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Point of care testing (POCT) Present and Future

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To quote Alain Mérieux, “without diagnostics, medicine is blind.” The paradox is that only 2% of total healthcare costs go towards in vitro diagnostics but 60% of treatment decisions are made based on in vitro information. The dominant model of laboratory testing throughout the world remains the centralized laboratory in which most of the analytical processes are nowadays automated to enable the analysis of large numbers of samples at relatively low cost. This trend is well established in biochemistry and hematology and is now extending to other disciplines including microbiology and anatomical pathology. However, healthcare is changing, due to economic pressure and shortage of personnel, economic growth of developing countries, and a shift towards a more patient-centered approach. There is always the urge to provide even faster results allowing rapid decisions and need to facilitate testing of chronically ill patients or patients with limited access to medical care. All the above together with the recent advances in technology have contributed to the development of point of care testing (POCT)[1]. The term “point of-care testing” (POCT) describes the clinical laboratory testing that is carried out at patient’s bedside or in the direct proximity of the patient. A generally accepted definition has still not been agreed. Other terms used are: “near-patient laboratory testing”, “remote rapid testing”, “bedside testing”, “decentralized testing”, “Patient self-management”[2]. The National Academy of Clinical Biochemistry (NACB) defines POCT as “clinical laboratory testing conducted close to the site of patient care, typically by clinical personnel whose primary training is not in the clinical laboratory sciences or by patients themselves (self-testing)[3]. POCT refers to any testing performed outside of the traditional, core, or central laboratory”. The typical characteristics of POCT[1] are the following: the test is performed near the patient; no sample preparation is required; whole blood can be used; no pipetting steps; “Ready-to-use” reagents; single sample measurement; no medical technical expertise is required; the results are available in short time. **What are the criteria for the selection of a POCT system[2]?** The clinical and operational needs along with facility needs. The clinical needs include the evaluation of POCT results on patient management (i.e., improved TAT compared to laboratory testing) and the requirements for POCT implementation in clinical practice. The operational needs include staffing needs, storage, space, and temperature requirements, information technology requirements (i.e., patient and QC data, LIS, HIS), and cost (financial justification of POCT vs laboratory testing). **What characteristics of a POCT system should be evaluated?** Test complexity, QC mode, type of device, operator management, type of connectivity, type of specimen, training, cost assessment, and users training. Sufficient concordance between POCT results and results obtained in the central laboratory must be assured within acceptable tolerance limits with accuracy relative to lab reference method, precision, and specificity[4].

The primary goal of POCT is “reducing turnaround time without compromising the quality of information on which clinical decisions are based” resulting in faster diagnostic and therapeutic processes, shorter length of stay (LOS), lower total medical cost, clinician and patient’s satisfaction.

Additionally, epidemics, pandemics, disasters are confronted and health care in rural areas is supported.

Medical device legislation for POCT[5,6]**.** All systems or devices used in the field of POCT are classified as IVDs (in vitro diagnostics). For this classification according to the IVD Directive, it is irrelevant whether the devices used for diagnosis are applied in medical laboratories by trained personnel (laboratory diagnostics devices), on the ward (POC diagnostic devices) or for self-testing (home diagnostic devices, "home-use"). Since May 2017, IVDs have become subject to a separate, directly applicable European regulation, Regulation (EU) 2017/746. There are perspectives for establishing a new product category named "devices for near-patient testing", certainly to account for the increasing importance of POC diagnostics. This category will contain devices which are not intended for self-testing, but for the application outside the (conventional) laboratory environment, generally near or at the patient. Product information (labeling, instructions for use, identification) will be provided in the language(s) of the Member State(s) in which the intended users will be supplied with the device. Moreover, devices for near-patient tests are additionally tested on their performance in different medical environments (e.g., patient's home, emergency department, outpatient centers).

All sites that perform POCT are required to have a Clinical Laboratory Improvement Amendments (CLIA) certificate[7]. According to CLIA regulations in 1988, tests are divided as follows:

- ✓ Tests of low complexity that are "simple tests with an insignificant risk of an erroneous result", so-called **"Waived tests"**. The CLIA Waived Testing Requirements include a Certificate of Waiver (CW), a fee certificate, and conformity to manufacturers' instructions. Waived Tests evolved from a few to hundreds.
- ✓ Tests of medium and high complexity so-called "No waived tests".

POCT does not specifically refer to CLIA-waived tests but also includes a wide variety of non-waived medium complexity tests.

POCT accreditation

"All laboratory tests regardless of their location require the same accreditation criteria" EN ISO 22870 was the first international standard on quality management for POCT, 2006 and modified at 2017. The requirements of the ISO 22870 included: Quality control, establishment of a committee, training programs management, and quality assessment. In the present, accredited POCTs exist in several European countries and in the USA[8].

Quality assurance

Instrument and method validation[9]

a. Calibration studies: accuracy, linearity, repeatability • Record keeping (calibration, maintenance, washing, storage, troubleshooting)
b. Supplier selection • Maintenance and control of reagent supply / storage, expiration dates • Record keeping

POCT regulatory requirements focus on two areas: training and competency of the personnel doing the testing and verification of strict adherence to the manufacturer-specified procedure for each test.

Internal quality control twice daily (with exception of waived tests) and external quality assessment.

Quality management manual

a. Educational programs • Training and certification test • Continuing education and testing • Keeping user registers
b. Instructions for use next to the instrument• Procedures for recording the results• Procedures for infectious waste disposables • Recording of compliance with other security procedures[10]

The ideal requirements for a POCT System are the following: First results in a minute or less, portable instruments with consumable reagent cartridges, single-step operating protocol, capability of analysis on non-processed samples (whole blood, CSF, urine, and stool), simple operating procedures, flexible test menus, results comparable with those of the central laboratory, built-in/integrated calibration and quality control, ambient temperature storage for reagents, result storage and transmission, and low instrument cost.

