2013 Diagnostics Forum



UPPSALA BIO
LIFE SCIENCE PATHFINDER



Welcome

to Diagnostics Forum 2013!

For innovation to take place we all know that cross-fertilization of ideas and experiences are necessary. Meeting people from different organizations, with another background than your own, is therefore a necessary ingredient in efforts to promote innovation.

To promote innovation in life sciences is one of Uppsala BIO's prime objectives. For this purpose and as part of our offerings, we develop arenas for networking.

Diagnostics Forum, previously known as BIO Ångström, is created in that spirit. And we sincerely hope that this day will be an arena for you to meet, to share experience and knowledge and to find partners for new collaborations.

Diagnostics Forum starts with the needs for diagnostics in healthcare, from a patient and a health-economics perspective, and ends with a look at new technologies and solutions.

My vision: Bring your results and ideas and leave with new ideas and connections!

Enjoy your day!

Anna Ridderstad Wollberg
Project Manager Uppsala BIO
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Did we live up to our goals? We would appreciate if you would like to share your experience from the Diagnostics Forum Conference with us. Therefore we have asked two MBA students, Eva Ludvigsen and Hillevi Englund to explore this. They will distribute a survey via e-mail to all participants after the conference. They will also perform a few interviews. The results from this survey will be a part of their master thesis in business administration. And it will help us to make the next Diagnostics Forum even better!

Program 2013 Diagnostics Forum, February 13

8:00	**Start hanging posters**
8:30	**Registration opens**
9:00	**Welcome and introduction** Erik Forsberg, Associate Professor, Managing Director, Uppsala BIO

Session 1 Societal needs and health economic aspects

9:15	**Connecting health assessment to business decisions** *Key Note Speaker* Professor Terry P. Young, Chair of Healthcare Systems, Brunel University, London, UK
9:45	**An innovative system for health technology evaluation of devices and diagnostics** *Key Note Speaker* Mr. Mark Campbell, M.Phil. Associate Director, Medical Technologies Evaluation Programme, NICE (National Institute for Health and Clinical Excellence), Manchester, UK
10:15	**Coffee break**
10:45	**Validation of biomarker candidates and molecular diagnostics of tumours adapted for analysis of fine-needle biopsies** Bo Franzén, Department for Oncology and Pathology, Karolinska Institutet
11.00	**Throwing the baby out with the bathwater. Is innovation inadvertently killing innovation and return on investments in the diagnostics industry?** Dale Charlton, Optima pharma GmbH, Schwäbisch Hall, Germany
11.15	**Validation and implementation of an enzyme-linked immunosorbent assay for C-peptide analysis in Cameroon, a sub-Saharan developing country** Farah van Genderen, Vrije Universiteit, Brussel
11.30	**Poster session and lunch**

Session 2 Clinical needs

12:45	**From identification to large-scale clinical implementation of multiple biomarkers in prostate cancer** *Key Note Speaker* Professor Henrik Grönberg, Head of Department of Medical Epidemiology and Biostatistics, Coordinator of the Cancer Risk Prediction Center, Karolinska Institutet
13:15	**Challenges of CDx Partnering** Birgit Reitmeier, Associate Director, Global Business Development, Merck KGaA, Darmstadt, Germany
13:40	**Measurement quality a route to improved patient outcome** Hanna Ritzén, Mercodia AB, Uppsala
13:55	**Contrast agent for early diagnostics and monitoring of progression of liver cancer (hepatocellular carcinoma)** Dmitry Grishenkov, Division of Medical Engineering, School of Technology and Health, Royal Institute of Technology, Sweden
14:10	**High throughput, standardized data collection in medical practice** David Zakim, Institute of Digital Medicine Foundation, Stuttgart Germany and Mill Valley, CA, USA
14:25	**Coffee break**

Session 3 New technologies, including biomarkers, detection systems, read formats

15:00	**High-performance tools for research and diagnostics** *Key Note Speaker* Professor Ulf Landegren Department of Immunology, Genetics and Pathology, Molecular tools, Uppsala University
15:30	**Development of multiplexed MSIA (Mass Spectrometric Immunoassay) – SRM assays for proteins associated with Alzheimer's disease and application to clinical samples** *Key Note Speaker* Dr. Mary F Lopez. Director, BRIMS – Biomarker Research Initiatives in MS, Thermo Fisher Scientific, Cambridge MA, USA
16:00	**Visualization of tissue heterogeneity with single cell resolution – mutation analysis *in situ*** Elin Lundin, Department of Biochemistry and Biophysics, Stockholm University
16:15	**Biomarkers for Alzheimer's disease** Andrea Armstrong, Linköping University
16:30	**Proteome screening identifies galectin-1 as a negative predictor for malignant mesothelioma in pleural effusions** Filip Mundt, LabMed, pathology, Karolinska Institutet
16:45	**Concluding Discussion** *On the podium:* Keynote speakers and Moderators
17:15	**End of scientific program**

17:15–17:45 **Refreshments available in the Lobby** Hosted by Thermo Fisher Scientific

Professor Terry P. Young
Chair of Healthcare Systems, Brunel University, London, UK

Connecting health assessment to business decisions



As the market for medical devices becomes more difficult with the economic climate, and more sophisticated under an increasing emphasis on costs and outcomes, the MATCH-programme has been researching the decisions made by those who bring medical technologies to market, and those who purchase them.

Most new products progress along a development cycle which contains a series of decision point – sometimes called business reviews or even stage gates. The information available and analysis performed at these events contribute greatly to the quality of the product that emerges. Under MATCH, we have looked at user needs and economic evaluation – both of which have the potential to reduce risk and speed the development of high quality products that are more likely to find acceptance in the market.

This presentation will review progress and show how these approaches may be integrated, particularly at the early stages of development.

Mr. Mark Campbell
M.Phil. Associate Director, Medical Technologies Evaluation Programme,
NICE (National Institute for Health and Clinical Excellence), Manchester, UK

An innovative system for health technology evaluation of devices and diagnostics



In the UK, the National Institute for Health and Clinical Excellence (NICE) has introduced a new guidance programme the aim of which is to promote adoption in the UK NHS of medical technologies which offer demonstrable advantages over standard care. The programme includes innovative methodologies which were designed specifically to take into account the characteristics of devices and diagnostics. These include: ways of identifying and selecting technologies for evaluation; methods for the economic evaluation of the resource consequences of adoption; and a framework for generating further high-quality clinical and proof-of-concept evidence where gaps in the literature prevent its independent advisory committees from making comprehensive recommendations about the use of otherwise promising technologies. The presentation will describe the first two years' experience, during which time 20 pieces of guidance have been published.

Professor Henrik Grönberg
Head of Department of Medical Epidemiology and Biostatistics,
Coordinator of the Cancer Risk Prediction Center, Karolinska Institutet, Solna, Sweden

From identification to large-scale clinical implementation of multiple biomarkers in prostate cancer



Prostate cancer will be used as an example on how structured validation of new biomarkers can be used to change healthcare strategies in the diagnosis and treatment of this cancer. Several examples on how new and emerging technologies in genomics and proteomic will dramatically increase the possibilities to identify new biomarkers will be given.

However, from initial identification of a novel and promising biomarkers there are many obstacles until these markers will reach the clinic. The STHLM3 trail will serve as example how to overcome these obstacles. In this trial we will include over 100.000 men aged 50–69 in Stockholm to evaluate a combined biomarker panel in a prospective randomized trial.

Professor Ulf Landegren
The Rudbeck Lab, Department of Immunology, Genetics and Pathology,
and SciLifeLab, Uppsala University. Se-751 85 Uppsala, Sweden

High-performance tools for research and diagnostics



Sensitivity and specificity of protein detection are key limiting factors in basic research, in the search for new biomarkers, and for advanced diagnostics. Proximity ligation or proximity extension assays can offer improved performance over earlier assay formats, they expand the scope for parallel measurements from small sample aliquots, and they can help visualize interacting proteins reflecting activity steps of cells and tissues.

The proximity assays are conducted using affinity reagents, typically antibodies, which are modified by attaching short DNA strands. Upon proximal binding by pairs of reagents the DNA strands are made to undergo ligation or polymerization reactions for amplified detection. The assays allow measurement of protein molecules present in plasma, in isolated single cells, or *in situ* directly in tissue sections, revealing cellular heterogeneity at the protein level, and functional responses to cell stimulation and inhibition.

Two new classes of reagents, unfolding probes and superRCA probes, can further enhance molecular detection reactions and provide new tools for diagnostics.

