In vitro diagnostic platforms of the future; technological possibilities and challenges

Furuberg, Liv and Borch, Stig Morten

***Abstract*—Micro- and nanotechnologies are utilized in the new generation of diagnostic platforms for in vitro analyses. Complex analyses that need a sequence of manual or instrumental process steps in the laboratories can be automated by controlling microfluidic flows of samples and reagents in polymer chips. The biochemistries are adapted to small volume reactions. New and sensitive detection principles will be able to detect down to a few copies of biomarkers per sample. We present examples of platforms analyzing patient samples for proteins and nucleic acids, and some of the challenges involved in volume manufacturing of these platforms.**

I. Introduction

In vitro diagnostic methods are used for analysing patient samples such as blood, salvia or urine for disease markers. The disease markers may for example be proteins or a piece of DNA, hormones, lipids or glucose.

Advanced analyses that involve a sequence of operations such as sample preparation, separations, concentrations, washing, pipetting, mixing with reagents and heat cycling are usually performed in professional laboratories. Analyses may be performed by large automated equipment that analyse a large batch of different patient samples simultaneously, or by the skilled personnel in a number of manual processing steps. Patient samples that are drawn at the local doctor's office are sent to the laboratory for analysis. The patient and doctor have to wait several days for the analysis result, increasing anxiety and delaying treatment.

In contrast, point-of-care (POC) diagnostics is performed at the doctors' office, by the bedside in hospitals, or in the homes of the patients. Today the POC marked is 15% of the total in vitro diagnostics marked, but it is expected to increase to 30% in 2014 [1]. Use of lab on a chip, microfluidics and new biosensors based on micro- and nanotechnology will lower the price and increase the sensitivity of POC tests [2].

There have for some years been available fast and simple dipstick tests for e.g. pregnancy measuring the hormone human chorionic gonadotropin and diabetes measuring glucose levels. These analyses are based on simple lateral flow in cellulose strips and colorimetric or electric measurements to obtain the result.

However, new easy-to-use diagnostic platforms are now being developed for more complex analyses. Often the analytes are proteins or nucleic acids. The targeted use is both home care and doctors' offices; some bench top platforms for doctors' office are already commercially available. A point of care diagnostic platform typically consists of an instrument and corresponding disposable polymer chips / cartridges.

Diagnoses relevant for doctors' office are e.g. inflammations, infectious diseases, cardio vascular diseases, cancer etc, as listed in table 1. The doctor will perform tests for diagnostics, screening and treatment monitoring applications.

Table I
Examples of clinical use of point-of-care diagnostics

Clinical use	Diseases
Risk screening	Cancers, cardio vascular diseases…
Detection	Inflammations, acute phase reactions…
Specific diagnosis	Diabetes, infections, cardio vascular disease (e.g. AMI), cancer, celiac disease, allergies…
Disease monitoring	Diabetes, celiac disease…
Treatment monitoring	Cardio vascular diseases (coagulation), diabetes, rheumatic disease, cancer…
Relapse	Infections, cancer...

For the doctors' offices, certain platform requirements are important. The instrument needs to be compact to fit into the small avilalable bench space, and it must be flexible to perform different analyses, having a wide parameter menu. In practice, different disposable cassettes, cartridges or chips with different integrated reagents are used for different analyses, and the instrument actions are specific for each analysis. Often it is desirable to perform several analyses simultaneously in one chip. If blood is used as sample, the analysis must be based on finger prick blood of volumes smaller than 15 microlitres. The reliability and robustness of the results are crucial because there will be no laboratory personnel available to evaluate the result. The instrument must also be simple and safe in use, typically described by

Manuscript received January 10, 2010. This work was supported in part by the European Commission under contract no. IST-NMT-CT-2005-017319 and contract no. IST-027175.

