



Half-day ANALYTICA Conference 2000 "BioChips in Medical Diagnostics"

On April 12th, 2000, BNLD organized a lecture about the very actual topic "BioChips in Medical Diagnostics" ; it was held in the scope of the "Half-day ANALYTICA Conferences 2000" in Munich. The interest among the visitors of the exposition was great and already early in the morning the organizers (Dr. A. Rühlmann, Magdeburg, PD Dr. N. Gässler, Hildesheim) could realise that the number of participants exceeded the number of chairs in the auditorium. In brilliant recitations the lecturers presented the possibilities of different kinds of biochips in medical diagnostics, but they did not forget to talk about the limitations of this technology as well. The following abstracts will give you an insight into this event.

Introduction - "BioChips in Medical Diagnostics"

Dr. A. Rühlmann, Magdeburg, (BNLD)

Since the first heart beats of Molecular Biology could be detected, this new discipline was always good at initiating and developing revolutionary methods resulting in a knowledge explosion unthinkable just a few decades ago. The development of Molecular Biology rivals that of the computer revolution including information technologies. Robotics, microfluidic systems and nanostructures, three other scientific blockbusters, are now joining to form a science super league to produce products collectively coined BioChips. BioChips, similar to microchips, are miniaturised high throughput analytical devices harbouring a ,probe'-microarray like nuclear acid arrays (e.g. DNA chips), protein arrays, tissue arrays or microfluidic networks suitable for separation techniques, PCR reactions or even multistep chemical reactions. These devices are already starting to be used in clinical laboratories and

will have a tremendous impact on new diagnostic tools for genotyping or drug monitoring. With the advent of advanced genotyping tools, sequence collection and collation allows a pharmacogenetic approach to correlate genetic profiles with therapeutic drug responses. With this impressive and exiting deployment of BioChips ready to invade molecular diagnostics a continuous and critical monitoring of these methodologies is urgently needed.

This morning session, although only a glimpse of the field of BioChips with important implications to medical diagnostics, will start with a critical introduction into genetic testing followed by prominent representatives of current R&D in BioChips with a gradual move from a currently more research based to ready to use BioChips. During the break there will be reasonable time for informal discussions. We have the privilege to witness or even be a part of today's and upcoming BioChips use and developments.

Genetic testing: easy and straightforward

Professor Jean-Jacques Cassiman, Center for Human Genetics, University of Leuven, Leuven, Belgium

Molecular biological methods and diagnostic tools are slowly but steadily invading all specialties and subspecialties of medicine. DNA diagnosis for rare inherited diseases has become molecular diagnosis for genetic diseases, molecular oncology for malignant diseases and molecular medicine for strictly inherited traits or for predisposing factors. Even molecular forensic medicine has become a new and thriving discipline.

The human genome project and the intensive academic and private research initiatives have generated and are continuously generating massive amounts of new information on DNA, genes, transcription factors and animal models, while biotechnological companies bring almost daily new diagnostic tools and equipment to the market, which are gradually revolutionizing the laboratory procedures such as the microarray systems, the megasequencers, the quantitative PCR systems and the mass-spectrometric analyses.

One can envisage with some imagination that in the coming years, automated systems will be available which will analyze a substantial part of an individual's genome or the expression profile of a particular tissue, in a few hours.

Before it comes to this however, we will need to figure out the precise significance of mutations or polymorphism for the phenotypic properties of monogenetic diseases but in particular and none the least in the frequent multifactorial diseases. We will have to design appropriate controls to monitor the correctness of the different and multiple assays and we will have to find time to explain the relevance and precise significance of these findings to our patients.

In the mean time, we should be careful in validating any new assay before introducing it in clinical practice and we should design strategies to monitor our daily diagnostic practice in order to identify any shortcoming in the procedure.

Finally, we should carefully follow up those who received results of genetic tests in order to better understand the impact of our services on the patients and to optimize them in the future.

