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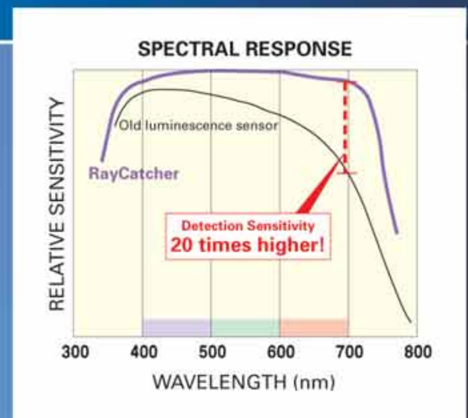


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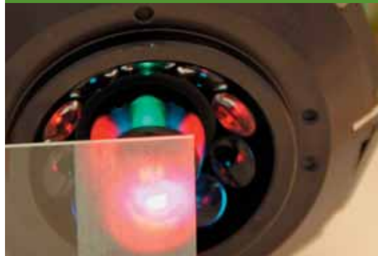


26

## FOCUS


BIO-ELECTRONICS

**2 Bioelectronics Today**
Current approaches for bioelectronic devices and their effects on medical and biological applications.

**6 Testing Diagnostic Tests**
Electronics meets point-of-care applications.

VACCINES

**10 A new Dimension in Vaccine Development – Adjuvent Systems**
Novel adjuvents from GlaxoSmithKline Biologicals (GSK)

SYSTEMS BIOLOGY

**13 Applying Systems Biology in Drug Development and the Clinic**
Facing the challenge of combining data from highly diverse sources.


## SPECIAL SECTION

*Partners of VPM (Vakzine Projekt Management GmbH) display their development inputs to VPM on the occasion of the 2nd VPM Vaccine Development Days.*


**16 Capgemini Deutschland GmbH**
**18 Vakzine Projekt Management GmbH**
**21 Vibalogics GmbH**
**22 Geneart AG**
**24 Innogenetics NV**
**26 ProBioGen AG**


## MAGAZINE


BUSINESS

**28 Biopharmaceuticals, Small Molecules, and Vaccines**
An interview with Steven Projan, Head of Biological Technologies at Wyeth

**32 Managing the Supply Chain**
How does Roche, with 17 global production sites, manage its supply chain?

**34 Jan van Koeveringe**
The Roche Head Global Technical Pharma Operations anwers BioWorld *EUROPE*s questions.

**35 The Asian biofuels industry**
What will the impact of Asia be on the global biofuels industry?

**38 Lab Work in Flux**
The «Trend-Survey – Lab Work».


## SOLUTIONS


PRODUCTS & APPLICATIONS

**42 The Wave FlexReactor™**
**44 MS-based Monitoring of Phosphorylation Sites**
**46 Microarray Measurements in Routine Applications**


## SERVICE

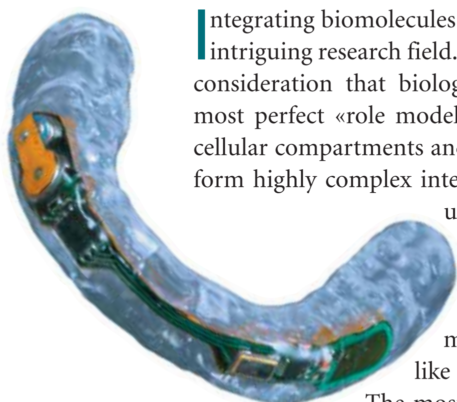

EVENTS

**50 Calendar**
**52 Imprint**

# Bioelectronics Today

Given the electronic character of many biological processes the integration of electronics with the biology is quite «natural». The possibilities for bioelectronic devices seem to be unlimited. The article discusses current approaches for bioelectronic devices and their effects on medical and biological applications.

TEXT | BERNHARD WOLF



Integrating biomolecules with electronic substrates is an intriguing research field. Even more so when taking into consideration that biology itself offers the oldest and most perfect «role model» for electronic devices. Cells, cellular compartments and molecules are capable to perform highly complex interactions that work following a ubiquitous principle: the transfer of electrically charged ions and particles causes structural and functional changes in molecules triggering cascade-like signal transduction pathways. The most obvious example of course is the building and propagation of electrical potentials over cellular membranes to pass on sensory, muscular or hormonal signals. Fascinating enough, a quite simple shift of intra- and extracellular ion concentration orchestrates our complete nervous system including our intellectual capacities. Biological processes, i.e. membrane fusion, might even be triggered by the shift of a single electron, as discussed in a recently published review (1).

Given the electronic character of many biological processes the integration electronics with the biology is quite «natural». The possibilities for bioelectronic devices seem to be unlimited. An unaccountable number of natural and technically engineered biomolecules can be combined with different electronic materials and substrates, e.g. electrodes, piezoelectric elements or semiconductors (2). This article will discuss current approaches for bioelectronic devices and their effects on medical and biological applications.

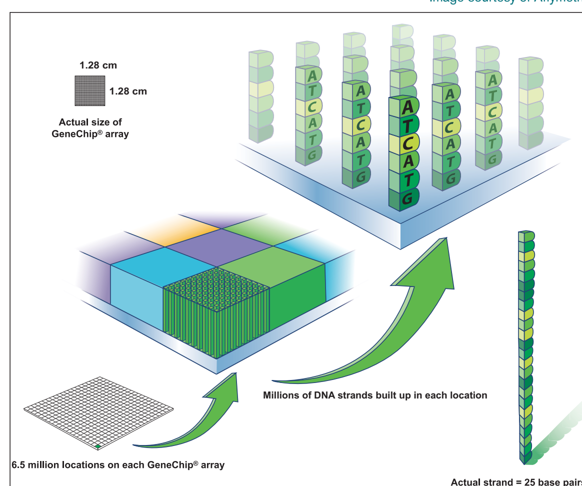
**INFO**

*Bernhard Wolf* (1948) studied biology, chemistry, and physics at Universität Freiburg/Breisgau (DE) where he obtained his venia legendi in Medical Physics and Biophysics in 1988. In 1998 he moved to Universität Rostock (DE) where he held the Chair of Biophysics until 2000 when he was nominated full professor at TU München and holds the Heinz Nixdorf Chair of Medical Electronics. His research interests are in Bio- and Cellular Sensorics and Screening Systems.

### Current biochip developments

Although the term «biochip» implies the utilization of a semiconductor substrate along with an integrated circuit, most of the analytical devices with this label are not directly based on electronic components. The most widespread biochips are DNA and protein chips integrating and parallelizing DNA and protein identification on a very small space, i.e. a 1-Eurocent-coin (picture 1). Other approaches have even succeeded to pack complex analytical routines on a miniaturized lab, ranging from sampling up to isolating the reaction products. To analyze hybridization results on the chips, most micro-arrays use a combination of fluorescent dies and optical read out methods. However, as organic dies tend to bleach, optical detection techniques may deliver false results. Current developments therefore aim to use electrical signals instead of optical detection methods. These «electrical biochips» identify DNA binding by redox reactions or by examining the electrical conductivity of DNA labeled with gold or other nanoparticles. Some chip developments try to measure electrical activities without markers, i.e. using the changing impedance following DNA hybridization as a detection signal. The deployment of field effect transistors (FETs) offers an electronic method to identify double stranded DNA, taking advantage of the electrically charged DNA phosphate backbone and measuring charge changes after hybridization. Especially marker

Image courtesy of Affymetrix




Picture 1: A single feature on a DNA microarray.

free detection methods provide for highly resolved DNA identification even without DNA amplification making these chip platforms a promising tool especially for routine diagnostics in medicine as well as food analytics.

### Cell Chip Technologies

Living cells are extremely complex microstructures composed of different functional subunits. These compartments are linked together by complex biochemical and physical pathways. An emerging biohybrid detection method based on semiconductor technology might be the key for analyzing cells as a coherent system: Sensor based cell chip devices work with living cells growing on semiconductor substrates and acting as signal transducers for different kinds of reactants (3). Current chip developments use a set of different optical and electronic sensors to measure pH, $pO_2$, impedance and electric activity of the cells on the chips, allowing the simultaneous monitoring of different parameters under realistic conditions. The results are read out by ICs embedded in substrates like silicon, glass or ceramics (4, 5) (picture 3). Cell chips can be used in different analytical scenarios: either as detection systems in food or environmental analytics using cell types that are sensitive to certain toxins or other substances or as experimental systems in pharmacological research or medical diagnosis, i.e. to allow high content screening of potential drug components or examine chemosensitivity of tumor biopsies before starting therapy. In the same way, neurons and nerve tissues can be cultivated directly on a sensor chip to form spontaneously active neuronal networks. These cell clusters can be incubated for several weeks to examine the effects of neurotoxins or neurodegenerative proteins e.g. in Alzheimer's or Parkinson's disease.

### Bioelectronic implants

However, bioelectronic devices are not limited to external analytical applications. A growing number of electronic implants is being developed for medical therapy. A few examples shall show the range of current and future appli-

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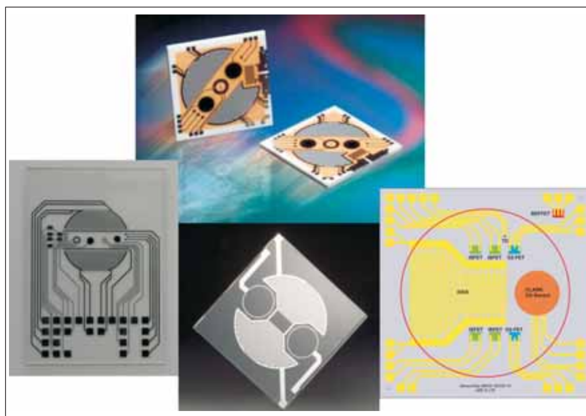

Picture 3: Sensor Cell Chips made of ceramics (top), glass (left and middle) and silicon (right).

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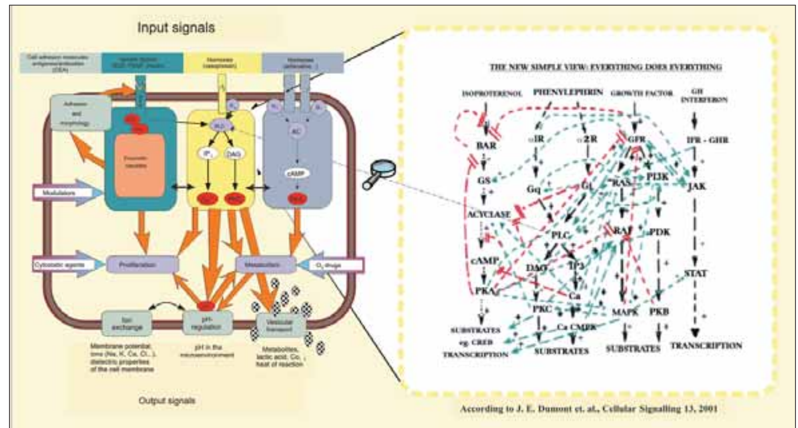


Picture 2: Cellular signaling processes are highly parallelized and cross-linked to each other. Genetic expression patterns are modified metabolically.

cation fields. Besides supportive implants, pacemakers or ICDs for cardiac disorders, implants that replace or restore organic functions are gaining more and more importance. Cochlear implants have been available for more than 20 years and help deaf patients to obtain different amounts of hearing capabilities by electrically stimulating the acoustic sensory cells. So far the cochlear implant is the only neuroprosthetic device used in medical therapy. Another approach for a neuroprosthesis is an artificial sensory system for patients with retinitis pigmentosa, a disease caused by the decay of photoreceptors in the retina (6). First studies with retinal implants show promising results, reporting partially restored vision in formerly blind patients. The retinal implant used in this study consists of a chip with 1.500 microelectrodes and an equal number of transistors that stimulate the intact retinal nerve cells.

### From bioelectronics to mechanics

Besides medicine, bioelectronics will play an important role in nanoscale devices. In nature, biological motors are specialized protein machines in the nanometer scale optimized for their specific task as switches, pumps or transporters. Current research work includes experiments with linear motors, e.g. with actine and myosine filaments or kinesine and microtubules respectively. A rotation motor was formed by combining the Gamma-unit in proton transporter ATPase to a propeller made of nickel. Although there are some promising results with linear and rotational motors, the problems of instable artificial molecules and motion control (start, direction, end of movement) still remain to be solved. Regarding actuators, the situation is similar: there are experiments that can be seen as «proof of concept», for example with the crystalloid P-protein. This protein acts as a valve regulating the flow of cellular liquids in the tubular sieve cells of some plants. The uptake of calcium ions leads to reversible morphological changes in the crystal. In an experimental setting protein crystals were brought into a very thin glass capillary. The switch function was achieved by adding calcium ions for closing and

EthyleneDiamine Tetraacetic Acid (EDTA) for opening the switch. The open question to be answered is the external control of this mechanism – this problem solved the P-protein could be used as a valve in microfluidic applications.

DNA is another candidate molecule for switches, as a research group at Lucent Technologies Bell Labs was able to demonstrate: Artificial, partially hybridized DNA strands can be opened and closed by the hybridization of another DNA strand, thereby forming molecular tweezers. The advantage of DNA based switches lies in the selectivity the tweezers can be operated with. In the future individual switches might be coupled to more complex structures, enabling molecular construction and positioning systems.

Looking at the current situation in nanobiological mechanics, technical or even economical application fields aren't clearly visible yet, but might be found in the controlled transport of vesicles or molecular building blocks as well as the utilization in biomechanical valves and pumps. A potential application scenario is the integration of biomolecular transport mechanisms into the microfluidics of lab-on-chip-systems.

## Conclusion and perspectives

The approaches and – partly – marketable devices presented in this article can only show a small section of current research and development in the field of bioelectronics. Some innovative work was left out, e.g. DNA computing, although especially the latter subject has gained some attention in the media. Despite large investments the results in DNA computing the results achieved are limited to interesting theoretical and experimental approaches. From today's perspective DNA computers are unlikely to take the place of modern silicon computers.

The fusion of biology, bioengineering and electronics has generated a very lively worldwide marketplace with numerous small and larger companies commercializing bioelectronic systems. So it can be expected that major achievements in biolectronics will not remain closed within laboratory walls, but make their way into business plans, to investors and finally to marketable applications for a variety of industries. Eventually, the deployment of electronic applications in biomedical research might bring about a substantial efficiency increase in the health system – a scheme that in the past has managed to revolutionize other industries like the automotive or the telecommunication sector. □

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# Testing Diagnostic Tests

Electronics meets point-of-care applications.

