



Analytical methods, biosensor technology

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Originating from the simple hand-held blood glucose meters and the first blood gas analyzers of the 1960s, POCT technology has spawned a myriad of analytical methodologies and applications [14]. As the areas of application are so diverse, it is not possible to exhaustively present all analytical methods; nevertheless, general detection principles will be explained in brief. These are in particular:

- Electrochemical methods (e.g. electrophoresis, potentiometry, amperometry)
- Mass change methods (e.g. quartz crystal microbalance)
- Optical methods (e.g. spectrometry, refractometry) and
- Chromatographic methods.

- Transducer unit
- Electronic amplifier
- Sample application/fluidic unit.

POCT systems also evolved from rapid tests (e.g. pregnancy test). In these simple lateral-flow systems, the human eye assumes the role of the transducer in reading out the signal. All other more complex analytical systems are defined as sensors if their signal is read by the transducer and converted into an electrical signal. The underlying principle is referred to as chemosensor and/or biosensor technology [24]. ■ Fig. 5.1 illustrates and explains a sensor flow cell.

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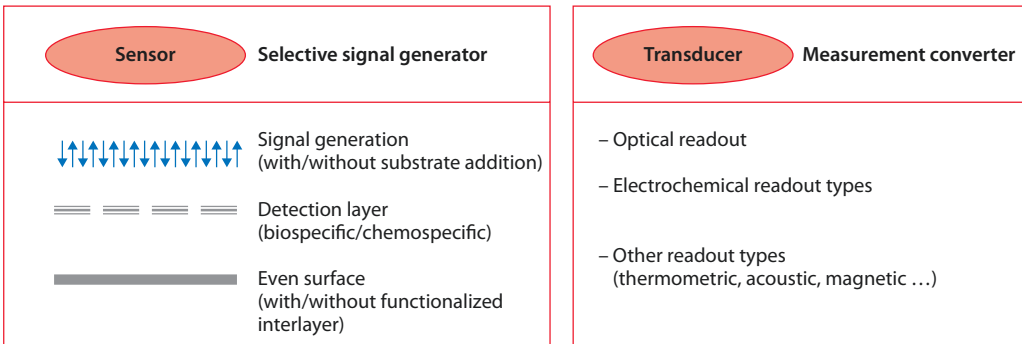
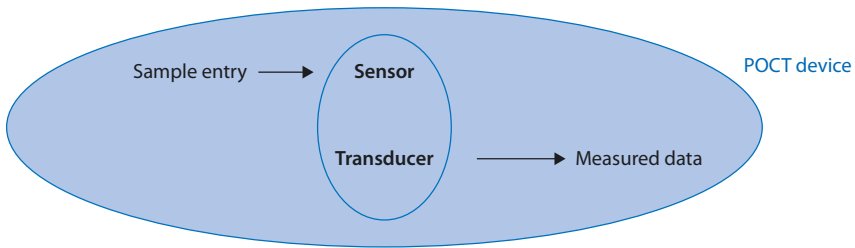
5.1 Biosensor technology

The setup of analytical systems based on biosensor technology provides a helpful overview [13]:

- Surface-immobilized, biologically active sensor

5.1.1 Sensor (bioreceptor)

The sensor can be regarded as a selective signal generator. That is the case when a specific ligand layer is immobilized on the surface of a sensor/transducer system. This ligand can have either biospecific or chemospecific properties. The surfaces are made of various plastic mate-



■ Fig. 5.1 Basic setup of a biosensor

■ **Tab. 5.1** Properties of a biosensor

Signal generation	Micro-reflectometry and micro-refractometry (label-free optical surface analysis)	Spectroscopy	Microgravimetry	Electro-chemistry
	Ellipsometry Reflectometric interference spectroscopy Surface plasmon resonance Diffraction grating Photonic crystals Mach-Zehnder interferometry	Fluorescence (Chemi)luminescence Total internal reflection fluorescence Reflection Absorption Turbidimetry Optical movement analysis	Quartz crystal microbalance (QCM) Surface acoustic wave (SAW)-based systems Microcantilever	Amperometry Potentiometry Conductivity Coulometry
Sensor surface	In micro-reflectometric and micro-refractometric methods	In spectroscopic methods	In electro-chemical methods	In mass-sensitive methods
Self-assembled monolayers (SAM), polymers or hydrogels on different carrier materials	Silicon wafer, glass or various plastic materials Nobel metal surfaces Glass or plastic materials with optic gratings Glass, silicon-wafer with integrated optic	Glass or plastic materials (often microtiter plate and microscope slides), in TIRF also in integrated optic or optical gratings Silicon wafer Homogeneous phase	Nobel metal electrodes	Quartz crystal, coated with a metal film Glass or plastic materials in microcantilever sensors
Ligand layer on the sensor surface	Biospecific		Chemospecific	
	Antibodies Antigens Receptors Ligands Other binding proteins (e.g. anticalins)	Enzymes (substrate conversion, enzyme activation/inhibition) Aptamers DNA/RNA oligomers (Protein) scaffolds Living cell	“Molecular imprints” Indicators Ion-selective membranes (in electro-chemical methods)	

