

Global and special tests of plasma coagulation are used in large numbers in everyday clinical practice. The small blood count only records the number of platelets in the peripheral blood. Defects in primary hemostasis (defects in adhesion and aggregation of platelets in the event of injuries) are usually not measurable or only measurable to a limited extent in routine diagnostics. Specific investigations of plasmatic or thrombocytic coagulation disorders are usually carried out in special laboratories. The available methods can be divided into the analysis of plasmatic coagulation, the analysis of platelet function, and the combined recording of plasmatic coagulation, platelet function, and fibrinolysis (viscoelastic methods). When diagnosing blood coagulation, several interfering and influencing variables from the sample must be taken into account. Depending on the detection method, different instruments can react differently to variations in HC, to the influence of colloids, and to the formation of microaggregates in the circulation. The effects of specific metabolic conditions (e.g. acidosis) and environmental conditions (e.g. hypothermia) on hemostasis diagnostics are often not systematically clarified. Whole blood methods are sensitive to interfering and influencing factors from the sample matrix.

The specificity of the molecular recognition of antigenic structures by antibodies is the basis for the immunoassay technology as well as for immunosensors with the antibodies on a solid phase. The most important analytical problem areas for the selective recognition of the antigen–antibody complex are the bioconjugation chemistry and the orientation of the bound antibodies, the specificity of which must not be compromised by the binding. The immunosensors include electrochemical sensors (potentiometric, amperometric, conductometric, or capacitive), optical sensors, microgravimetric sensors (quartz microbalances), and thermometric sensors. All types can be used both as direct (unlabeled) and indirect (labeled) immunosensors. The direct sensors are able to track physicochemical changes during the immune complex formation, while the indirect sensors mostly use fluorescent or chemiluminescent markings and thus enable a high level of sensitivity. There are simple strip tests for numerous analytes, which can be read off visually. Alternatively, for a less extensive analysis spectrum, smaller automatic detectors are used to read the test strips and quantify the results. Automatic detectors are mainly used in the clinical area. The spectrum of methods includes fluorescence and chromatographic detectors as well as enzyme immunoassays. The examinations usually use not only whole blood, saliva, or urine but also serum or plasma. A major problem with blood examinations with test strips is the use of capillary blood. The concentration can change when the blood is drawn so that the measured concentration of the analyte does not represent the concentration in the blood. This is particularly problematic in the case of analytes whose qualitative detection is at the analytical limit.

Numerous systems are available for the metrological determination of classic laboratory parameters. If the samples are not to be centrifuged to obtain serum or plasma, a step to eliminate cellular components from the whole blood must be integrated into the analysis process. Usually, a few microliters of samples are sufficient. The actual analysis takes place within minutes. Common measuring systems usually have an interface to export data and measured values to laboratory or hospital information systems. Clinical-chemical analysis systems represent a downsizing of established laboratory systems and can analyze a wide range of parameters. Other measuring devices enable clinical-chemical analyzes in addition to other measurements. These include devices for blood gas analyzer (BGA), on which electrolytes, glucose, lactate, creatinine, bilirubin, and other parameters can be determined using electrodes or photometric detection. There are also test systems that are specially designed for individual procedures, e.g. to determine the lipid status or lactate.

1.3.2.2 Determination of Vitamins

The determination of vitamins in the human organism is becoming increasingly important, as a lack of vitamins is associated with numerous diseases. Depending on the type of vitamin that is missing, a deficient vitamin supply causes specific deficiency diseases so called avitaminoses, which have been known for centuries under names such as "Beri–Beri," "Scurvy," "Pellagra," etc. With a varied and balanced healthy diet, an adequate supply of all vitamins is always guaranteed. Vitamins are found in relatively small amounts in most plant and animal foods. They are indispensable for the human organism because they functionally intervene in almost all metabolic processes in the body. Vitamins are divided into fat- and water-soluble vitamins according to their solubility. These two groups have different functions in the human body. Due to the excretion and degradation processes, there is a constant need so that they have to be returned to the organism with food. The fat-soluble vitamins include vitamins A, D, E, and K. Vitamin B1, B2, B6, and B12, niacin, folic acid, and pantothenic acid as well as the most well-known vitamin C belong to the water-soluble vitamins.

The analytical determination of the content of the individual vitamins is usually very difficult. Since various vitamins belong to very different chemical substance groups and for the most part do not have any common chemical properties, it is not possible to determine a large number of vitamins with a few – let alone a single – analytical method. At best, a few vitamins can be quantified together in small groups in certain cases. Very few vitamins can be determined with relatively simple processing and examination methods. This includes, for example, the enzymatic analysis of vitamin C. Some vitamins, however, are extremely difficult to determine because very complex work-ups are necessary and they do not run reproducibly. For vitamins B6, B12, niacin, pantothenic acid, and folic acid, microbiological methods are recommended for their determination.

Methods for determining vitamin A have been known for a long time. Bessey et al. described in 1946 an optical method for the determination of vitamin A in small amounts of blood; the vitamin A absorption was measured at 328 μm [111]. More recent methods have been described by Xuan et al. They used an SPE-based method to extract vitamin A from 200 μl serum; the metrological determination was carried out by means of high-performance liquid chromatography (HPLC) [112].

The determination of vitamin C in blood and urine has also been known for a long time. A photometric method was described by Roe et al. in 1942; vitamin C was determined after derivatization with 2,4-dinitrophenylhydrazine [113]. Detection limits of up to 0.2 µmol/l can be achieved for blood samples using reversed-phase HPLC and fluorometric detection [114].

Current methods mainly use mass spectrometric methods to determine the vitamins in blood and urine after the samples have been prepared accordingly. For example, the determination of B vitamins using LC/MS/MS in concentration ranges from 0.42 to 5.0 µg/l has been reported [115]. Numerous methods have also been described for the determination of vitamin D and its metabolites. Thus, among other things, the quantitative determination of 25-hydroxyvitamin D metabolites (25OHD3, 25OHD2, and 3-epi-25OHD3) from dried blood samples after extraction and derivatization using LC/MS/MS [116]. Alternatively, solid-phase extractions can be used for the extraction of vitamin D; the automation of this process was described by Bach et al. [117].

As early as 1936, Schønheyder described a method for the quantitative determination of vitamin K from blood in connection with studies on vitamin K deficiency in chicks [118]. Here, too, HPLC-based methods are used today, which enable detection limits of 0.04 ng/ml vitamin K in plasma [119].

1.3.2.3 Determination of Drugs of Abuse

Another large group of parameters to be determined is narcotics. Narcotics is a group of centrally effective drugs and substances, which are heavily regulated and controlled by drug and health authorities to prevent abuse and protect the population from adverse effects and addiction. Certain narcotics – for example, many potent hallucinogens – are prohibited or may only be used for medical or scientific purposes with a special permit from the authorities. Some of the substances are also referred to as "psychotropic substances" in the Narcotics Act. Structurally, narcotics are very heterogeneous. However, different groups can be distinguished within this class (see Table 1.2). The most important narcotics include opioids, benzodiazepines, barbiturates, amphetamines, and medicinal

Table 1.2 Selection of classic narcotics.