Mary F Lopez
Thermo Fisher Scientific BRIMS Center, Cambridge, MA, USA

Development of multiplexed MSIA (Mass Spectrometric Immunoassay)-SRM assays for proteins associated with Alzheimer's disease and application to clinical samples



Several protein biomarkers are known to be associated with Alzheimer's Disease (AD). The leading theory of AD patho-physiology is the Amyloid Cascade Hypothesis, which was originally focused on the extracellular deposition of beta amyloid peptides (Aß) in large fibrillar aggregates. To date, several variants of Aß have been found in brain and cerebrospinal fluid (CSF) while data from blood and plasma are more ambigous. Another possible biomarker of AD, apolipoprotein E (Apo E) may also play a central role in the AD amyloid cascade leading to cognitive decline. Apo E binds to Aß and both promotes Aß fibril formation in the brain and Aß clearance from the blood. The apparently contradictory fibril promotion and clearance roles of Apo E might be due to the different binding capacities of Apo E isoforms (Apo E2,3,4) for Aß. Disturbances of copper homeostasis may also contribute to the neurodegeneration associated with AD and the level of the copper enzyme ceruloplasmin (CP) is increased in the CSF of AD affected individuals. In order to further investigate the relationship of these markers to AD, we developed multiplexed, MSIA-SRM assays that allow quantification of CP, APOE monitoring and isoform-specific peptides and several Aß peptides in human plasma. The MSIA technology provides rapid enrichment for low abundance analytes from fluids such as plasma, serum and cerebrospinal fluid (CSF). We used these assays to interrogate a small cohort of clinical plasma samples from patients with AD and matched controls. Our results demonstrated, (for the first time), quantitative MS detection of Aß and other peptides in human plasma.

Mary F Lopez[1], Alejandra Garces[1], Bryan Krastins[1], David A Sarracino[1], Maryann Vogelsang[1], Scott Peterman[1], Amol Prakash[1], Gouri Vadali[1], Joachim Struck[2], Bruno Darbouret[2], Johan Gobom[3], Erik Portelius[3], Josef Pannee[3], Eric Niederkofler[4], Urban Kiernan[4], Dobrin Nedelkov[4], Madalina Oppermann[1], and Kaj Blennow[3].

[1] Thermo Fisher Scientific BRIMS Center, Cambridge, MA, USA

[2] Thermo Fisher Scientific, Biomarkers, Henningsdorf, Germany

[3] The Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

[4] Thermo Fisher Scientific LCD, Tempe, AZ USA

Session 1

Societal needs and health economic aspects

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2	**Throwing the baby out with the bathwater. Is innovation inadvertently killing innovation and return on investments in the diagnostics industry?** Dale Charlton. *Optima pharma, Germany*	Oral and Poster	p. 13
3	**Validation and implementation of an enzyme-linked immunosorbent assay for C-peptide analysis in Cameroon, a sub-Saharan developing country** Farah van Genderen. *Vrije Universiteit Brussel Belgium*	Oral and Poster	p. 14
4	**Clinical evaluation of the TailorDose™ approach for individual dose adjustment of cytostatic drugs.** Per Rydberg. *Department of Oncology-Pathology CCK, Karolinska Institutet, Stockholm*	Poster	p. 15
5	**The need for genetic testing in families with multiple endocrine neoplasia type I** Jakub Piatkowski. *Department of Endocrinology, Jagiellonian University Medical College, Krakow*	Poster	p. 16

Session 2

Clinical needs

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Session 3

New technologies, including biomarkers, detection systems, read formats

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13	**PGE2 metabolite levels as a biomarker for HIE score and outcome after birth asphyxia** Lars Björk. *Department of Women´s and Children´s Health, Pediatric division, Karolinska University Hopsital and Karolinska Institutet*	Poster	p. 24
14	**Development of diagnostic lab-on-a-chip systems for ligation-based mutation detection** Annika Ahlford. *Uppsala University, Dept. of immunology, genetics and pathology, Rudbeck laboratory, Uppsala*	Poster	p. 25
15	**Fucose on a chip: a lab-on-a-chip device for screening cancer and liver disease** Per Erlandsson. *The Department of Physics, Chemistry and Biology at Linköping University, Sweden*	Poster	p. 26
16	**Homogenous assay for real-time and simultaneous detection of thymidine kinase 1 and deoxycytidine kinase activities** Per Stålhandske. *cSens AB, Uppsala*	Poster	p. 27
17	**A software platform for genomic signature analysis in bacteria** Bo Segerman. *National Veterinary Institute, Sweden*	Poster	p. 28
18	**nFold probes: New tool for biomolecular analysis in research and diagnostics** Rachel Yuan Nong. *Department of Immunology, Genetics and Pathology, the Rudbeck Laboratory, Sci Life Lab, Uppsala University*	Poster	p. 29
19	**Urinary prostaglandin E tetranor metabolite is a biomarker of inflammation and respiratory dysfunction in respiratory syncytial virus-bronchiolitis** Eric Herlenius. *Department of Women´s and Children´s Health, Pediatric division, Karolinska University Hopsital and Karolinska Institutet*	Poster	p. 30
20	**Noble metal nanoparticles for diagnostic applications** Ron Gill. *Nanobiophysics Group University of Twente, Netherlands*	Poster	p. 31

21	**Separation of polypeptides by isoelectric point focusing in electrospray-friendly solution using novel multiple-junction capillary fractionator** Thorleif Lavold. *Biomotif AB, Sweden*	Poster	p. 32
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24	**Development of a fully automated IDS-iSYS inactive Matrix Gla Protein (MGP) immunoassay** Dagmar Kasper. *Immunodiagnostic Systems (IDS), United Kingdom*	Poster	p. 35
25	**Determination of glargine and its metabolites M1 and M2 by using a combination of insulin assays of different specificity** Annika Carlsson. *R&D, Mercodia AB, Uppsala*	Poster	p. 36
26	**Automatic malignancy grading of prostate cancer with image analysis** Jimmy Azar. *Centre for Image Analysis, Uppsala University, Sweden*	Poster	p. 37
27	**Label-free bio sensors based on graphene transitors** Malkolm Hinnemo. *Department of Engineering Sciences, Division of Solid State Electronics, Uppsala University*	Poster	p. 38
28	**Mapping post translational modifications and protein interactions in cancer using the *in situ* proximity ligation assay** Karin Grannas. *Department of Immunology, Genetics & Pathology, SciLife Lab, Rudbeck lab, Uppsala University*	Poster	p. 39
29	**Inhibitory sample material? Let´s go template fishing!** Mats Isaksson. *SVA Uppsala, Sweden*	Poster	p. 40
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31	**Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides** Mats Nystrand. *Technology Development, Thermo Fisher Scientific, Uppsala*	Poster	p. 42
32	**A new sensitive anti-drug antibody screening assay with high drug tolerance** Camilla Eriksson. *Technology Development, Thermo Fisher Scientific, Uppsala*	Poster	p. 43

Bo Franzén and Gert Auer
Department for Oncology and Pathology, CancerCenter Karolinska, Z5:02,
Karolinska Institutet and University Hospital, Solna, 171 76 Stockholm

Validation of biomarker candidates and molecular diagnostics of tumours adapted for analysis of fine-needle biopsies

It is currently very important to increase the diagnostic accuracy in patients with suspected cancer, which is a prerequisite for the selection of treatment strategy. Unfortunately, there is currently a severe shortage of pathologists and cytopathologists. A panel of validated diagnostic biomarkers could therefore serve as a valuable complement to today's practices.

For more than a decade, studies on tumour material using 2D gel electrophoretic analysis have been conducted at our department. Several hundred analyzes were performed using fresh tissue and standardized protocols. We have found that a number of biomarker candidates show a recurrent expression pattern, that is, tumours analyzed from e.g. breast, prostate and ovary, identified same subset of biomarker candidates with significant difference in expression between benign and malignant tumours. Our preliminary review of published work during the period shows that about 40-60 different biomarker candidates express promising profiles.

The challenge of this project is to validate biomarker candidates and to translate these into a methodological platform that can be used in daily routine diagnostics. The medical need for new diagnostic markers is widely recognized and if diagnostic panels of biomarkers are combined with patient-friendly sampling techniques such as needle aspiration biopsy, we may add significant impact for the cancer diagnostics.

Dr Dale Charlton
Optima pharma GmbH, Schwäbisch Hall, Germany

Throwing the baby out with the bathwater. Is innovation inadvertently killing innovation and return on investments in the diagnostics industry?