TEXT MARK G. DOBSON, PAUL GALVIN, DAVID E. BARTON

**INFO**

*Mark G Dobson* (1976) earned a degree in Genetics from the University of Newcastle upon Tyne, UK in 1998 and completed a Ph.D. in the same institution in 2003. He was awarded a Marie-Curie fellowship in 2001 which involved a period of work at the National Microelectronics Research Centre (now the Tyndall National Institute) in Cork, Ireland. Prior to his doctorate Mark researched the genetics of diabetes; his Ph.D. involved the development of a novel MEMS resonant mass biosensor. He has also been involved in the development of miniaturized PCR systems. Mark is a senior research associate on the SNiP2CHIP project and lives and works in Dublin, Ireland.

*Paul Galvin* (1969) obtained from National University of Ireland Cork a BSc Honours Zoo (2H1) in 1990, and a PhD (Science) in 1995. In 2000 he joined Tyndall National Institute at Cork as research scientist where he established a Nanobiotechnology research activity. He is today Senior Staff Researcher and leader of the Strategic Research Programme for Biochips in the EU FP6 Nano2Life Network of Excellence.

*David E. Barton* (1958) has a BA (Mod.) Microbiology from Trinity College, Dublin, Ireland and received his Ph.D. in Medical Genetics from Queen's University of Belfast, Northern Ireland in 1983. Until 1988 he was Associate Research Scientist in the Department of Human Genetics at Yale University School of Medicine, and then Head of the Molecular Genetics Laboratory, East Anglian Regional Genetics Service at Cambridge, UK. Since 1995 he is Chief Scientist of the Molecular Genetics Laboratory, National Centre for Medical Genetics in Dublin. He has worked in a number of professional and international bodies on molecular genetics quality including the UK National External Assessment Scheme in molecular genetics and EU and OECD expert groups.

Medical science is currently taking the first steps into a new era of personalized medicine that will be based on an individuals genetic profile [1]. In order that this vision may be fully realized it will be necessary to create instruments that can perform fast and convenient genetic analysis in a point-of-care (POC) setting such as on a hospital ward on in a doctor's surgery.

This article will briefly consider the current state-of-the-art in POC genetic testing instruments, the applications for which these instruments may be used in medicine and some the requirements of these instruments.

### 1st versus 2nd generation POC genetic testing instruments

The first generation of POC genetic testing instruments is nearing the market. These instruments incorporate of a means of DNA sample preparation followed by a quantitative-fluorescent PCR (qfPCR) assay that simultaneously amplifies and quantifies the amount of one or a few DNA sequences. The best example of a first generation system is the Cepheid GeneXpert System®; this is the only system currently for sale that is suitable for POC genetic analysis. The company has four assays available for sale in the EU (as of July 2007) that have been CE marked as being suitable for in-vitro diagnosis and may thus be used at the point-of-care.

A major reason that QF-PCR systems were the first to be released is that they represent some of the simplest systems that that can be built with proven technology. After DNA preparation the majority of current and currently-planned systems require PCR to be performed. A system, such as one based on QF-PCR, that can detect DNA sequences during PCR rather than in a subsequent stage will benefit from a simpler protocol with a reduced number of fluidic manipulations which thus enables comparatively simpler instrumentation and a reduced assay time.

A serious limitation of this approach is that in a multiplex PCR, the detection of different DNA sequences is accomplished in separate optical channels; thus the maximum number of DNA sequences that may be detected is limited by the number of optical channels that are available for detection, typically four. Since various controls must be run the maximum number of DNA sequences that may be detected is typically two per PCR.

The first examples of second generation POC instruments are under intensive development. The majority of these will detect DNA sequences using a microarray which relies on the spatial encoding of different DNA sequences. This means that the number of different DNA sequences determined in a single assay is essentially unlimited which significantly increases the applications for which the instrument may be utilized.

Further information on current and emerging systems for POC genetic analysis is available in a recent review by the authors [2].

### Current applications of POC genetic testing

Many applications exist for automated genetic analysis such as monitoring bioterrorism, use in forensics, food production and veterinary science. The most significant application however will be in healthcare where genetic analysis could be used for the identification of infectious

agents and for pharmacogenetics and pharmacogenomics (commonly abbreviated to PGx).

*Identification of infectious organisms*

The largest current application of point-of-care genetic testing is for the identification of infectious organisms. Cepheid, for example, has several microbial identification tests available for sale for their GeneXpert System® that are intended to be used by healthcare professionals that are not trained in molecular biology.

The major advantage of genetic systems for the identification of pathogens is their speed; the Cepheid tests take around an hour whereas typical lab-based methods of microbial identification would take at least a day or two. This speed advantage enables earlier treatment and greater patient convenience.

Microbial identification is an application that is well suited to the 1st generation of POC assays as it can be accomplished by the quantitative identification of a single DNA variant. Even when one considers the required controls this can done using a single qfPCR reaction. An advantage of a first generation approach for pathogen identification is that quantitative information is important and qfPCR tends to have a higher dynamic range than that of array based systems. This is important as it is necessary to know whether the organism is present in a trace amount of if there is a serious infection. That said, second generation systems still have advantages for microbial identification as, for example, an assay may simultaneously test for a number of pathogens or pathogen subtypes.

*Pharmacogenetics and pharmacogenomics (PGx)*

PGx can be considered to be «the science and technologies associated with dividing patients into groups on the basis of their therapeutic requirements using a genetic test» [3]. To put it more simply the aim of PGx is to be able to more accurately predict the response of an individual to a particular drug by taking genetic factors into account. Frequently this involves analyzing variation in the genes that produce the enzymes that are directly involved with the metabolism, transport or targets of the drug of interest; this variation can remove reduce or increase the activity or the quantity of an enzyme of interest.

A small number of pharmacogenetic tests are already in use; the best example is probably the test associated with the reverse transcriptase inhibitor Abacavir. A small number of patients are hypersensitive to this drug (with sometimes fatal consequences) as a result of a defined genetic variant, HLA-B*5701.

A number of genes have been identified that influence a large number of drugs; examples of this are the cytochrome P450 genes CYP2D6 and CYP2C19. These are members of a very large group of genes which, incidentally, is found in all organisms and is thought to be around 3.5 billion years old. Of interest to the current discussion is the fact that the enzymes made by CYP2D6 and CYP2C19 are thought to metabolize 25% of all drugs [4]. The Roche AmpliChip CYP450 (http://www.amplichip.us/) is a microarray system that analyzes 30 genetic variations in the two genes and classifies patients by their metabolism rate. The system must be used in a laboratory owing to the fact that it does not have the ability to prepare DNA or perform PCR. Nevertheless, the AmpliChip CYP450 is the first PGx microarray system to obtain FDA approval.

Certain drugs have a reasonable amount of PGx information; one example is warfarin. The amount of this drug prescribed to patients at the extremes of the dosing range varies over 120 fold. It has been estimated that 25% of this variability can be attributed to 10 polymorphisms of a single gene, VKORC1.

In general, however there are insufficient clinical data linking particular DNA sequences with particular drug responses. A large amount of research is underway to resolve this lack of understanding. An example is the Pharmacogenetics research network, a collection of several groups who maintain a database of pharmacogenetic related information (www.pharmgkb.org) and in addition are performing primary research on the genetic basis of drug response in diseases such as asthma depression cardiovascular disease and cancer [5].

INFO

**THE ESSENTIAL REQUIREMENTS OF THE IVD DIRECTIVE**

- The device must be designed in such a way that its safety (and therefore performance) is equivalent to the state for the art.
- The device must attain the sensitivity and specificity that is specified on the instructions; this performance should be retained during the specified storage and transport conditions.
- Risks associated with the device must be eliminated as far as is possible (by design), those that cannot be eliminated must be controlled for and the user should be informed of any residual risk.
- Any control materials used should be traceable.

**TABLE 1**

**THE COMMON TECHNICAL SPECIFICATIONS OF THE IVD DIRECTIVE**

- Performance evaluations must be carried out against a CE marked device.
- Specificity must be at least 95.5% and should be determined on low-positive specimens.
- Devices should be tested to establish the effect of interfering substances.
- Each nucleic acid test sample should have a state of the art internal control as far as is possible throughout the entire process.
- Validation should be performed according to the European Pharmacopoeia validation guidelines.

**The most critical points of the essential requirements of the IVD directive POC genetic testing devices.**

## Requirements of a POC instrument

*Hardware Requirements*

For an instrument to be successfully utilized in a POC situation certain attributes are necessary [6]:

- The cost per assay is critical and must be as low as possible as it is the cost to benefit ratio that will determine whether POC genetic testing becomes widespread.
- Results must be determined from samples without user-intervention in order that a minimal level of expertise is required to perform the assay (this may also increase assay reproducibility through the elimination of human-introduced errors and variation). Therefore, a sample in, result out, "black box" design will be favoured.
- Assay speed is critical; POC instruments should be as fast as possible. An assay time of under an hour is desirable (and achievable).
- The number of DNA sequences that can be detected in a single assay must be sufficient for the intended use. For example a first generation POC instrument will not be suitable for determining CYP450 status unlike a scalable 2nd generation instrument.
- Finally, the most important aspect of any genetic testing method is the accuracy and reproducibility of the genotypes provided. Performance levels similar to those achieved in a traditional laboratory are likely to be expected of a POC instruments although this will ultimately be determined by the clinical use to which a test result is put.

*Regulatory requirements*

POC genetic testing devices will have to carry a CE mark indicating that they conform to the appropriate legislation. In the EU the current medical-related legislation for such devices is the *in-vitro* diagnostics directive (IVDD) [7].

The IVDD is separated into different categories depending on the perceived level of risk associated with the use of the device. The level of risk influences the way that conformity to the IVDD is assessed (from a full design and production audit to a manufacturer declaration) and whether the device needs to comply with the common technical specifications. Currently, the majority of POC genetic assays would be considered low-risk devices but in the authors opinion this is unlikely to continue indefinitely.

The directive sets-out that the «essential requirements» be met in addition to the common technical specifications (where relevant). In practice the best way to achieve compliance is to follow the appropriate harmonized standards; a full list of these is available on the European Commission website but the most critical standards are ISO 13485:2003 (quality management), ISO 14971:2000 (risk management) and EN 13612:2002 (performance evaluation).

It is worth pointing out that the reason that few instruments have passed the relevant regulatory hurdles is most likely due to an insufficient level of accuracy. An accuracy greater then 95% is required; this is not a trivial accomplishment in a genetics laboratory and is harder in an automated device.

It should be noted that the EU is lagging behind the USA in terms of legislation and regulation regarding PGx; so more stringent regulation should be expected to be introduced in the coming years.

Other, non-medical, safety standards will be applicable to POC instruments; for example, electrical equipment carrying a CE mark must be compliant with the electromagnetic compatibility directive or the low voltage directive. In addition depending on the means of transfer of blood between the patient and the device certain provisions of the medical device directive could be required, most notably related to sterility.

INFO | **DISCLOSURE**

The authors are part of the EU 6th framework consortium, SNiP2CHIP, which is developing a second generation system for the point-of-care determination of genetic polymorphisms. The system will be fully automated and able to prepare DNA from a blood sample, perform PCR and perform microarray detection by magnetic and/or optical means. The consortium is using cystic fibrosis as a model system thus the prototype device will be capable of determining around 30 genotypes although the system is designed to be easily scalable (http://www.tyndall.ie/projects/snip2chip/index.html).

## Electronic methods of nucleic acid detection

The most common means of DNA detection is the detection of fluorescent dyes, either covalently bound to the DNA or intercalated into the double helix. Electronic detection by comparison has a number of advantages in that it does not require a camera (which tend to be expensive and bulky) or a light source which can also be expensive (if a laser source is used) or have a high heat output (if an incandescent source is used). This hardware advantage makes it likely that future biological sensor systems will increasingly use electronic detection.

Several methods of electronic-based DNA detection have already been demonstrated; two examples were discussed in more detail in our recent review [2]. Integrated Nano-Technologies developed a detection method which relies on the metallization of a DNA strand which is stretched between two electrodes; the presence of the strand may be determined from conductivity measurements. Also discussed were Osmetech who have a method based on the amperometric detection of ferrocene labels in conjunction with an elegant self-assembled monolayer.

Another electronic-based DNA detection technology is the detection of magnetic beads to which DNA is attached. These are being developed by several groups including the authors' SNiP2CHIP project. The spin valve sensor is the most common type of what are known as "giant magnetoresistive" sensors and is the sensing transducer used in current hard disk drives. The electrical resistance of such a sensor is dependent on whether its

| System | Integrated DNA prep | Integrated PCR | No. of channels | No. of positions on array | Assay time | Assay format | Detection method | Format |
|---|---|---|---|---|---|---|---|---|
| Cepheid GeneXpert | yes | yes | 4 | na | 30-40 min | QF-PCR | fluorescence | desktop |
| CombiMatrix Electrasense | no | no | 1 | 12544 | 5 hours *D* | microarray | electrical | mid sized |
| Directif Lab on a Chip | yes | yes | ukn | 99 | 2 hours | microarray | electrical | desktop |
| GeneOhm ePlex | no | ukn | 20 | 20 x 96 | ukn | microarray | electrical | desktop |
| Idaho Technology RAZOR | no | yes | 1 | na | 30 min | QF-PCR | fluorescence | handheld |
| Integrated Nano Technologies | yes | not required | 1 | ukn | 20 min | microarray | electrical | ukn |
| Ionian Technologies | ukn | not required | ukn | ukn | ukn | ukn | ukn | handheld |
| IQuum Liat Analyzer | yes | yes | 4 | na | 30 min | QF-PCR | fluorescence | handheld |
| Nanogen NC400 | no | no | 2 | 400 | variable* *P,D* | microarray | fluorescence | desktop |
| Nanomix 'Sensation' technology | no | no | 1 | ukn | ukn | microarray | electrical | ukn |
| Nanosphere Verigene system | no | not required | 1 | ukn | <35 min *D* | microarray | densitometry | desktop |
| Osmetech eSensor | no | no | >1 | 36 | 20 min *P,D* | microarray | electrical | desktop |

**Table 2. The most critical points of the common technical specifications of the IVD directive for POC genetic testing devices. A summary Table of current, and emerging genetic analysis systems; adapted from reference [2]. Key: P: not including PCR, D: not including DNA/RNA prep, B: excluding PC, C: roughly, ukn: unknown, na: Not applicable.**

constituent layers are magnetized in a parallel or antiparallel orientation. This can be influenced by an external magnetic field when magnetic materials with different resistance to polarization by such a field are used [8].

The methods of DNA detection just discussed (both fluorescent and electronic) are complicated by the requirement for DNA labelling. One of the first to suggest that is should be possible to read unlabelled and unsorted nucleic acids directly were Kasianowicz et al. in 1996. Most attempts at the development of this technology use an apparatus that measures the conductance change between two compartments separated by a nanopore through which the DNA is electrophoretically driven. The conductance changes subtly as a result variation in size of the DNA bases and therefore, as a result, the sequence can be discriminated. The nanopores must be around 2nm is diameter to ensure that only one DNA strand can fit through the pore at once; frequently the bacterial pore protein α-hemolysin is use. However, non-biological pores have also been used.