Opioids	Alfentanil, buprenorphine, codeine, fentanyl, heroin, hydrocodone, methadone, morphine, oxycodone
Benzodiazepines and Z-Drugs	Alprazolam, bromazepam, diazepam, flunitrazepam, lorazepam, zolpidem alprazolam, bromazepam, diazepam, flunitrazepam, lorazepam, zolpidem
Barbiturates	Butalbital, pentobarbital, secobarbital
Amphetamines and other stimulants	Aminorex, amphetamine, dexamphetamine, cathine, cathinone, cocaine, methamphetamine, methylphenidate, phentermine
Medicinal drugs	Cannabis, coca leaves, cath, opium
Hallucinogens	Dimethyltryptamine (DMT), hallucinogenic mushrooms such as *Psilocybe semilanceata*, Ibogaine, LSD, Mescaline, Peyote, Psilocybin, *Salvia divinorum*, San Pedro
Other examples	Gamma hydroxybutyrate (GHB), Dronabinol (THC)

drugs. Narcotics are often structurally related to the body's own substances, such as neurotransmitters. Natural narcotics like opium, cannabis, and coca leaves have been used for thousands of years. The narcotics legislation is relatively young. The first regulations came into force at the beginning of the twentieth century, and in Switzerland in the 1920s. Narcotics have, among other things, analgesic, psychotropic, hallucinogenic, stimulating, euphoric, sedating, calming, and sleep-inducing properties. The drug targets are located in the central nervous system, i.e. in the brain and spinal cord. The body's own ligands, which interact with the same target structures, are known for many active substances. Typical areas of application for narcotics are pain, sleep disorders, psychiatric disorders, and anxiety, as well as attention-deficit hyperactivity disorder (ADHD). They are also used in the treatment of dry coughs (e.g. codeine) or in anesthesia. Table 1.2 shows an overview of classic groups of narcotics and their representatives.

Typical dosage forms include tablets, capsules, drops, transdermal patches, and injection preparations. As is well known, narcotics are also illegally produced, cultivated, distributed, and traded. They have a high potential for abuse. They can be used as intoxicants, party drugs, stimulants, hallucinogens, smart drugs, for suicides and poisonous murders.

The selection of suitable analytical methods for the determination of narcotics depends on the type of substances to be determined. Detection and quantitative determination are usually carried out using blood, urine, or saliva. In addition, a determination in hair, nails, and teeth is also possible for numerous compounds. Blood is very well suited for testing for drugs and medication. It contains the substances to be determined from the time it is administered and transports it to all tissues, including the sites of action and the organs that remove it from the organism. Blood cannot be manipulated, its composition is quite uniform. The concentration of the active substance is in dynamic equilibrium with the concentration of absorbed substances in the central nervous system and thus, at least to a limited extent, in relation to an effect. For many questions, urine is the test material of the first choice. For other questions, the examination of urine is an important addition. As a test material, urine has the advantage that it can usually be given off in large quantities by the test person without invasive techniques. In general, the foreign substances or their metabolites are present in higher concentrations than in the blood and can be detected for longer. The broader metabolite profile can also provide additional information. The disadvantage, however, is that it can only be compared to the blood result to a limited extent. In most cases, measurable concentrations can be found in the blood immediately after consumption, while the degradation process of the drugs in the body means that detection in the urine is not yet possible or is hardly possible. As a test material, saliva offers an informative statement on current drug effects. Similar to blood samples, more up-to-date references to the time of drug consumption and the degree of effect can be made than with the examination of urine.

Immunoassays in the form of test strips or test cassettes are usually used as preliminary tests for the consumption of narcotics. All assays are based on the principle of the antigen–antibody reaction, according to which the substances compete with antigens for binding with specific antibodies. The number of immune complexes formed from antibodies and analytes allows a statement to be made about the concentration of the analyte in

the sample. However, the antibody–antigen binding is not directly accessible analytically in most immunoassays. Coupling one of the two components, the antigen or the antibody, with an easily detectable marker substance, e.g. with an enzyme (biocatalyst) or a dye (Fluorophor), solves this problem.

A conclusive quantitative determination of various drugs from a complex matrix such as the serum requires the use of a selective method. The low concentrations in the nanogram range of drugs not only in the blood but also in the saliva, make the use of complex analytical methods of determination necessary, which also enable measurements close to the detection limit. Numerous methods for identification and quantitative determination from physiological sample materials are described in the literature, the combination of liquid chromatography or gas chromatography with mass spectrometry with stable isotopes being preferred as internal standards. Gas chromatography–mass spectrometry (GC/MS) has long been known as a "definitive method" that is characterized by being "correct" and specific; it provides a definitive (correct) value as the best approximation of the "true value." The GC/MS is also listed as a "confirmatory drug test" in the "Mandatory Guidelines for Federal Workplace Drug Testing Programs" in the USA. Furthermore, liquid chromatography, HPLC, especially coupled with a mass spectrometric detector (LC/MS), is increasingly being used as a definitive method. A high throughput method for the determination of benzodiazepines in human urine was described by Zweigenbaum et al. [120]. Using LC/MS/MS, 1000 samples could be examined within 12 hours. The LC/MS/MS method is also used in the determination of saliva, with detection limits of 0.5–5 ng/ml for different benzodiazepines being achieved [121]. In some cases, electroanalytical-based approaches are also used for the determination of benzodiazepines [122]. Numerous methods have been reported for the determination of cannabinoids in blood and urine. Using uHPLC/MS/MS, it is possible to detect Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THC-COOH) in whole blood with detection limits of 0.05–0.2 ng/ml [123]. An SPE-based method with subsequent LC/MS/MS analysis has been described for the determination of cannabinoids and their metabolites in urine [124]. A method for the simultaneous determination of 75 narcotics (benzodiazepines, amphetamines, opiates, opioids, cocaine, etc.) in hair samples has also been developed and validated [125]. The quantitative determination of opioids from dried blood spots is achieved by coupling on-line SPE and LC/MS/MS with detection limits of 0.1 to 50 ng/ml fentanyl [126].

1.3.2.4 Requirements

Clinical examinations today are characterized by an increasing number of samples and a steady increase in the number of parameters to be determined. Depending on the type of analytes to be determined, this can require extensive processing steps, in particular, due to the complex matrix structure. Human sample material such as blood, plasma, or urine contains numerous matrix components that can interfere with the determination of the actual analytes. However, since there are only a few different matrices, it was possible to develop suitable, generally applicable methods for the initial preparation of the samples. In

general, blood samples are initially mixed with organic solvents in order to achieve protein precipitation. At the same time, this step also serves to separate the analytes from the proteins. For example, vitamin D binds to vitamin-binding proteins. Protein precipitation using methanol, acetonitrile, 2-propanol or sodium hydroxide is used to detach vitamin D from its transport protein and to remove the proteins from the sample [127, 128]. Zinc sulfate can also be used to improve the release from protein binding [129]. After centrifugation to separate the solid constituents, the resulting supernatant is used for further processing. Numerous processes use methods which includes complete evaporation of the solutions followed by a resuspension. This requires the use of stirrers for optimal re-dissolving of the evaporated compounds. Solid-phase extraction methods are also increasingly being used, which, in addition to separating matrix components, also enable a change in the solvent. This eliminates the step of evaporation and resuspending of the sample from the process flow. However, an appropriate system is then required for the solid phase extraction (see Chapter 7). Figure 1.2 shows an example of the process for determining vitamins in the blood.

Corresponding standard procedures have also been established for the preparation of urine. In the simplest case, the original urine sample is simply diluted. However, this leaves high salt loads which, under certain circumstances, can affect the measurement systems used. Other methods include enzymatic hydrolysis of urine using solid or liquid-solid-ß-glucuronidase (Helix pomotia, H-1) [130].

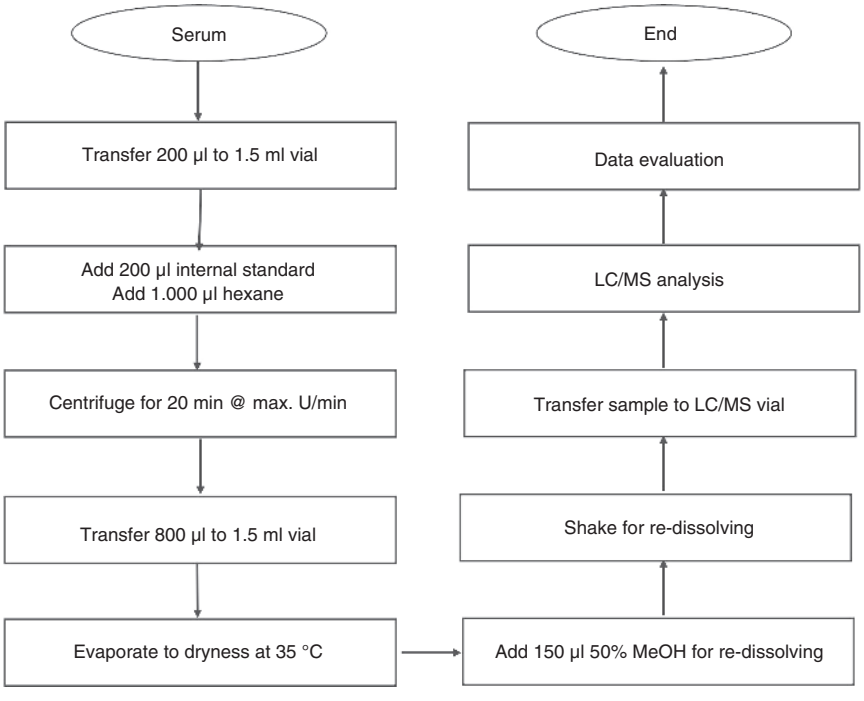

Figure 1.2 Sample preparation process of blood for the determination of vitamin D with classical protein precipitation.