There is a considerable amount of research on-going to develop innovative diagnostics solutions to meet the needs of society with e.g. more home testing and treatments, molecular diagnostics tools and bio-markers and to meet the growing opportunities in developing countries with more PoC products, to mention but a few. This requires innovation and development and the uptake of those new technologies for clinical diagnostic products. Whilst this innovation reflects the incorporation of new technology, market needs and customer needs? It can sometimes overlook one fundamental partner in all this, the manufacturing environment. Production engineers are often left out of initial discussions and only after prototyping and perhaps even bench-scale production born in R&D departments are the production engineers asked to look at developing manufacturing operations that can match the expectations of marketing to make the product at reasonable cost. There are many examples of products being too complex for manufacturing and returns of investment not met. This paper sets out, with references, to look at several examples of innovation in production machinery that was required to match the expectations of the product developers. Reference to lateral flow, microfluidic devices, immunoassay micro-plates, nucleic acid purification cartridges and even the humble filling and closing machine will be discussed.

Farah van Genderen
Diabetes Research Centre, Brussels Free University, Belgium

Validation and implementation of an enzyme-linked immunosorbent assay for C-peptide analysis in Cameroon, a sub-Saharan developing country

The need for proper diabetes classification and the absence of the necessary diagnostic methods, led us to validate and implement an ELISA method (Mercodia) for measuring C-peptide levels in plasma and serum of diabetic patients in Cameroon. The assay is performed on 25 µL samples for a dynamic range between 0.32 µg/L (functional sensitivity) up to (at least) 7.09 µg/L. The inter- and intra-assay percentage coefficients of variation were 2.9–9.9 %, and 5.2–9.4 %, respectively. Recoveries of added peptide were 81–94 % in serum, and 93–98 % in buffer. Comparison with an electrochemiluminescence immunoassay (Roche) yielded a good correlation coefficient (R2 = 0.98). Cross-reactivity by proinsulin, and interferences by lipids, bilirubin and haemoglobin were negligible. C-peptide was stable at room temperature for 24h and up to 7 freeze-thaw cycles for medium (1–6 µg/L) and high (> 6 µg/L) levels (< -15°C and < -70°C). The reference range for C-peptide in Cameroon was 0.38–3.63 µg/L. Local technical staff was trained during one week with this technology at the Biotechnology Centre of Yaounde I University. ELISA results for plasma C-peptide analyzed in Yaounde correlated well with those analyzed in Brussels (R2 = 0.99). This method requires relatively low investments and is suitable for low-income countries.

Per Rydberg, PhD and Hans von Stedingk, PhD
Dept of Oncology-Pathology, CCK, KI, Solna, Sweden

Clinical evaluation of the TailorDose™ approach for individual dose adjustment of cytostatic drugs

Chemotherapy is used for the majority of women treated for breast cancer. Different dose regimes are given which normally includes the nor-nitrogen mustard agent cyclophosphamide (CPA). Doses are adjusted according to standard protocols based on the body surface area (BSA) of the patient. This measure, BSA, does not consider well known interindividual variability regarding metabolism and rate of clearance of cytostatic drugs. For CPA which is a prodrug which is converted (activated) in the liver, the difference of the cytotoxic (and toxic) active metabolite "phosphoramide mustard" (PAM) can differ by up to a factor 10 at the same given BSA dose. It has been estimated that 30 % of the patients who receive cytostatic drugs, in general, are being under- or over dosed with the current standard, BSA dosing.

By using a new patented analytical method, the "adduct FIRE-procedure™" (FIRE), individual doses of PAM can be obtained in patients treated with cyclophosphamide from a single blood sample. FIRE offers thereby a genuine short-cut to obtain the over-time accumulated blood dose of the cytotoxic active principle, the AUC dose. This is obtained as FIRE measures chemical modifications (adducts) to hemoglobin which are caused by cytostatic drugs which are chemically reactive. A benefit with FIRE is a documented high sample throughput, high sensitivity and excellent specificity by mass spectrometry detection (LC-MS/MS).

FIRE is now being clinically evaluated as a new tool for improved dosing of cytostatic drugs. This new approach, called TailorDose™, is intended to be used by oncologists for individual dose adjustments. We are now recruiting patients from Radiumhemmet and Danderyds hospital who are given CPA in combination with two other cytostatic drugs. Early data are very encouraging and will be presented at the meeting. The TailoDose™ approach can be exploited as a general diagnostic tool for other cytostatic drugs belonging to the family "alkylating cytostatic drugs" such as: temozolomide, chlorambucil, ifosfamide, lomustine and melphalan.

Skalniak Anna, **Piatkowski Jakub**, Jabrocka-Hybel Agata, Stefanska Agnieszka, Pach Dorota, Przybylik-Mazurek Elwira, Sokolowski Grzegorz, Hubalewska-Dydejczyk Alicja
Department of Endocrinology, Jagiellonian University Medical College, Krakow, Poland

The need for genetic testing in families with multiple endocrine neoplasia type I

The MEN1 syndrome includes different combinations of over 20 endocrine and non-endocrine tumors. Hyperparathyroidism occurs in about 90 % of patients, endocrine pancreatic tumors in 60 % of patients, and pituitary adenomas in 40 % of patients. The overall median age at death for MEN1 patients is 47 years.

All these manifestations can be caused by mutations in one single gene – MEN1, encoding the protein menin. The disease is inherited in an autosomal dominant manner. Molecular genetic testing of MEN1 detects mutations in approximately 80 %–90 % of patients with familial MEN1 syndrome and in about 65 % of sporadic cases. Early detection affects management, and people at risk should undergo regular surveillance. Thus, genetic testing is a very important aspect for family members of affected people. Mutations in the MEN1 gene are specific for a given family, and virtually any position within the sequence can be subject to a mutation. Therefore sequencing of the gene remains the golden standard in mutation analysis. Approximately 1 %–4 % of patients without a mutation in the coding region of MEN1 or in splicing sites harbor large gene deletions.

At the Department of Endocrinology at the University Hospital in Krakow we introduced the possibility to perform genetic testing for MEN1-affected patients and their families.

The expression of the disease is variable, even within families. Our understanding of the function of the MEN1 gene product has increased significantly. However no clear genotype-phenotype correlation has been established. Based on our own experience, we present the most interesting mutations, which cause severe phenotypes despite being located in regions of the gene that would not suggest this level of severity.

Hanna Ritzén and Robert Gunnarsson
R&D, Mercodia AB, Uppsala, Sweden

Measurement quality – a route to improved patient outcome

Biomarker research and their clinical utility has been a great interest in the past years. Biomarkers are used in research, diagnosis, screening and predictions of a disease, monitoring treatment response and complications. Biomarkers are required and essential for decision-making in trials and have a potential to make a trial more cost efficient and to save time to get new therapies to patients.

Biomarker research involves extensive amount of work in discovery, qualification, verification and validation for an intended clinical use. In all stages some sort of measurements are needed. Making the right interpretation of collected data ultimately rely on the expertise of the scientist and the quality of the measurement methods used.

Unfortunately the measured value usually deviates more or less from the true value. The information of measurement errors is highly important, which is why a lot of effort is put into international guidelines, standardization and harmonization of methods. Lack of precision and inaccuracy in measurement methods might for example cause one to underestimate the correlation of a biomarker with the outcome i.e. a disease.

Topics that will be discussed are the responsibilities around measurement quality. The producer should provide a validated and robust method that gives accurate results. The scientist need to assure that the analytical performance given by the chosen method is fit for purpose. Examples will be given on how the needs of a measurement method will vary based on the nature of the study.

To provide measurement quality to scientists is a great responsibility for assay developers today and still remains a great challenge for the future.

Dmitry Grishenkov
Division of Medical Engineering School of Technology and Health Royal Institute of Technology, KTH Alfred Nobels allé 10 SE-141 52 Huddinge, Sweden

Contrast agent for early diagnostics and monitoring of progression of liver cancer (hepatocellular carcinoma)

Evaluation of liver lesions is a challenge to every radiologist. Novel microbubble (MB) based targeted multimodal contrast agent, support several diagnostic approaches, including ultrasound, MRI and SPECT.

Hepatocellular carcinoma (HCC) is the most frequently diagnosed type of liver cancer and is the second most common liver lesion after cirrhosis. Size, staging, and surgical radicality severely affects the prognosis. Today it is impossible to mix different contrast agents, requiring diagnostic tests such as perfusion ultrasound, SPECT, MRI, biopsy to be made in different days. In clinical practice this means that the time from suspected HCC to diagnosis can be several weeks. Novel procedure makes it possible to perform these tests in a single day with immediate response. Moreover, MBs functionalized with ligands specific to HCC, allow specific diagnosis even on sub-cm lesions, which is currently difficult or even impossible. This would have a significant impact on healthcare. Unsuccessful treatment can then be given up earlier, in favor of more aggressive treatment or surgical approaches.