"Accuracy does Matter – Genotyping by Mass Spectrometry in Medical Diagnostics"

*Karsten Schmidt, Sequenom,
Hamburg/SanDiego*

SEQUENOM's mass spectrometry-based system, MassARRAY™, will be presented as a tool for error-free genotyping in a high-throughput environment. This results from a combination of automated assay design which maximizes mass differences seen among differing genotypes; a high precision "launching pad" inserted into the mass spectrometer; the discriminating power of the MALDI-TOF mass spectrometer itself; and software capable of automated data analysis and genotype-calling.

Preparation of Arrayed Libraries by Micro Wet Printing

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Efficient production of arrayed libraries of oligonucleotides or other substances on small chips, suitable for substrate specific on chip interaction reactions, is an important prerequisite for novel miniaturized multiparameter assay systems. With the potential to make a large number of analytical questions accessible in a single assay, optimized analysis systems based on array technology open up new dimensions for medical diagnostics and therapy monitoring, biomedical research, agriculture, food quality control and environmental monitoring. For the production of large numbers of such arrays with the required quality (reproducibility and standardization), appropriate means of *in-situ*-synthesis or immobilization on solid support are required. Various successful methods have been described so far to match this goal (1, for review 2). Micro Wet Printing (μ WP) provides a method to build up highly diverse substance libraries by on chip *in situ*-synthesis (3). The distinguished features of μ WP are: - not limited to one type of chemistry allowing the use of well known chemical synthesis reactions - parallel processing in wafer scale is feasible - high potential for miniaturization - compatibility with standard methods in microsystems technology. The method is based on a masking technique. The mask is built based on a silicon wafer as frame holding the mask membrane. The membrane is patterned in such a way that selected areas of the surface remain accessible to the reagent. Using the latest mask-aligner technology the mask and the chip substrate wafer are brought in contact and the accessible areas are exposed to the synthesis reagent. After this step the mask is removed from the wafer and another mask is put in place at a position predefined by the chosen synthesis or immobilization algorithm. At present this technology is optimized for large-scale synthesis of on chip libraries.

- 1) Fodor, S. P. A., Rava, R. P., Huang, X.C., Pease, A.C., Holmes, C.P. and Adams, C.L. (1993). *Nature* 364, 555-556.
- 2) Ermantraut, J., Wölfl, S. and Saluz, H.P. (1998) *Microsystem Technology: a powerful tool for biomolecular studies*, M. Köhler and H.P. Saluz (eds.) Birkhäuser Verlag, Basel.
- 3) Ermantraut, E., Schulz, T., Tuchscheerer, J., Wölfl, S., Saluz, H.P., Thallner, E. and Köhler, J.M. (1998) *Micro Total Analysis Systems '98*, D. J. Harrison, A. van den Berg (eds.) Kluwer Academic Publishers, Dordrecht.

The Use of Disease Management Chips: the example of the p53 gene

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Disease Management Chips are designed to assist in the clinical management of a particular disease; the p53 Gene Assay Chip, for instance, is intended to identify mutations in the p53 gene in cancers or in pre-cancerous conditions. Such information, in combination with additional histopathological and clinical features i) may hint to possible cause(s) of the cancer, ii) may indicate its mono- or polyclonality, iii) may have prognostic value for some cancers, and iv) in the future might play a role in choice of therapy. There are over 10.000 p53 mutations recorded in the IARC Database of p53 gene mutations which were identified from sequencing the gene of more than 40.000 human tumors. So-called 'carcinogen-specific fingerprints' in the p53 gene are evident for skin, liver and lung cancer of individuals heavily exposed to sunlight, aflatoxin B1, and tobacco smoke, respectively. The mutation patterns that may indicate a role for most other exogenous or endogenous cancer risk factors remain largely undefined, however. In order to define additional chemical class-specific 'fingerprints', or spontaneous (background) or endogenous mutation patterns, new experimental systems such as the human p53-knock-in ('hupki') mouse are needed. While mutated p53 is usually overexpressed and stimulates aberrant gene expression and growth of cancer cells, overexpressed wildtype p53 sends cells into apoptosis; it is therefore conceptually tempting to correct the aberrant conformation of certain mutated p53 protein complexes by designer drugs which would specifically eradicate tumor cells and leave normal cells untouched; such approaches are underway. In relation to the above considerations, we initiated investigations on human cell lines with known p53 mutations using Affymetrix Chip technology. Our experience with DNA target preparation and results obtained will be described.