rials, glass, silicone or noble metals. Metal surfaces can be prepared with or without a functionalized interface.

The signal can be generated in different ways, either with or without substrate addition. There are optical surface analytical methods, such as ellipsometry or surface plasmon resonance, which generate specific information from the surface. Other spectroscopic methods can be employed, such as absorption, fluorescence, (chemi)luminescence or even optical motion analysis. Furthermore, electrochemical methods, such as amperometry and potentiometry, or microgravimetry, such as quartz crystal

microbalance (QCM), are utilized. The properties of the aforementioned sensors are summarized in ■ Tab. 5.1.

Biospecific ligands are particularly noteworthy [12]. These are often immobilized antibodies or enzymes. Antibodies serve as a biospecific ligand in the reaction with an analyte. Enzymes, by contrast, utilize biospecific catalytic reactions after substrate addition. Examples are glucose sensors (► Chapter. 12). Binding proteins can be used as “recognition elements“, e.g. in coagulation analysis [19].

Chemospecific ligands include all kinds of ion- and molecule-selective membranes, mole-

cular imprints or indicator molecules that are suitable as ligands because of their selectivity to the analyte.

Electrochemical sensors

■ ■ Potentiometric sensors

The Nernst equation [3] is the foundation for all potentiometric transducer types. According to this equation, the changes in the potential in a membrane at zero current show a logarithmic proportionality to the specific ionic activity. Potentiometric transducer electrodes are classified by their methodology:

- **Transmembrane potential.** This transducer is based on the accumulation of a potential along a sensor membrane. Ion-selective electrodes (ISE) utilize ion-selective membranes that generate a charge separation between the sample and the sensor surface.
- **Electrode potential.** This transducer is similar to the transmembrane potential sensor; however, the electrode itself provides the surface for the biochemical detection reaction, whereby the electrode potential changes as a function of analyte concentration
- **Field effect transistor (FET).** As a semiconductor, FET is able to analyze the smallest charge changes on the surface of an electrode (“gate”), which is positioned between the source and drain electrodes. In ion-selective FET (ISFET), which is a combination of ISE and FET, the measuring principle is based on the change in the field effect (development of a space charge zone). This field effect can be observed, depending on the concentration of ions in the sample between source and drain when, instead of an electrical contact at the gate, an ion-selective layer is applied and brought into contact with the solution to be measured. This causes a change in the source-drain current that is directly proportional to the change in the analyte concentration and can be measured very accurately.

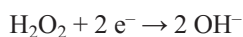
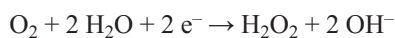
The advantage of potentiometric sensors is seen in the simplicity and robustness of their measuring system. Advantageous is also the small sensor size, which is important for POCT systems. All potentiometric sensor methods show problems with sensitivity and non-specific effects, which are reflected in a poor signal-to-noise ratio.

Parameters for potentiometric sensors are pH, pCO₂, Na⁺, K⁺, Ca²⁺ and Cl⁻.

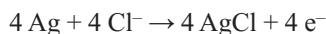
■ ■ Amperometric sensors

Amperometric sensors measure the current that is generated by an electrochemical reaction at constant voltage. The application of amperometric sensors is only successful if an analyte can also function as redox partner in an electrochemical reaction. Such an analysis system was first described in 1956 by L.C. Clark [4]. He described an oxygen electrode which consisted of an electrolyte-containing chamber, a platinum sensor cathode (polarized at -0.7 V) and an Ag/AgCl reference electrode. The chamber was covered with an O₂-permeable membrane.