Areas of application: The main areas of application of POCT are within the hospital (Emergency Department (ED), Intensive Care Unit (ICU), Operating Room/Reanimation, Delivery Room/Neonatal Ward, CT scanning/ Invasive Radiology, Diabetic Care Ward, Dialysis unit) and outside the hospital (physician's office, pharmacy, home care and nursing care, ambulances and emergency vehicles, health centers, patient's home, disaster and pandemic locations)[1].

The clinically analytes or parameters in use for POCT include blood gases, electrolytes, metabolites, coagulation factors, enzymes, hormones, drugs and narcotics, hematology factors, cardiac markers, viruses etc. POCT emergency parameters should be provided in all emergency departments and ICUs, to achieve fast answers. The most common parameters are blood glycose, electrolytes, blood gases, cardiac markers, coagulation parameters, β-HCG.

POC diagnostic market

POCT systems make up an important segment of the in-vitro diagnostic (IVD) market, nearly the 30%. The global POC diagnostic market is anticipated to show rapid growth over the next years and is estimated to reach USD 50.6 billion by 2025 from USD 29.5 billion in 2020, at a CAGR of 11.4%. because it offers significant growth potential for product manufacturers. Additionally, technological evolution in POC devices, rising incidence of infectious diseases, and increased investments by key players are the main reasons for POC diagnostics growth. It is a multi-billion-industry with high competition and various sectors. By product, the glucose monitoring products will dominate, by platform the lateral flow assays segment will be the largest share, by end user the home care setting segment will be the fastest-growing end-user segment, by region North America will account for the largest share of the market, and by mode of purchase the over the counter (OTC) products segment will have the highest growth rate. The most important Key Market Players are the following: Abbott (US), Roche (Switzerland), Siemens (Germany), Danaher (US), Becton Dickinson and Company (US), Johnson & Johnson (US), Instrumentation Laboratory (US), PTS Diagnostics (US), Quidel (US), Chembio Diagnostic Systems (US), Sekisui Diagnostics (US), Nova (US), EKF Diagnostics (UK), AccuBioTech (China), Trinity Biotech (Ireland), etc[10,11].

Types of POCT devices

POCT devices according to their size are classified into small handheld devices and large bench-top devices[1,11].

POCT handheld devices first appeared in the form of paper strips many years ago for the testing of urine. Test strips as we know them today were introduced in the 1940s and 1950s through companies such as Ames and Roche, while the first disposable immunochemical test could be

deemed to be the relatively rapid (2 hours) pregnancy test presented by Wampole in 1970. This type of devices ranges from the simplest form, the dipstick, to sophisticated, small cartridge devices. Unprocessed whole blood can be applied directly to the device without any prior preparation. Dipsticks are of a single use, give qualitative or semiquantitative and can detect one or up to 10 analytes; each strip is composed of several layers each with designated functions such as separation of plasma from the red cells, spreading or support. Chemical or immune reactions take place. The measuring signal can either be read off directly or recorded on a simple read-out device. The sensors are incorporated into the test strip. The spectrum of available tests varies greatly and depends on the sensors employed. Dry chemical methods are implemented, e.g., glucose-converting enzymes that are immobilized on reagent strips. Any calibration in these devices is usually replaced by electronic or physical standards, more complex cassette devices are equipped with automated calibration programs that run at set time intervals. There are devices which are designed for a singular parameter only (e.g., Bayer's Ascensia Contour for glucose, Roche Coaguchek for INR) and device-based systems that can measure multiple parameters using different strips or cassettes (e.g., Nova's StatStrip for glucose and creatinine, Alere's Triage for multiple parameter groups). Immunostrips are **immunosensors** where the recognition agent is an antibody that binds to the analyte with detection by **reflectance or fluorescence spectrophotometry**. Lateral flow designs where the separation takes place as the sample moves along a solid phase are much more common, and in fact are the dominant technology. The utility of lateral flow strips can be extended from qualitative to quantitative measurement by small reader devices that incorporate multichannel light detectors. The latter are often a charge-coupled device (CCD) or CCD camera which can measure much lower light signals than a conventional reflectometer. There are two **types of detection systems**: photometric or electrochemical. The latter detection systems have enabled the design of strips that are less subject to interferences. The more sophisticated handheld devices are the so-called **integrated cartridges** best exemplified by the i-STAT. After placement of a small sample of whole blood the cartridge is inserted into a reader for measurement. The cartridge utilizes thin-film sensors in combination with microfluidics, and cartridges are produced in various formats for different analytes. Their popularity is due to the extensive critical care testing menu that is available on a single device. The i-STAT represents an economical way to provide relatively low numbers of critical tests. The Epoc critical care testing system is also a handheld POC device but of a different construction being based on so-called Smart Card Technology. Here the biosensors and microfluidics are printed on a 35 mm format Once again, different cartridges are available to provide a range of POC tests.

POCT Large Bench-Top Devices

This type of instruments presents more complex built-in fluidics and mimics miniaturized automated analyzers. They are multiple use instruments, for single-sample measurements. Reagents and solutions are contained in prefabricated disposable disks or cartridges with different combinations for multiple selections. 100 microliters are enough for 10 analyses. Self-calibrators and controls are integrated. After transferring the sample onto the uptake point, the disc is inserted into the analyzer drawer where the sample is moved by centrifugal and capillary force to the appropriate site inside the cuvette where the reaction occurs. In this way, diluents and reagents also flow to the "correct" reaction site. After a few minutes, the analysis is performed photometrically; self-calibration and continuous quality controls are integrated in the analyzer. Small desktop instruments are also available to measure a wider range of general chemistry analytes such as the Piccolo express which uses a small disposable rotor that contains all the required reagents and diluents to perform a particular group of tests. The major difference of the cartridge-based systems here compared to the hand-held devices is that the sensors are reusable. Constructed using thick-film technology, the sensors for each of the tests and all the required reagents for calibration and washing are packaged into a single cartridge pack which is inserted into the instrument. The lifespan of the cartridge when inserted into the machine is based on the number of samples analyzed and the life-duration of the cartridge[11,12].