Diagnostic imaging is not only important from the healthcare perspective but also from its economic potential. The medical device market is expected to grow 9 % annually. According to a report by Global Industry Analysts the imaging agents market is assumed to exceed 11 billion euro by 2015. The market is primarily driven by broadening areas of applications. The US is still the largest market for contrast agents worldwide, although, developing Asian countries are considered to play major role in the nearest future. Keeping in mind that HCC is widely diagnosed in Asia, the current project can potentially meet healthcare needs with market possibilities and trends.

Birgit Reitmeier, Associate Director
Global Business Development, Merck KGaA,
Frankfurter Str. 250, 64293 Darmstadt, Germany

Challenges of CDx Partnering

For a pharmaceutical company finding the right partner for companion diagnostic development has several challenges. Not the least of these is the internal challenge of figuring out what the Rx company's expectation from a companion diagnostic actually is. Product development teams often become lost within the multitude of available technologies that can analyze a huge variety of alterations. Whereas biomarkers used in an exploratory fashion are a great source of scientific knowledge and are not of interest to regulators, the development of a true companion diagnostic is so tightly regulated that there are in reality not many degrees of freedom. To date few companion IVDs have obtained a pre-market approval (PMA) and these were developed by well known diagnostics companies. Once the product team has aligned the clinical and regulatory strategy of the product with the pre-clinical data obtained for a potential companion diagnostic, discussions with potential CDx partners can begin. The main challenge arising at this point is the starkly different value propositions of Rx and CDx products. Although Rx and CDx partners have to work in very close collaboration to ensure co-launch of Rx and CDx, there are several asymmetries that present challenges to agreement drafting and the later partnership. The Rx partner has to adjust to the idea of paying for the development of an asset that is subsequently owned by the CDx company. Once the project is kicked off there is hardly any room for changing partners, technologies, or measured alterations, and at the end of the co-development the Rx partner depends on the approval and commercialization of the CDx by the CDx partner. In essence the Rx partner needs the commitment from the CDx partner all the way to commercialization of both products. Locking into a co-development with long timelines, at the end of which the CDx product will be in immediate competition with laboratory developed tests (LDTs) presents commercial challenges for the CDx partner. While the Rx business is predicated on the exclusivity that can be obtained in the market, the CDx aims to become the standard application for a number of drugs and is therefore usually partnered non-exclusively. Thus, the main topics of discussion between Rx and CDx developers tend to be exclusivity, IP split, termination and commercialization.

David Zakim
Institute of Digital Medicine Foundation, Stuttgart Germany and Mill Valley, CA, USA

High throughput, standardized data collection in medical practice

Standardized data collection to control for confounding variables is the bed-rock of experimental science and is no less important for medical practice and clinical research. Yet physicians do not adhere to these principles of data collection. We therefore are formalizing medicine as software [with the eponym CLEOS®] to enable computerized collection of detailed, standardized clinical data by direct interview of patients. We show that extensive amounts of knowledge can be built into such a system. CLEOS®, for example, has 16000 decision nodes in 400 problem-specific decision graphs, is expandable through addition of new modules, but functions as a single program at run-time. The average patient populates 385 of > 28000 data fields. Standardization of the interview protocol means, however, that unpopulated fields are empty for valid medical reasons and are not potential confounding variables. Standardization of data collection thus provides data sets that are comparable for all patients. Laboratory data up-load automatically to a patient's file and are parsed according to significant cut-points. CLEOS® is web-based. To facilitate diagnosis and management, CLEOS® reports findings as narratives and differential diagnoses for acute and chronic problems. To facilitate clinical research, all data are stored as time-stamped codes in defined data fields. Codes can be searched singly or in combination across populations. Machine learning algorithms can be applied to the stored data. Clinical testing shows that CLEOS® out-performs physicians in a major teaching hospital both in history-taking across all facets of clinical medicine and in adherence to management guidelines. CLEOS® is usable by all but the sickest patients and operates even in this setting with nursing help.

Elin Lundin
Department of Biochemistry and Biophysics, Stockholm University, Sweden

Visualization of tissue heterogeneity with single cell resolution – mutation analysis *in situ*

Background: Advances in large-scale sequencing techniques have permitted in-depth mutational analysis of numerous tumor samples from different types of tissues. These studies have revealed extensive heterogeneity between individual tumors and evidence of considerable genetic intra-tumor heterogeneity is emerging. In bulk analysis of cancer tissue homogenates, rare mutations may drown in noise and hence *in situ* mutation analysis of cancer tissue could work as a complement to existing methods to obtain a more comprehensive picture of the mutation profile.

Method: We present a method for performing mutation analysis *in situ* with single molecule and cell resolution. Initially, mRNA is converted to cDNA using LNA primers which anchor the cDNA strand to the mRNA. Then the cDNA is targeted and detected by target primed rolling circle amplification (RCA) resulting in a localized detection of single transcripts. The method was evaluated for scoring of the KRAS mutation status in clinical samples. We also genotyped mutations in PIK3CA, KRAS, EGFR and TP53 to investigate expression patterns and whether some mutations are co-localized or occur in different cell populations.

Results: Mutation analysis of PIK3CA double mutants indicate that the two mutations occur in different cell clones. Differences in expression levels and transcript localization were observed for EGFR mutant transcripts and for TP53 wildtype and mutant transcripts. The difference in transcript localization would not have been observed in a bulk analysis. Our method can visualize mutation status, expression and localization *in situ* which provide a tool for stratification of patients and selection of treatment strategy.

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Biomarkers for Alzheimer's disease

Introduction. Accurate diagnosis of Alzheimer's disease is not possible until post mortem and disease modifying therapies is so far lacking. Since Alzheimer's is a neurodegenerative disease with severe loss of neurons, early diagnostics is crucial so that future treatment can be started before the brain is irreversible damaged. Therefore reliable biomarkers for Alzheimer's disease are highly needed in the clinic.

The cerebrospinal fluid (CSF) provides a natural source for Alzheimer biomarkers and we have via a targeted Western blot screen discovered six new, unpublished proteins which are overexpressed in CSF from Alzheimer's patients.

Aim of BIO-X project. Our aim is to develop a diagnostic tool for Alzheimer's disease.

We will develop a quantitative method consisting of ELISA kits to quantify approximately six proteins for the use of diagnosing Alzheimer patients.

Our diagnostic tool has the potential to be used for the following purposes: (1) diagnosis, (2) selection of pure Alzheimer cases for clinical trials, (3) as a read out for the outcome of clinical trials (4) monitoring of disease progression, (5) development of new treatment strategies and (6) monitoring of future treatments.

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Proteome screening identifies galectin-1 as a negative predictor for malignant mesothelioma in pleural effusions

Background: Malignant mesothelioma is an asbestos induced malignancy of the pleura. To reach a conclusive diagnosis is difficult and the median survival time is around one year. The diagnosis relies on morphological examination of cells and is often conclusive at an advanced stage. The additional measure of soluble biomarkers has not been extensively adapted by the clinical routine; partly due to the lack of predictability of existing biomarkers.

Aim: Using mass-spectrometry to screen pleural effusions from patients with mesothelioma, lung cancer and mesotheliosis, we aim to discover new biomarkers to add diagnostic value and improve standard diagnostics.

Methods: Pleural effusions from 6 mesothelioma patients, 6 lung cancer patients and 3+4 (2 pools) patients suffering from benign mesotheliosis were selected for this study. To decrease variance and increase proteome coverage the effusions were depleted of high abundant proteins (MARS-14 affinity purification, Agilent®), trypsinized, mass tagged (iTRAQ™) and subsequently pooled as well as fractionated (ultra narrow isoelectric focusing and nano-liquid-RP HPLC) before screened with the LTQ Orbitrap Velos (Thermo Fisher Scientific, San Jose, CA, USA). Analyses of the results were done using Significant Analysis of Microarrays (univariate: SAM v. 3.11; Stanford Tools), SIMCA (multivariate: Umetrics®, Principal Component Analysis; PCA and Partial Least Squares; PLS) and Ingenuity Pathway Analysis (IPA; Ingenuity®, network analysis). Candidates were validated using ELISA assays on a larger group of patients.

Results: More than 1300 proteins were identified in the screened patients (FDR<5 %). Univariate analyses (SAM) identified proteins with high respectively low expression in effusions from mesothelioma patients. The findings include a number of known mesothelioma biomarkers (mesothelin, osteopontin and apolipoprotein-CI) as well as several novel candidates; though, with rather high q-values. Multivariate analysis and modeling (PCA and PLS) identified galectin-1.

Validation showed that galectin-1 is a good negative predictor of a mesothelioma. When comparing to metastatic adenocarcinomas, galectin-1 has a sensitivity of 73 % at 100 % specificity, exceeding tested reference biomarkers. Additional validations show proteins with promising prognostic information and network analysis (IPA) gives further information about this disease and the system we work with.