L. Furuberg and S. M. Borch are with SINTEF, Department of Microsystems and Nanotechnology, P.O Box 124, Blindern NO-0314 Oslo, Norway, (corresponding author to provide phone: +47 22 067587; fax: +47 22067300; email: liv.furuberg@sintef.no)

the US CLIA-waived approval requirements [3]. The test procedure should include a maximum of three handling steps; "add sample – activate - read result". In addition, in order to get the results within the time frame of a typical consultation, the results should be available within a few minutes. All these requirements have in some cases been met for protein analyses, such as in the Afinion platform [4], but diagnostics and screening based on nucleic acid analyses that include an amplification step is normally slower, more than 30-60 minutes.

In this paper we will first describe the successful commercial platform for blood protein analyses, the Afinion platform from Axis-Shield. We also give an overview of disease related protein markers in blood, and show how their relevant concentrations for diagnostics vary over ten orders of magnitude. For a broader panel of diagnostics in the doctors' offices in the future, microfluidic chips and new sensitive biosensors will be crucial.

Then we will move to nucleic acid based POC analyses and an example of a prototype platform for nucleic acid analysis used for multiplex HPV virus diagnostics from cervical smear specimen.

In order to develop a new diagnostic platform, not only the instrument with pumping mechanisms, heaters and biosensor read-out must be created, but also the disposable cartridges or chips. These chips need biological surface layers and stored dry and wet reagents, and sometimes sensitive biosensors may be included on-chip. In the last section we describe how the challenges of manufacturability of systems that combines very different technologies can be approached.

II. POC DIAGNOSTICS BASED ON PROTEIN ANALYSIS

For protein based analyses, there are already some point-of-care in-vitro diagnostic products on the marked. These are in general not based on microfluidics or nanotechnology sensors, but larger, disposable polymer cartridges that have integrated reagents and wash buffers. For each new test to be performed, a new cartridge is opened, the patient sample is sucked into the cartridge and the cartridge is inserted into the instrument that performs the analysis and shows the result in a panel.

One example is the Axis-Shield platform Afinion (see figure 1), widely used in local doctors' offices [4]. So far three protein analyses are available in the system; CRP (C-reactive protein), an acute phase protein indicating inflammation, HbA1C (glycated haemoglobin), for monitoring diabetes, and ACR (albumine-creatnine ratio), a marker for early kidney damage. The CRP and HbA1c assays are based on finger prick samples blood (1.5 µL) rather than ml of blood drawn from veins. Also, the analysis time is remarkably fast, less than 4 minutes. Typical relevant concentrations for CRP during infections are in the range of 5 to 200 mg/L. The current detection principle is based on colorimetry.

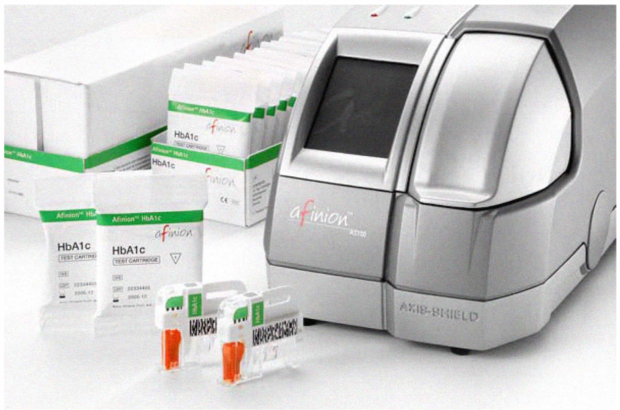

Fig. 1. The Axis-Shield platform Afinion (from www.afinion.net). Disposable polymer cartridges have all reagents necessary for analysis on-board. The patient blood sample is collected in a capillary tube which is a part of the cartridge. The cartridge is inserted into the instrument and the result can be read out on the display.

In table 2 we list some more proteins relevant for point-of-care diagnosis and the diseases related to those proteins. In figure 2 the concentration levels of the proteins are plotted. The concentration ranges relevant for diagnostics vary from just a few protein molecules per sample (e.g. 10^{-18} g/L) to relatively high concentrations (up to g/L quantities). New and more sensitive sensing principles will be needed for the low concentration proteins. A review of recent advances in immunoanalytical systems can be found in [5], where different optical, electrochemical, magnetic or mechanical protein detection methods are described.