One group attempting to directly read DNA sequences, and the only one so far to have entered the Archon X PRIZE for Genomics, is Reveo. A talk given by the Reveo CEO, Sadeg Farres, at Nanotech 2006 went into some detail of the technology.

In short, DNA detection relies on the measurement of a current produced when nucleotides base pair to a DNA strand being sequenced. The strand is electrophoretically stretched and is approached by an array of probes which have a nozzle with a sub nanometre aperture through which the nucleotides may be dispensed. The probes must be moved with sub Angstrom motion which is to be achieved using piezoelectric actuation. There is no doubting the technological expertise of this group: one of Dr. Faris's previous inventions is what is claimed to be the world's fastest oscilloscope, the Pico-second Signal Processor (PSP-1000).

## Conclusion

A current generation of PCR-based instruments for detecting genetic variation at the point-of-care is currently on the market and is being targeted towards the identification of pathogens. There are good reasons for targeting the devices towards this market, as there is currently insufficient clinical data to enable widespread PGx use. A great deal of research is currently under way both in private companies and in academia in order to understand the PGx relationship between genetic variants and drug response. A second generation of instruments able to determine a greater number of genetic variants is also nearing the market and these will increasingly use electronic detection methods. □

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# A New Dimension in Vaccine Development – Adjuvant Systems

TEXT NATHALIE GARÇON

For over 80 years vaccine adjuvants have been known for their ability to increase the immune response against a given antigen, as first demonstrated by G. Ramon in 1926 [1] and later by P. Glenny [2]. With the emergence of new diseases and new breakthroughs in immunology there is now a need for more tailored adjuvants/antigens formulations: (i) to better target the effector response (humoral and cellular); (ii) to induce long-term persistence of protection with a higher level of immune response as well as an improvement of the immune memory; (iii) to overcome a weakened immunity, as seen in immunoscenescence and immunosuppression; (iv) and to allow for immunomodulation.

**INFO**

*Nathalie Garçon* received her Pharm.D degree in 1982 and PhD (immunotoxicology, immunopharmacology) in 1985, after which she spent one year in England as a postdoctoral research fellow, studying liposomes in vaccines at the Royal free Hospital in London. She then spent 4 years in the United States (Baylor College of Medicine, Houston Texas, USA) first as postdoctoral research fellow, then as assistant professor, working on vaccine delivery systems and immunopotentiators. Dr Garçon joined SmithKline Beecham Biologicals –now GlaxoSmithKline- in 1990 where she set up and lead the vaccine adjuvant and formulation group. She is now vice president, head of research and north America R&D.

Classical adjuvants – such as aluminium salts – have demonstrated safety and efficacy in humans for decades. As a result, there are now numerous aluminium-based vaccines. Additional classical adjuvants such as emulsions, liposomes and virosomes have also been licensed for use in human vaccines.

## Developing Adjuvant Combinations

Today, thanks to improvements in immunological and biochemical tools, scientists can now draw on a large variety of classical adjuvants and new immunomodulator molecules ("immunomodulators"), which may be used to improve antigen-specific protection for a given population. At present dozens of new adjuvants are being evaluated in vaccines intended for human use. A few examples of novel immunomodulators include CpG-containing oligodinucleotides (CpG), monophosphoryl lipid A (MPL), and QS21.

While these novel compounds may be used as standalone adjuvants, vaccine developers are also looking at ways to combine classical adjuvants and immunomodulators in order to optimize the immune response. One such combination, which serves as a next step in this evolution process, is the immunostimulating complex ISCOM. Considered to be a single adjuvant but possessing multiple properties, these cage-like complexes are made up of cholesterol, lipid, immunogen, and saponins.

Further, a better understanding of immunogenicity pathways (e.g. the interactions of innate and adaptive immune pathways) has opened the door to greater rational design of vaccines using adjuvants chosen for the synergistic effects achieved through their combination. With a growing number of novel adjuvants to choose from, one of the greatest challenges for vaccinologists is finding the best-suited combination to achieve an optimally effective and safe formulation in which each part of the vaccine - antigen and adjuvant(s) - works together to produce an appropriate and lasting immune response.

## Adjuvant Systems – The GSK Biologicals Experience

GlaxoSmithKline Biologicals (GSK) has devoted more than 15 years to the study of adjuvants and has pioneered the development of novel adjuvant combinations. The company currently maintains a broad portfolio of proprietary Adjuvant Systems and the following examples serve to illustrate their added value in vaccines and vaccines candidates that have been designed for a specific pathogen and/or target population.

### *AS04-formulated vaccines*

The Adjuvant System AS04 consists of the immunomodulator MPL, a stimulant of the immune system, adsorbed on different aluminium salts. AS04 has already been evaluated in various vaccines and vaccine candidates intended to protect against viral infections/diseases including herpes simplex viruses, hepatitis B, the Epstein-Barr virus and human papillomaviruses (HPV).

The first AS04-formulated vaccine approved for use in humans is hepatitis B surface antigen (trade name: FENDrix™), developed specifically for patients with end stage renal diseases with a high risk of hepatitis B infection. This tailored hepatitis B vaccine adjuvanted with

**TABLE** **Adjuvanted Vaccines Licensed for Human Use**

| Adjuvant | Description | Selected Examples |
|---|---|---|
| Aluminium compounds | Aluminium salts (e.g. aluminium hydroxide or phosphate). These are the most widely used adjuvants in human vaccines today. | Diphtheria, pertussis, tetanus, and HBV, HAV vaccines, etc |
| MF-59 | Microfluidized oil/water emulsion, including squalene and surfactants Tween 80 and Span 85. | An influenza vaccine for elderly licensed in parts of Europe |
| Virosomes | Immunopotentiating reconstituted influenza virosomes (IRIV) – influenza H1N1 surface glycoproteins intercalated in natural and synthetic phospholipids | Two examples include an hepatitis A vaccine registered in several countries around the world, and an influenza vaccine |
| Exotoxins | Bacterial ADP-ribosylating exotoxins (bAREs) | The only licensed bARE adjuvanted vaccine was an intranasal virosome-based influenza vaccine (since withdrawn) |
| AS04 | An adjuvant system consisting of aluminium salt and MPL, a purified, detoxified derivative of bacterial lipopolysaccharide. | An HBV vaccine for haemodialysis subjects and an HPV, cervical cancer vaccine licensed in Australia and submitted for licensure in other areas of the world. |

AS04 allows the induction of higher specific antibody titers, enhanced cell-mediated responses, and increased seroprotection rates, with fewer vaccine doses needed compared to the classical hepatitis B vaccine adjuvanted with aluminium salts only. Clinical data also suggest that protective antibody levels persist longer with the AS04-adjuvanted vaccine with less boosters needed. [3].

Now approved for use in Australia and awaiting approval elsewhere, a cervical cancer vaccine that targets HPV types 16 and 18 (trade name: Cervarix™) is an AS04-formulated vaccine indicated to prevent persistent oncogenic HPV infection that could lead to cytological abnormalities and cervical cancer. The AS04 containing vaccine induces a stronger and more sustained immune response than the same virus antigens formulated with aluminium hydroxide alone, as manifested by a rapid and vigorous onset of antibody production that persists at a high level over time. In addition, the AS04 adjuvanted vaccine induces not only high antibody levels but, more importantly, functional antibodies, as evaluated by their long-lasting virus neutralization capacity [4].

The AS04 formulation has been evaluated over 15 years, involving over 30,000 subjects. The AS04-based vaccines are generally well-tolerated, but solicited local symptoms are usually reported at a higher rate than with alum-adjuvanted vaccines. This, however, may reflect a stronger immunological stimulation, with higher involvement of cell-mediated immunity that is induced by AS04 adjuvantation.

*AS02-formulated vaccines*

The AS02 Adjuvant System is the combination of an oil-in-water emulsion with MPL and QS21. The latter is an bark of a South American tree, Quillaria saponaria. Originally developed for use in a malaria vaccine candidate, AS02 has been used in a number of other vaccine candidates, where a strong T-cell response is needed to obtain effective protection – including HIV, tuberculosis and cancer immunotherapy.

Development is ongoing on an AS02-formulated malaria vaccine. Malaria, a major health problem in endemic areas, is caused by multi-stage protozoan parasites of the genus Plasmodium, with P. falciparum being responsible for the most severe disease and accounting for the highest mortality (1 child dies every 30 seconds from malaria infection). AS02 was selected for the candidate malaria vaccine in order to: (i) generate an effective antibody response; and (ii) elicit a cell-mediated immune response in order to interfere with the intra-hepatic stage of the parasite. The AS02-adjuvanted vaccine is the only clinically evaluated vaccine candidate to date that has been shown to elicit a protective immune response against P. falciparum infection and prevent disease in children living in malaria endemic regions [5]. Several additional phase 2 trials are now ongoing in various African countries to evaluate a number of vaccine parameters including the vaccine candidate's performance in younger age groups. A large multi-centre phase III clinical trial is planned to start in late 2008, in 8 to 10 sites across sub-Saharan Africa.

In clinical studies performed to date, the AS02-adjuvanted vaccines have been well-tolerated, with the most frequent solicited local adverse events being mild to moderate swelling and pain at the injection sites and the most common general solicited adverse events being mild to moderate headache, fatigue and myalgia.

*AS01-formulated vaccines*

Other vaccines may need different immunomodulators in order to induce effects other than those provided by the AS02 or AS04 formulations. The AS01 Adjuvant System has been developed using an alternative formulation based on the combination of liposomes, MPL and QS21.

To date, the immune response induced by the current AS02-adjuvanted candidate malaria vaccine allows for an unprecedented protection against P. falciparum infection and malaria clinical disease, bringing hope for a new tool in our preventative armoury. To further increase the immune response and protect a higher percentage of people, the AS01 Adjuvant System has also been evaluated in the malaria candidate vaccine. A comparative challenge study in humans has demonstrated the superiority of AS01-adjuvanted candidate malaria vaccine when compared with the AS02-adjuvanted candidate vaccine in terms of antibody titers, T cell-mediated immunity, and protection level. Based on these results the AS01 formulation is now also being evaluated in field studies, both in adult and paediatric populations.

Pre-clinical safety evaluations have demonstrated a safety pattern similar to that observed with other Adjuvant Systems.[6] To date, AS01 has been administered to a limited number of clinical trial volunteers, including to young children, and the overall safety profile appears similar to the profile of AS02 containing vaccines. Further clinical evaluation will be needed to confirm the safety and tolerability profile of this Adjuvant System.

## Conclusions

The development of adjuvants has progressed from a primarily empirical based approach to an increasingly rational design, largely enabled by advances in our understanding of the immune system and the improvement of analytical, chemical and immunological tools. It is now possible to combine a specific adjuvant system with the most appropriate antigen with the objective to design effective vaccines that are expected to provide an immune response tailored to the pathogen and to the target population. Persistent unmet medical needs demand increasingly sophisticated vaccine design. One area for improvement is in the development of vaccines that are designed for elderly populations, those whose immune systems are frequently less responsive to vaccination than younger populations. The emerging area of therapeutic vaccines, which are designed to treat, rather than prevent disease, is another example where novel adjuvants and/or combinations of adjuvants may be needed to elicit an antigen-specific immune response that would be capable of halting the disease process. These and many other areas of unmet medical need may one day be met through advancements in vaccine adjuvant science. □

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# Current Limitations and Future Prospects for Applying Systems Biology in Drug Discovery and the Clinic

Most Systems Biology scenarios advocate the use of data recently delivered by post-genomic technologies (microarrays, proteomics, etc.), but it is also important to consider data generated during the past decades of molecular biology, biochemistry, pharmaceutical and clinical research. The technical challenge is to somehow combine data sources as diverse as the scientific literature, DNA microarrays, high-throughput screens, toxicology studies or clinical trials to make usable predictive systems.

GORDANA APIC, ROBERT B. RUSSELL | TEXT

Systems biology is the buzzword of the moment. Like all buzzwords it is highly fashionable, promises a great deal, and nobody is really sure what it means. Most, however, would agree that it describes «the next big thing», and that it largely constitutes a synthesis of years of research in order to arrive at usable, predictive models of biological systems. Drug discovery is no exception. One can scarcely attend a conference without hearing something about Systems Biology and how it might be applied to different aspects of the drug pipeline, from target selection, through lead design and into toxicology and the clinic. However, there are a number of technical and organisational hurdles that will probably need to be managed before its promises can come to fruition.

**Combining heterogeneous data sources pursuing multiple goals at different points in time**

Modelling requires data, and models of biological systems need a great diversity of data from many different experiments. Most Systems Biology scenarios advocate the use of data recently delivered by post-genomic technologies (microarrays, proteomics, etc.), but it is also important to consider data generated during the past decades of molecular biology, biochemistry, pharmaceutical and clinical research. The technical challenge is to somehow combine data sources as diverse as the scientific literature, DNA microarrays, high-throughput screens, toxicology studies or clinical trials. Even if this runs seamlessly, one has to deal with the hard fact that there are almost never sufficient data available to model a biological system fully, and most everybody would agree that it will take decades for a near complete dataset to be available. This means that one must adopt innovative solutions that can cope with missing data to make predictive models that are useful now.