The cathode reactions occur as follows:



The anode reaction is:



There are a number of enzymes with high catalytic conversion rates (>10³ s⁻¹), which ensure a substrate transformation in the amperometric systems [7]. In addition to the oxygen generated from H₂O₂ by catalase, there are further amperometrically detectable compounds, such as ferrocene derivatives, In²⁺ salts [1] or the redox polymer PVP-Os(bipyridyl)₂Cl that can be co-immobilized with antibodies in immunosensors [11]. Enzymes such as horseradish peroxidase (HRP), glucose oxidase, glucose dehydrogenase and others have been successfully implemented in amperometric sensors [16].

Amperometric sensors have excellent analytical sensitivity. By contrast, the transport rate

limitation inherent to the system can have an adverse effect on the redox partners on the electrode surface.

Parameters for amperometric sensors are pO_2 , glucose and lactate. ► Chapter 12 (diabetes diagnostics) offers more detailed information, especially for glucose analysis.

■ ■ Conductometric and capacitive sensors

These sensors measure the change in electric conductivity in a solution at constant voltage. The change results from biochemical reactions that produce or reduce ions in a specific way. The capacity changes are measured via an electrochemical structure where the bioactive element is attached to a pair of Au or Pt electrodes. Except for hematocrit measurements, there are only a few clinical applications, as the high ionic strength in whole blood or serum makes it difficult to measure the relatively low conductivity changes in a signal generating reaction [2].

Optical sensors

The methods that are utilized in these sensors are categorized into three groups:

- Optical detection
- Optodes
- Optical surface measurement

■ ■ Optical detection methods

The most frequently used optical sensors are part of the first group and measure light absorption or light reflection of an analyte as long as the parameter is present in at least millimolar concentration. The absorption of light as electromagnetic radiation at a given energy or wave length is subject to the Beer-Lambert law if the analyte is distributed homogeneously in the solution. The use of multi-wavelength photometers in oximetry is presented in ► Chapter 14. If the analyte is expected in a range far below a millimolar concentration, other spectrometric methods such as nephelometry, turbidimetry, fluorescence or (chemi)luminescence are needed as with immunoassays. However, this requires the use of appropriately labeled tracer substances.

■ ■ Optodes

Fiber optic chemosensors, called optodes, represent a further group of optical sensors, which in the future may enable continuous intra-arterial monitoring of various parameters (electrolytes, blood gases) for example. They utilize fluorescence dyes that are bound onto ion-selective (ionophore) or gas-permeable membranes. When these membranes only encase the fiberoptic, determination of electrolytes such as Na^+ , K^+ and Ca^{2+} or the measurement of pO_2 , pCO_2 and pH is possible. After excitation with a particular excitation wavelength at constant energy, the intensity of the emitted fluorescence is directly proportional to the concentration of a cation present or to the partial pressure of a gas.

■ ■ Optical and surface analytical methods

The use of label-free optical surface techniques in POCT [6] is rarely employed despite their high analytical power. Conversely, optical movement or picture analyses have already been utilized quite frequently in coagulation analyzers to detect the formation of blood clots. As an example, the pattern of movement of paramagnetic ferrous oxide particles under the influence of fibrin formation can be observed in an orthogonal oscillating magnetic field using a photodiode [17].

Total internal reflection spectroscopy (TIRS)

This optical sensor comprises two materials with different reflection indices (RI), where the light strikes the sensor surface entering through the layer with the higher RI under total internal reflection conditions. Thus, an evanescent wave (as electrical light vector) is formed in the material with the lower RI. This wave propagates (to ca. $\frac{1}{2} \lambda$, equivalent to a few 100 nm) into the medium at an exponentially decaying amplitude. Hence, biomolecules that are immobilized at the sensor surface can interact with these evanescent waves. This leads to a reduction of the reflected density intensity. By modifying this technique, a total internal reflection fluorescence measurement (TIRF) can also be achieved by applying fluorescent substances [6].

Reflectometric Interference spectroscopy (RIfS)

RIfS is a detection method based on the interference of white light at thin films (layers). In practice, it is used to investigate molecular interactions. The underlying principle of multiple reflections at thin layers corresponds to that of the Fabry-Pérot interferometer. The shift in the characteristic interference spectrum of the respective layer system allows biochemical binding events to be observed over time [5].

Ellipsometry Ellipsometry measures the change in polarization of light upon reflection on a surface with an immobilized sample. In general, linearly or circularly polarized light is used. After reflection, it is elliptically polarized, hence the name of this method. The orientation of the ellipse depends on the incident radiation angle, the orientation of the polarization of the incident light and of the sensor surface reflection properties (RI, density of the biologically modified surface).