Table 1.3 Enzymatic determination of ingredients in food.

Compound class	Compounds	Wave length (nm)
Acids	Formic acid, D/L malic acid, succinic acid, citric acid, acetic acid, D/L lactic acid, gluconic acid	340
	Ascorbic acid	578
	L-Glutamic acid, D-3-hydroxy butyric acid	492
	Oxalic acid	580
	Tartaric acid	520/546
Sugars	ß-Glucan	546
	D-glucose, D-fructose, lactose/D-galactose, maltose/sucrose, raffinose, saccharose, starch	340
Others	Acetaldehyde, ammonia, ethanol [132], glycerine [133], urea, nitrate	340
	Cholesterol	405
	Iron, copper	580
	D-sorbitol/xylitol	492

1.3.3 Classical Analytical Applications

1.3.3.1 Food Analysis

In food analysis, often enzymatic assays are used, e.g. for the determination of ingredients such as sugar, acid, or alcohol. These methods are used, in particular, for the analysis of wine, beer, fruit juice or milk and dairy products. The detection of the compounds of interest is based on an enzymatic reaction that causes a color change. Suitable test kits are often available. Sample preparation and analysis are easy to implement in this case. Automation in this area leads to a significant reduction in the required sample volumes [131]. Table 1.3 gives an overview of classic enzymatic reactions in food analysis.

Enzymatic substrate determinations are very specific and sensitive (measurements in the ppm range are possible). The determination of the enzyme activity is also used as an indicator of the freshness status [134].

In addition to the above-mentioned simple compounds, numerous other substances in food must also be determined qualitatively and quantitatively. These include pesticides in high-fat foods, acrylamide, furans, etc. Usually, simple enzymatic processes cannot be used here, as they are either too complex, have too little specificity or are too cost-intensive. Numerous chromatographic methods have been developed which, often in combination with mass spectrometric detection, enable the unambiguous identification of compounds. Many of these methods are now implemented in DIN and EPA norms as standards in food control and monitoring. Table 1.4 provides a selection of the most important standards and determination methods.

Table 1.4 Parameter and method in food analysis.

Parameter	Matrix	Method	Standard
Organic compounds			
Acesulfam-K, Aspartam, and Saccarin	Food	HPLC	DIN EN 12856
Neohesperidin-Dihydrochalcone	Food	HPLC	DIN CENT/TS 15606
Acrylamide	Food	LC-ESI-MS/MS	DIN EN 16618
	Coffee and coffee products	HPLC-MS/MS, GC-MS	DIN EN ISO 18862
Benzene	Soft drinks, other beverages, baby food	HS-GC-MS	DIN EN 16857
Benzo[*a*]pyrene, Benz[*a*]anthracene, Chrysene, and Benzo[*b*]fluoranthene	Food	GC/MS	DIN EN 16619
		HPLC-FD	DIN CEN/TS 16621
Chlormequat, Mepiquat	Lowfat food	LC-MS	DIN EN 15054
Cyclamate	Food	HPLC	DIN EN 12857
Dithiocarbamate and thiuramdisulfide residues	Lowfat food	UV spectrophotometric xanthate method	DIN EN 12396-1, DIN 12396-2
		GC	DIN EN 12396-2
Domoic acid	Raw shellfish, fish, cooked mussels	RP-HPLC, UV detection	DIN EN 14176
Ethylcarbamate	Fruit brandies and other spirits	GC-MC	DIN EN 16852
	Low-fat food	HPLC	DIN EN 14185-2
Furane	Coffee and coffee products	HS GC-MS	DIN EN 16620
Histamine	Fish and fishery products	HPLC	DIN EN ISO 19343
Melamine, cyanuric acid	Food	LC-MS/MS	DIN EN 16858
3-Monochlorpropan-1,2-diol	Food	GC/MS	DIN EN 14573
N-Methylcarbamat residues	Low fat food	HPLC, SPE	DIN EN 14185-1
Pesticides and PCB	High fat food		DIN EN 1528-4, DIN EN 1528-2; DIN EN 1528-1, DIN EN 1528-3
	Vegetable food Vegetable oils	LC-MS/MS	DIN EN 12393-1, DIN EN 12393-2, DIN EN 12393-3 DIN CEN/TS 17062

Table 1.4 (Continued)

Parameter	Matrix	Method	Standard
Saturated mineral oil hydrocarbons (MOSH)	Vegetable oils	HPLC-GC-FID	DIN EN 16995
Sucralose	Food	HPLC	DIN EN 16155
Xanthophyllen (Astaxanthin, Canthaxanthin)	Fish	HPLC	ONR CEN/TS 16233-1, DIN CENT/TS 16233-2
Toxins			
Aflatoxin B1 and sum Aflatoxin B1, B2, G1, G2	Cereals, nuts, and related products	HPLC	OENORM EN ISO 16050
	Food for infants and young children	HPLC-UV	DIN EN 15851
	Hazelnuts, peanuts, pistachios, figs, paprika powder	HPLC with after column derivatization	DIN EN 14123
Algae toxins (okadaic acid group toxins, yessotoxins, azaspiric acids, pectenotoxins)	Shellfish and shellfish products	LC-MS/MS	DIN EN 16204
Citrine	Food	LC-MS/MS	DIN EN 17203
Deoxynivalenol	Cereals and products, food for babies and young children	HPLC-UV, immunoaffinity purification	DIN EN 15891
Emethic toxin (cereulide)	Food	LC-MS/MS	DIN EN ISO 18465
Ergot alkaloids	Cereals and product	dSPE, LC-MS/MS	DIN EN 17425
Fumonisin B1 and B2	Corn products	HPLC	DIN EN 14352
	Food for infants and young children		DIN EN 16187
Ochratoxin A	Wine, beer	HPLC, purification immunoaffinity column	DIN EN 14133
	Barley, roasted coffee		DIN EN 14132
	Currants, raisins, sultanas, dried fruits, dried figs		DIN EN 15829 DIN EN 15835 DIN EN 17251 DIN EN 17250
	Pork and pork products	HPLC-FD	
	Paprika, chili, pepper, cocoa, etc.	HPLC-FLD	
Phomopsin	Food	LC-MS/MS	DIN EN 17252
Saxitoxin group toxins	Shellfish	HPLC	DIN EN 14526
Zearalenone and trichothecenes including deoxynivalenol and acetylated derivatives	Cereals and products	LC-MS/MS	DIN EN 17280
	Baby food	HPLC-FD	DIN EN 15850
	Edible vegetable oils	LC-MS/MS	DIN EN 16924
	Cereals and products	LC-MS/MS	DIN EN 17280

(Continued)

Table 1.4 (Continued)