Conclusion: In this study, proteomic screening of pleural effusions have provided candidates for diagnostic and prognostic use for malignant mesothelioma. The validation of galectin-1 sheds light on a novel biomarker to discriminate a malignant mesothelioma from metastatic adenocarcinoma, which might help clinics to reach an earlier diagnosis.

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PGE2 metabolite levels as a biomarker for HIE score and outcome after birth asphyxia

Background: Cerebral hypoxic-ischemic injury (HII) as a result of peripartal asphyxia is a major cause of neurological impairment and later disabilities in human infants. Acute anoxic exposure *in vivo* increases the prostaglandin E2 (PGE2) production in neonatal mice cortex and brainstem. We hypothesize that central PGE2 also is released in humans during birth asphyxia and that it might be used as a biomarker for degree of insult.

Objective: To determine levels of PGE2 metabolite (PGEM) in cerebrospinal fluid (CSF), its correlation to the HIE score and final outcome and neurological sequel.

Design/Methods: 66 full terms (>37 GA) at the NICU at Karolinska University Hospital were initially enrolled in the study and 37 had lumbar puncture (LP) available for analysis of PGE2 and PGEM. Control group consisted of term infants with suspected infection but negative cultures from blood and CSF, no WBC and normal protein levels in CSF, and no CNS pathology. Hypoxic Ischemic Encephalopathy (HIE) was classified as mild, moderate or severe (HIE I-III). Neurological assessment of surviving patients was done at 3, 6 and 18 months of age. Included patients had LP performed within 24 hours after birth and in some cases an additional LP. PGEM were analyzed using an enzyme immunoassay.

Results: PGEM where in general higher in the early LP samples in comparison to later time-points. This may reflect the immediate release and rapid turnover following the birth asphyxia. Higher HIE correlated to higher PGEM levels and the HIE-III cases had significantly higher PGEM levels (227 pg/mL +38 SD) compared to controls (35 pg/mL +35 SD) and the HIE-I group (46 pg/mL +32 SD). Furthermore, low Apgar score at 5 min (<6) correlated to higher levels of PGEM. These levels also correlated to the severity of the final clinical outcome. Overall the results indicate that PGEM levels in CSF correlates too severity of the perinatal asphyxia and in order to ensure that the levels correlate to the perinatal event rather than the delayed secondary inflammatory response we suggest that it is important to perform the LP within 24 hours postnatal.

Conclusions: Our data indicates that PGEM is a biomarker for degree of hypoxia damage in human infants that have been exposed to birth asphyxia. Moreover, the relative concentration of metabolites of PGE2 may be instructive to determine acute therapeutic interventions and useful as a biomarker for predicting degree of insult and long term outcome.

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Development of diagnostic lab-on-a-chip systems for ligation-based mutation detection

Lab-on-a-chip technology is promising for improved point-of-care diagnostics providing rapid, automated, highly accurate and sensitive tests at low cost. Lab-on-a-chip for molecular diagnostics has however had limited application success beyond research partly due to lack of appropriate device materials, the difficulty to establish complex molecular assays compatible with microfabricated formats and the challenge to integrate multi-step modules into a functional process. Molecular assays using padlock probe ligation with subsequent rolling-circle-amplification (RCA) can with favor be implemented into integrated tests as the isothermal nature of the RCA reaction reduces hardware complexity and high specificity and detection sensitivity are achieved with exponential circle-to-circle amplification (C2CA). We are working in three collaborative projects developing integrated microfluidic devices based on C2CA.

We are developing two alternative systems for prediction of treatment response for minimal invasive and personalized cancer diagnostics by detection of mutations in the KRAS oncogene in circulating tumor DNA. The first one is a stop-flow protocol using a microchip with two interconnected chambers for incubation of the biochemical reactions. The second is a continuous flow protocol where reagents are moved through multiple temperature zones. In both systems we use magnetic beads as solid support for performing the reactions and fluorescent read-out of the amplified products. In the third project multiple platforms for point-of-care testing of infectious disease and antimicrobial resistance are being developed which include functional modules for: sample collection, extraction and amplification and signal detection.

Our main tasks in the projects are to adapt the molecular protocols for the microfluidic format, to participate in the design of microfluidic devices, and to implement and test the reactions in the integrated system.

In collaboration with: DTU Nanotech, Technical University of Denmark, DENMARK, KTH Royal Institute of Technology, Dept. of Micro and Nanosystems, SWEDEN, Partners within the 7th Framework funded European projects DiaTools and Rapp-ID

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Fucose on a chip: a lab-on-a-chip device for screening cancer and liver disease

Here we demonstrate a prototype device for detecting specific carbohydrates in biological samples; the current design detects free fucose in urine, a promising candidate for screening against cancer and liver disease.

The device consists of a disposable PDMS/glass microfluidic platform with multiple channels, each containing a competitive binding assay using a recombinant form of the lectin Aleuria Aurantia (AAL) as receptor and a fluorescent glycoprotein as reporter ligand.

Glycosylation of cell associated proteins and lipids, has been shown to change during certain disease conditions. In cancer patients, all tumor cells show altered glycosylation. Liver disease and several types of cancer all give rise to an increased production and degradation of biomolecules glycosylated with the monosaccharide fucose. The degradation of these structures increases the concentration of free fucose in blood and gives rise to fucose in urine, which can serve as a diagnostic marker for liver cirrhosis and stomach, liver and rectal cancer [1].

Traditional tests for free fucose have been time-consuming and costly, making the prospect of screening for these diseases impractical. However, an inexpensive and disposable device could make routine measurements of fucose in urine viable. Lab-on-a-chip (LoC) devices; microfluidic systems carrying out chemical and biological experiments at a miniature scale, are becoming robust enough for medical diagnostics and could provide ready-to-use assays. In this device we utilize the highly specific binding of fucose to a peptide based on a recombinant form of AAL [2] in a competitive binding assay to detect free fucose in urine.

Our future goal is to design fully autonomous devices suitable for point-of-care use. In order to make an inexpensive test system our aim is to integrate active sample transport directly on the microfluidic chip and remove the need for external pumps [3]. Implementation of low cost optical components and ubiquitous instruments for excitation and detection, such as PDMS prisms and cell phone cameras, could promote decentralization of fluorescent measurements traditionally carried out in a laboratory [4].

References

[1] Sakai, T.; Yamamoto, K.; Yokota, H.; Hakozaki-Usui, K., et al., Clinical Chemistry 36. 474-76 (1999).

[2] Olausson J, Åström E, Jonsson BH, Tibell L and Påhlsson P Glycobiology. 21:34-44 (2011).

[3] Erlandsson, P. G.; Robinson, N. D., Electrophoresis 32, 784-790 (2011).

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Homogenous assay for real-time and simultaneous detection of thymidine kinase 1 and deoxycytidine kinase activities

Measuring activities from the salvage pathway enzymes deoxycytidine kinase (dCK) and thymidine kinase 1 (TK1) may be useful in cancer disease management. dCK is the rate limiting enzyme in the activation of nucleoside analogues drugs and is implicated in the development of resistance to these drugs during treatment. TK1 is a prognostic marker, in particular for hematological cancers. A one-step homogenous assay for real-time determination of TK1 and dCK was developed by combining enzyme complementation with primer extension and fluorescent signal generation using a quenched probe oligonucleotide system at 37 °C. In the dCK/TK complementation step, for the production of dCTP/TTP from nucleoside substrates, dTMP kinase and/or CMP kinase and nucleoside-diphosphate kinase was used. dNTP's produced were continuously used for primer extension in enzyme sequence specific and fixed oligonucleotide primer, template and probe system(s). Fluorescent signal was generated by the 5'-exonuclease activity of Taq DNA polymerase for relief of fluorescent quenching, in analogy with TaqMan®. Fluorescence was captured at 1 min intervals using a real-time PCR instrument. Raw fluorescence was normalized against background to obtain rfu. A horizontal threshold line, crossing all sample rfu values at the level of the rfu of the blank sample at the end of the assay (i.e. after 60 or 90 minutes of fluorescence capture), was drawn obtaining rfu measurement data in minutes for each sample. Duplex proof-of-principle was demonstrated by the independent determination of different of amounts of dCK and TK1 in combination. R2 values of 0.9 were demonstrated with TK-REA U/l reference values obtained from lymphoma diagnosed canine serum samples and from human blood donors and pathological serum samples.