TABLE 2.
SOME PROTEINS RELATED TO DISEASES

Protein	**Main diagnostic use**
Albumin	Liver diseases
Fibrinogen	Coagulation disorders
CRP	Inflammations, infections
Ca-125	Ovarian cancer
D-Dimer	Thrombosis
Myoglobin	Myocardial infarction
Ferritin	Iron deficiency
PSA	Prostate cancer
CK-MB	Myocardial infarction
Procalcitonin	Severe sepsis
Troponin I	Myocardial infarction
NT-Pro-BNP	Heart failure
TSH	Thyroid diseases
BNP	Heart failure
IL-6	Inflammations, infections

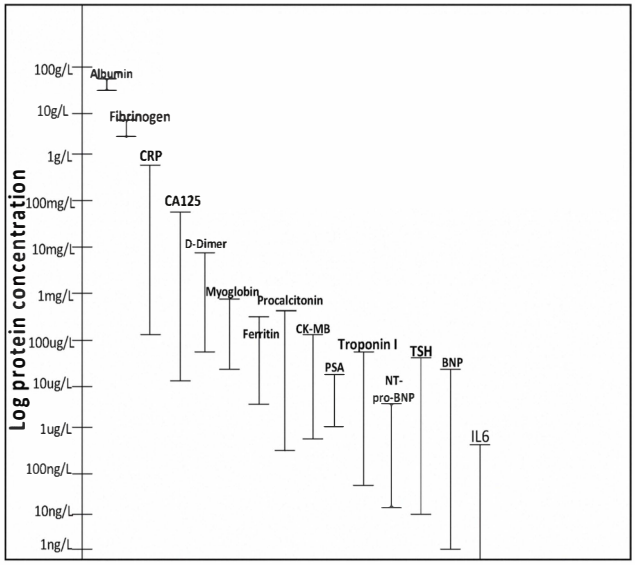

Fig. 2. Relevant plasma concentration ranges (Log scale) for some proteins frequently measured in hospital laboratories.

The Verigene ® System by Nanosphere [5] is an example of new systems that meets the challenge of detecting nucleic acids and proteins with a very high sensitivity. The system utilizes patented gold nanoparticle technology to obtain detectable signals from a few molecules.

III. POC Diagnostics based on nucelic acid analysis

At present, POC testing for nucleic acid strands is most relevant for infectious diseases. In the future, also personalized medicine will be a large marked. While more sensitive detection techniques, like Verigene's, will open for direct nucleic acid detection, the common procedure is to create millions of copies of the marker DNA sequence before detection. This is done by enzymatic amplification techniques, like polymerase chain reactions (PCR). The amplification requires purified nucleic acids to avoid inhibition of the reactions. Due to the complexity and diversity of patient samples and the number of steps involved in nucleic acid extraction, the main hurdle of POC DNA analysis is the sample preparation [7]. POC systems based on microfluidic chips are thus mainly on the patent and prototype stage, with exceptions such as Cepheid's GeneXpert cassette [8].

In the European funded project MicroActive a new prototype platform for automated mRNA based diagnosis was established [9]. In the project, the developed system was tested successfully for the "proof-of-principle" detection of biomarkers indicative of cervical pre-cancer. Specifically the system was designed for the ion of transcription of oncogenic HPVE6/E7. The analysis detects mRNAs, which are indicative of a biologically significant HPV (Human Papillomavirus) infection. The system can detect up to 7 different HPV viruses in one chip.