POCT in Clinical Biochemistry

The wide analytical spectrum offered in clinical chemistry laboratories is equally accessible to POCT. Full-scale clinical biochemistry analyzers are essentially miniaturized versions of conventional laboratory systems to analyze a wide range of parameters, either singularly or in parallel. Wet and dry biochemistry analyzers fall into this group. A range of sample types can be used including capillary, venous and arterial blood. To avoid the need for centrifugation, the assay must either use whole blood or integrate an additional process step to eliminate cellular blood components from the whole blood. Usually, a few micro-liters are sufficient for analysis, whereby the actual analysis is completed in a few minutes. The commonly used testing systems can store data and are equipped with an integrated printer or IT interface that allows the results to be sent directly to the LIS (laboratory Information system) or HIS (hospital information system.

There are also POC devices designed specifically for singular parameters, e.g. , devices for lipid profile, lactate acid or glucose. Total bilirubin in newborns is often performed by direct spectrometry of non-diluted serum or plasma in a “bilimeter”. Special systems, e.g., Cholestech LDX by Alere, are used to determine patients’ lipid status. In sports medicine, lactate measurements in blood or saliva play an important role (e.g., AccuTrend Plus by Roche). It should also be mentioned that the intra-partum fetal scalp lactate assessment can give important information about the fetal condition during birth, a fast creatinine check in capillary blood is important in many radiological and interventional settings and ammonia test is critical in patients with cerebral and neuromuscular dysfunctions concurrent with liver cirrhosis. Small POCT devices for measuring blood ammonia are available from different manufacturers[13,14].

Blood gas analyzers are the dominant application of the POCT market. For decades, blood gas analysis (BGA) has been established in intensive care, anesthesiology, and emergency medicine as well as in lung function diagnostics and is therefore primarily conducted at the POC. The electrochemical sensor consists of a pH glass electrode, a pCO2 electrode and pO2 electrode. These sensors use gas-selective membranes that act as chemo-specific detection layers. The measuring principle is based on electrochemical reactions that occur between electrode surface and blood. The electric current (pO2) or tension allows a total analysis time of less than 60 s at a sample volume of 400µl. pO2 is determined by an excitable fluorescent dye, whose luminescence lifetime is modulated by the O2 concentration in the blood sample.

The menu of these devices has been now extended to electrolytes, urea, creatinine, glucose, lactate, and bilirubin as well as hemoglobin derivatives by cartridge-based technology. Blood gas analyzers use wet chemistry to perform clinical chemistry analyses and selective electrodes for electrolyte analysis[15].

POCT in hematology

POCT devices for blood count analysis are equivalent of miniaturized conventional laboratory analyzers. They can measure specific parameters, e.g., Hb or total leukocyte count. Direct measurement of Hb is usually carried out by photometry.

Various POC Prothrombin Time (PT) analyzers operate with different analytical principles of clot formation including optical, electromechanical, and electrochemical clot detection. Venous or capillary blood measurement of the PT in the POC setting is most often reported as the International Normalized Ratio (PT/INR). The analyzers utilize an enclosed system of disposable test strips or cartridges with an in-built quality control test. Other parameters include aPTT for monitoring heparin in POC settings, D-dimer, and activated clotting time (ACT) which has proven beneficial in the setting of cardiothoracic surgery, extracorporeal membrane oxygen ratio (ECMO), and hemodialysis, where high doses of heparin are used[15].

Thromboelastometry: The method is based on the kinetics of a clot from its creation to its degradation. A blood sample is placed in a container along with a sensor rod. Either the container or the rod rotates gently to form a clot. The change in velocity and the rate of thrombus change is measured on a computer. The ROTEM platelet module measures platelet function and aggregation by electrical impedance based on impedance aggregometry. When activated platelets adhere to the surface of two sensor wires, the electrical resistance between them increases significantly. This can be recorded and plotted graphically (impedance vs. time)[16]. **Impedimetric detection**

Impedance-based approaches are generally label free, which can differentiate different types and subtypes of blood cells by measuring cellular impedance and enumerate cells by electrical signals **optical detection Optics-based methods** utilize light signals for quantification such as scattered light, fluorescence, and absorbance. A microfluidic cartridge allows automatic blood sample preparation and visual cell counting. It shows results comparable to those obtained by flow cytometry and the hemocytometer, exhibiting potential use in POCT.

Biosensors

Biosensors are analytical devices that convert a biological response into an electrical signal. Biosensors must be highly specific, independent of physical parameters such as pH and temperature and should be reusable. Parts of a biosensor: a surface-immobilized, biologically active sensor (nucleic acids, proteins, antibodies, enzymes, bacteria, cell, tissues), a Transducer (optical, electrochemical, mass-based), and a Processor. The surfaces are made of various materials, plastic, glass, silicone, or noble metals.