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A software platform for genomic signature analysis in bacteria

The effects of the tremendous technological development in the field of high throughput sequencing is sweeping through the life science research fields and is also entering into the diagnostic laboratories. Today, sequencing machines can produce data amounts that were unimaginable ten years ago. The research community is fed with huge quantities of information. In the field of microbiology, genomic sequencing can efficiently be used as a high resolution genotyping tool in diagnostic labs. It has become evident that bacteria have plastic and variable genomes. A somewhat simplified, but nevertheless useful depiction is that a bacterial species has a “core genome” consisting of conserved housekeeping genes and a variable amount of “accessory genes”. Bacteria reproduces asexually and new trait combinations are formed by lateral gene transfer channels. Lateral transfer of resistance genes and virulence factors can have a major impact on the clinical importance of different stains. Genomic sequencing can reveal such changes but many of the underlying pathogenicity mechanisms are often poorly understood. We have developed a software tool, Gegenees, that can compare large genomic datasets and identify regions with conservation patterns that correlates with traits related to clinical importance. Up to several hundreds of bacterial can be compared and “genomic signatures” can be defined representing diagnostically important target groups. The signatures can be explored graphically or tabular and new strains can be compared with the signatures. In summary, we are developing a graphic user interface software platform for identifying diagnostically important genomic signatures in bacteria and for matching draft sequences from clinical isolates against such signatures.

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nFold probes: New tool for biomolecular analysis in research and diagnostics

It is likely that future biomarkers for disease diagnostics will be selected from a wide catalogues of molecules. For examples, biomarker candidates may represent point mutations, copy number variations and chromosome aberrations at the genomic level; coding or non-coding RNAs at the transcriptomic level; the effects of splicing variation, post-translational modification, and complex formations at the level of proteins; and other entities such as cellular communicators exosomes, circulating tumor cells, as well as microbes and viruses.

In order to meet this need for improved diagnostics, and in particular to enable analyses at the point-of-care it is important to establish technologies that enable comprehensive and effective analyses of a broad range of molecular entities. Currently, specific methods are available for detection of individual classes of molecular targets, however no single techniques is currently available with the same performance for scalable, rapid and precise detection of a combination of multiple molecular entities.

We are addressing the needs for one-for-all molecular measurements by building a radically new probing strategy, named nFold probes. nFold probes are designed to offer tunable (n>1) specificity in molecular detection by requiring necessary number of target recognition events for generating one correct signals, with minimized efforts for reconfiguration for different sets of targets or sample types as assays entities are varied, scaled and combined.

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Urinary prostaglandin E tetranor metabolite is a biomarker of inflammation and respiratory dysfunction in respiratory syncytial virus-bronchiolitis

Background: The proinflammatory cytokine interleukin IL-1ß impairs respiration during infection via PGE2. Infection, with associated eicosanoid release, is the main cause of respiratory disorders in preterm infants. Apneas are common among young infants with bronchiolitis and some patients require intensive care due to respiratory failure. Traditional inflammatory markers don't correlate well with symptoms in viral bronchiolitis. We hypothesize that PGE2 metabolites in urine (uPGEm) correlate to inflammation and breathing disturbances in infants with respiratory syncytial virus (RSV) bronchiolitis.

Objective: To determine levels of uPGEm in infants with RSV bronchiolitis and controls and investigate its correlation to need for therapeutic intervention.

Design/Methods: Eligible were infants (age<1 year) hospitalized at the pediatric university hospital with RSV-bronchiolitis. Healthy age-matched controls were recruited during routine out-patient checkups. CRP, blood and morning urine was sampled, all journal data including saturation, apneas and level of therapeutic support was reviewed. A liquid chromatography and detection by tandem mass spectrometry (LC-MS/MS) method was used to quantify the uPGEm subsequently normalized with patient weight as well as urinary-creatinine. In a subgroup, urinary hypoxanthine levels, a biomarker for prolonged hypoxia, was also analyzed.

Results: Infants with RSV-bronchiolitis have uPGEm levels that are drastically increased compared to control subjects. High and increasing levels correlate to need of respiratory support and intensive care. Hypoxanthine levels in urine did not differ between RSV-bronchiolitis infants and controls, indicating that it is the degree of inflammation and not hypoxia per se that induces the uPGEm in RSV-infants.

Conclusions: uPGEm is rapidly increased in the urine during inflammatory events and might be used as a diagnostic tool sensitive to viral as well as bacterial induced inflammation and is correlated to autonomic dysfunction.

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Noble metal nanoparticles for diagnostic applications

Nanoparticles of noble metals such as gold and silver have unique physical and chemical properties that make them potentially interesting for use in medical diagnostic applications. Several commercial applications have already been realized, such as their use in lateral flow assays and in DNA detection. These applications are all based on properties of single nanoparticles. At the university of Twente we are looking into the properties of two or more nanoparticles interacting with each other and with nearby dye molecules. In parallel we are looking at possible benefits for diagnostic applications of such interactions, for improving sensitivity, specificity and compatibility with current instruments.

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Separation of polypeptides by isoelectric point focusing in electrospray-friendly solution using novel multiple-junction capillary fractionator

Success of deep-proteome analysis often depends upon fractionation of protein mixture and/or peptide digest. Ideally, the fractionation device should operate fast, have a large sample capacity (>10 µg) and require no additional sample clean-up after fractionation. Here we describe an online multiple-junction capillary isoelectric focusing fractionator (OMJ-CIEF) for the separation of biological molecules in solution by pI that satisfies the above criteria. In OMJ-CIEF, the separation capillary is divided into seven equal sections joined with each other via tubular Nafion membrane insertions. Each junction is communicated with its own external electrolytic buffer which is used both to supply electrical contact and for solvent exchange. The performance of the fractionator was explored using protein and peptide samples covering broad pI range. Separation is carried in ionic and ampholytic buffers, including ammonium formate, ammonium hydroxide, histidine and arginine, and typically takes one hour. By maintaining electric potential across upstream segments of the capillary after the focusing stage, selective release of downstream analyte fractions could be achieved. The selective release mode circumvents the problem of peak broadening during mobilization and enables convenient comprehensive sampling for orthogonal separation methods. Using single-component ampholyte buffers with well-defined pI cut-off values, controlled separation of protein mixture into basic and acidic fractions is demonstrated. Peptides can be fractionated into 6–8 fractions with less than 50% overlap between the fractions. With a 6-fraction separation, proteome analysis to the depth of >7,000 proteins is achieved. The device is simple in operation and straightforward in interfacing to hyphened analytical platforms. OMJ-CIEF has a potential of becoming a practical add-on unit in a wide range of bioanalytical set-ups, in particular as a first-dimension separation in mass spectrometry based proteomics or as a preparative tool for analyte purification, fractionation and pre-concentration.

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Discovery of novel prognostic and diagnostic biomarkers in cancer cell-derived vesicles

Exosomes are the newest family of bioactive vesicles that function as a vehicle for cell-free intercellular communication. These endosome-derived vesicles are actively secreted by virtually all cell types under normal as well as pathological conditions. Following the discovery that cancer cells secrete excessive amounts of exosomes compared to normal cells, it became evident that these vesicles can be used as a source of circulating diagnostic, prognostic and predictive biomarkers. We have isolated and characterised vesicles from the serum of cancer patients (e.g. breast, prostate cancer). Our results confirm that cancer patients (breast, prostate cancer) have higher levels of circulating exosomes circulating in the blood. Furthermore, molecular analysis of the vesicles isolated form the plasma of these cancer patients has revealed a set of putative biomarkers that will be further validated in the clinic. Overall, identifying cancer specific markers in vesicles isolated from patients, offers a novel, non-invasive diagnostic tool for cancer.

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Rapid antibiotic susceptibility testing for urinary tract infections

Background: Antibiotic drug resistance has become an increasing problem in terms of morbidity, mortality and treatment costs. Thus, it is important to develop rapid assays for antibiotic susceptibility testing (AST), not only for more effective treatment, but also to slow down the spread of antibiotic resistance and to reduce health care costs.

Methods: We have developed a new rapid phenotypic AST method for UTI based on DNA detection. The assay principle relies on short bacterial culture in the presence or absence of antibiotics. The bacterial growth is quantified by species-specific measurement of 16S rDNA using padlock probes and rolling circle amplification (RCA). Padlock probes are linear oligonucleotides with target-complementary arms which are circularized and ligated upon target recognition. Once a closed circle is formed, the target-specific sequence is amplified by RCA. Fluorescence-labeled RCA products are counted in a high performance fluorescence detector. Bacteria susceptible to a certain drug will yield a lower signal in cultures containing that substance compared to cultures without.

Results: Our assay successfully detected 1 000 colony forming units and distinguished between Escherichia coli, Pseudomonas aeruginosa and Proteus mirabilis. Ninety minutes of culture time was sufficient to discriminate antibiotic resistant strains from susceptible strains, with a total assay time of less than 3 hours. A clinical extended-spectrum beta-lactamase strain was successfully tested using our newly developed assay.