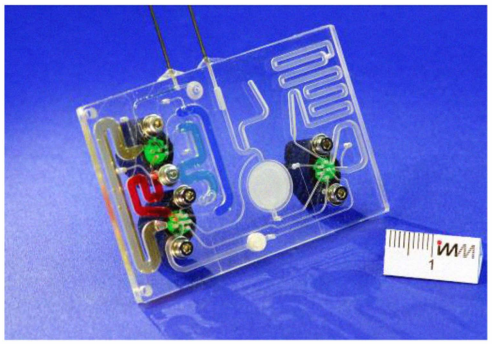

Fig. 3. The MicroActive device accepts 3 ml of sample and performs the extraction in a disposable polymer chip of credit card size. Tests performed using cancer cell lines and cervical liquid based cytology specimens confirm the extraction of HPV-mRNA by the system. Courtesy of IMM.

The project developed one microfluidic chip and instrument for the sample preparation and another chip and instrument for mRNA amplification and detection. The two instruments were designed to be merged into one, but in the clinical testing procedure one manual step of transferring the output of the sample preparation chip to the amplification chip was performed. The extraction procedure implemented in the sample preparation chip was based on adsorption of the DNA onto bare silica in the presence of a chaotropic salt [10].

3 ml of the buffer with patient cervical smear containing cells and mucus from a standard test container was inserted into the sample preparation chip. The extraction of mRNA, with a sufficient high quality for later NASBA (Nucleic Acid Sequence Based Amplification) [11], was performed automatically in the chip and the output was about 50 microliter of eluate [12]. This elute is mixed with reagents and then transferred to the amplification/detection chip in the second instrument where the liquid is automatically pulled into the chip, and split into separate amplification volumes. The mRNA for a specific HPV is amplified if the patient sample was positive for that active HPV virus, and then a fluorescent signal is monitored real time in the reaction chambers. From the signal a HPVE6/E7 mRNA positive result is determined.

Using 300 clinical cervical cytology smear specimens from the an established biobank, for the development of the platform, finalyy the functionality of the developed nucleic acid extraction with following on-chip amplification was demonstrated with 30 specimens and compared to "gold standard" laboratory procedures. Both instruments and chips are at present further developed for production and for future use in a commercial system by the Norwegian SME NorChip [13].

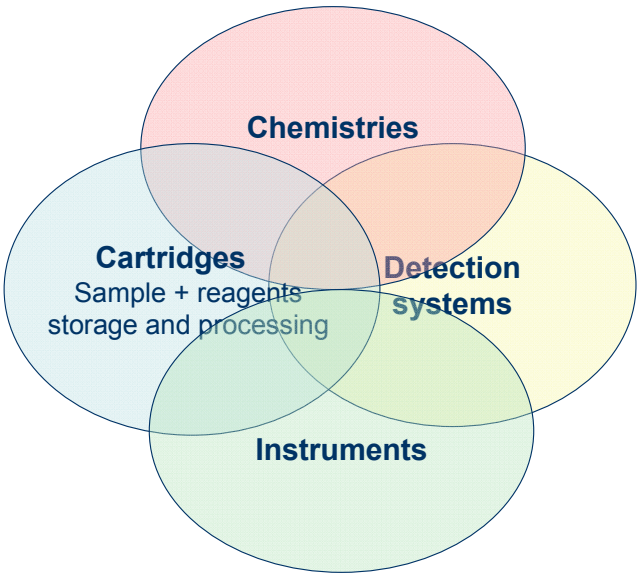

Fig. 4 Point of Care In Vitro Diagnostic platforms of the future typically will be built and characterized by 4 key elements. The Chemistries including reagents and assay principle for specific identification of the analytes or clinical parameters, the Cartridges for storing reagents and processing the samples and reagents according to the assay sequence, the Detection system for measuring the response of the analytical assay and the Instrument for processing the cartridge, operating the detection system and communicating and storing the results.

IV. Manufacturing Technologies

The manufacturability and cost of the platform instrument and disposable chips is of crucial importance for a potential commercial product. In particular, the microfluidic chip manufacturing will involve micro- and nanostructuring of polymers combined with microchannel surface treatment supporting both liquid control and biochemical reactions. Also special procedures for drying nanolitre sized volumes of reagents and wet buffer storage on chip must be developed.