Parameter	Matrix	Method	Standard
Vitamins			
D-Biotin	Food	HPLC	DIN EN 15607
Niacin	Food	HPLC	DIN EN 15652
Vitamin A All-E-Retinol, 13-Z-Retinol	Food	HPLC	DIN EN 12833-2 DIN EN 12823-1
Vitamin B1	Food	HPLC	DIN EN 14122
Vitamin B2	Food	HPLC	DIN EN 14152
Vitamin B6	Food	HPLC	DIN EN 14164, DIN EN 14663
Vitamin D	Food	HPLC	DIN EN 12821
Vitamin E	Food	HPLC	DIN EN 12822
Vitamin K	Food	HPLC	DIN EN 14148
Metals and inorganic components			
Total arsenic	Food	HGAAS	DIN EN 14546
Arsenic	Seafood	GFAAS, microwave digestion	DIN EN 14332
Inorganic arsenic	Marine food, vegetable food	Anionenaustausch-HPLC-ICP-MS	DIN EN 16802
Mercury	Food	AAS cold steam technology, pressure digestion	DIN EN 13806
Methyl mercury			DIN EN 16801
Organomercury	Food marinen Ursprungs	Isotope dilution GC-ICP-MS	DIN EN 17266
Lead, cadmium, zinc, copper, iron	Food	AAS after microwave digestion	DIN EN 14084
Tin	Food	ICP-MS	OENORM EN 15765
Nitrate/Nitrite content	Meat products	Ion chromatography	DIN EN 12014-4
	Vegetables and products		DIN EN 12014-2

1.3.3.2 Environmental Analysis

Numerous analytical meteorological examinations and regulations are also exist in the environmental sector. This applies to the determination of inorganic and organic compounds as well as the quantitative detection of metals. Classic matrices for environmentally relevant issues are air, water, and soil. In addition, studies of waste and natural useful materials such as wood are also important. The aims of the investigations are both individual substances and sum parameters. Important compounds and groups of compounds in the environmental analysis are heavy metals (e.g. cadmium, lead, mercury), polychlorinated dibenzodioxins and dibenzofurans, polychlorinated biphenyls (PCB), pesticides (e.g. DDT,

lindane, toxaphene), mineral oil hydrocarbons (MKW), volatile halogenated hydrocarbons (LHKW), sulfur dioxide, nitrogen oxides, greenhouse gases (e.g. carbon dioxide, methane, etc.), ozone and fine dust. The most important total parameters include adsorbable organic halogen (AOX), chemical oxygen demand (COD), total organic carbon (TOC), and polycyclic aromatic hydrocarbons (PAHs).

The modern environmental analysis uses the entire spectrum of available analytical devices. Gas and liquid chromatography (GC and LC) for separating organic substances and ion chromatography (IC) for separating ions are used to separate the substances. The actual determination and quantification of the compounds are carried out, depending on the issue, by means of element-selective (AAS, OES, ICP-MS) or structure-selective (MS, UV/vis, fluorescence spectroscopy, IR spectroscopy) methods.

Table 1.5 shows a selection of classic parameters that are required in environmental analysis for the investigation of soil, air, and water samples as well as of the building fabric.

1.3.3.3 Requirements

Analytical applications from the above-mentioned areas differ significantly in their requirements from those of biological tests and screening methods. This results on the one hand from the type of compounds to be determined. In biological applications, it is generally known which reactions are taking place; the investigations should provide information about the activity of different compounds. In the areas of food monitoring, environmental analysis or general quality controls, on the other hand, it is first necessary to clearly identify a compound and then to carry out a quantitative determination. For the unambiguous identification of the substances, complex analytical measuring systems often have to be used. Thus, mass spectrometry is the method of choice in a variety of applications. The type of measurement system used also places demands on the samples to be examined. In general, no aqueous solutions are examined in gas chromatography, while in liquid chromatography no non-polar organic solvents are used for the most part. This requires the compounds to be determined to be present in a suitable solvent.

Extensive pre-analytical process steps are therefore required for the transfer of the analytes (see Figure 1.3). Depending on the application, the matrix can be of different complexity. Gaseous samples make certain demands on the sampling, at the same time, a suitable transfer into the measuring systems is necessary. However, they are not matrix-loaded and thus do not require any separation steps. Liquid samples can have different matrix loads. In addition to high salt contents (e.g. in water samples), this can also include organic matter such as proteins in blood or other biological samples. The range of the matrix contamination increases further with the transition to solid samples. In soil samples, the analytes to be determined are integrated into the complex soil matrix. In addition to pure inorganic soil components, there are also large amounts of humic acids in the soil, which may bind the pollutants to be determined. Complex mixtures of fats, proteins, and carbohydrates can also be found in food samples.

Different methods are used for preanalytical sample preparation. Liquid–liquid extraction is used to separate organic compounds from liquid samples and is often used for the preparation of water samples. Solid–liquid extraction is used to separate analytes from solid matrices, e.g. from soil or food samples. If the compounds to be examined are thermally stable and do not have too high boiling points, headspace technology can also be used. In

Table 1.5 Frequent parameters in environmental analysis.

Parameter	Standard Germany	U.S. EPA method
Adsorbable organic halogen (AOX)	DIN 38414-18	1650
Total organic halides (TOX)		9020B
Aromatic hydrocarbons	DIN 38407-44, DIN EN ISO 15680	
Chrome VI	DIN 38405-52	7196A
Total cyanide and easily releasable cyanide	DIN EN ISO 14403-1	9014; 335.4
Landfill gases CH_4, CO, CO_2, O_2, N_2, H_2S	VDI 3860	
Dissolved organic carbon	BS ISO 20236	415.3
Extractable organic halogen	DIN 38414-17	9023
Formaldehyde	DIN EN 1243	1667
Highly volatile compounds	DIN ISO 16000-6	8260
Low volatile compounds	DIN ISO 12219-6	310
High volatile halogenated hydrocarbons	DIN 38407 F4/F5	8010A; 8020A; 8021A
Solvents	DIN 38407 F9	310B
Anionic surfactants	DIN EN 14668, DIN EN 14669, DIN EN 14880	5540C; 425.1
Polyaromatic hydrocarbons	DIN 38407–3	610; 550.1
Polycyclic chlorinated biphenyles (PCB)	DIN 38414–20	8082A
Phenols	DIN 38407–27, U.S. EPA 8250	528; 604
Heavy metals	AbfKläV, DIN EN 14084	200.7; 3050B
Total organic carbon	ASTM D 7573a	415.3
Pestcides	DIN EN 1528–2	1699; 508
Dioxines	DIN EN 16190, ISO 13914	23
Chlorophenols	DIN EN 12673	1653; 528
Chlorobenzenes	DIN EN ISO 6468	8260C
Organic tin compounds	DIN EN ISO 22744-1, DIN EN ISO 22744-2	8323
Explosives	DIN EN 13631-16	8095; 529
Oils, gasoline, diesel	DIN EN 15721	4030
Pyrethroids and piperonyl butoxide	VDI 4301 Blatt 4	1660

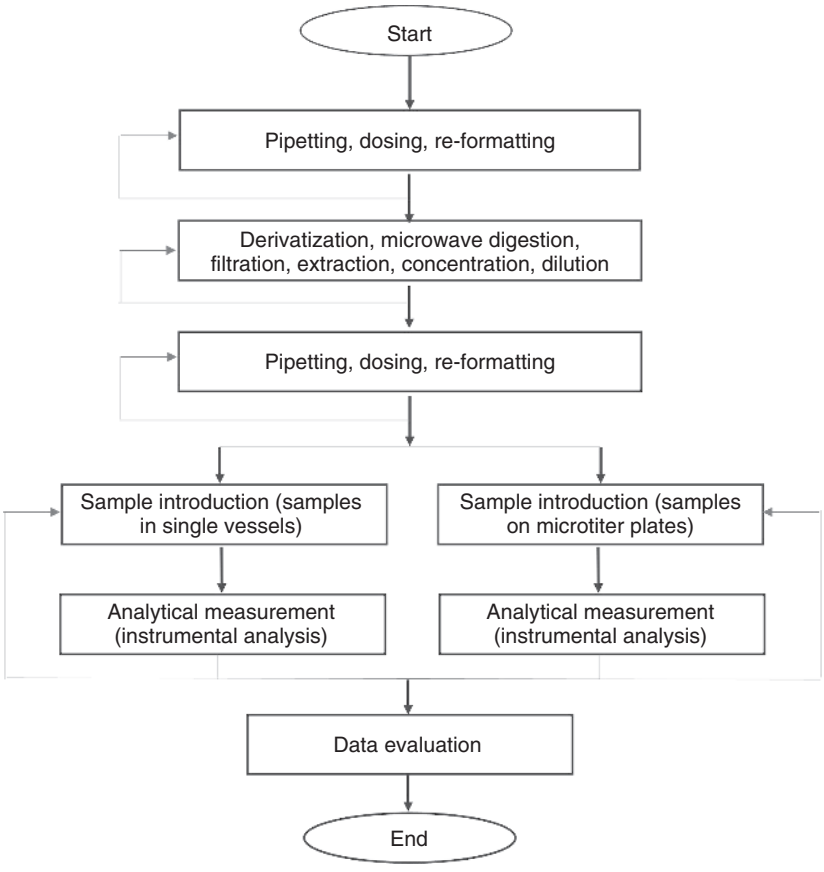

Figure 1.3 Process flow for classical analytical applications.