Many POCT systems use common computer or smart phone systems (Windows Phone, iOS, Android) or Internet-based apps. All analyses calculations (calibration etc.) and data management (storage of quality control measurements, patient results etc.) are stored in the device. The main types of biosensors are Magnetic, Piezoelectric (quartz crystal microbalance and surface acoustic wave device), Optical, Electrochemical, Potentiometric, Amperometric, Immunosensors, Thermometric, and Acoustic biosensors. The immunosensors include Electrochemical, Optical sensors, Microgravimetric sensors (quartz crystal microbalance), and Thermometric sensors. Fluorescence and chemiluminescence techniques are mainly used for signaling with labeled primary or secondary antibodies or labeled tracers. The application of paramagnetic nanoparticles coated with specific antibodies has engendered immunoassay procedures with high analytical sensitivity[17].

Microfluidics is the science of manipulating fluids at the micron scale. Microfluidic systems are devices that include a set of miniaturized components allowing the study and analysis of chemical or biological samples. Those "microprocessors for biology" can replace bulky and expensive instruments. They rely on the continuous flow of liquid through prefabricated channels with the help of external pressure or built-in pumps or electromotive mechanisms. Some of the functions of a microfluidic device are sample preparation, separations of liquids, detection, and fluid manipulation. The ability to extract or purify, label, or separate the sample within the device help to reduce analysis time. Pumps, valves, and mixers are added onto the device to help in manipulating the fluids. Samples are then sensed and detected by biosensors. Microfluidic technology offers three main advantages to POCT: smaller sample volumes, lower test cost, and faster turn-around time. Microfluidic systems have made POCT more affordable and accessible, especially in developing nations[18].

The integration of biosensors with microfluidic systems offers an integrated and miniaturized alternative to the conventional laboratory analyzers offering significant reduction in sample and reagent volume, energy consumption, and waste production. Moreover, the microfluidic biosensors can decrease the cost, and increase the specificity and detection sensitivity limit compared to the

regular detection methods. Due to the small size of micro-systems, a single microfluidic biosensor can perform all the spectrum of analysis including continuous sampling, sample separation and mixing, and pre-concentration and treatment. Furthermore, these microfluidic biosensors offer enhanced analytical performance, high throughput, real-time detection, fast reaction rates and portability making detection adaptable to POCT. Overall, the integration of biosensors with the microfluidic systems creates a powerful analytical tool that will be an advanced step towards the home-testing approach which will benefit both developing and developed countries.

Immunochromatographic tests/ lateral flow assays (LFA)

A label coated antibody is immobilized at the conjugate pad. A primary antibody against the analyte is immobilized over the test line. A secondary antibody against the labeled antibody is immobilized over the control zone. The sample is applied to the sample application pad. At the conjugate pad, the analyte is captured by the immobilized labeled antibody and a labeled antibody /analyte complex is formed. This complex now reaches at the nitrocellulose membrane and moves under capillary action. At the test line, the label antibody /analyte complex is captured by the primary antibody. The excess labeled antibody will be captured at the control zone by the secondary antibody. Intensity of color is measured with an optical strip reader. Appearance of color at control line ensures that a strip is functioning properly. Although lateral flow technology dominates, there is difficulty of multiplexing, namely the ability to measure multiple analytes on the same strip and limited sensitivity and this has been brought into focus by the need for better technology for infectious disease testing, particularly in the developing world. This technology is based on molecular methods[20,21].

POCT based on molecular techniques

Molecular point-of-care tests utilize the same methodology as the laboratory molecular assays, automating a varying number of the steps required.

Selection criteria for point-of-care molecular biological diagnostic systems are the following: Suitable material must be processable directly without prior preparation, Hands-on time 5–10 min, Run time ≤ 50 min, Low complexity, transfer to the LIS. Unequivocally interpretable qualitative result (e.g., positive or negative pathogen). The careful selection of POCT molecular system adapted to the setting protects against diagnostic errors and reduces stress for the health care professionals.

Molecular testing has become a mainstay mainly in infectious disease diagnostics. Classic microbiological diagnostics usually takes at least 36–48 hours before the first results are available. A long delay can be fatal for the patient. Depending on the severity of the infection, often broad-spectrum antibiotics are given, without prior confirmation of the causative pathogen or its resistance. **PCR** offers rapid and targeted analysis and the advantage to analyze multiple factors simultaneously. In the age of multidrug-resistant (nosocomial) pathogens, rapid and reliable molecular biological differentiation is becoming increasingly important given the urgency indicated to effectively isolate affected patients at the earliest. POCT apparatus that address infectious disease control, especially for the developing countries, should follow the “ASSURED” criteria: Affordable, Sensitive, Specific, User Friendly, Robust and rapid, Equipment free, Deliverable to the final user.

For PCR single-use systems integrated cartridges are used. The reagents are pre-packaged and ready-to-use in these test cartridges. Hands-on work for the user is limited to loading the sample and starting the PCR run. The sample is mixed with the lyophilized reagents and digested. Fluid movements cause the reagent mixture to move through plastic arrays or channels into the reaction chambers where the next PCR process steps occur (DNA amplification and signal detection). By varying the reagents in the test cartridges different pathogens may be detected. Tests using smaller

aliquots tend to suffer from limits to the pathogen detection sensitivity because of heterogeneous sample material.

The first FDA approved tests were in January 2015, Influenza A&B by Alere i Abbott and in June 2015 Strep A at cobas Liat System by Roche. In the LIAT system (LIAT, lab in a tube) test cartridge is shaped like a small tube which contains the necessary reagents within small chambers arranged in rows. After insertion, the sample passes through these chambers step by step. The fluid column of the reagent mix moves up and down, allowing it to reach the different temperature zones of the PCR. Thanks to this simple movement, the PCR requires only a small space, making the reaction significantly faster. The io system by Atlas Genetics also provides an accelerated PCR cartridge by a specific electrochemical detection[22].