The microfluidic chip format introduces the opportunity of integrating low cost micro technology based biosensors on-board. The integrated sensor may be optical (e.g. Surface Plasmon Resonance based, waveguide, ring resonator), an electrode array or a mechanical structure like a cantilever [14]. The sensing element surface is coated by antibodies for specific binding of protein antigens or single stranded nucleic acid sequences for hybridization of the target nucleic acid sequences. The attachment of a target molecule will result in a chance of light wave phase, an electrochemical current or a change in electric resistance. These sensors are sensitive enough to avoid labelling of the target molecules by gold particles or fluorescent beacons.

Starting a volume production of chips is a challenge, because many different manufacturers must be involved and collaborate. The European funded FP6 project microBUILDER [15] have made available key technologies related to microfluidic based cartridge and sensor designs and production.

V. Conclusions

Microfluidic based POC diagnostic platforms have a large potential for commercial success, due to low production cost, small sample volumes, quick reactions, automatic procedures, robustness and safety in closed and compact systems. An instrument suited for the doctors' offices must be able to perform a significant number of relevant analyses. That implies handling a diversity of patient samples, such as blood, urine or cell smears. The same platform must be able to measure molecular concentrations from a few molecules up to g/L. The samples have to be prepared for different protein based, nucleic acid based and low molecular weight substance based analyses. Currently blood-plasma separation for protein analysis is at a more commercial level than nucleic acid extraction.

References

[1] Yole developpment, "Point of care testing applications of microfluidic technologies", marked and technology analysis, March 2010
[2] P. Yager, T. Edwards, E. Fu , K. Helton K , K. Nelson, M. R. Tam, B. H. Weigl BH, "Microfluidic diagnostic technologies for global public health", Nature, 442 (7101), pp 412-418, Jul 2006
[3] http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm124105.htm
[4] http://www.afinion.net/
[5] C. A. Marquette and L. J. Blum,"State of the art and recent advances in immunoanalytical systems", Biosensors & Bioelectronics, 21 (8), pp 1424-1433, Feb 2006
[6] http://www.nanosphere.us/VerigeneSystem_4411.aspx
[7] M.A. Dineva, L. MahiLum-Tapay, H. Lee, "Sample preparation: a challenge in the development of point-of-care nucleic acid-based assays for resource limited settings", Analyst, 132 (12), pp 1193-1199, Dec 2007
[8] http://www.cepheid.com/
[9] L. Furuberg, M. Mielnik, A. Gulliksen, L. Solli, I. R. Johansen, J. Voitel, T. Baier, L. Riegger, F. Karlsenn, "RNA amplification chip with parallel microchannels and droplet positioning using capillary valves", Microsystem Technologies, 14 (4-5), pp 673-681, Apr 2008
[10] K. A. Wolfe, "Toward a microchip-based solid-phase extraction method for isolation of nucleic acids", Electrophoresis, 23, pp 727 , 2002
[11] J Compton, "Nucleic Acid Sequence-Based Amplification", Nature, 350 (6313), pp 91-92, Mar 1991
[12] T. Baier, T. E. Hansen-Hagge, R. Gransee, A. Crombe, S. Schmahl, C. Paulus, K. S. Drese, H. Keegan, C. Martin, J. J. O'Leary, L. Furuberg, L. Solli, P. Gronn, I. M. Falang, A. Karlgard, A. Gulliksen, F. Karlsen, "Hands-free sample preparation platform for nucleic acid analysis", Lab on a Chip, 9 (23), pp 3399-3405, 2009
[13] http://www.norchip.com
[14] S. Carrara, "Nano-Bio-Technology and Sensing Chips: New Systems for Detection in Personalized Therapies and Cell Biology", Sensors, 10 (1), Jan 2010
[15] http://www.microbuilder.org