It is clear that one can't make the same mistakes that Bioinformatics made during the past decade. It too promised a great deal, most notably in large, integrative systems that promised to be something of a panacea for managing data in the pharmaceutical industry. However, for the most part these systems were obsolete before they were finished, and almost always ran into problems when new innovations came along. Whatever systems are in place need to adaptable, and need to adapt quickly. Probably a greater degree of modularity is the key – modu-

**INFO**

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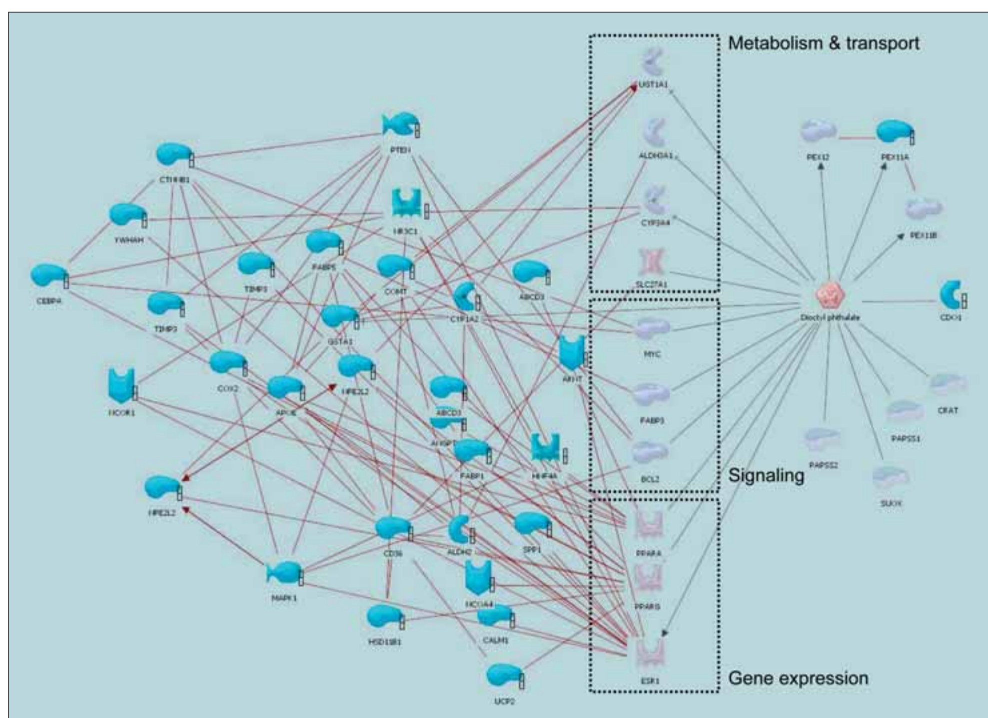
*Robert B. Russell* (1968) received a B.Sc. (hons) in Chemistry from Queen's University, Kingston, Ontario (CA), and a D.Phil. in Biochemistry from the University of Oxford (UK) in 1993. For two years he worked at the Laboratory of Molecular Biophysics, Oxford, to move on to the Imperial Cancer Research Fund, London, in 1994. From 1997 until 2000 he was Senior Investigator Bioinformatics with SmithKline Beecham Pharmaceuticals (UK). He joined the European Molecular Biology Laboratory in 2001 where he is today Group Leader, Structural Bioinformatics. Rob Russell retains numerous scientific and professional positions. He is Editor of PLoS Computational Biology, and FEBS Letters, and serves as a consultant to several companies, e.g. Celera Genomics (US), Cellzome (DE) and Novexin (UK).



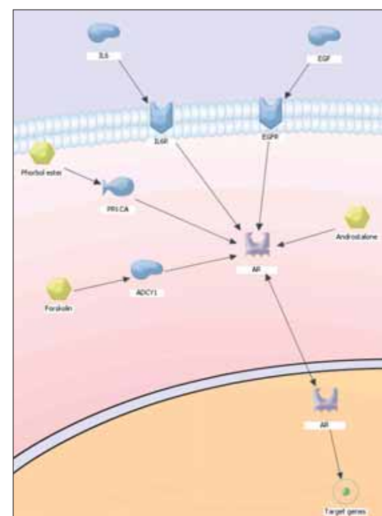

**Relating microarray data to chemistry via protein-chemical interactions**
**Selection of genes affected in Rat liver affected upon treatment by diethylhexyl phthalate (blue). Protein-chemical interactions extracted form the literature show that the chemical likely induces changes in gene levels by first binding to a nuclear hormone receptor (PPARA/G or ESR1), which is, in turn, responsible for turning the genes on or off in the living system. Classes of proteins/genes interacting with the chemical and other genes in the microarray study are boxed. Microarray datasets are from the EDGE (Environment, Drugs and Gene Expression) database; protein chemical interactions and data integration/visualization was performed using ToxWiz (Cambridge Cell Networks Ltd., UK).**

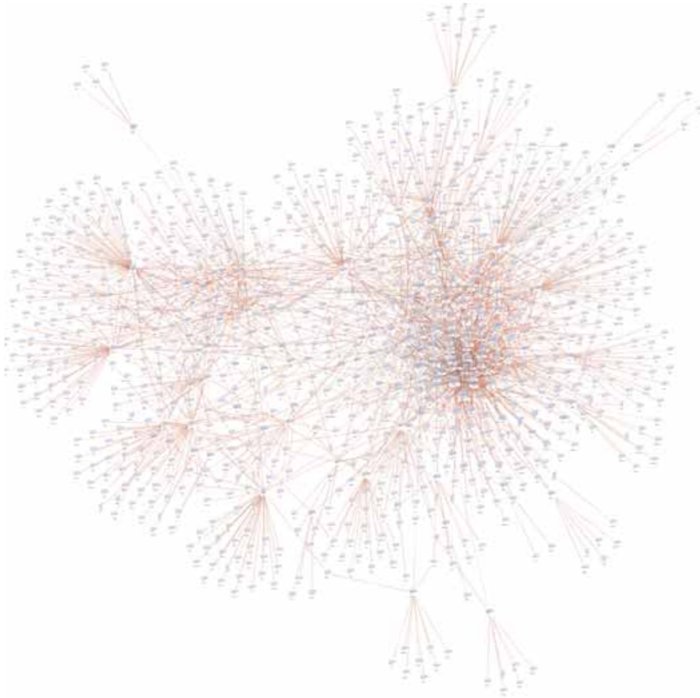
lar systems that communicate with one another, and which can be readily adapted without being overly disruptive.

Nevertheless, better systems for data integration might just overcome some of the perceived failures of 'omics' technologies, for example microarrays or proteomics – many of which similarly promised a great deal for drug discovery, and often came up short. Microarrays, for example, when used in isolation suffer from problems of disease heterogeneity, limited sample availability and experimental variation that can lead to strange or ineffective biomarkers. They, like many 'omics'-technologies, also suffer from an 'everything and nothing phenomenon' – providing too many data points to be understood; the answer to the question is probably there, but it is difficult to see through a fog of data. Almost anybody with experience now advocates the use of a modicum of prior knowledge to make full use of these and other similar technologies (e.g. Russo et al, 2003; Ein-Dor et al, 2006). Even the simplest example shows that this makes very good sense. If one wishes, for instance, to use microarrays to study the molecular basis of response to a toxic chemical, then incorporating prior knowledge in the form of protein-chemical interactions can provide mechanistic suggestions very quickly (e.g. Figure). Simple integration of data can thus prove very rewarding. Combining omics datasets both with each other and the abundance of prior knowledge from the literature or generated inside companies will clearly make them more effective in delivering on their promises.

### Modern computational tools – current and future

Modern computational tools that allow systems to be perused and studied are in abundance. There are now pathway analysis tools & datasets available from several companies (e.g. Ingenuity Systems, Cambridge Cell Networks, Genego) and academic sites (e.g. Reactome, KEGG, Biocarta, Cytoscape). The fact that these cover a wide range of processes and often contain genes, proteins in addition to chemicals, permits people in different parts of an organisation to understand things faster, and to speak the same language to some extent. There are also a number of software packages that allow systems to be modelled and thus predictions to be made. The E-cell and Silicon Cell projects, for example, are international consortia attempting to create usable models of biological processes, and such models have met with some success in certain applications (e.g. Nakayama et al, 2005; Hornberg et al, 2007). However, these applications can require a more complete set of parameters than is often available for many systems. Missing data is not always a critical issue as it is increasingly possible to use simpler networks directly to make predictions. Most often these predictions are of biomolecular interactions that have not yet been reported (e.g. Linding et al, Cell, 2007), but methods are emerging that use networks directly to predict macroscopic phenomena, such as toxic endpoints (e.g. ToxWiz). These concepts show certain parallels with internet sites such as Amazon, which





can, for example, predict book preferences despite not understanding everything about each customer.

### Beyond in silico

The management challenge is how to get people, who traditionally have been rather insulated from one another, communicating without experiencing a breakdown in efficiency. A prominent aspect of Systems Biology is that it is probably the most interdisciplinary subject ever created. It requires a greater degree of coordination not just between laboratory subjects as diverse as molecular biology and biophysics, but across entire disciplines that don't have the best history of communication: biology, chemistry, engineering & mathematics. This means that integrated systems have to cope with not just data diversity, but often baffling differences in terminology. At the same time, management must cope with getting people at different parts of a traditionally partitioned pipeline to communicate more continuously. In order, say, to select targets or lead molecules in the context of a biological system, it might be necessary to get early stage discovery scientists engaged with toxicologists and even clinicians. To some extent, here too, modular systems are needed. Traditional departmental or division barriers can't be too strictly enforced if such multi-disciplinarity is to be achieved. Several of the larger pharmaceutical companies have introduced flatter, project-based organisational structures and are promoting multi-disciplinarity throughout discovery projects.

So how do we address these challenges? Perhaps we might not need to look any further than the internet. The term Web 2.0 describes the new, more interactive and intelligent world-wide web, and in many ways it is the direct internet parallel to Systems Biology. It too suffers from being consider hype, lacks an agreed upon definition, but nevertheless it too is also something that everybody acknowledges to be the next best thing. Recent years have witnessed the birth of some of the most impressive internet sites ever, from intelligent customer suggestions in Amazon (mentioned above), to the breathtaking speed with which wikipedia has seemingly replaced traditional encyclopaedias with those written dynamically by a network of thousands of contributors. Wikis are among the very best ways of capturing what sits inside the heads of clever people, permitting them to define and refine not just what is inside the system, but how it is represented and its relationships to other data. It is fitting that perhaps Web 2.0 holds some of the answers to the Systems Biology challenges. Integrative, intelligent systems capable of seamless communication between different people, in different sites, generating different data, with different problems and using different terminology, are probably just what are needed. □

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# A Dazzling Horizon for the Vaccines Industry

TEXT **MARC REINHARDT, JEAN-FRANÇOIS GOUZER**

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After having been overlooked for decades, vaccines have revived as a major growth opportunity and companies have considerably increased their investment in this field. Not many in the pharmaceutical industry may have predicted such a turnaround. Up to the late 1990s, vaccines were far less attractive than most other pharmaceutical segments: Returns achieved with vaccines were generally lesser than those generated with small molecules, in particular when compared to 'me-too' products. This mature product group, protected by a limited set of technologies, would not generate significant revenues anymore. Government control was strict on prices and in some cases on manufacturing capabilities. And countries that needed vaccines most could only afford small volumes as local payers could not subsidise and deliver large immunization programmes. Investment in the vaccine industry was limited, and consequently, technological and therapeutic innovation lagged behind other pharmaceutical sectors.

**Revived innovation has woken up the vaccines market.**

The value of vaccines has dramatically risen as new vaccines have been replacing older generations, and innovative products have been addressing unmet medical needs. The increase in pricing in high-income markets over the last two decades was spectacular. The case of diphtheria, tetanus, and pertussis immunization is characteristic as its price rose from few dozens of cents in the 1980's to now USD 20, partly due to the new acellular pertussis antigen. Vaccine manufacturers are now taking advantage of their innovations, with examples of vaccines such as Wyeth's Prevnar® or Merck's Gardasil® priced above USD 300 per immunization. As a result, the vaccine industry is now cataloguing its first blockbusters: Prevnar® was the first vaccine to rank among the best launches for a major pharmaceutical company, and positioned itself in a few years among Wyeth's top sellers with revenue nearing USD 2 bn in 2006. And a number of blockbuster candidates are anticipated to emerge by 2010 in indications spanning cervical cancer (HPV), rotavirus, meningitis prophylaxis, pneumococcal disease, and herpes simplex.

**Industry consolidation and global market opportunities have permitted scale multiplication.**

Consolidation of the vaccine industry into an oligopolistic structure has strengthened its economic competitiveness. Mergers and acquisitions have concentrated local and specialist companies into five key global players with large-scale manufacturing and marketing capabilities: GlaxoSmithKline, Merck, Sanofi-Pasteur, Novartis/Chiron, and Wyeth. These companies have reached a critical size to achieve significant returns on vaccines, and have seen the profit margins of their vaccine divisions rise significantly to compare with those in their core pharmaceutical businesses. As the therapeutic and cost effectiveness of prophylactics for many preventable diseases became evident, public and non-profit institutions worldwide have taken action to reshape the vaccines landscape. As a result, the economics of developing and dispensing neglected vaccines have improved and a global vaccine market has emerged. In high-income countries, the public sector has been taking substantive steps to speed up the development of new vaccines and to strengthen the capacity for existing ones. While pandemic flu initiatives and bioterrorism encourage private-sector investment, public institutions have established long-term procurement programmes, and increased subsidies made available for R&D. In lower income countries, public and non-profit supports have secured sustained assistance to vac-

Mercer, 2006

| 2006 | 2007 | 2008 |
|---|---|---|
| **Rotavirus** GSK (Rotarix) | **HPV** GSK (Cervarix) | **S. pneumoniae** GSK (Streptorix) |
| **Rotavirus** Merck (RotaTeq) | **Meningitis P.** GSK | |
| **Shingles** Merck (Zostavax) | | |
| **HPV** Merck (Gardasil) | | |

**Major current vaccine launches**

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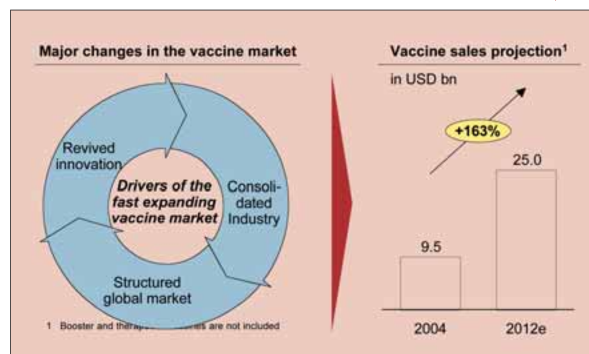
cine procurement and dispensing in lower-income countries. This led to the creation of a viable and attractive market. The non-profit sector has fostered public-private partnerships to ensure vaccines penetration in low-income countries. For example, GAVI and the Gates Foundation partnered with GSK in the Malaria Vaccine Initiative to develop a malaria vaccine. These public and philanthropic initiatives have introduced policies that stimulate investment and therapeutic innovation by sharing or taking over the burden of expensive and risky vaccine development programmes.

### The horizon is clear for this fast growing segment.

The confluence of a technology push and a market pull has motivated pharmaceutical and biotechnology companies and the specialist investment community to prioritize investments in this segment. The increase of the number of IPOs and attractive deals validate this prioritization, as Intercell's history illustrates. Furthermore increased R&D investments have helped fill the pipelines of the industry. Promising vaccines are being developed in a number of disease areas, including herpes simplex virus, HIV, HPV, influenza, and SARS. In addition, companies are working on defence-related products, such as anthrax and smallpox vaccines.

Bear Stearns, 2006




**Dramatic value and scale changes have boosted the vaccine market.**

As the vaccine market is anticipated to more than double by 2012, vaccines are expected to account for a growing share of the total revenue of the key players. This summer, Merck posted revenues from vaccines of nearly USD 2 bn for the first semester alone, more than the entire 2006 period. Over the prior four years, Merck's vaccine sales were essentially flat with annual sales rounding the USD 1 bn landmark. But last year, Merck received market authorization for three new vaccines, including Gardasil®, the breakthrough vaccine for HPV.