the case of liquid samples, the vapor pressures of the respective matrices must be observed. Solid-phase extraction is increasingly finding its way into preanalytical sample preparation. It not only enables an optimal separation of the analytes from interfering matrix components, but also a change of the solvent in order to create optimal conditions for the subsequent analytical determination. The measurement of metal contents in solid samples usually requires total digestion with strong acids. For example, for the determination of mercury in wood, corresponding digestions with concentrated nitric acid are required [135]. However, to avoid damage to the measurement system, appropriate dilution steps are then required.

Sampling has a very decisive influence on the quality of the measurement results. It is important to ensure that a sufficiently large sample is taken to obtain a really representative sample. Due to often large inhomogeneities of the sites to be sampled, larger sample quantities are required than in biological or clinical analysis.

The processes mentioned usually consist of several sub-steps and are not easy to automate. In addition to a large number of different solvents with sometimes difficult properties

(e.g. high vapor pressure, strong acids or alkalis, corrosive properties, etc.), the handling of solid compounds is also necessary in some cases. Since not all analytes can be determined in their own form by measurement technology, it is sometimes necessary to convert them into a measurable form. A wide variety of derivatization processes are used here, and the derivatization agents used are often unstable, have an intense odor, or are even sensitive to oxygen.

The automation of classic processes in environmental analysis is currently still in its infancy. In some cases, automation systems have been described for special applications. Korenaga and Yono described a system for automation in environmental water analysis [136]. The system used an *RV-M1* (Mitsubishi, Chiyoda, Japan) as the central robot as well as commercially available systems (automatic conductometric detector, automatic oxidation–reduction potential titrator, automated burettes, etc.).

1.4 The Goal of this Book

The automation of life science processes has numerous advantages. The type of process to be automated and the desired number of samples to be processed automatically is of the greatest importance. In addition, numerous other factors influence the selection of suitable automation devices and systems.

This book attempts to classify devices and systems in the life sciences that cover essential process steps for applications in the life science laboratory. The underlying principles as well as the requirements for automated systems are presented. Exemplary examples of devices are presented for each area, without claiming to be complete.

The following Chapter 2 gives an introduction to general automation concepts. In addition to partial automation, the main focus will be on different variants of full automation.

Due to the numerous different applications and uses in the field of life sciences, there is a multitude of formats to be handled for sample vessels and labware. Chapter 3 gives a systematization and shows the automation requirements associated with the different formats.

The core and main feature of almost all processes in the life sciences is the dosing of different liquids. Chapter 4 is devoted to this topic for the dosing of larger amounts of liquid in the range >1 µl and gives an insight into the basics of liquid dosing and the factors influencing the liquid handling results. Different examples of parallel automated dosing are presented on the basis of specific commercially available systems.

With increasing parallelization and miniaturization, new requirements arise for the dosing of ever-smaller amounts of liquid. Classic dosing methods as described in Chapter 4 cannot be used here. Thus, Chapter 5 describes the principles of the dosing methods that can be used for dosing amounts of liquids in the nl and pl range.

The dosing of solid substances is also increasingly playing an important role in various life science applications. The underlying principles are complex and are presented in Chapter 6 as well as suitable automation solutions.

Classic biological screening processes, which can be either enzyme-based or cell-based, represent very simple process sequences which, in addition, can be automated very well and easily due to the standard format of the microtiter plate. In contrast, other life science

processes require extensive sample preparation procedures. These include automated heating and cooling, mixing of samples, incubation of sample material, centrifugation for material separation, pouring, and filtration of samples, solid-phase extraction methods, the treatment of samples with ultrasound or the evaporation of solvents. Numerous manually operated devices are available for this. The subject of Chapter 7 is the description of the requirements that exist for corresponding devices with regard to the automation of these processes.

Full automation requires both, automated sample delivery to the sample processing subsystems and the transport of samples and labware between different devices and automation systems. Robotic components are predominantly used for this today. Chapter 8 mainly deals with modern developments in the field of so-called collaborative robotics. A second focus is a possibility of using mobile robots in highly complex automation systems. Solutions and possibilities for this are also presented.

After the sample preparation, an analytical examination of the samples is usually carried out to determine a wide variety of parameters. Optical reader technologies are mainly used for this in the biological field. This enables the fast determination of selected parameters, even in highly parallel applications. The disadvantage is the limited information content, which usually does not allow a clear identification of components. Mass spectrometric methods are available here, which enable both element- and structure-selective measurements to be made in a highly selective manner. Principles and examples of different analytical measurement methods are dealt with in Chapter 9.

To implement automated processes, continuous tracking of samples and labware is required throughout the entire process. Both optical and electrical identification methods, which are the subject of Chapter 10 of this book, are suitable for this purpose.

System integration includes the connection of different devices and systems to complex automation systems. An important point here is the problem of interfaces. In contrast to computer technology, which is already characterized by a high level of standardization, there are often different, sometimes even proprietary interfaces for classic devices in the life sciences. Chapter 11 presents important interfaces and their distribution in laboratory automation. Current standardization efforts are also discussed.

The final Chapter 12 is devoted to automation software. For the automation process, different levels have to be taken into account, from process control software and complex control systems to Laboratory Information Management Systems (LIMS) and electronic laboratory diaries.

The number and scope of process steps in life science applications are very extensive and lead to numerous automation solutions for individual steps. The aim of this book is not to provide an all-encompassing description of all available devices. This is not possible due to the size of the market and the very dynamic development in this area. Rather, an overview of automated device systems and components should be given as well as an introduction to physical principles that are used. The reader should be able to select the suitable system structure and suitable components for their own automation projects. This book aims to be both: an introductory textbook for everyone who is dealing with the topic of automation of life sciences laboratories for the first time. On the other hand, it is also an aid in the selection of suitable components for upcoming automation projects.