Conclusion: We demonstrated a new rapid assay for AST for UTI which can be employed in a clinical setting. To broaden the diagnostic significance, the final assay will be validated for the antibiotic drugs ampicillin, ciprofloxacin and trimethoprim.

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Development of a fully automated IDS-iSYS Inactive Matrix Gla Protein (MGP) immunoassay

Matrix Gla protein (MGP) is a member of the Gla protein family, which includes Osteocalcin and a number coagulation factors. MGP is secreted by chondrocytes and vascular smooth muscle cells and acts as an *in vivo* calcification inhibitor. This activity is exerted after the gamma carboxylation of five glutamate residues and this activation step depends on the availability of vitamin K. Moreover, to be adequately secreted, MGP must undergo further phosphorylation. Recently, a conformation-specific dual antibody assay enabled the specific detection of circulating desphospho-uncarboxylated MGP (dp-ucMGP) or Inactive MGP. It was demonstrated that Inactive MGP is an independent risk factor for vascular calcification, especially in chronic kidney disease. We developed an Inactive MGP assay for use with the fully automated IDS-iSYS system.

The IDS-iSYS Inactive MGP assay uses two monoclonal antibodies. The biotinylated antibody is coupled to Streptavidin magnetic particles (MP). The second antibody is coupled to an acridinium derivative. After an incubation of 50 µl of sample with the Streptavidin-MP coupled to MAb Biot and the second antibody followed by a washing step, triggers are added and the measured luminescence is directly proportional to the dp-ucMGP concentration present in the sample. The first result is available after 62min. The assay measurable range is 40–5000 pM. limit of detection ≤30 pM and precision <15%. The IDS-iSYS Inactive MGP assay yields good correlation with the VitaK assay (R=0.97): IDS-iSYS Inactive MGP = 0.91 x ELISA – 15.5 pM.

The results indicate that the fully automated IDS-iSYS Inactive MGP test method is a sensitive and reproducible assay with good correlation with the VitaK lab developed dp-ucMGP assay and a potential marker for monitoring progression of vascular calcification in CKD patients.

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Determination of glargine and its metabolites M1 and M2 by using a combination of insulin assays of different specificity

Background & Aim: Insulin analogs are standard therapies for treatment of both type 1 and type 2 diabetes. Specific measurement of each analog is of great importance in many studies. Glargine is a long acting insulin analog, with a substitution of glycine for asparagine at A21 and two arginines added to the carboxy terminal of the insulin B chain. After injection, glargine is enzymatically transformed to its metabolites M1 and M2. The cross-reactivity of both glargine and its metabolites in insulin assays makes specific measurement of glargine a complex issue. The aim of this study was to find a method for determination of glargine and its metabolites along with endogenous insulin in human samples.

Methods: We used two well characterized, commercially available ELISAs to develop a method for determination of insulin, glargine and its metabolites in human samples. An alternative protocol was developed for one of the assays, where the cross-reactivity to glargine and its metabolites would be reduced, but endogenous insulin still detected – an "endogenous insulin ELISA". In the second assay, the cross reaction of glargine and its metabolites was determined – a "total insulin ELISA". The recovery of endogenous insulin, glargine, M1 and M2 was determined in human samples, using the two assays.

Results and conclusion: The "endogenous insulin ELISA" was highly selective, with 100 % cross reactivity to insulin, and "non-detectable" cross-reactivity to glargine, M1 and M2 within physiological insulin range. The "total insulin ELISA" showed a cross reactivity of 44 % to glargine, 41 % to M1 and 28 % to M2. The recovery of endogenous insulin was 80–102 % using the "endogenous Insulin ELISA" in samples spiked with glargine, M1 or M2. Using the "total insulin ELISA", the recovery of glargine in spiked samples was 100–106 %, the recovery of M1 was 91–102 %, and M2 was 90–102 %. We conclude that measurement of insulin, glargine and its metabolites in human samples is possible by using two different insulin ELISAs with different specificity. The method described may be a valuable tool to determine glargine or its metabolites in patient samples.

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Automatic malignancy grading of prostate cancer with image analysis

Prostate cancer is the leading cause of death in men. The diagnosis is based on Gleason grading of tissue samples, which is the most widely used method for determining the severity of prostate cancer. However, Gleason grading is highly subjective with significant variation between experienced pathologists. Today about 70 % of patients with localized prostate cancer receive aggressive treatment that does not prolong life but often results in debilitating side effects. The aim of this research is to replace subjective diagnosis of prostate cancer with automatic and objective severity grading based on morphological features that correlate to disease outcome.

Several attempts have been made to use image analysis to quantify and standardize Gleason grading. However these methods use tissue stains that are developed for visual examination, not for automatic analysis. We describe a technique to quantitatively evaluate the efficacy of stains for automation based on their cluster separability performance. We also describe a new stain that is well suited to automatic, color-based discrimination that uses immunohistochemical staining of basal cells to discriminate invasive cancer while increasing the color contrast between glandular epithelium, stroma, and nuclei. Finally we describe a color decomposition method that removes inter- and intra-specimen color variations in stained tissue due to tissue preparation factors and that yields one accurate density map for each stained tissue type. The new tissue stain and density maps from the color decomposition will be the basis for automatic extraction of morphological features that are known to be linked to cancer.

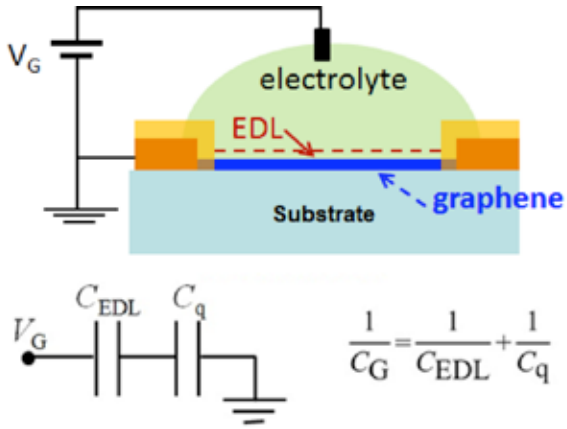
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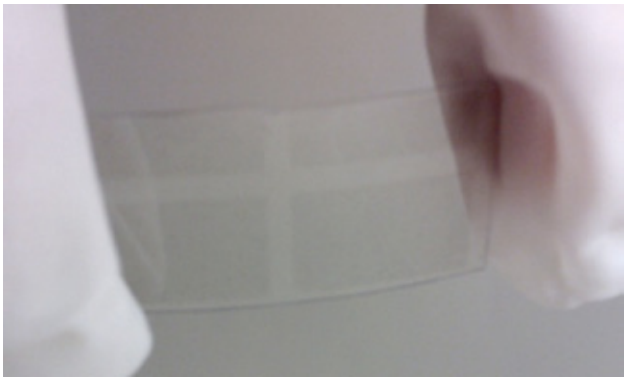
Label-free bio sensors based on graphene transitors

We are developing and investigating an Immunological Ion Sensitive Field Effect Transistor (Im-ISFET) based on graphene. This Im-ISFET allows us to measure both the current through the graphene and the capacitance on its surface. Through C-V measurements, the potential drop over the Electrical Double Layer (EDL) and the capacitance of it are obtained simultaneously. This gives more information about the adsorption of molecules on the graphene, and will forward our understanding of the binding of targets to the graphene surface which in turn will give us a deeper understanding of what the measured signals mean.

Doing C-V measurements with Si-based structures is difficult since a thick oxide layer is necessary to prohibit uncontrolled redox reactions at the surface. This oxide layer has a smaller capacitance than the EDL. When measured in series with the EDL capacitance, the oxide capacitance dominates and renders the EDL capacitance undetectable. The use of graphene as the channel material overcomes this challenge. Graphene is much more chemically inert and does not need passivation. A capacitance measurement then only contains two terms: the quantum capacitance and the double layer capacitance. These terms are in the same order of magnitude and this enables capacitance measurements of the binding process.

Our focus in this work is on DNA sensors. To make a DNA sensor it is necessary to bind the complementary DNA probe to the graphene via a linker molecule. We utilize 1-pyrenebutanoic acid succinimidyl ester (PYR-NHS) as a linker. This has a pyrene group that binds to graphene through pi-pi stacking, and a carboxylic group activated by a succinimidyl ester. The functionalization is done through wet chemistry in room temperature and can be combined with *in situ* electrical measurements to characterize the coverage of the PYR-NHS. The coverage is also measured by AFM, XPS, SEM, and fluorescence measurements.