Surface plasmon resonance The underlying concept of surface plasmon resonance (SPR) is based on a quantum-physical phenomenon that takes place on the surface of gold. In the gold layer, plasmons (fluctuations in the density of the swinging electrons in the metal, quantum-mechanically treated as quasiparticles) are generated by irradiation with monochromatic and polarized light under total reflection conditions. The plasmons dissipate energy in the form of an evanescent wave (like TIRS) and have a shadow-like effect (loss of intensity) on the reflected light. The reflectance angle of the light changes under otherwise constant optical parameters. This happens because of the interaction of molecules with a biochemical layer along the underside of the chip as the RI of the medium changes slightly due to a different surface composition [10].

The principle of refractometry and reflectometry are presented in Fig. 5.2.

Micro-gravimetric sensors

In mass-sensitive sensors, three different signal transduction methods are adopted: Quartz

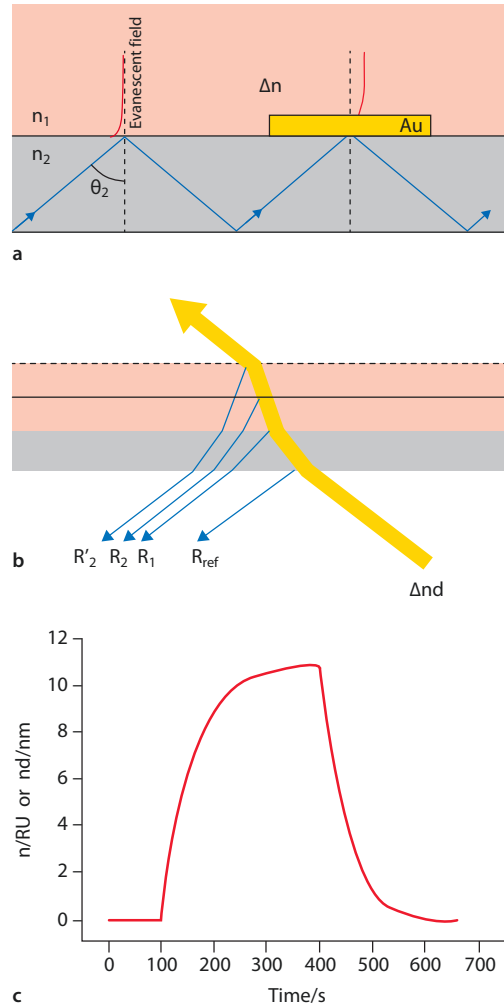


Fig. 5.2a–c Label-free optical methods. **a** Refractometry (evanescent field methods) with evanescent field in general (e.g. grid couplers) and surface plasmon resonance (SPR); **b** Reflectometry as reflectometric interference spectroscopy (RIfS); **c** Typical binding curve of a biomolecular reaction as seen in label-free methods

crystal microbalance, surface acoustic wave-based systems and microcantilevers.

Quartz crystal microbalance (QCM) In the QCM method, the oscillating quartz crystal is covered with a thin metal film (usually Au) and brought into its resonant frequency by alternating voltage. This resonant frequency is indirectly proportional to the mass of the material

that is adsorbed on the surface. These label-free sensors react very sensitively to every change in the solution. Therefore, such sensors need a reference system that enables them to distinguish between interactions with the target analyte and non-specific bonds or viscosity changes at the sensor surface [9, 18].

Surface acoustic wave (SAW)-based systems

SAW-based sensors consist of micro-electro-mechanical systems (MEMS), which are also based on vibration. The acoustic waves are restricted to the sensor surface and decay exponentially to the surface distance [22]. Surface waves are excited electrically by interdigital transducers (IDT). One SAW system suitable for POCT is the Love Wave Surface Acoustic Wave (LW-SAW) immunosensor that minimizes the acoustic losses which are affected by the thickness of the aqueous layer above the sensor. The analytic sensitivity of the device can be increased into the nanomolar range [20].

Microcantilevers The microcantilever is also a MEMS device that consists of a miniaturized “trampoline”. Here, a piezo-resistive bulk material loaded with a biological layer is only fixed to a platform on one side so that the other end of the elongated carrier can swing freely. The analyte binding to the sensor surface causes the microcantilever to bend. This can be measured either as an electrical (e.g. capacitive), piezo-resistive or optical effect [15]. Microcantilever sensors are already used in POCT applications [21].