Isothermal amplification

This method operates at a stable temperature eliminating the use of thermocyclers. Real-time reading of amplification products is possible by turbidimetry or visual inspection of color change. This capability eliminates the need for gel electrophoresis. IA method needs lower energy, and can be integrated into simple, compact systems[23].

Multiplexed POCT (xPOCT) is a simultaneous on-site detection of different analytes from a single specimen and has gained increasing importance for clinical diagnostics, with emerging applications in resource-limited settings. In bead-based systems, three different conceptual approaches are primarily favored for multiplexing: (i) distinction of beads by either their size/shape or color (ii) labeling of beads with different enzymes, (iii) metal ions or spatial separation of beads in different channels[24].

Infectious diseases are caused by pathogenic microorganisms as virus, bacteria, parasites, and fungi and can be transmitted between individuals and populations thus threatening the general, public health and potentially the economy. Efficient diagnostic tools are needed to provide accurate and timely guidance for case identification, transmission disruption and appropriate treatment administration. Point of care (POC) tests provide actionable results near the patient and thereby serve as a personal “radar”.

Almost all the molecules or cells involved in the infection process can be used as biomarkers, such as proteins, nucleic acids, and antibodies. The treatment of life-threatening or highly acute infections such as sepsis are considerably facilitated by POCT, as the immediate HIV results (rapid HIV) for antiretroviral treatment or the intrapartum detection of group B streptococci in women in labor or the detection of plasmodia spp., at patients with suspected malaria infection. POCT is also useful for guiding treatment of viral infections and prevent their spread (transmission prophylaxis).

The FilmArray system by bioMérieux is a prime example of testing for “syndromic diagnosis”, differentiating patients’ symptoms, caused by various pathogens. The arrays allow the detection of numerous pathogens, which can cause respiratory or gastrointestinal infections with simultaneous detection of resistance markers. This parallel detection, however, leads to the greater need for interpretation of results[25].

Over 300 million patients every year in tropical areas (such as subSaharan Africa) suffer from malaria. According to the World Health Organization (WHO), effective malaria management relies mostly on early diagnosis and therapy. The last decade has seen significant development of malaria rapid diagnostic tests (RDTs), to enable fast and reliable testing in remote settings.

Over 40 million people are affected by HIV worldwide. About 85% of them are living in developing countries, where clinical diagnostics and antiretroviral therapy (ART) are limited. FDA approved

fourth-generation HIV-Ag/Ab assays combo Ag/Ab EIA (Bio-Rad Laboratories and Walter Reed Army Institute of Research), and Vitros HIV combo assay (Ortho Clinical Diagnostic). Commercially available HIV Rapid Diagnostic Tests (RDTs) such as Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories), HIV 1/2/O rapid test device (ABON), Determine HIV 1/2 (Alere), OraQuick Rapid HIV-1/2 Antibody Test (OraSure Technologies) and DPP HIV 1/2 (Chembio) can detect and sometimes differentiate between antibodies to HIV-1/2 in the POC setting.

Implementation of the traditional Pap smear in national screening programs is not sustainable in under-resourced LMIC settings with limited number of skilled cytologists and where, despite a high prevalence of cervical cancer loss to follow-up and poor adherence to treatment are major impediments for programmatic success. The current WHO recommendation for HPV testing as a primary cervical cancer screening tool is adopted by countries such as Kenya, where it forms part of the national cancer screening guidelines.

The Non-structural protein (NS1) is released from the infected cells into patients' blood, However, if the patient is tested 7 days after the onset of the fever or if he has been infected with dengue virus in the past, then his serum contains anti-dengue antibodies which compete with the monoclonal antibodies of the rapid method and affect the result. The rapid method is more susceptible to primary infections[26,27]

Continuous measurement methods

In the abdominal area or the upper arm, a minimally invasive micro-dialysis catheter is placed in the subcutaneous tissue for continuous measurement of metabolites in the interstitial fluid. An aqueous perfusion solution with low flow rates is carried in and the highly water-soluble metabolites with low molecular weight are carried out. This applies to important parameters such as glucose, lactate, creatinine. The results are transmitted 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. *In-vivo* needle biosensors with integrated glucose oxidase monitoring systems are used or extracorporeal in-vitro-biosensors (electrochemical via glucose oxidase). **Free-style Libre** by **Abbott** measures glucose in the interstitial fluid at regular intervals and alerts at abnormal values, useful for the detection of subclinical hypoglycemic states, particularly during sleep[28].

Direct to consumer testing A new healthcare market has emerged in Europe over the past years, that of direct-to-consumer testing (DTC). By eliminating the physician, the patient can choose from a selection of generally available DTC tests. The “quantified-self ” movement allow knowledgeable and empowered patients and consumers to answer questions about their own health and exercise-related habits. In this respect, POCT technology offers exactly that option of delivering minimally invasive performance data about one’s own body (as per the motto: “self-knowledge through numbers”). The potential opportunities and disadvantages of these novel applications must be viewed carefully considering that the patients themselves are responsible for carrying out and interpreting the tests. DCT tests are to be regarded with equal importance whether they are over-the-counter pregnancy tests or specimen collection kits for genetic analyses, purchased on the internet[29,30].

No invasive analyses include **Hb measurement by** transcutaneous reflection spectroscopy, **glucose** measurement by patient's skin illumination with infrared light and measurement of the reflected light, **bilirubin** measurement by densitometry of the reflection of the yellowish discolored skin on the forehead or above the sternum with corrections for the native color of the skin, and **arterial oxygen saturation by** pulse oximetry.

Rapid and practical point-of-care testing (POCT) devices are also used in blood donation centers for determining Hb concentrations.

Other devices rely on photoplethysmography, which is the study of volume changes in the body. They detect the relative magnitude of the photoplethysmographic signal at different times of the cardiac cycle using different wavelengths of light. Separate photodetectors detect this signal and utilize it to determine the hemoglobin content and hematocrit of blood[31].