References

1 Hitomi, K. (1994). Automation – its concept and a short history. *Technovation* 14 (2): 121–128.
2 Veillette, P.T. (1959). The rise of the concept of automation. In: *Automation and Society* (ed. H.B. Jacobsen and J.S. Roucek). New York: Philosophical Library.
3 Bright, J.R. (1958). Automation and management. *Library of Congress Catalog Card No. 58-5968*.
4 Munekata, M. (1989). *Theory of Technology (in Japanese)*. Tokyo: Dobunkan.
5 Olsen, K. (2012). The first 110 years of laboratory automation technologies, applications, and the creative scientist. *Journal of Laboratory Automation* 17 (6): 469–480.
6 Stevens, T.M. (1875). Rapid and automatic filtration. *American Chemistry* 6 (3): 102.
7 Mitchel, T.A. (1912). Automatic filter feed. *Journal of Industrial and Engineering Chemistry* 4 (8): 613.
8 Lathrop, E.C. (1917). Simple device for the automatic and intermittent washing precipitates. *Journal of Industrial and Engineering Chemistry* 9 (5): 527–528.
9 Horne, W.D. (1893). An automatic extractor. *Journal of the American Chemical Society* 15 (5): 270.
10 Squibb, E.R. (1894). Automatic zero burette. *Journal of the American Chemical Society* 16 (3): 145–148.
11 Greiner, E.A. (1894). New automatic pipette. *Journal of the American Chemical Society* 16 (9): 643–644.
12 Hibbard, P.L. (1930). Apparatus for percolation at a uniform rate and automatic collecting device. *Industrial and Engineering Chemistry, Analytical Edition* 2 (4): 404.
13 Palkin, S., Murray, A.G., and Watkins, H.R. (1925). Automatic devices for extracting alkaloidal solutions. *Industrial and Engineering Chemistry* 17 (6): 612.
14 Automatic coal crusher and sampler. *Mining Science* 48 (1737): 342 (1913).
15 Dennis, L.M. (1913). *Gas Analysis*. New York: Macmillan.
16 Taylor, G.B. and Hugh, S. (1922). Automatic volumetric analysis carbon monoxide recorder. *Journal of Industrial and Engineering Chemistry* 14 (11): 1008.
17 An automatic chemist. *Popular Science Monthly* 98 (1): 84 (1921).
18 Shakespear, G. and Weaver, E. (1920). Notes and correspondence: automatic methods of gas analysis depending upon thermal conductivity. *Journal of Industrial and Engineering Chemistry* 12810: 1027.
19 Balch, R.T. and Paine, H.S. (1928). Factory operation of automatic electrometric pH control of cane juice defecation. *Industrial and Engineering Chemistry* 20 (4): 348–353.
20 Holven, A.L. (1929). Experimental application of automatic pH recorders to sugar-refinery alkalinity control. *Industrial and Engineering Chemistry* 21 (10): 965.
21 Experiments made by robot chemist. *New York Times* 20 (1929).
22 Hickman, K. and Sanford, C.R. (1933). Automatic titrating devices. *Industrial and Engineering Chemistry, Analytical Edition* 581: 65.
23 Olsen, K. (1997). Rosie the robot, laboratory automation and the second world war, 1941 to 1945. *Laboratory Robotics and Automation* 9 (3): 105–112.
24 Ferguson, B. (1942). Semiautomatic fractionation: a rapid analytical method. *Industrial and Engineering Chemistry, Analytical Edition* 14 (6): 493.

25 Tamele, M., Ryland, L., and Irvine, V. (1941). Potentiometric determination of mercaptans in aqueous alkaline solutions. *Industrial and Engineering Chemistry, Analytical Edition* 13 (9): 618.

26 Van Duuren, B. (1960). Automatic solvent addition funnel for paper chromatography. *Analytical Chemistry* 32 (6): 732.

27 Lingane, J.J. (1948). Automatic potentiometric titration. *Analytical Chemistry* 20 (4): 285.

28 Frediani, H.A. (1952). Automatic Karl Fischer titration. *Analytical Chemistry* 24 (7): 1126.

29 Kelley, M.T., Stelzner, R.W., Laing, W.R., and Fischer, D.J. (1959). Automatic coulometric titrator for Karl Fischer determination of water. *Analytical Chemistry* 31 (2): 220.

30 Ewing, G. (1960). Automatic titrators. *Journal of Chemical Education* 37 (7): 380.

31 Patterson, G. (1955). Review: automatic operations. *Analytical Chemistry* 27 (4): 574.

32 New equipment. *Chemical and Engineering News* 26 (45): 3372 (1948).

33 C&EN REPORTS: Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (1952). New device makes analysis and types out results. *Chemical and Engineering News* 30 (11): 1092.

34 Cerda, V. and Ramis, G. (1990). *An Introduction to Laboratory Automation*, 255–287. New York: Wiley.

35 SLAS. (2009). The history of automated liquid handling. *Labautopedia*. http://diyhpl.us/~bryan/irc/labautopedia-history-of-automated-liquid-handling.html (accessed 21 January 2020).

36 Schnitger, H. (1961). Vorrichtung zum schnellen und exakten Pipettieren kleiner Flüssigkeitsmengen. *Device for fast and exact pipetting of small liquid volumes*. Patentschrift Patentschrift 1 090 449, Deutsches Patentamt, München.

37 Gilson, R., Gilson, W. (1974). Adjustible pipett. US Patent 3827305, filed 24 October 1972 and granted 6 August 1974.

38 Rosenfeld, L.A. (2000). A golden age of clinical chemistry: 1948–1960. *Clinical Chemistry* 46: 1705–1714.

39 Plumb, R.K. (1956). *Science in Review: Clinical Chemists Report on New Methods and Devices in the Field of Medicine*, E13. *New York Times*.

40 Streitberg, G.S., Bwititi, P.T., Angel, L., and Sikaris, K.A. (2009). Automation and expert systems in a core clinical chemistry. *Journal of the Association for Laboratory Automation* 14 (2): 94–105.

41 Durner, J. (2010). Clinical chemistry: challenges for analytical chemistry and the nanosciences from medicine. *Angewandte Chemie International Edition* 49: 1026–1051.

42 Kricka, L.J. and Savory, J. (2011). International year of chemistry 2011. A guide to the history of clinical chemistry. *Clinical Chemistry* 57: 1118–1126.

43 Maclin, E. and Young, D.S. (1987). Automation in the clinical laboratory. In: *Fundamentals of Clinical Chemistry*, 3e (ed. N.W. Tietz), 160–192. Philadelphia, PA: WB Saunders Co.

44 Boyd, J. (2002). Robotic laboratory automation. *Science* 295 (5554): 517–518.

45 Felder, R. (2006). The clinical chemist: Masahide Sasaki, MD. *Clinical Chemistry* 54 (4): 791–792.

46 Sarkozy, L., Simson, E., and Ramanathan, L. (2003). The effects of total laboratory automation on the management of a clinical chemistry laboratory. Retrospective analysis of 36 years. *Clinica Chimica Acta* 329: 89–94.

47 Sasaki, M., Kageoka, T., Ogura, K. et al. (1998). Total laboratory automation in Japan past, present and the future. *Clinica Chimica Acta* 278: 217–227.

48 Felder, R.A. (1998). Modular workcells; modern methods for laboratory automation. *Clinica Chimica Acta* 278 (2): 257–267.

49 Hamilton, S. (2002). Introduction to screening automation. In: *Methods in Molecular Biology, Vol. 190. High Throughput Screening: Methods and Protocols* (ed. W.P. Janzen), 169–193. Totowa, NJ: Humana Press.

50 Pereira, D.A. and Williams, J.A. (2007). Origin and evolution of high throughput screening. *British Journal of Pharmacology* 152 (1): 53–61.

51 Major, J. (1998). Challenges and opportunities in high throughput screening: implications for new technologies. *Journal of Biomolecular Screening* 3 (1): 13–17.

52 HighTech Business Decision (2002). *High-Throughput Screening 2002: New Strategies and Technologies*. High Tech Business Decision http://www.hightechdecisions.com/pdf/hts.pdf.

53 Mayr, L.M. and Fuerst, P. (2008). The future of high-throughput screening. *Journal of Biomolecular Screening* 13 (6): 443–448.

54 Hamilton, S.D. (1986). Robotic assays for fermentation products. In: *Advances in Laboratory Automation Robotics*, vol. 4 (ed. J. Strimatis and G.L. Hawk), 1–23. Hopkinton, MA: Zymark.

55 Freifeld, K. and Kindel, S. (1985). The one-armed chemist. *Forbes* 135: 116.

56 Bente, P., Shuman, M., and Schliefer, A. (1986). A robotic microarray system: enzyme linked immunosorbent assays in a 96-well plate format. In: *Advances in Laboratory Automation Robotics*, vol. 4 (ed. J. Strimatis and G.L. Hawk), 201–216. Hopkinton, MA: Zymark.

57 Moses, V., Springham, D.G., and Cape, R.E. (1999). *Biotechnology: The Science and the Business*. CRC Press.

58 Akowitz, A. (1997). High throughput screening: a simplified protocol to verify pipetting accuracy. *Laboratory Automation News* 2 (2): 33–35.