Application oriented low to medium density "BioChip-Array-Technology" and high throughput production of BioChips

Roland Toder, PhD., GeneScan Europe AG, Freiburg

Different application fields of microarrays are based on various requirements concerning integration densities, design and quality of biochips as well as different ways of data analysis. One can identify the following fields of (DNA/RNA) chip applications: gene ID, gene expression, DNA identification, CGH- and SNP-analysis.

BioChip Technologies, a 100% "daughter" of *GeneScan Europe AG* is focusing on low- to medium-density biochips. Within this field, there are several biochips produced and developed from *BioChip Technologies*. The "ScienceChip" represents a custom made microarray for individual laboratory / research requirements. This "Custom made biochip has densities, according the customers specifications, of up to 2500 dots/ cm². There is a high flexibility of what kind of biomolecules are to be immobilized on the chip surface (e.g. oligonucleotides, PCR products, cDNAs, plasmids, cosmid and BACs etc. as well as peptides and proteins).

The "NutriChip" is one of the standard application chips developed by *BioChip Technologies*. This biochip is designed to detect pathogens in food and water. The NutriChip allows the identification of pathogens like *Shigella*, *Salmonella*, *E.coli* (EHEC) as well as *Campylobacter*. This system is flexible enough to adjust the setup to the needs and requirements of various fields in the food processing industry. Several standard biochips in the field of medical-diagnostic are in development, since there are applications in medical diagnostics where low- to medium-density microarrays are required for high throughput standard analysis. Together with (in Collaboration with the "Frauenhofer Institute IPM developed") an inhouse analyser, BioDetect 645, it is possible to provide analytical laboratories with an efficient and affordable "diagnostic tool" (chip and reader).

"Lab-on-a-Chip Technology: Quantitative and Qualitative DNA / RNA Analysis for Molecular Biology"

*Götz Frommer, Agilent Technologies,
Waldbronn, Germany*

The determination of fragment size, quality and concentration of nucleic acids is one of the fundamental steps in molecular biology. Traditional methods of nucleic acid analysis such as gel electrophoresis, capillary electrophoresis and mass spectrometry will now be complemented by Lab-on-a-Chip Technology. Lab-on-a-Chip technology integrates electrophoretic separation and fluorescence detection in one analytical instrument. Samples are directly pipetted onto a glass chip with microfabricated channels, which is simply placed into the Agilent 2100 bioanalyzer system. Automated electrophoresis as well as simultaneous data acquisition occurs without further user intervention. The advantages of the LabChip™ technology are: easy operation, high sensitivity, small sample size (1 µl), digitized data storage (gel-like image and electropherogram) and a short run time per sample of 90 seconds (a total of 30 min for 12 samples). This new analytical technique for nucleic acids has the potential to simplify and speed up molecular biology experiments.

The Development of Biochip Array Technology in Clinical Pathology

Jean Wilson, UK

Biochip Array Technology was developed to enable the simultaneous detection of a panel of diagnostic parameters. Our objective has been to construct biochips where a single biochip can provide a means of determining the full panel of analytes necessary for the accurate diagnosis of a particular clinical condition.

The technology can be applied to panels to determine infectious diseases, cardiac markers, tumour markers, fertility hormones, thyroid function tests, drugs of abuse and allergen testing.

A biochip consists of a 1 cm² substrate on which discrete test regions have been constructed, each test region representing a different analyte. The principle of the biochip assays is immunoassay

based and both competitive and sandwich assay formats have been validated.

Chemiluminescence is used as the method of detection. The light signal generated from individual test regions is determined at an imaging station capable of imaging up to 9 biochips simultaneously, with typical exposure times of 4 seconds. The light signals expressed in relative light units (RLU) is then converted by imaging software to provide the concentration of individual analytes present on the biochip. The time taken to generate the first result is typically 15 to 30 minutes.

Biochips for fertility hormones, tumour markers and drugs of abuse have been evaluated against conventional competitor methods and results show that multiple immunoassays can be carried out simultaneously on a single biochip with good agreement being achieved with current technologies.

C. Kaiser