In Self-monitoring the test is performed by patients with chronic conditions (e.g., diabetes), or by individuals to screen their own state of health. The quality of self-testing depends on the precision of the test and the adequate user guidance and capability. Patients who are self-monitored should be offered appropriate training, follow-up, and access to quality assurance. Urine glucose is only detectable when the individually variable renal excretion threshold is exceeded. As a result, self-monitoring of urine glucose is no longer applied although the costs are considerably lower than for the alternative, blood glucose self-monitoring. Nevertheless, this form of glucose self-monitoring is still used to a large extent internationally due to cost considerations. Prothrombin time, measured in seconds, is converted to INR based on the sensitivity of thromboplastin used in the test and calibrated against an international WHO reference thromboplastin. INR is therefore independent of method and laboratory. In most patients treated for almost all indications such as thromboembolism, atrial fibrillation and status post heart valve replacement, the target INR nowadays is usually 2.5 with a target range of 2.0–3.0. corrected for hematocrit, not influenced by heparin[32].

Smartphones are the ideal new generation POCT devices and will play an important role in the future of mobile and affordable mobile healthcare (mH) and personalized health (pH). Smartphones with their camera with small modifications become detectors, result processors or signal converters for optical or electrochemical biosensors. They can also be converted into microscopes or cytometers. Cloud technologies will ensure the secure transfer, storage, and retrieval of data. Smartphone-based applications for POCT devices have been developed for lateral flow and immunoassays, for electrochemical and colorimetric assays, as well as for microscopy and flow cytometry, spectrophotometry and SPR-based biosensors. Other applications link smartphones with lenses to create a compact and light-weight device for applications based on light, fluorescence, dark field, transmission and polarizing microscopes[33].

Wearable devices are real-time and noninvasive biosensors allowing for the continuous monitoring of individuals, and thus provide sufficient information for determining health status, and even preliminary medical diagnosis. In addition, wearable biosensors allow health care providers to monitor the physiological traits of patients after therapeutics or treatments. Wearable devices have gradually been developed in the form of accessories, integrated clothing, body attachments, and body insertions. Wearable devices connect to other smart devices via Bluetooth, infrared, radio-frequency identification (RFID) and near-field communication (NFC) technology. Together, this connectivity has led to the development of wearable systems for remote and long-term patient monitoring in homes and communities[34].

POCT IN THE HOSPITAL SETTING

Too many cooks… spoil the broth! •dozens of spaces• hundreds of devices • thousands of users • The spaces, the devices, the users, and the load of tests create conditions where rare mistakes turn to be common and regular.

POCTs have existed for years in hospitals without cost study or evaluation. An irrational or unjustified use of POCTS has impact on health care. In hospitals of many countries there are specific

procedures for the supervision of POCTS. The main responsibility is up to hospital management (determines the medical goal, provides the means, staff, and resources) and the core laboratory that contributes to the selection and evaluation of POCTS as well as quality assurance. According to ISO 22870 the management of the hospital creates a Committee. The POC coordinator is the key person who installs and oversees the POCT, creates procedures and rules, studies proposals for new POCTs. He organizes the training programs of the users, co-ordinates and supervises POCTS users, ensures that all systems are compatible with departments' needs and analyzes the quality control data[35,36].

Connectivity

Data management and computerization are recommended for all POCT devices. The instrumented devices can capture a result at the time of testing and link that result to the device serial number, date, time, operator, and the quality control. The control and patient results can be transferred automatically to the data management system. **The data management system** ensures the capture and documentation of all patient and control results, automates billing, ensures the generation of control statistics and patient reports and the documentation of operator training/competency (Operator and control lock-out assures that QC is properly analyzed) and compares populations of patients for quality measures and monitor hospital treatment goals (e.g., glucose averages for intensive insulin protocols). The **POCT data manager** forms the interface between POCT devices and hospital's IT system. Connects POCT devices to hospital IT network, POCT findings are integrated in the laboratory report and/or the HIS-based electronic medical record, sets POCT coordination and organization (devices, operator, patients, quality controls, reagents, device control, device reports etc.), accesses control and electronic communication with LIS, serves as a repository for testing locations, instrument serial numbers, instrument service history and software versions. Larger manufacturers of POCT systems frequently supply a matching POCT data manager with their devices. If several devices from various manufacturers are used together, it may be necessary to run several POCT data managers in parallel or use a device-independent software product.

With the adoption of mobile devices such as tablets and smartphones, web-based data management applications can be accessed from virtually anywhere to exchange information and manage systems, including in some applications the ability to send remote commands to the devices[37].

Advantages

The health cost benefit of POCT is beneficial to the facilities that utilize it. The speed in which a clinician receives an answer, provides a diagnosis, and makes a treatment plan is increased significantly. Patient's length of stay in a healthcare facility is reduced, the physician and other clinical staff provide care to other patients too while reducing the cost of healthcare for each patient. Readmittance is significantly reduced by wearable monitoring and testing devices when patients are discharged with them. The total expected cost of using POCT to deliver a routine Health Check in the primary care setting is lower than the laboratory-led pathway. Laboratory-led pathway offers patients more opportunities to miss subsequent Health Check-related appointments or to exit the care pathway. Using POCT could be more convenient for patients and offers GPs the ability to act as a 'one stop shop' by delivering a complete Health Check in a single sitting. Using POCT in routine general practice could likely reduce overall costs.

Disadvantages

Increased workload for the users**,** potential errors due to insufficient training of the users, cost of user-training and user-evaluation, insufficient quality control, inadequate storage of results, billing problems.