At the same time, an emerging class of biotechnology companies is very active on a number of innovative solutions in the field of vaccines and is also increasingly pushing these closer to the market. These companies have different economics from the big players and often can profitably serve technological or therapeutic niches in a variation of the «specialty pharmaceutical» business model. While many biotechnology companies have taken a leading discovery role as opportunities arise, others are creating new formulations, production methods or delivery mechanisms. Innovative players are also applying vaccine technologies to non-traditional therapeutic areas. The advent of «therapeutic vaccines» could expand the paradigm. Under the heading of «immunotherapy», companies increasingly develop the knowledge and technologies to harness our immune system to fight diseases, even after they have broken out. Since the immune system remains the most potent pharmaceutical laboratory existing, the buzzword of «individualized medicine» gets a whole new dimension in this therapeutic class.

In a quick turnaround, the vaccine segment has become one of the most attractive pharmaceutical industries sector. Market dynamics have been reverted by a dramatic increase in value – driven by new vaccines introduced to replace older generations and innovative products that are addressing unmet medical needs. At the same time, industry consolidation and global market opportunities have permitted a handful of companies with large-scale manufacturing and marketing capabilities to control a growing, well structured market, and an emerging class of innovators with the ambition to partner or even compete on niche areas. As further opportunities arise in fields of immunotherapy and therapeutic vaccines, the horizon for the vaccines industry is dazzling.

For the last decade, Capgemini has been helping both leading and emerging vaccines players take advantage of these trends along the entire value chain. At the same time, Capgemini is working with Governments and payers to develop effective strategies to leverage these changes and optimize healthcare systems. □

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# The VPM way – Shorter time to market with an attractive portfolio for licencing

Starting from market needs VPM is scouting and managing promising product developments across a broad range of institutions in Germany – to be taken further down the pipeline by industry partners.

TEXT ALBRECHT LÄUFER

VPM

**VPM – a team dedicated to creating value for partners (left to right): Dr. Hans-Heinrich Henneike-von Zepelin (Clinical Project Manager), Dr. Albrecht Läufer (CEO), Dr. Bernd Eisele (CSO), Dr. Jörn Möller (CFO), Dr. Heiner Völk (Project Manager, Manager Business Development), Dr. Leander Grode (Project Manager).**

Innovative academic research still takes too long to reach industry – at least, in the case of vaccines research. In recent years well-minded enrepreneurs and university technology transfer agencies have indeed sped-up translation, but not enough. A central limitation is the focus on an individual firm resp. a project from an individual institution. Vakzine Project Management GmbH, VPM, has another approach. VPM starts from market needs and looks then for product developments across a broad range of institutions in Germany.

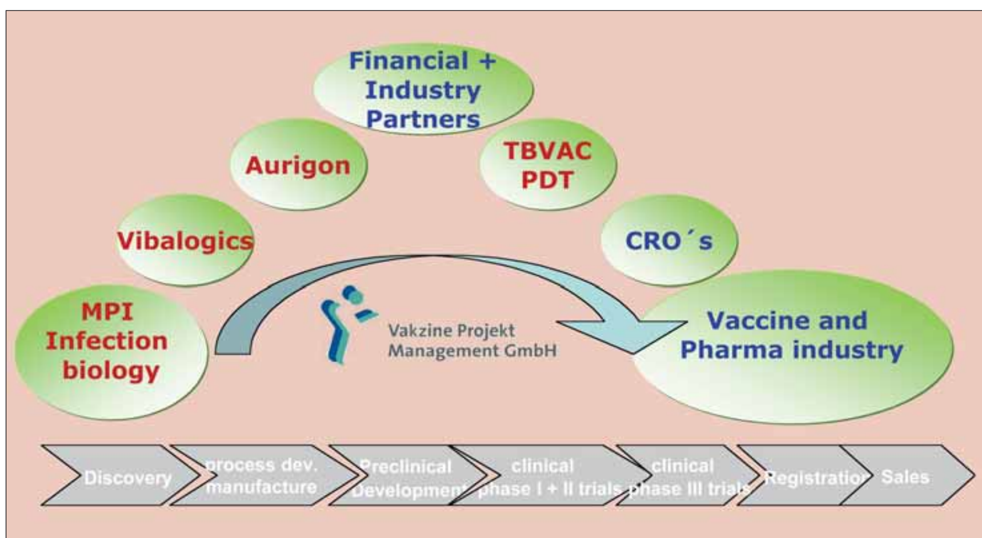


**Figure 1: Partnering with experts along the value chain of development**

A database of vaccine projects in Germany, more than 180 projects in around 50 institutes, has been established as a screening tool, the «German Vaccine Database». This database is continuously updated to provide benchmarks for screening and selection of commercially most interesting research. Projects are evaluated with respect to patents (applied for, granted), medical need, market potential (i.e. market for outlicencing of phase I or II results) and development costs and time. The most promising projects are then licenced from the academic institution and then process development, manufacturing, preclinical and early clinical developent start.

### Development Experience at Work

Experienced pharma executives work with VPM committed to create value

from the beginning. Since VPM was founded in 2002, they have put together an an attractive product portfolio for vaccines and biopharmaceuticals. At issue is steering preclinical and clinical development of its existing pipeline as well as looking at potential new compounds for in-licensing. As part of its business concept VPM is outsourcing most of the functions of development. This does not bind capital in fixed assets and allows the company to flexibly manage its burn rate and enhance the potential return for its investors.

Currently, collaboration and service contracts exist with more than 20 research institutes and companies, some of them being represented in this issue. This allows VPM to maintain a very lean team.

#### Viable Assets ready for Industry Partners

Today, viable assets have been created and are further readied for further development by industrial partners:

- One clinical-stage immunotherapeutic against prostate cancer, VPM4001:
  - → Strong Phase II data with 81% prolongation of PSA doubling time and 73% responder rate in progressive hormone refractory patients.
  - → Billion dollar potential market.
  - → License from Memorial Sloan Kettering Institute, New York.
- One preclinical-stage biopharmaceutical: Soluferon®, a 2nd generation beta-interferon with better bioavailability for better effectiveness and tolerability
  - → Ideal life cycle extension product to branded beta-interferons.
  - → IP protection until 2018.
  - → Billion dollar potential market.
  - → License from Fraunhofer Society, München.
- One preclinical preventive vaccine against tuberculosis: VPM1002
  - → One of the most promising live recombinant Tb vaccine candidates poised to replace BCG.
  - → IP protection until 2018.
  - → License from Max Planck Society, München.
  - → Huge medical need, sizable market.
  - → To enter phase I end 2007 / early 2008.
- An attractive pipeline of preclinical vaccine products and
- A unique database on vaccine projects, called the German Vaccine database, available upon request free of charge.
- Experienced product development team with deep expertise in development of immunologicals.
- Strong management team with track record in international clinical development and successful international pharma operations, in both vaccines and therapeutics.
- Strong network of research and development partners for each product.

VPM4001 is a novel prostate cancer immunotherapeutic directed at hormone-refractory prostate cancer. It has the potential to access a significant percentage of worldwide market for prostate cancer therapies.

VPM4001 is a proprietary allogeneic immunotherapeutic consisting of γ-irradiated human LNCaP cells that have been genetically modified to permanently secrete interferon-γ and interleukin-2 (LNCaP/IL-2/IFN-γ). Interferon-γ enhances presentation of tumor antigens, whereas interleukin-2 stimulates T cells. In open-label Phase 1/2 trials in thirty progressive, hormone refractory prostate carcinoma (HRPC) patients, clinical proof of concept (PoC) was demonstrated. VPM4001 showed an 81% increase in median doubling time of PSA (prostate specific antigen), as seen in Fig. 2 and a median survival time of 981 days. 73% of the patients responded to the treatment. VPM4001 was well tolerated in this study. The study was carried out by Prof. Bernd Gänsbacher at Technical University München. VPM enjoys continued good collaboration in research concerning VPM4001 with Prof. Gänsbacher.

Soluferon® (1) is a novel and proprietary synthetic beta-interferon for treatment of multiple sclerosis (MS) featuring improved bioavailability. Nine hydrophobic amino acid residues on the outer surface of the natural human β-interferon molecule have been substituted with more hydrophilic moieties, leading to a 6-fold higher bioavailability providing for higher possible plasma levels and thus potentially for higher effectiveness. Its more hydrophilic moieties make Soluferon® also an ideal candidate for the development of improved galenic formulations for eg sustained release or for inhalative applications. Soluferon® is an ideal life cycle management product for the branded β-interferons and has the potential to capture 25 % or more of the $4 billion current annual market for beta-interferon products, the efficacy of which has been demon-

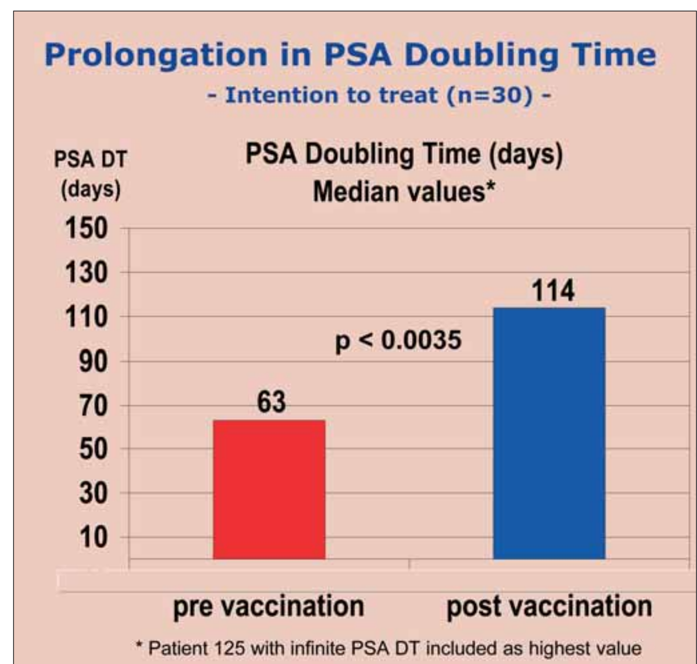

Fig 2. chart prolongation in PSA doubling time

strated over more than ten years in hundreds of thousands of multiple sclerosis patients. By showing equivalence or even superiority with respect to tolerance and side effects of Soluferon® to existing beta-interferons, VPM intends to streamline the regulatory path and speed Soluferon® to the market.

INFO **VPM AT A GLANCE**

VPM is a private company created within the vaccine initiative of the German Ministry of Education and Research to foster vaccine development and commercialization. Shareholders are two non-profit foundations (Deutsche Stiftung Impfstoffforschung, Hannover; Förderverein of Helmholtz Center for Infection Research, Braunschweig) and CEO Dr. Albrecht Läufer. VPM´s task is to bridge the gap between academic science and the industry and to carry out the successful transfer of vaccines and biopharmaceuticals from academic institutions through clinical trials into the market. VPM is seed financed through a € 25.6 million grant from the German Ministry of Education and Research. One objective of this grant is that VPM obtains funding from the private sector through licensing or partnering their projects and will be established as a sustainable "accelerator" for transfer of highly promising research projects from academia to industry. A second objective is to develop a network of collaboration partners for development of vaccines. Third objective is to increase awareness for development principles. VPM has 8 employees and is based in Hannover, Germany.

Soluferon was licensed from Fraunhofer Society, München. Original research for this compound has been carried out at Fraunhofer Institute for Interface and Bio-Engineering, Stuttgart/Hannover, and VPM continues a research collaboration with the institute.

VPM1002 is a recombinant *Mycobacterium bovis* BCG (Bacille Calmette Guerin) which contains a lysteriolysine gene and an urease gene knock-out. Lysteriolysine is a protein derived from listeria bacteria which causes bacteria taken up by phagosomes of the macrophages to pass through the phagosomal walls. This makes the mycobacterial antigens accessable to the MHC class I presenting pathway to improve stimulation of CD8 T cell response which is assumed to be necessary for better protection against tuberculosis infection. Although today a preventive vaccine against tuberculosis exists and is applied since more than 80 years (BCG, Bacille Calmette Guerin) this vaccine only prevents tuberculosis in infants, and not against pulmonal tuberculosis. Thus a much more efficacious vaccine is urgently needed as more than 2 million people die of tuberculosis every year. VPM has licensed this construct from Max Planck Society, where it had been invented at MPIIB (May Planck Institute for Infection Biology), Berlin. There is a research collaboration with MPIIB under which various challenge experiments are carried out. VPM has recently become partner of the European consortium TBVAC, which is carrying out research and development on TB vaccines with over 40 partners under the EU framework 6 program. Successful process development and GMP manufacturing has been achieved together with Vibalogics GmbH, Cuxhaven. The preclinical work is being carried out at Aurigon Life Science GmbH, Tutzing. All partners are shown in Fig 1, which highlights the principle of organization of VPM´s management work.

### The Networks grows

VPM started as part of the initiative of the German Ministry of Education and Research to foster vaccine development and commercialization. In the next few years VPM will transform the public-private partnership and increasingly open up to new investors ready to benefit from growth generated by VPM managed projects. Today, a larger network for collaboration partners for development of vaccines has been put in place in Germany and as part of TBVAC also in Europe.

Much has to be still done. Licence or finance partners for the development products need to be found. The network has to be stabilized with more projects and expanded further. Awareness within academia for development principles has to be raised. The impact of evolving research in the vaccines field has to be discussed among those involved. This is not a task for an individual, but for all interested in creating value in the sector. In October 2007 again, the VPM Vaccine Development Days will bring together experts from academia, process development and manufacturing, analytics, regulatory, financing, to exchange their thoughts and develop new ideas and collaborations in a stimulating conference program as part of the conference program of BioTechnica in Hannover (www.biotechnica.de/vpmdays e). □

**(1) Soluferon® is an internationally registered trademark owned by VPM**

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# GMP-compliant manufacturing of prophylactic and therapeutic Vaccines

**DR. STEFAN BEYER** | TEXT

The solution provider Vibalogics supports companies in designing and developing production processes, and manufactures vaccines and biologics for use in clinical trials. Indeed, the company helps to bridge the gap between discovery and the first clinical trials in humans.

A combination of specific know-how, a high-class facility (typically on a biosafety level 2) and specially trained operators are required to manufacture vaccines. Unfortunately, from the perspective of a small biotech company, access to such a facility and pool of experience is often limited or unavailable and can only be found in mid-sized and large organizations developing, manufacturing and marketing their own products. But this demand is becoming more crucial with regard to process development and GMP-compliant production for early clinical trials as many biotech companies recognize the medicinal and economic potential of prophylactic and therapeutic vaccines.