Sensors for hemostaseological and hematological analyses

For coagulation analyses, known principles that are adopted in mechanized coagulation analyzers are utilized to detect blood clot formation. This is in addition to the above-mentioned optical movement sensors. Electrochemical methods are also employed, for example, to detect electrochemically generated active fibrinogen cleavage products due to the effect of thrombin. For hematological POCT analyses, the principles of particle counting

(Coulter principle) analogous to the mechanical hematology analyzers are adopted. These are based on the measurement of electrical conductivity changes when cells pass between two electrodes in a sensitive aperture. Alternatively, there is a quantitative buffy coat method, based on an electro-optical principle. The buffy coat layer is formed by centrifugation of a coated microhematocrit capillary. This layer is analyzed with an optical system.

Further details can be found in ► Chapters 15 and 16.

5.1.2 Transducers, electronic amplifiers

The transducer is part of a biosensor and is the equivalent of a transformer that allows optical, electrochemical or other readings (thermometric, acoustic, magnetic etc.) The transducer converts biological/chemical reactions into a physically measurable quantity that can be changed into an analog or digital signal and then be further used in various ways. The signal processing system conditions the sensor signal by electronic smoothing and noise filtration.

For the processing of signals from the transducer, many POCT systems use common computer or smart phone systems (Windows Phone, iOS, Android) or Internet-based apps. Thereby, not only (quantitative) results become visible (display, printer etc.), but also all analyses calculations (calibration etc.) and data management (storage of quality control measurements, patient results etc.) are carried out in the device. Many devices have a built in Wi-Fi-enabled network card, which allows network-compatible communication in the hospital.

5.1.3 Sample application/fluidic unit

The sample application in POCT devices can be very simple in contrast to the complex loading methods used in clinical-chemical analyzers. POCT devices often utilize a fluidic unit that

mostly consists of single-use components. The fluidic unit format can be defined as follows:

- Systems with absorbent materials, flow cells, lateral flow or (immuno)chromatography,
- Cassettes, capillary, tube or centrifugal systems.

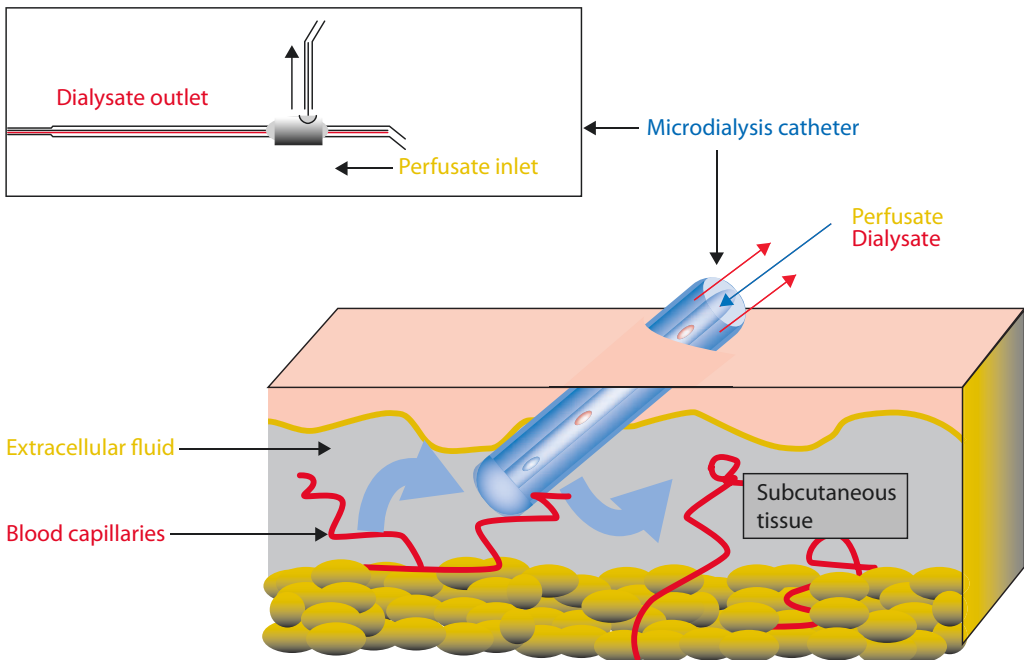
The flow is maintained using vacuum, pumps or centrifugal force; alternatively, the absorbent properties of many materials as well as capillary force and electroosmosis are utilized. Many fluidic unit systems are cassettes which provide all reagents in segments where the sample material can be easily loaded through an opening.