The pros and cons of point-of-care testing vs laboratory testing

There are situations where POCT can provide a definite advantage to the treating clinician, have minimal risk, better cost savings, and provide a quality healthcare experience for the patient. However, core laboratory testing is more advanced, follows the process and science for laboratory testing, and is fully integrated with the technology necessary to ensure that results are accurate, analyzed, validated, and recorded. There are

clearly situations where each method excels. Nonetheless, the gaps are quickly being closed and the two methodologies are merging through software technology[38,39].

Future perspectives

The laboratory testing decentralization is an extension and not a replacement of laboratory services. POCT would not overtake conventional laboratory diagnostics or even replace it. POCT and the central laboratory can be complementary. The central laboratory will continue to dominate the processing of large-scale analysis series and complex specialized tests. POCT is an innovation because it offers better and easier access to health care for every patient. New markers should add interest in the POCT systems. Rapid tests, particularly for cardiovascular and infectious diseases as well as ovulation and pregnancy testing hold considerable future potential. A new area for POCT growth, called companion diagnostics and liquid biopsy, may also come to the fore. Companion diagnostics refers to (stratified) tests that help to decide if a planned treatment is suitable for patients in a particular disease stage or have a known predisposition to it. The enormous developments in chip and microfluidic technologies and multiplexed analysis will be used for POCT. However, their clinical diagnostic value has still to be proven in studies. Long-term development will also be influenced by new diagnostic technologies, such as the continuous **"inline" measurement** of metabolites, **digital ELISA** (based on an array of femtoliter wells, antibody-loaded nanoparticles detect the antigen and beads are loaded into nano-well arrays with nanoparticles and immunoreaction signaling), **miniaturizarion** (open Lab-on-a-chip).

Geospatial Science and Point-of-Care Testing create solutions for population access, emergencies, outbreaks, and disasters[40,41,42].

POCT and COVID-19

The pandemic set the requirement for rapid, reliable, and sensitive diagnostic methods for widespread testing at an early stage of disease in emergency departments, airports, and home care facilities etc. where ultrafast screening with high accuracy is necessary. The most promising SARS-CoV-2 POC detection methods are immunoassays for antibody and antigen detection, RT-PCR as the gold standard, isothermal amplification, and CRISPR-Cas methods as a new emerging technique. Due to the increasing number of confirmed COVID-19 cases throughout the world, fast and reliable POC tests for early detection are greatly needed. A reliable POC diagnostic device could reduce transportation needs, risk of spreading infection, strain on the healthcare system, and cost of care for both individuals and the government. Despite of the outbreaks caused by viral infectious diseases such as MERS, SARS, and Ebola, the existing POC diagnostic platforms were not ready enough to promptly address the COVID-19 viral threat. However, during 2020 many efforts have been made in the field of POCT to enhance COVID-19 detection[42,43] .

REFERENCES

1. Nichols JH, Alter D, Chen Y, Isbell TS, Jacobs E, Moore N, Shajani-Yia Z. AACC guidance document on management of point-of-care testing. *J Appl Lab Med.* June 4, 2020, 1-20.

2. Peter B. Luppa · Ralf Junker Eds. Point-of-Care Testing Principles and Clinical Applications. Springer. February 2018, 1-445
3. James H. Nichols, Robert H. Christenson, William Clarke et al. Executive summary. The National Academy of Clinical Biochemistry Laboratory Medicine Practice Guideline: Evidence-based practice for point-of-care testing. Clinica Chimica Acta. April 2007, (379) 14-28
4. Mark Shephard. A Practical Guide to Global Point-of-Care Testing. CSIRO. 2016, 1-471
5. WORLD HEALTH ORGANIZATION GENEVA. MEDICAL DEVICE REGULATIONS Global overview and guiding principles, 2003
6. Medical device legislation and POCT. Point-of-Care Testing pp 251-260
7. CMS.gov. How to Apply for a CLIA Certificate, Including International Laboratories. January 2020.
8. European accreditation EA-4/20 G:2014. Guidance for the Assessment of Laboratories against EN ISO 15189 and EN ISO 22870 Point-of-Care Testing.
9. Pedro E. Castenada. Secrets for Success in Managing Point-of-Care Testing Quality Assurance. AACC.org *Clinical Laboratory New.* July August 2020.
10. Fortune Business Inside. Point of care diagnostic market. *December 10, 2020*
11. Reportlinker.com "Global Point of Care (POC) Diagnostics Market 2020-2024" Dec. 08, 2020 - https://www.reportlinker.com/p05770957/?utm_source=GNW
12. Andrew St John and Christopher P Price. Existing and Emerging Technologies for Point-of-Care Testing. *Clin Biochem Rev*. 2014 Aug; 35(3): 155–167
13. Richard F, Louie Zuping Tang, David G. Shelby, Gerald J. Kost. Point-of-Care Testing: Millennium Technology for Critical Care. *LABORATORY MEDICINE.* VOLUME 31, NUMBER 7 JULY 20 0 0
14. Peter B. Luppa. Point-of-care testing at the interface of emerging technologies and new clinical applications. De Gruyter 2020
15. James H. Nichols. Contemporary Practice in Clinical Chemistry. 2020, Pages 323-336
16. Matthijs Oyaert , Tom Van Maerken , Silke Bridts , Silvi Van Loon , Heleen Laverge , Veronique Stove. Analytical and pre-analytical performance characteristics of a novel cartridge-type blood gas analyzer for point-of-care and laboratory testing. *Clin Biochem*. 2018 Mar;53:116-126.
17. Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. *Anesth Analg*. 2008 106:1366–1375
18. Alexandr E. Urusov, Anatoly V. Zherdev and Boris B. Dzantiev *Biosensors*. 2019, 9(3)89
19. Becker H, Klemm R, Dietze W, White W, Hlawatsch N, Freyberg S, Moche C, Dailey P, Gärtner C. Modular microfluidic cartridge-based universal diagnostic system for global health applications. *Proc SPIE*. 2016, 9705:970514
20. Mekonen AA*, Abebe SA and Adali TJ. Microfluidics Devices Manufacturing and Biomedical Applications. *Biosens Bioelectron*. 2019, Vol 10(1): 265
21. Weijian Qin, Yafei Hou, Kun Xiao, Wenqiang Yan, Can Wang. The Application of Lateral Flow Immunoassay in Point of Care Testing: A Review. *Nano Biomedicine and Engineering*. September 2016 8(3)
22. Luppa PB, Sokoll LJ, Chan DW (2001) Immunosensors – Principles and applications to clinical chemistry. *Clin Chim Acta.* 2001, 314: 1–26
23. Goldmeyer J, Kong H, Tang W (2007) Development of a novel one-tube isothermal reverse transcription thermophilic helicase-dependent amplification platform for rapid RNA detection. *J Mol Diagn.* 2007, 9: 639–644
24. Cohen DM, Russo ME, Jaggi P, Kline J, Gluckman W, Parekh A Multicenter clinical evaluation of the novel Alere i Strep A isothermal nucleic acid amplification Test. *J Clin Microbiol.* 2015 53(7):2258–2261
25. Spindel S, Sapsford K (2014) Evaluation of optical detection platforms for multiplexed detection of proteins and the need for point-of-care biosensors for clinical use. *Sensors.* (Basel) 14: 22313–22341
26. Babady NE. The FilmArray respiratory panel: an automated, broadly multiplexed molecular test for the rapid and accurate detection of respiratory pathogens. *Expert Rev Mol Diagn.* 2013 13(8):779–788
27. Hui Chen, Kengku Liu, Zhao Li, Ping Wang. Point of care testing for infectious diseases, *Clinica Chimica Acta.* Volume 493, June 2019, Pages 138-147
28. Bissonnette L, Bergeron MG Infectious disease management through Point-of-Care personalized medicine Molecular Diagnostic Technologies. *J Pers Med.* 2012, 2:50–70
29. Vashist SK Continuous Glucose Monitoring Systems: A Review. *Diagnostics.* 2013 (Basel) 3:385–412
30. Lippi G, Favaloro EJ, Plebani M Direct-to consumer testing: more risks than opportunities. *Int J Clin Pract.* 2011, 65:1221–1229
31. AACC. Direct-to-Consumer Laboratory Testing Date: DEC.16.2019