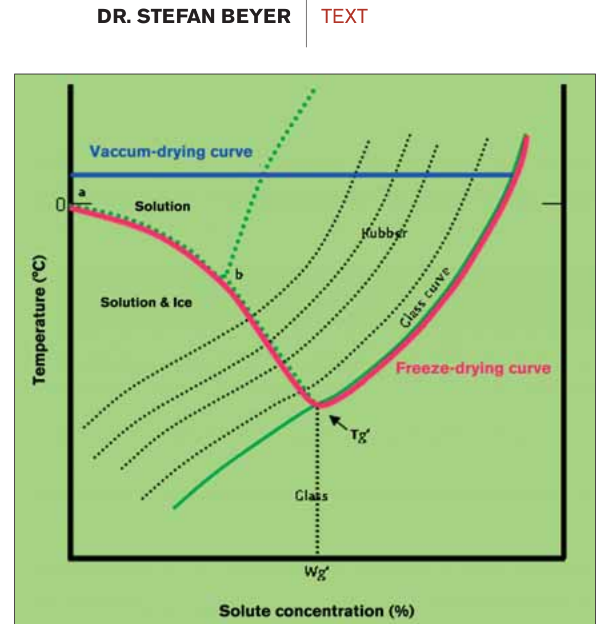
But in fact, Vibalogics is filling the above-mentioned gap! Triggered by the request of Vakzine Projekt Management GmbH for the GMP-compliant production of a novel tuberculosis vaccine, Vibalogics has meanwhile moved to being an established contract manufacturer of clinical quantities for human vaccines.

One challenge often ignored on the long and cumbersome road to developing a new investigational medicinal product (IMP) is the transcription of the laboratory protocols with their proven effectiveness for research purposes into manufacturing and testing instructions and procedures according to the Good Manufacturing Procedure (GMP) requirements. The GMP production process must become robust, reproducible and result in a safe and high quality product with respective biological activity. This cannot only be achieved by paper work alone, which admittedly constitutes a considerable work load, but also sometimes entails extensive laboratory work for both production and analytical testing issues. It commences with choosing certifiable raw materials and does not simply end with the scale-up of the manufacturing process.

Vibalogics operates a GMP facility of 1,100 $m^2$ under biosafety level 2. Inactivated and live vaccines based on bacteria and viruses are produced in three independent manufacturing suites. In addition, a fill and finish suite with a filling line and two lyophilizers allow the delivery of ready-to use IMPs. A very high standard of the central utilities ensures the manufacturing of high quality and safe products.

At a first glance, Vibalogics seems to be just another new service provider trying to establish itself in this highly competitive market of contract manufacturing. But Vibalogics can be simply distinguished from those companies. It offers a more comprehensive service, thus contributing a great deal more to the success of their partners' projects. Vibalogics took over and is still profiting from the long-standing experience of its sister company Lohmann Animal Health (LAH) which is one of the largest poultry vaccines producers worldwide. Even if LAH is active in the veterinary instead of the human product business as Vibalogics is, both companies have to obey the same rules, namely GMP. The close cooperation is particularly obvious in all aspects of quality control and assurance, and for the production of various bacteria. Viruses are manufactured using cell cultures and bioreactors, cell factories or wave bags and working with pathogenic organisms is more the rule rather than the exception. For downstream processing mobile cross-flow filtration units, a continuous ultracentrifuge and a chromatography system are ready-to-use. To keep flexibility and avoid any extensions of deadlines or validation procedures, Vibalogics integrates «single use» equipment and material whenever possible.

Vibalogics continues to focus on its market, i.e. contract manufacturing of vaccines for clinical trials which are based on recombinant and non-recombinant mycobacteria, salmonella and other bacterial strains as well as on attenuated or recombinant viruses used for gene therapy or as oncoloytic acting drugs. Vibalogics partners place great emphasis on competence, frankness and reliability, as does Vibalogics. At the end of the day, this is the nucleus of success! □




**Vibalogics is well trained in the development of freeze-drying and vacuum-drying processes. The phase diagram shows the difference between both processes. The vacuum process could be advantageous for live microorganisms and viruses since material does not freeze.**

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# GENEART – Vaccine Development by DNA Engineering

TEXT RALF WAGNER

GENEART plays a leading role as an integrated solution provider to facilitate early steps in the development of innovative biopharmaceuticals and biomolecules. The outstanding value of GENEART services in the field of DNA vaccine development and DNA engineering results from a unique combination of product and service features: The DNA is produced fast and completely in-house by a fully DIN EN ISO 9001-certified process by simultaneous connection of proprietary customer know how and conceptual needs along with GENEART's sound experience regarding gene and vector design. Optimum gene performance relies upon the proprietary GeneOptimizer™ platform, an optimization tool improving mRNA stability and translational efficacy combined with to the user's individual needs by a proprietary algorithm. The flexibility of GeneOptimizer™ also enables addressing sequence based biosafety issues during the multi-parameter one-step optimization (Fig.1).

Geneart

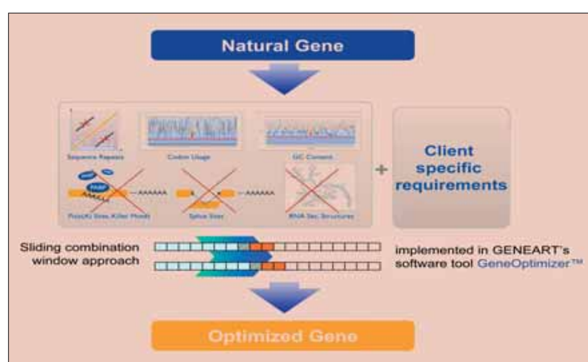


**Figure 1: The art of optimizing genes: a multi-parameter challenge**

## DNA Vaccines

In recent years, DNA-based immunization has proven its potential to be an effective alternative to conventional vaccination. Major economical advantages of DNA as a biopharmaceutical product include manufacturing, characterization, storage and transportation. Important biological benefits arise from a very close mimic of a real infection by endogenous antigen expression after DNA immunization. However, limited access to efficient DNA vaccines against a lot of worthwhile pathogens and diseases is still an issue. Major challenges of vaccine development are the insufficient efficacy of antigen expression, the unavailability of the antigen itself, and biological safety.

## DNA synthesis and engineering open up new routes to efficacy and safety

Being one of the first companies to emerge in this young industry, GENEART has a long track record of comprehensively mastering synthesis and engineering of DNA molecules. The spectrum of GENEART's proprietary technology platforms ranges from the manufacture of RNA- and codon optimized synthetic genes to the production of DNA-based pharmaceuticals, with special emphasis on DNA vaccines. DNA synthesis more and more becomes an essential resource for most DNA-based research applications, and classical molecular biology technology will continually be replaced by outsourcing of DNA engineering and processing. Nearly unlimited design and construction options combined with a high-throughput, high-capacity (2 Mb per month) and fast-turnaround synthesis platform (several kb in only weeks) provide a quick and reliable source for genetic research material at reasonable cost, lifting biological research as well as production of biomolecules to a new level of speed, predictability and efficacy. Today, an increasing number of biopharmaceutical companies rely on synthetically produced DNA for their molecular biology processes in general,

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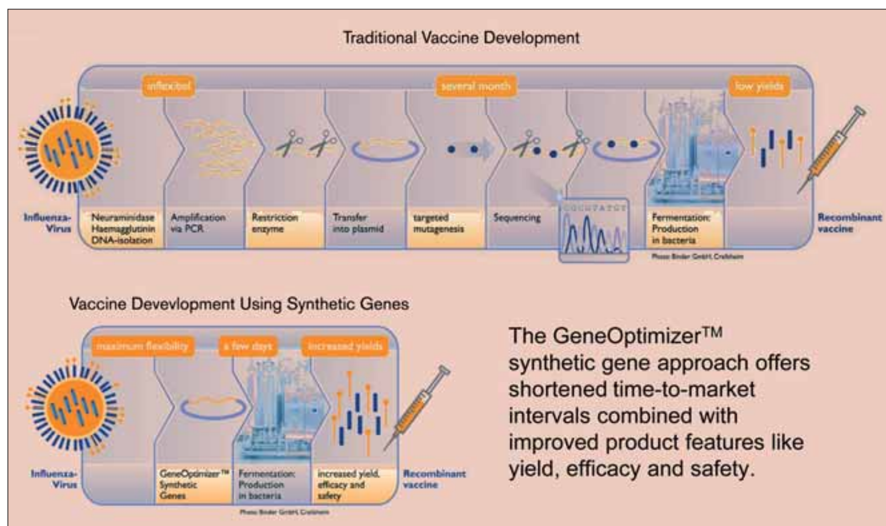


**Figure 2: Improvements by optimized synthetic DNA vaccine development**

GENEART
THE GENE OF YOUR CHOICE

and vaccine development in particular to benefit from shorter time-to-market intervals. In addition, use of de novo synthesized genes allows for maximum level of flexibility regarding gene or vaccine designs (Fig.2).

Challenges to efficacy and safety were addressed by Ralf Wagner, CSO of GENEART, and his lab at RIMMH, University of Regensburg since the late 90s. The research focus was to improve expression of HIV-1 group specific antigen (gag) for a DNA vaccine based on a synthetic DNA template.

The optimized synthetic HIV-1 construct (*syngag*) resulted in a significant enhancement of both humoral and cellular immune response compared to the wild-type sequence when tested in mice (Fig.3). In addition, expression rates increased by an order of magnitude and were clearly related to increased *syngag* mRNA availability (Fig.4), visibly demonstrating the need of considering not just translational efficacy when striving for optimized expression.

Deml et al., J Virol. 2001

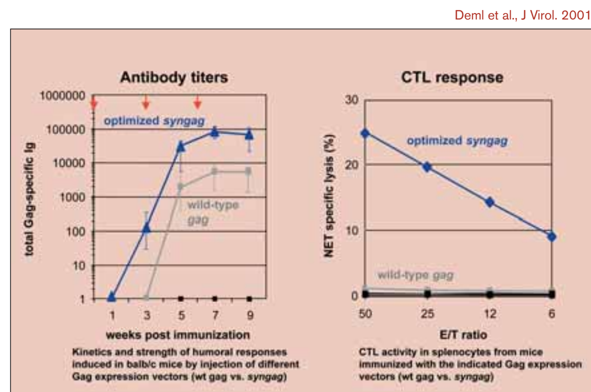


**Figure 3: Optimized HIV-1 syngag: Increased efficacy compared to the wild-type**

The successful outcome of these studies unraveled a novel approach to gene optimization and de novo synthesis, which evolved into the marketable gene synthesis services of GENEART GmbH in 1999. Major enhancements since then have been the switch to in house oligo production by establishing a proprietary and easily scaleable oligo synthesis platform and the coverage of all services by DIN EN ISO 9001:2000 certification.

During the last 5 years, GENEART has broadened its patent portfolio now including IP on novel strategies for autonomous gene evolution in vitro and in vivo, advanced bioinformatic tools for DNA-optimization and viral and non-viral vector platforms. Proprietary or in-licensed techniques as well as completion of the service chain together with strong partners in the field of antibody production and GMP grade DNA manufacturing create an entire freedom-to-operate status, and offer the opportunity to operate as a comprehensive one-stop shop.

Together, these assets have helped to establish GENEART's leading position on this young market by serving academic research groups, renowned public institutions, biotech firms and all of the 20 major pharmaceutical companies and leading firms from chemical industry in eager shares. The extraordinary success and increase in services has led to a growing team of currently more than 150 staff in Regensburg and at the company's sales office in Toronto, Canada.

### A trusted and proven partner

GENEART is a reliable partner – not only for individual firms (Powdermed, Epivax, Polymun, Sanofi-Pasteur and Vaxin to name a few) and reputed science institutions (eg US NIH), but also for targeted national and international research consortia like ForIngen or BioChancePlus in Germany, EUROVACC, THERAVACC, DEC-VAC, HIVAB across Europe, and global networks like the Gates Foundation. Other challenging partnerships serviced by GENEART involve organizations specialized to translate academic research into commercial projects like the International AIDS Vaccine Initiative (IAVI).

GENEART holds a basic patent/-application covering distinct HIV-1 clade C polypeptides and their use as vaccines. RNA- and codon-optimized genes encoding these and related artificial HIV genes were designed, synthesized and provided to EuroVacc for non-commercial vaccine development in early 2000. Since then, several clinical products such as NYVAC-C (New York Vaccinia Virus) and DNA-C have been developed. They were produced according to GMP guidelines within the EU networks EUROVACC II and III through Sanofi-Pasteur and University of Regensburg, respectively, and successfully tested in phase I clinical trials. In brief, these vaccines turned out to be safe and, if combined in a prime boost regimen, extremely immunogenic by inducing cellular immune responses closely mimicking those seen in long term non progressing HIV infected individuals regarding their magnitude and function. Today, partners benefit of GENEART for tomorrow's products. □

Graf et al., J Virol. 2000

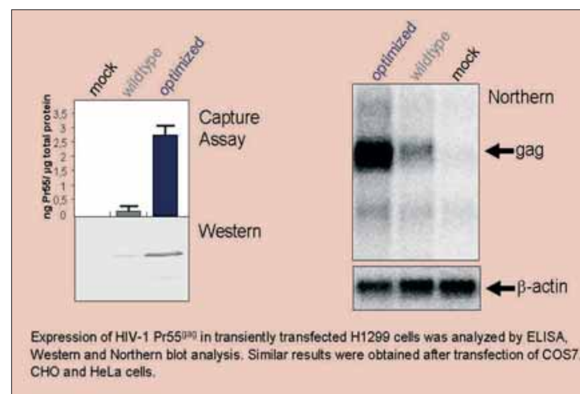


**Figure 4: Optimized HIV-1 syngag: Increased expression rates compared to the wild-type**

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# Innogenetics Research and Contract Manufacturing Services

Innogenetics is a Belgian biopharmaceutical company based at Gent. The Biologicals Group works in ultra-modern facilities and is capable of carrying out Contract Manufacturing Services requiring very special expertise including non-standard projects. Innogenetics' successful formula of facilities, expertise, organization, and quality has repeatedly been leveraged for many blue-chip pharmaceutical companies, and can certainly also be harnessed for your success.