59 Swank, P.R., Lewis, M.L., and Damron, K.L. (1991). A computer program for microplate reader interface and analysis of kinetic, microclot lysis assays. *Thrombosis Research* 64 (2): 235–242.

60 Attene-Ramos, M.S., Miller, N., Huang, R. et al. (2013). The Tox21 robotic platform for the assessment of environmental chemicals – from vision to reality. *Drug Discovery Today* 18 (15–16): 716–723.

61 Godfrey, O., Raas, A., and Landis, P. (1987). The application of a robotic work station to the handling of microbial colonies. In: *Advances in Laboratory Automation Robotics*, vol. 4 (ed. J. Strimatis and G.L. Hawk), 161–174. Hopkinton, MA: Zymark.

62 Blow, N. (2008). Lab automation: tales along the road to automation. *Nature Methods* 5 (1): 109–111.

63 Warburg, O., Christian, W., and Griese, A. (1935). Wasserstoffübertragendes Co-Ferment, seine Zusammensetzung und Wirkungsweise. *Biochemische Zeitschrift* 282: 157.

64 Balcke, O. (1961). Die theoretischen Grundlagen des optischen Tests nach O. Warburg. *Pure and Applied Chemistry* 3 (3–4): 465–472.

65 Gerlier, D. and Thomasset, N. (1986). Use of MTT colorimetric assay to measure cell activation. *Journal of Immunological Methods* 94 (1–2): 57–63. https://doi.org/10.1016/0022-1759(86)90215-2.

66 Hansen, M.B., Nielsen, S.E., and Berg, K. (1989). Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of Immunological Methods* 119 (2): 203–210.

67 Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65 (1, 2): 55–63.

68 Pereira, S.A.P., Dyson, P.J., and Saraiva, M.L.M.F.S. (2020). Miniaturized technologies for high-throughput drug screening enzymatic assay and diagnostics – a review. *Trends in Analytical Chemistry* 126: 115862.

69 Zang, R., Li, D., Tang, I.-C. et al. (2012). Cell-based assays in high-throughput screening for drug discovery. *International Journal of Biotechnology for Wellness Industries* 1 (1): 31–51.

70 Kumar, A. (2017). *What Are the Different Types of Cell-Based Assays?* Enzo Life Sciences: Technote.

71 Schroeder, H.W. and Cavacini, L. (2010). Structure and function of immunoglobulins. *Journal of Allergy and Clinical Immunology* 125 (suppl. 2): S41–S52.

72 Zhou, X., Hu, W., and Qin, X. (2008). The role of complement in the mechanism of action of rituximab for B-cell lymphoma: implications for therapy. *The Oncologist* 13: 954–966.

73 Courtis, A., Gac-Breton, S., Bethou, C. et al. (2012). Complement dependent cytotoxicity activity of therapeutic antibody fragments is acquired by immunogenic glycan coupling. *Biotechnology of Human Disorders* 15 (5): 7.

74 Meyer, S., Leusen, J.H.W., and Boross, P. (2014). Regulation of complement and modulation of its activity in monoclonal antibody therapy of cancer. *mAbs* 6 (5): 1133–1144.

75 Serrano, M.M., Rodriguez, D.N., Palop, N.T. et al. (2020). Comparison of commercial lateral flow immunoassays and ELISA for SARS-CoV-2 antibody detection. *Journal of Clinical Virology* 129: 104529.

76 Ehmen, C., Medialdea-Carrera, R., Brown, D. et al. (2021). Accurate detection of ZIKA virus IgG using a novel immune complex binding ELISA. *Tropical Medicine and International Health* 26 (1): 89–101.

77 Samsonova, J.V., Osipov, A.P., and Kondakov, S.E. (2017). Strip-dried whole milk sampling technique for progesterone detection in cows by ELISA. *Talanta* 175 (1): 143–149.

78 Kolosova, A.Y., Shim, W.-B., Yang, Z.-Y. et al. (2005). Direct competitive ELISA based on a monoclonal antibody for the detection of aflatoxin B1. Stabilization of ELISA kit components and application to grain samples. *Analytical and Bioanalytical Chemistry* 384: 286–294.

79 Wang, L., Zhang, Q., Chen, D. et al. (2011). Development of a specific enzyme-linked immunosorbent assay (ELISA) for the analysis of the organophosphorus pesticied

fenthion in real samples based on monoclonal antibodies. *Analytical Letters* 44 (9): 1591–1601.

80 Sreeranjit, C.V.K. and Lal, J.J. (2003). Glucose: properties and analysis. In: *The Encyclopedia of Food and Health*, 1e (ed. B. Caballero, P. Finglas and F. Toldrá), 2898–2903. Oxford: Academic Press.

81 Hirt, B. (1967). Selective extraction of polyoma DNA from infected cell cultures. *Journal of Molecular Biology* 26 (2): 365–369.

82 Chomczynski, P. and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry* 162 (1): 156–159.

83 Birnboim, H.C. and Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Research* 7 (6): 1513–1523.

84 Zeugin, J.A. and Hartley, J.L. (1985). Ethanol precipitation of DNA. *Focus* 7 (4): 1–2.

85 Crouse, J. and Amorese, D. (1987). Ethanol precipitation: ammonium acetate as an alternative to sodium acetate. *Focus* 9 (2): 3–5.

86 Lee, S.B., McCord, B., and Buel, E. (2014). Advances in forensic DNA quantification: a review. *Electrophoresis* 35: 3044–3052.

87 Zhao, Y., Xiang, S., Dai, X., and Yang, K. (2013). A simplified diphenylamine colorimetric method for growth quantification. *Applied Microbiology and Biotechnology* 97: 5069–5077.

88 Goldshtein, H., Hausmann, M.J., and Douvdevani, A. (2009). A rapid direct fluorescent assay for cell-free DNA quantification in biological fluids. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine* 46 (6): 488–494.

89 Gerdes, L., Gürtler, P., Iwobi, A., and Pecoraro, S. (2017). Digitale PCR. *Journal of Consumer Protection and Food Safety* 12 (Suppl 1): S53–S56.

90 Mullis, K.B. (1985). Process for amplifying nucleic acid sequences. US Patent US4683202A, filed 25 October 1985 and granted 28 July 1987.

91 Kleppe, K., Ohtsuka, E., Kleppe, R. et al. (1971). Studies on polynucleotides. XCVI. Repair replications of short synthetic DNA's as catalyzed by DNA polymerases. *Journal of Molecular Biology* 56 (2): 41–361.

92 Galbiati, S., Brisci, A., Lalatta, F. et al. (2011). Full COLD-PCR protocol for noninvasive prenatal diagnosis of genetic diseases. *Clinical Chemistry* 57 (1): 136–138.

93 Ying, Z., Ramshani, Z., Waggoner, J.J. et al. (2020). A non-optical multiplexed PCR diagnostic platform for serotype-specific detection of dengue virus. *Sensors and Actuators, B: Chemical* 310: 127853.

94 Bwire, G.M., Majigo, M.V., Nijro, B., and Mawazo, A. (2021). Detection profile of SARS-CoV-2 using RT-PCR in different types of clinical specimens: a systematic review and meta-analysis. *Journal of Medical Virology* 93 (2): 719–725.

95 van Kasteren, P.B., van der Veer, B., van den Brink, S. et al. (2020). Comparison of seven commercial RT-PCR diagnostic kits for COVID-19. *Journal of Clinical Virology* 128: 104412.

96 Cani, A.K., Hu, K., Kiu, C.-J., Siddiqui, J., Zheng, Y., Han, S. et al. (2021) Development of a whole-urine, multiplexed, next-generation RNA-sequencing assay for early detection of aggressive prostate cancer. *European Urology Oncology*. https://doi.org/10.1016/j.euo.2021.03.002.

97 Araujo, R.V., Feitosa-Suntheimer, F., Gold, A.S. et al. (2020). One-step RT-qPCR assay for ZIKV RNA detection in *Aedes aegypti* samples: a protocol to study infection and gene expression during ZIKV infection. *Parasites and Vectors* 13: 128.