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Mapping post translational modifications and protein interactions in cancer using the *in situ* proximity ligation assay

Cancer is caused by genetic alterations in oncogenes and tumor suppressor genes. These alterations often result in aberrant protein function and deregulation of signaling pathways. The activity status of a protein or signaling pathway, as reflected in posttranslational modifications (PTMs) and protein interactions, can be visualized using *in situ* proximity ligation assays (*in situ* PLA) using a pair of antibodies that target the interacting proteins or PTM. Each antibody pair is equipped with DNA strands that guide the creation of a circular DNA molecule, serving as a surrogate marker for the interaction. This DNA molecule can then be amplified by rolling circle amplification (RCA) and detected with single-molecule resolution in fixed cells or tissues. We have recently developed a multiplex version of *in situ* PLA simultaneous analysis of multiple protein complexes or PTMs. By combining *in situ* PLA with padlock probes for nucleic acid detection we can now simultaneously detect protein and RNA molecules, and monitor cellular activity status in clinical material within individual cells.

We are currently developing a panel of assays that target nodes in pathways that are commonly deregulated in colon cancer and Chronic Lymphocytic leukemia hoping to use these assays to mapping the molecular status of signaling pathways in cell lines and patient samples as wells as determine the molecular effects of drug candidates found in high-throughput screens of drug libraries.

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Inhibitory sample material? Let's go template fishing!

The use of short capture probes with locked nucleic acid bases incorporated in RNA stabilizing buffer allows for general or specific "fishing" of both DNA and RNA under stable conditions. Specific capture allows for large samples to be processed without risking high concentrations of non template nucleic acids. Faecal samples may contain up to 50 weight percent bacteria and may not be the target of your assay. If a general nucleic extraction method is used, the concentration of non template nucleic acid alone could be enough to inhibit the PCR reaction.

Based on capture probes we developed a semi automated magnetic bead based capture probe DNA extraction method and real time PCR assay that was implemented in the Swedish surveillance programme for the zoonotic and deadly tapeworm Echinococcus multilocularis. The programme includes mtDNA extraction and taqman PCR detection of the eggs and worms of Echinococcus multilocularis in 3 ml fecal samples from 4 000 red foxes (manuscript in progress).

Current work is aimed at modifying the capture probe DNA extraction method to be usable also for RNA targets in a standardized format, mixing RNA and DNA targets in the same robot run or sample well. Initial tests with adenovirus show no differences in ct-values between spiked buffer (inhibitor free) and spiked samples from sludge from wastewater treatment plants, indicating that inhibition is not a problem. The sample size is 3 ml, but can easily be up scaled to 10 ml. We believe that the use of capture probes and automation is an excellent method to enrich DNA/RNA from viruses, bacteria or parasites from large sample volumes or inhibitory samples matrices.

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Evaluation of a non-commercial rapid point of care test for mast cell markers using tryptase as a model protein

Background: A rapid point of care test was investigated for its suitability to detect and quantify mast cell proteins in serum or plasma. Tryptase was used as a model protein. Tryptase is the most abundant protein in mast cells. Immature tryptase constitutively leaks into the plasma. Constantly elevated levels of tryptase may reflect an increased burden of mast cells and are associated with blood disorders such as mastocytosis. Upon mast cell activation (e.g. anaphylaxis), stored mature tryptase is released resulting in transiently elevated levels of tryptase with peak values typically between 15 and 120 minutes after the mast cell activation. The use of rapid point of care test with tryptase as a model protein was investigated and compared to a commercially available laboratory immunoassay for total tryptase (ImmunoCAP Tryptase).

Methods: A capillary flow membrane assay was established. An antibody specific for all forms of tryptase was immobilized as thin bands on nitrocellulose membrane strips (one band per strip). A different antibody, also specific for all immature and mature forms of tryptase, was coupled to gold particles and used as detection antibody.

Serum samples and tryptase calibrators, 20µL respectively, were applied to the strips and allowed to migrate by capillary flow. The gold conjugate and wash liquids were subsequently added to reveal red colored bands on the strips where the conjugate had bound. Evaluation of the assay was performed either visually or with a photometric color reader (ImmunoCAP Rapid reader).

Using the color reader, a calibration curve was established that could be used to determine the tryptase concentration in serum. The results were compared with a commercial tryptase assay (ImmunoCAP Tryptase).

Results and Conclusion: Proof of principle was shown for a new rapid point of care assay for the measurement of mast cell proteases in serum or plasma using tryptase as a model protein. Assay results could be obtained within 30 minutes using only 20µL serum or tryptase calibrators and showed excellent conformity to ImmunoCAP Tryptase.

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Validation of a multiplex chip-based assay for the detection of autoantibodies against citrullinated peptides

Introduction: Autoantibodies directed against citrullinated proteins/peptides (ACPA), i.e. against peptides post-translationally modified by conversion of arginine to citrulline, are highly specific and predictive for the development of rheumatoid arthritis (RA). Different subgroups of RA patients, which have different prognosis and may require different treatments, are characterized by different auto-antibody profiles. A microarray for detection of multiple RA associated autoantibodies, initially focusing on responses against citrullinated epitopes on candidate autoantigens in RA, has been developed.

Methods: The microarray is based on Phadia´s ImmunoCAP ISAC® system, where reactivity to more than 100 antigens can be analysed simultaneously, using minute serum volumes (< 10 µl). Twelve citrullinated peptides, and the corresponding native arginine-containing control peptides, were immobilized in an arrayed fashion onto a chemically modified glass slide, allowing a 3-dimensional layer with high binding capacity. The assay was optimized concerning serum dilution and glass surface, while each individual antigen was optimized concerning coupling chemistry, antigen concentration and selection of spotting buffer. The performance of each peptide in ISAC was compared with the performance in ELISAs. Serum from 927 RA patients and 461 healthy controls from a matched case-control study were applied onto reaction sites on glass slides, followed by fluorescent-labeled anti-human IgG antibody. Fluorescence intensities were detected with a laser scanner, and the results analysed using image analysis software. All patient samples had earlier been tested with a commercial ELISA test, anti-CCP2, which aim at collectively identify as many antibodies against citrullinated epitopes as possible.

Results: Strong correlations between ISAC and ELISA results were found for the individual citrullinated peptides (Spearman's ρ typically between 0.75 and 0.90).

Reactivity of RA sera with the peptides was seen mainly in the anti-CCP2 positive subset, but some additional reactivity with single citrullinated peptides was seen in the anti-CCP2 negative subset. Adjusting for reactivity against arginine-containing control peptides did not uniformly change the diagnostic performance for antibodies against the individual citrullinated peptides.

Conclusion: The multiplexed array, for detection of autoantibodies against multiple citrullinated epitopes on candidate RA autoantigens, will be of benefit in studies of RA pathogenesis, diagnosis and potentially as a guide to individualised treatment.

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A new sensitive anti-drug antibody screening assay with high drug tolerance

Anti-drug antibody (ADA) formation to biological drugs is a major concern during drug development and may interfere with the efficacy of the treatment and could, in rare cases, induce adverse reactions. The analysis of ADA in serum using immunoassays is often complicated due to high concentrations of free drug in the sample that may lead to false negative results due to inhibition. This is especially important in ADA screening assays using the bridging format where a positive test result is dependent on the capacity of multivalent ADA to bind both to drug attached to the solid phase and drug in the detection reagent. The aim of this study was to develop an ADA screening assay and compare the performance with a commercially available ELISA. The TNF alpha inhibitor drug, Infliximab, was used as a model.

The new ADA screening assay is based on an initial incubation step where ADAs in a sample first bind to biotinylated drug and then in a second step bind to drug immobilized on a solid phase. The drug-ADA-drug bridging complex is then detected with a streptavidin beta-galactosidase enzyme conjugate. All liquid handling, incubation steps as well as the fluorescence reading are fully automated. The ADA screening assay was preliminary validated using a mouse monoclonal antibody against human kappa light chain in rabbit serum and fifty ADA negative human serum samples.

The results show that with a sample dilution of 1:20, a sensitivity of 15 ng/ml was obtained and that ADAs still could be detected in the presence of 800 times molar excess of free drug (drug tolerance 1:800). The automated assay had an intra- and inter-assay variation of 4.2–6.9 % CV and 3.1–7.3 % CV, respectively. No interference of rheumatoid factor (RF) was observed using 6 RF positive samples (22–217 U/ml). The commercially available ELISA assay that was used in comparison had a similar sensitivity (50 ng/ml) and good precision but it was not possible to detect ADAs in the presence of 5–800 times molar excess of drug (drug tolerance <1:5).

The new fully automated ADA screening assay has a high technical sensitivity combined with a high drug tolerance and good assay precision. The assay may be used for screening of ADAs both in clinical trials during development of biological drugs and for monitoring individual patients treated with biologicals.

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