Simple unit-use POCT devices such as glucose meters utilize test strips that are inserted into the device manually. Then, the sample material is loaded manually onto an application pad and absorbed by capillary force into the strip. In contrast, blood gas analyzers suction the blood sample directly from the syringe or capillary with roller pumps and tube systems.

5.2 Continuous monitoring methods

5.2.1 Continuous monitoring methods

For some years now, the previously described in-vitro determination of metabolic parameters has been supplemented by the principle of continuous monitoring in the interstitial fluid of subcutaneous fatty tissue, mainly in the abdominal area or the upper arm over a limited time period (presently max. 14 days) [8, 23]. Via a minimally invasive microdialysis catheter placed in the subcutaneous tissue (■ Fig. 5.3), that can also be inserted by trained patients by themselves, continuous measurement of metabolites over a few days is possible. Microdialysis uses an aqueous perfusion solution with low flow rates and therefore highly water soluble metabolites with a low molecular weight are suitable for dialysis. This applies in particular to important parameters such as glucose and lactate but also creatinine. Determination of



■ Fig. 5.3 Principle of subcutaneously applied microdialysis

parameters from the dialysate is carried out using the analytical principles described in other chapters. As a matrix for analysis, dialysate is easier to handle than capillary blood, plasma or urine.

5.2.2 Continuous glucose monitoring (CGM)

At present in Germany, various technologies are approved that transmit results to an extracorporeal device to collect, process, store and present data at short intervals (<1 to 5 min). They provide immediate data access (on-line) for a needs-based therapy adjustment including alarm settings for hypo- and hyperglycemia. Examples include s.c. in-vivo needle biosensors (needle electrodes with integrated glucose oxidase monitoring systems) like those featured in the Guardian REAL-Time (Medtronic), FreeStyle Libre (Abbott) (■ Fig. 5.4) and SEVEN PLUS (DexCom, distributed by Nintamed in Germany) or GlucoDay S (Menarini Diagnostics) as extracorporeal in-vitro-biosensors (electrochemical via glucose oxidase). These features are combined with an s.c. microdialysis fiber whose filtrate volume of interstitial fluid is continually pumped to an ex-vivo biosensor for glucose monitoring.

Clearly, the main advantages of CGM methods primarily lie in the increased number of glucose readings with 500–1,500 per 24 h compared to 2–4 per hour with BGSM.

- This information yield is useful to identify subclinical hypoglycemic states, particularly during sleep,
- postprandial blood glucose profiles,
- overall glucose variability in 24 h for targeted therapy adjustments, including the prospect of connection to an insulin pump (“closed loop”).

Secondly, in this way, there is a prognostic potential for trend analyses such as for alarm functions mentioned above.

To date, all CGM systems share a common problem that glucose is measured from or in the



■ Fig. 5.4 FreeStyle Libre (Abbott Laboratories, Wiesbaden). The glucose sensor requires the insertion of a sensor filament under the skin. It can be left in situ for up to 14 days. An additional check of glucose values with a blood glucose meter is required when glucose levels change quickly as the levels in tissue fluid may not represent the true blood glucose levels. The same applies in situations when the system indicates hypoglycemia or a developing hypoglycemia or when symptoms do not correspond with the measured values of the system.

s.c. interstitial fluid and cannot be objectified by an independent method. Clinical evidence is based on glucose levels in blood and not in s.c. interstitial tissue. As a result, CGM readings are converted and given as blood glucose equivalents in mg/dL or mmol/L, based on a few parallel capillary blood glucose measurements using different adjustment processes. These are unknown to the operator and allow for physical and physiological time delays caused by glucose flux between blood and interstitial tissue. This approach also has a considerable potential for error due to anatomical and physiological variables that occur inter- and intra-individually over the course of diabetes as well as due to additional system-dependent errors. The resulting requirements for quality assurance of these CGM systems vary considerably from those of the glucose POCT single measurements. However, a regulatory legal framework for this does not yet exist in Germany.

Notwithstanding, the CGM systems user group is growing year on year, not only in the outpatient sector, but also in inpatient settings, in particular in specialized diabetes wards and

intensive care driven by the clinical advantages presented above. The use of such CGM systems, particularly in clinical settings, meets the hallmark criteria to count as POCT.

In contrast, **non-invasive glucose monitoring** systems (► Chapter 11) do not yet have market readiness and the required approval, despite considerable interest, very high investment in development and various technological approaches. It is currently expected that the development period for **non-invasive glucose monitoring** systems will be between 5–20 years.

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