TEXT ANNE VAN BROEKHOVEN

## Facilities and capabilities

Our top-class, Biosafety Level 2 facility is designed for customized special production of clinical products that may need a higher level of containment. In these ultra-modern cGMP units, we can express your (recombinant) proteins of interest in appropriately chosen microbial systems or in mammalian cell lines. We can also assist you in the design and validation of production processes as well. Our multipurpose production areas are designed according to European and US regulatory requirements, where they support and segregate different manufacturing processes simultaneously. Importantly, we can also offer aseptic filling and finishing services for drugs and live vaccines in our BL2 environment. This fill/finish capacity includes filling of either killed, attenuated, or live viruses.

sbA

## Expertise

As a result of having independently manufactured the biological materials needed for our in-house research and clinical studies, we have acquired an enormous amount of cell culture, fermentation, and downstream know-how you can readily use to match your needs. Our track record is truly outstanding evidenced by numerous successfully completed and ongoing biomanufacturing contracts for major U.S. and European pharmaceutical and biotechnology companies. In particular, we have unique expertise in manufacturing protein, plasmid DNA and viruses for vaccination purposes, as well as vaccines based upon polyepitope technology, for either preclinical studies or for clinical supply.

sbA

### INFO

*Annie Van Broekhoven* (1957) received a Ph.D. in biochemistry with a specialty in proteins from the University of Antwerp (BE). She did her postdoctoral studies at the Weizmann Institute, Rehovot (IL) and was a fellow of Leuven University. In 1981 she joined UCL-Fina Research, a consortium between the Catholic University of Louvain-le-Neuve (BE) and Total Fina, NV, Belgium. Since 1993 she is with Innogenetics where she is today Director Biological Operations and oversees development and manufacturing, QC and QA. She is a board member of Belgian Biotech Association Bio.be and a member of the Research Commission of the Belgian Federation of Chemistry. Since 1988 she has been appointed as a part-time professor at the University of Antwerp.

The Biologicals Group also has extensive experience in cell banking and large-scale culturing of keratinocytes, as well as in the production of epithelial sheets for wound treatment.

**Organization**

We have a level of organizational depth that translates into confidence. The advantage for interested pharmaceutical customers is that the efficiency and expertise of the Biologicals Group is backed up by the know-how of Innogenetics' Therapeutics R&D Group.

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This R&D group is responsible for discovery research, genetic engineering, and has long-standing experience in protein expression and purification, protein analysis, analysis of humoral response (ELISA development) and mAb development. It also features specialized know-how in cellular read-out (ELISPOT, CBA), *in vitro* immunological assays (DC based), microscopy (cellular assays and image analysis), analytical assays, pDNA and formulation.

Third parties can contract these technological tools and achieve deliverables for vaccine development and other immunological agents, either as a stand-alone service or integrated into process development and biomanufacturing.

sbA

For its part, the Biologicals Group, carries out optimization, scale-up of the fermentation, purification processes and formulation, as well as method development for in-process controls and for quality controls of the final drug substance and products. This approach ensures smooth process transfer and provide cost-effective, streamlined follow-through for on-time delivery.

**Quality**

Last not least, the unit is cGMP as well as EU 98/81 compliant. It has also been certified by the Belgian Ministry of Health (licence no. 641) and has an ISO 9001/2000 certified quality system. This quality excellence does not only assure compliance with cGMP guidelines, but provides for meeting any cGMP challenges customers may face. Whether you transfer your analytical methods to us, or, we develop the required assays in-house, methods and assays will be checked, qualified, and validated according to appropriate ICH guidelines within this cGMP environment. This allows us to deliver the highest quality of products and services to you.

Our formula – expertise, facility, organization, and quality – has succeeded each time. If you are considering outsourcing an important project in the near future, why not contact us to discuss how our Biomanufacturing Services might be useful to you. Let's talk about your next project! □

**Contract Manufacturing Services Offered at Present**

- Production of proteins in microbial systems such as E. coli, H. polymorpha, P. pastoris, S. cerevisiae, as well as BL-2 strains
- Production of proteins in a wide variety of mammalian cell lines
- Production of recombinant viruses including BL-2 to large scale
- Production of master and working cell banks, storage, stability studies
- Production of master and working virus seeds/banks, storage, stability studies
- Fermentation/cell culture process development, upscaling, and cGMP production - batch fermentation - perfusion - fed-batch fermentation - adherent and suspension cell lines
- Purification process development, upscaling, and cGMP production - plasmid DNA - proteins - monoclonal antibodies – viruses
- Formulation and filling, including live viruses (BL-2)
- Final QC - biochemical and biophysical testing - potency assays
- Storage of produced drug substance/product and stability studies

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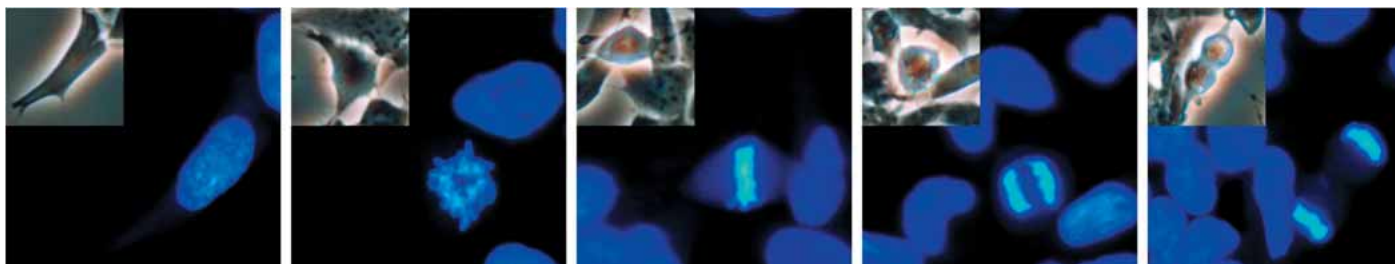

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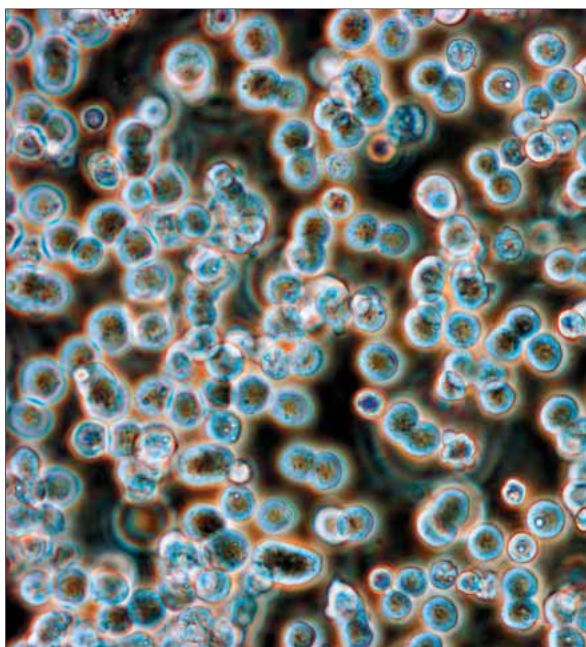
# An Avian Designer Cell Line for Vaccine Manufacture

TEXT INGO JORDAN*, ADRIAAN VOS+, ANDREAS NEUBERT+, VOLKER SANDIG*

Embryonated chicken eggs and chicken embryonic fibroblasts still are important substrates used in the production of human and animal vaccines. They are able to support the replication of a wide range of viruses, including unmodified strains (for production of inactivated vaccines) and attenuated strains (defective viruses that have impaired potential to replicate in human or mammalian cells for application as live vaccines). Generation and maintenance of attenuation usually requires continuous passage in eggs or chicken cells.



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Today, primary chicken cells not only serve as substrate for Pasteur-type vaccines (where life or inactivated pathogen serves as vaccine) but are also considered an essential tool for generation and production of modern approaches in vaccine and vector technology: Highly attenuated poxviruses (fowlpox, canarypox, and modified vaccinia Ankara (MVA)) are being developed to carry only the immunologically relevant genetic information of a given pathogen into the (human) vaccinee. Among the advantages of such an approach is the potential to generate therapeutic vaccines even against chronic infections. However, due to the high level of attenuation these poxvirus vectors require avian cells for replication.

The broad applicability and a long international track record have kept primary chicken cells as production technology alive despite clear disadvantages: like all primary animal cells, chicken embryonic fibroblasts suffer senescence and have to be freshly prepared for each manufacturing run. Maintenance of pathogen-free flocks, requirement for repeated testing, and dependence on cultivation on solid surfaces (typically in the presence of fetal calf serum) complicate the manufacturing process and substantially contribute to vaccine costs.

## Aim and approach

A permanent cell line proliferating in suspension in defined medium free of animal serum would overcome the disadvantages of primary chicken cells. Such a cell line could be derived from avian tumors or spontaneously transformed cells. However, tumor cells are not well defined and bear the risk of transmitting a tumor-inducing agent, gene or virus to the vaccinee.

Therefore, ProBioGen has taken an alternative approach: New cell lines were generated by transfection of defined immortalizing genes into primary cells derived from various tissues of a duck embryo. The flock of origin was established for vaccine production and is certified to be free of or vaccinated against a selected list of pathogens. For transfection, a combination of E1 genes (E1A, E1B 55k and E1B 19K) from human adenovirus type 5 was chosen for pharmaceutically safe immortalization: adenovirus type 5 causes common cold and despite widespread exposure virus or its E1 genes have not been associated with tumors. Indeed, E1A reverses the tumorigenic phenotype of human tumor cell lines and suppresses primary tumor growth (for example, see review by Frisch and Mymryk, 2002). E1B genes are unable to transform cells in the absence of E1A. The inserted genes represent

approx. 11% of the genomic DNA of adenovirus. No adenovirus is released by the cell nor can such a virus be rescued from these cells.
Our approach is consistent with the «defined risk» guidelines issued by the FDA for generation of new cell substrates for application in the production of live vaccines because the immortalizing genes are known and traceable.
Of the multiple cell lines established, two were shown to support the replication of MVA particularly well. These cell lines originated from somites (early body segments) and retina and were stabilized by continuous cultivation for over 2 years. All cells have maintained the expression of adenovirus E1 genes. As we did not apply any artificial selection pressure stable

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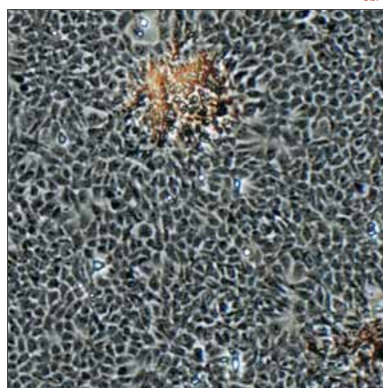

E1 gene expression indicates that – as expected – the transgenes (rather than a spontanous event) are responsible for the immortal phenotype. The retina cell line AGE1cr was adapted to growth as a single-cell suspension in fermenters (stir tank, Wave) without the requirement for animal-derived components in media, a requirement for convenient and safer large-scale virus manufacture. The culture can be expanded up to 100 fold/week and reaches densities of 5 - 9 x $10^6$ cells/ml. To further enhance susceptibility for attenuated virus strains the PIX gene of adenovirus 5 was stably transfected into the CR cell line to generate AGE1cr.pIX. This gene encodes a structural protein that stabilizes the adenoviral capsid but also cooperates with E1A in transcription activation and block of antiviral responses.

### Support of virus production

The CR cell line supports a wide range of viruses typically grown in avian cells. Besides poxviruses, (MVA and fowlpox) the spectrum includes members of the flavivirus, morbillivirus and parvovirus families. The cell line displays sialic acid linked to galactose in the alpha 2,3 as well as in the alpha 2,6-configuration, structures required for binding of avian and human influenza strains. Indeed, high yields have been observed for several influenza strains.
Highly attenuated poxvirus vectors such as MVA represent a very important application for the CR cell line. These viruses have generated high enthusiasm as viral vectors for vaccines against various pathogens: they exhibit co-stimulatory action to enhance the cellular immune response and at the same time are known to be safe even for application in immunocompromized individuals.
Towards serum-free MVA production a miniaturized shaker process was established and optimized with respect to MOI and starting cell density. In this system titers exceeding those observed in primary chicken cells are easily obtained.
When adherent, the cells are easily transfectable, when adherent they form confluent stable monolayers and allow the formation of well defined MVA plaques. This makes the cell lines highly suitable for generation and purification of recombinant viruses.

### Cell line characterisation

The entire development was carried out in a dedicated clean room environment. GMP Master cell banks have been established for CR cell lines. These banks are subjected to extensive safety testing to establish a Biologics Master File. In contrast to primary and transformed chicken cells which harbor endogenous retroviruses of the EAV type and release particle-associated reverse transcriptase, the new cell lines are free of retrovirus particles as demonstrated by TEM. RT activity was not detected in a research grade Q-PERT assay. Complete documentation including the flock/egg history adds to the safety profile. □

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**INFO**

*Volker Sandig* obtained his PhD in molecular biology from Humboldt University, Berlin. As an expert in viral vectors and gene regulation he worked at the Max Planck Society and he contributed to the HIV vaccine programme at Merck and Co Inc. In 2000 he joined ProBioGen where he is today VP Cell & Vector Biology and responsible for the company's cell line development programme. This includes the development of pharmaceutical high producer cell lines and creation of designer cell lines from primary cells of different origin which are customised for manufacture of vaccines and recombinant proteins.

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# Building Form to Function

Biopharmaceutical drugs will be just like small molecules, says Steven Projan, Head of Biological Technologies Wyeth Discovery Research. Developing and manufacturing drugs on three different technology platforms: biopharmaceuticals, small molecule drugs, and vaccines.

IINTERVIEW | **WOLF G. KRONER**

### How does RD impact biological manufacturing ?

Key is to understand what we want to end up with and where we think biopharmaceuticals in general are going to be in the near and medium future. Our general view is that biopharmaceutical drugs will be just like small molecules in terms of their use, study more targets accessible, or pricing. Many people are making the assumption that pricing for biopharmaceuticals is higher and you get bigger margins. Our assumptions are precisely the opposite. We think price pressures will be identical and that is how we are going to configure our manufacturing and, actually, what our benchmarks are in moving projects forward. So, if we don't think that we can come up with the cost of goods that's comparable to a small molecules drug, then we don't think we will be competitive.

We have actually put a lot of effort in this to enhance our development capabilities. In cell line development we achieved a superb expression platform to make monoclonal antibodies at scale. Publicly, we discuss 9.6 grams per liter. The mass you produce is half of the equation. The other is cost of media, where our development group is also decreasing our need to use serum in fermentation.

In terms of the discovery organisation of which my group is part of, very early on we have to come up with strains – at least genetic constructs – that have the ability to be produced in scale. The biomedical community become increasingly concerned about immunogenicity. One of the factors is the amount of aggregate one has in a given protein prep. If we end up with a strain producing a biopharmaceutical or proto-biopharmaceutical that is showing us some of these negative properties, it means back to the drawing board well before we proceed into the development phase. You might say the key is to have a very integrated drug discovery, early development – late development capability, where we don't throw something out, if we get relatively low levels of expression for instance. But if we're going to prioritize, we certainly will go with the compounds that have these properties early on. Before we go into even phase zero, we have already made this for the manufacturing capability assessment.

Wyeth

**Andover**

### You mention improvements in expression and media use. Where do you see major potential of cost savings – upstream or downstream?