98 Abe, K.T., Li, Z., Samson, R. et al. (2020). A simple protein-based surrogate neutralization assay for SARS-CoV-2. *JCI Insight* 5 (19): e142362.

99 Behijati, S. and Tarpey, P.S. (2013). What is next generation sequencing? *Archives of Disease in Childhood Education and Practice Edition* 98 (6): 236–238.

100 Hu, T., Chitnis, N., Monos, D., and Dinh, A. (2021). Next-generation sequencing technologies: an overview. *Human Immunology* 82 (11): 801–811.

101 Altmann, R.B., Prabhu, S., Sidow, A. et al. (2016). A research roadmap for next-generation sequencing informatics. *Science Translational Medicine* 8 (335): 335ps10.

102 Bronner, I.F., Quail, M.A., Turner, D.J., and Swerdlow, H. (2014). Improved protocols for illumina sequencing. *Current Protocols* https://doi.org/10.1002/0471142905.hg1802s79.

103 Kircher, M., Heyn, P., and Kelso, J. (2011). Addressing challenges in the production and analysis of illumine sequencing data. *BMC Genomics* 12: https://doi.org/10.1186/1471-2164-12-382.

104 Lindsay, S. (2016). The promises and challenges of solid-state sequencing. *Nature Nanotechnology* 11: 109–111.

105 Wu, Q., Lu, Z., Li, H. et al. (2011). Next-generation sequencing of microRNAs for breast cancer detection. *BioMed Research International* https://doi.org/10.1155/2011/597145.

106 Lehmann, R., Gallert, C., Roddelkopf, T. et al. (2016). 3 dimensional cell cultures: a comparison between manually and automatically produced alginate beads. *Cytotechnology* 68: 1049–1062.

107 Lehmann, R., Severitt, J., Roddelkopf, T. et al. (2016). Biomek® cell workstation: a variable system for automated cell cultivation. *Journal of Laboratory Automation* 21 (3): 439–450.

108 Rajaram, S., Boikos, C., Gelone, D.K., and Gandhi, A. (2020). Influenza vaccines: the potential benefits of cell culture isolation and manufacturing. *Therapeutic Advances in Vaccines and Immunotherapy* 8: 1–10.

109 Bisswanger, H. (2014). Enzyme assays. *Perspectives in Sciences* 1: 41–55.

110 Junker, R. and Gässler, N. (2018). Clinical chemistry parameters. In: *Point-of Care Testing* (ed. P.B. Luppe and R. Junker), 63–68. Berlin/Heidelberg: Springer.

111 Bessey, O.A., Lowry, O.H., Brock, M.J., and Lopez, J.A. (1946). The determination of vitamin A and carotene in small quantities of blood serum. *The Journal of Biological Chemistry* 166 (1): 177–188.

112 Xuan, R., Wang, T., Hou, C. et al. (2019). Determination of vitamin A in blood serum based on solid-phase extraction using cetyltromethyl ammonium bromide-modifies attapulgite. *Journal of Separation Science* 42: 3521–3527.

113 Roe, J.H. and Kuether, C.A. (1942). A color reaction for dehydroascorbic acid useful in the determination of vitamin C. *Science* 95: 77.

114 Speek, A.J., Schrijver, J., and Schreurs, W.H.P. (1984). Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with

pre-column derivatization. *Journal of Chromatography B: Biomedical Sciences and Applications* 305: 53–60.

115 Kahoun, D., Fojtikova, P., Vacha, F., Novakov, E., Hypsa, V. (2021) Development and validation of an LC-MS/MS method for determination of B vitamins and some its derivatives in whole blood. *bioRxiv*. https://www.biorxiv.org/content/10.1101/2021.01.18.427110v1.abstract.

116 Binks, M.J., Bleakley, A.S., Rathnayake, G. et al. (2021). Can dried blood spots be used to accurately measure vitamin D metabolites? *Clinica Chimica Acta* 518: 70–77.

117 Bach, A., Fleischer, H., Wijayawardena, B., Thurow, K. (2021) Optimization of automated sample preparation for vitamin D determination on a Biomek i7 workstation. *SLAS Technology*. https://doi.org/10.1177/2472630321103091.

118 SchØnheyder, F. (1936). The quantitative determination of vitamin K.I. *Biochemical Journal* 30 (5): 890–896.

119 Kamao, M., Suhara, Y., Tsugawa, N., and Okano, T. (2004). Determination of plasma vitamin K by high-performance liquid chromatography with fluorescence detection using vitamin K analogs as interna standards. *Journal of Chromatography B* 816 (1–2): 41–48.

120 Zweigenbaum, J., Heinig, K., Steinborner, S. et al. (1999). High-throughput bioanalytical LC/MS/MS determination of benzodiazepiens in human urine: 1000 samples per 12 hours. *Analytical Chemistry* 71 (13): 2294–2300.

121 Moore, C., Coulter, C., Crompton, K., and Zumwalt, M. (2007). Determination of benzodiazepines in oral fluid using LC-MS-MS. *Journal of Analytical Toxicology* 31 (9): 596–600.

122 Honeychurch, K.C. (2019). Review of electroanalytical-based approaches for the determination of benzodiazepines. *Biosensors* 9 (4): 130.

123 Jamey, C., Szwarc, E., Tracqui, A., and Ludes, B. (2008). Determination of cannabinoids in whole blood by UPLC-MS-MS. *Journal of Analytical Toxicology* 32 (5): 349–354.

124 Montesano, C., Sergi, M., Odardi, S. et al. (2014). A μ-SPE procedure for the determination of cannabinoids and their metabolites in urine by LC-MS/MS. *Biomedical Analysis* 91: 169–175.

125 Shin, Y., Kong, T.Y., Cheong, J.C. et al. (2019). Simultaneous determination of 75 abuse drugs including amphetamines, benzodiazepines, cocaine, opioids, piperazines, zolpidem and metabolites in human hair samples using liquid chromatography-tandem mass spectrometry. *Biomedical Chromatography* 33 (9): https://doi.org/10.1002/bmc.4600.

126 Verplaetse, R. and Henion, J. (2015). Quantitative determination of opioids in whole blood using fully automated dried blood sport desorption coupled to on-line SPE-LS-MS/MS. *Drug Testing and Analysis* 8 (1): 30–38.

127 Burgess, R.R. and Deutscher, M.P. (2009). *Guide to Protein Purification*, vol. Vol. 463. Academic Press ISBN: 978-0123-74536-1.

128 Otto, M. (2019). *Analytische Chemie*. Weinheim: Wiley ISBN: 978-3-527-34465-9.

129 Bruce, S.J., Rochat, B., and Beguin, A. (2013). Analysis and quantification of vitamin D metabolites in serum by ultra-performance liquid chromatography coupled to tandem

mass spectrometry and high-resolution mass spectrometry – a method comparison and validation. *Rapid Communications in Mass Spectrometry* 27: 200–206.

130 Dwiwedi, P., Zhou, X., Powell, T.G. et al. (2018). Impact of enzymatic hydrolysis on the quantification of total urinary concentration of biochemical markers. *Chemosphere* 199: 256–262.

131 R-Biopharm AG (2019) Enzymatische Foodanalytik. Firmenbroschüre.

132 Deutsches Institut für Normung 2021. DIN 10762: Untersuchung von Honig – Bestimmung des Gehaltes an Ethanol – Enzymatisches Verfahren, Ausgabe 2004–06.

133 Deutsches Institut für Normung 2021. DIN 10763: Untersuchung von Honig – Bestimmung des Gehaltes an Glycerin – Enzymatisches Verfahren, Ausgabe 2004–06.

134 Mattisek, R., Steiner, G., and Fischer, M. (2010). *Foodanalytik*, 4e, 365–370. Berlin: Springer.

135 Fleischer, H., Vorberg, E., and Thurow, K. (2014). Determination of total mercury content in wood materials – Part 3: miniaturization using ICP-MS. *American Laboratory* 46 (8): 16–20.

136 Korenaga, T. and Yono, Y. (1991). Robotics in automation of environmental water analysis. *Analytical Sciences* 7: 733–736.