32. Gamze Avcioglu , Cemil Nural , Fatma Meriç Yilmaz, Pervin Baran, Özcan Erel, Gülsen Yilmaz. Comparison of noninvasive and invasive point-of-care testing methods with reference method for hemoglobin measurement. *J Clin Lab Anal.* 2018 Mar;32(3):e22309
33. Olczuk D, Priefer R. A history of continuous glucose monitors (CGMs) in self-monitoring of diabetes mellitus. *Diabetes Metab Syndr.* 2018 Apr-Jun;12(2):181-187
34. Liu J, Geng Z, Fan Z, Liu J, Chen H. Point-of-care testing based on smartphone: The current state-of-the-art (2017-2018). *Biosens Bioelectron*. 2019 May 1;132:17-37.
35. Dunn J, Runge R, Snyder M. Wearables and the medical revolution. *Per Med.* 2018 Sep;15(5):429-448.
36. Lewandrowski K, Gregory K, Macmillan D (2011) Assuring quality in point-of-care testing: evolution of technologies, informatics, and program management. *Arch Pathol Lab Med*. 135:1405–1414
37. AACC Guidance Document on Management of Point-of-Care Testing JUN.4.2020 // Source: AACC Academy
38. Jang D, Shin SY, Seo DW et al. (2015) A smartphone-based system for the automated management of point-of-care test results in hospitals. *Telemed J E Health*. 21:301–305.
39. Tyrone Oliver Lightbody, Peter Nightingale, Vinay Reddy-Kolanu, and Elizabeth Sapey. The Benefits and Limitations of Point of Care Testing for Patients with Diabetes. *J Diabetes Metab*. 2017,8:9
40. Florkowski C, Don-Wauchope A, Gimenez N, Rodriguez-Capote K, Wils J, Zemlin A. Point-of-care testing (**POCT**) and evidence-based laboratory medicine (EBLM) - does it leverage any advantage in clinical decision making? *Crit Rev Clin Lab Sci.* 2017 Nov-Dec;54(7-8):471-494.
41. Kost GJ. Geospatial Science and Point-of-Care Testing: Creating Solutions for Population Access, Emergencies, Outbreaks, and Disasters. *Front Public Health*. 2019 Nov 26;7:329.
42. Vashist SK, Luppa PB, Yeo LY, Ozcan A, Luong JHT (2015) Emerging technologies for next-generation Point-of-Care Testing. *Trends Biotechnol* 33(11):692–705
43. Luppa PB, Bietenbeck A, Beaudoin C, Giannetti A (2016) Clinically relevant analytical techniques, organizational concepts for application and future perspectives of point-of-care testing. *Biotechnol Adv*. 34(3):139–160
44. CDC. Guidance for SARS-CoV-2 Point-of-Care and Rapid Testing Updated Mar. 12, 2021
45. Point of Care Diagnostics in the Age of COVID-19. Meysam Rezaei, Sajad Razavi Bazaz, Sareh Zhand, Nima Sayyadi, Dayong Jin, Martin P. Stewart, and Majid Ebrahimi Warkiani. Diagnostics 2021, 11, 9.