It's both sides. Where I really don't see any cost reduction potential is in the physical plant itself.

### The potential for improvement in downstream processing is largely exploited?

There are strategic improvements. We have tried to build our facilities to have a good degree of flexibility, not just for manufacturing single drugs. Take Grange Castle outside of Dublin. Originally, the prime focus was to make Enbrel. When we started to build that plant, Enbrel was not even on the market in Europe and barely scraping by in the US. Now, the TNF-anatgonists as a family are probably going to pass this year erythropoietin-type based drugs. Once the process matures it probably pays to actu-

**INFO**

*Steven J. Projan* (1952) earned his PhD from Columbia University, New York (US) in 1980. Thereafter he worked first as post-doctoral fellow, then as an Associate Scientist at The Public Health Research Institute in New York City. In 1993 he became a Group Leader to Lederle Laboratories (American Cyanamid) which in 1994 became what is now Wyeth Research. He is currently Vice-President and Head of Biological Technologies at Wyeth Discovery Research in Cambridge MA.

| TABLE | Wyeth's Six Major Biopharma Manufacturing Sites (Wyeth, July 2007) | | | | |
|---|---|---|---|---|---|
| Site | Location | Space [m²] | Empl. | Capabilities | Major Products Manufactured |
| Andover | Mass. (US) | 99.000 | 1.800 | Commercial drug substance manufacturing; Process and product development; Pilot-scale laboratory; Clinical manufacturing, fill/finish | BeneFix® BMP-2 Herceptin |
| Grange Castle | Dublin (IE) | 108.000 | 1.100 | Commercial drug substance manufacturing; Process and product development; Pilot-scale laboratory; Commercial fill/finish; Protein research | Enbrel® Prevenar® Tygacil™ |
| Sanford | N. Carolina (US) | 7.200 | 1.400 | Commercial vaccines manufacturing; Process and product development; Full-scale development facility | Prevenar® ibTITER® |
| Pearl River | New York (US) | 41.000 | 900 | Fermentation and purification; Bulking and formulation; Syringe and vial filling | Prevenar® |
| Havant | Hampshire (UK) | 46.500 | 500 | Assembly, storage and worldwide cold chain distribution; New product launch; Cold chain warehousing, shipping, and validation | N/A |
| Algete | Madrid (ES) | 162.000 | 200 | Aseptic processing; Lyophilization; Kits packaging and distribution; Solid dose manufacturing | Refacto® InductOs™ Prevenar® |

ally make a much more streamlined process as well as having the equipment built solely to manufacture that particular product.

### What is the role of disposables, then?

I've seen some very interesting technology using bags, plastic containers, even fermenters. But, at the end of the day for a dedicated facility to be making a drug, stainless steel is still going to be king and the reason is very simple: The costs are going to be less instead of buying a new disposable bag again and again for a product you are going to make over and over again. Despite the fact that one can use radiologic means of sterilization for plastic, there's, frankly, going to be a leaching issue with disposables and that has not been well considered. I can understand a small company going that route especially in a contract mode: small scale and getting enough material more rapidly for a clinical trial process. But if you can mix it in stainless steel – despite the alleged higher energy costs – it's going to be less cost in terms of hardware.

Wyeth

Grange Castle

offers more flexibility in what you can do in cell culture. Think of the impeller height. It can be adjusted in a stainless steel tank. We discovered in one fermentation process having something 6 inches from the bottom it did not give us good levels of expression. Raising it up another foot all of a sudden expression was better. What's the best way to mix? You have limited capability for erasion in most plastic fermentation vessels. And that's why I am concerned about leakages coming out from plastic materials, because each and every fermentation process is different. The microbes, mammalian cells will probably produce something at some point that will interact with an organic surface and it would not interact with a stainless steel inorganic surface. That's my concern.

### There's a lot talking about better integrating upstream and downstream and optimizing manufacturing processes. What are you doing in Wyeth?

In Andover Massachusetts Wyeth BioPharma put a group in place called BRITT – Biopharma Research Interface Technical Team. BioPharma designates manufacturing, Research means R&D. This team is pushing the downstream processes all the way to the beginning of the discovery effort.

### Could you describe that in more detail?

Let's start with TRU-015. It was discovered by Trubion Pharmaceuticals. They had their compound in hand which is targeting B-cells through CD20, the same molecular target as rituximab. They had a process for basically getting compounds into development, however not for a much larger amount of material we would need. We actually would not have been able to produce material to

Wyeth

Stanford

enter phase 3 clinical trials using the original development capability that they had at Trubion. So, literally, the development group in Andover had to go back and re-engineer this from scratch and came up with a process that is probably at least fourfold more efficient. Now, that's an example of when something comes late stage and isn't built properly. Our attitude is that the final process doesn't have to be in phase 3.

Bapineuzumab for the treatment of patients with mild to moderate Alzheimer's disease is a monoclonal that targets A beta. This is the opposite example. In this collaboration with ELAN we integrated our work on the antibody together with our development group very early in the process. And so we were able to progress this antibody very rapidly into phase 1. Less than 12 months after making the decision we already get produced clinical trial material, when we filed the IND.

### How did you achieve it?

It was a murine monoclonal. During the time we were doing the humanization work, we were testing every single one of those humanized versions for their ability to produce levels of aggregation. For example, we were starting to figure out what the right vector systems were. In general for monoclonals, we have our own proprietary vector systems. But it's not the same for every single one. Some depends on what (…. chain) you're using. It's like a brewmaster. Even though we're trying to get as much of the process patented, so much is tied up in the internal expertise. Thus we will have alternate antibodies – even if we see those working, we haven't quit trying to come up with better ones. Another tenet of Wyeth's faith is that, if you put one into development, you better put two or three into the same molecular target. This is different to what Pfizer did. They had a great molecular target and approach but only had a single compound they tested and failed. They never are going to know, if their lack of efficacy was because of the compound or because of the target.

Wyeth

Pearl River

### How's about manufacturing small molecules?

We're certainly moving into peptides and modified peptides. To me, Mylotarg is the highest form of a pharmaceutical. It is a small molecule which was actually a natural product called Calicheamicin that has been fused on to a monoclonal antibody for specifically targeting leukemic cells. That's something you can only do in an organization that has these multiple platform capacities – a medicinal chemist can talk to colleagues in Biopharma and ask: 'How do we come up with the ideal molecule.' We demonstrate that we will build form to function. In some cases we try multiple approaches such as a small molecule

INFO

### IMPACTING BIOPHARMA MANUFACTURING

The company operates six global biotechnology manufacturing facilities in which 5.900 employees (Wyeth Pharmaceuticals total: more than 43.000) work on some 464.000 m2. In 2006 one third (US$5.7; €4,1bn) of revenues of the Pharmaceuticals Division was generated from biotechnology products. Since 2000 Wyeth invested more than US$3bn (€2.8bn) into its biotechnology manufacturing facilities. The Grange Castle plant at Dublin is one of the most advanced boipharma manufacturing facilities with capabilities to produce biopharmaceuticals, small molecules and vaccines at a single site. In 2006 Adsorbed pneumococcal saccharide conjugated vaccine (trade name: Prevenar) was the best selling vaccine in the world with 42 million doses manufactured and net sales of US$2bn (€1,45bn) or 30% over the previous year.

Wyeth says that these strategic investments have enabled the company «to overcome the barriers and risks inherent to biotech manufacturing and have allowed the company to maximize the use of its biotech facilities.» Drug discovery as well as Wyeth's R&D in general is impacting manufacturing. Major inputs in the past were production of cell lines with outputs three times above ndustry standard, establishing two-step chromatography protein purification with more than 10 tonnes of product from a single site. *wk*

for a protein versus the same molecular target.

### Isn't it a challenge to scale-up not only proteins and small molecules, but also vaccines?

Wyeth is integrated across all three platforms: proteins, small molecules, vaccines, and indeed our strategy as a company will continue to be in as many platforms as possible. We build form to function.

Wyeth

**Havant**

### What are you focusing on in vaccine manufacturing beyond immunogenicity issues?

We're moving more to a component vaccine rather than one whole organism or attenuated microorganisms. This means each and every vaccine presents its unique challenges. Prevenar is a conjugate vaccine harvested from multiple strains of streptococcus pneumonia where each and everyone of those strains produces their capsular polysaccharide differently. Some of them are more difficult then others and not everybody can manufacture it. One of our competitors has actually dropped its attempt at making a capsular polysaccharide conjugate vaccine. To date there are no well established rules for positive immunogenicity. For instance, why does aluminum work as an adjuvant? Frankly, beyond an understanding that T-cell epitopes are going to be longer then 7 to 8 aminoacids, we have a very rudimentary knowledge about what generates an immune response.

### Which major trends do you see in CHO manufacturing.

I would like to see much more cell culture tissue manufacturing of vaccine components. That is an empiric field that's going lag behind. Another issue is building better expression platforms. We've seen that picchia pastoris can be a viable expression platform for human type proteins. I personally don't think that EPO HI is the best place, specially for something that's requiring glycosylation, although people will continue to use it, because of the perception of speed and lack of expense. I don't expect that we will see any plant-based system work for pharmaceuticals for a variety of reasons – a lot of them around purification. Then, CHO and e. coli with yeast probably are replacing e.coli in certain ways. So, Merck bought Glycofi which has an interesting platform for manipulating glycosylation patterns. However, the investment required won't be any less expensive than what Wyeth co-invested in CHO technologies. We will probably produce some of the less expensive therapeutic proteins in CHO. We elected to build on our mammalian cell expertise not because that's what our original expertise was. We still do fermentation for some of our projects in e.coli, for example.

### Which bacteria can be better for use in fermentation?

I would use a bacillus where I can secrete as opposed to where which makes (a narrower drop of glycosaccharide) or E. coli endotoxin. Some biotechs were actually trying to establish a bacillus expression platform and they failed to get it properly up to scale, but I think that's a reflection of the amount of investment in this technology. We invested heavily in CHO cell technology mainly because it would get us an appropriate glycosylation pattern. We figured we wouldn't have to re-engineer downstream or enzymatically modifying monoclonals to appropriately glycosylate. So, there was a logic for starting with this. Once we've committed to it, then resources have been put in mainly by our team in Andover to make this into a world class expression platform. The good news there is: Very little has to be re-folded. Almost everything is secreted, and this is across the board for most of the proteins we're trying to develop. But we will always be cost conscious because that makes the best way to deliver value to the patients as well as to our shareholders. □

Wyeth

**Algete**

# Depleting Success – Managing the Supply Chain

Roche's oseltamivir is a good example of an efficient and commercially successful drug. In the wake of the avian flu sales of Tamiflu unexpectedly tripled. Demand was in excess of Supply. How did management cope with this situation? Insights collected from a conversation with Jan van Koeveringe, Head of Global Technical Pharma Operations with Roche.

TEXT **WOLF G. KRONER**

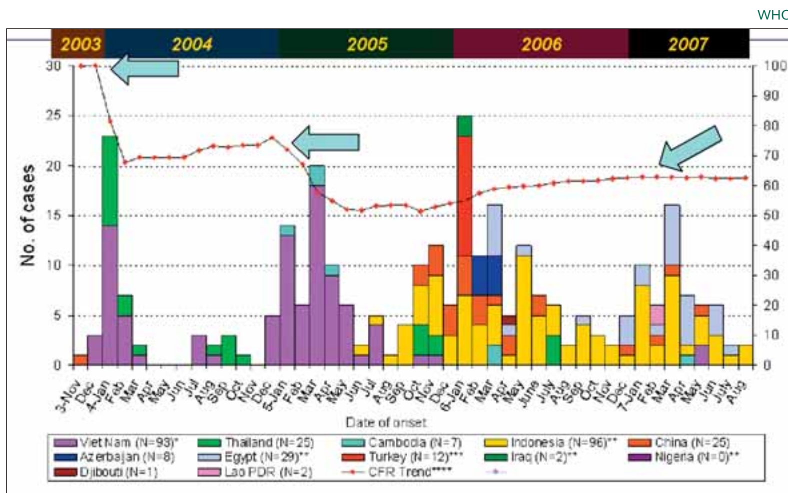
Jan van Koeveringe is heading Global Technical Operations with Hoffmann-La Roche responsible for the entire pharma manufacturing in all dosage forms and disease indications for which the company provides drugs. He manages the supply chain from procurement of raw materials for production to manufacturing APIs, galenical formulation and packaging up to logistics. Today, 8.500 employees at 17 different manufacturing sites all over the world ensure that Roche's medicines are made available to patients around the globe.

Roche

From star anis (left) to manufacturing oseltamivir capsules.

Access to Roche's drug suddenly became a public issue in 2004/05, when the H5N1 virus first detected 1996 in China killed 46 people (see chart) against 4 in the previous year. Moreover, it became clear, that the virus would not stop at the borders of Asian national health systems, but spread to Europe and other parts of the world as well. Good news were that neuraminidase inhibitors (oseltamivir, zanimivir, peramivir), a relatively new class of anti-viral drugs against both Influenza A and B, can stop (albeit not eliminate) the H5N1 virus to bud from the host cell. While peramivir (BioCryst Pharmaceuticals) is still at an experimental stage, GSK's zanimivir (trade name: Relenza) and Roche's oseltamivir (trade name Tamiflu) were available, the latter offering a crucial advantage over the competitor: Oseltamivir can be administered as liquid suspension and is therefore suitable treating infected children even at age 1. Therefore, Roche experienced an unexperienced rush for Tamiflu, when the avian flu threatened to grow into a pandemic.

WHO




Human avian influenza A (H5N1) cases by onset date and country (Aug 2007).

### Shaky forecasts, lack of scientific knowledge, but high public expectations

A major task in supply chain management is to ensure continuous supply of raw material to be fed into manufacturing according to detailed schedules. Oseltamivir production relies on EEC-epoxide which in turn initially depends on supply of shikimic acid, a natural product extracted from a species of star anis. «Originally we had one supplier in China from whom we got shikimic acid isolated out of star anise. While this is a very intelligent way to get a high-quality natural product with some optical purity» recalls van Koeveringe, «it is a very unreliable technology. Once per year you can harvest star anise and that means you don't have the flexibility for long-term planning. You can't order in May such quantity, and in April or October such one. You are dependent on how and when it grows and when it is harvested.» Today, Roche also uses shikimic acid produced by fermentation.This was a crucial building block in the strategy to become less dependent of naturally derived raw material that was but one problem to be solved.

Another question to be answered was: What is the demand for Tamiflu in order to plan procurement, production, stockpiling, and distribution? At the time knowledge about the H5N1-virus was still scarce. The genome was sequenced (Viseshakul N et al 2004), but it was not always obvious from symptoms if a diseased person had avian flu. Rapid detection tests still had to be

developed to support physicians' diagnosis. Most of all, the flu itself was not understood. It was far from clear, if and how humans are infected apart that somehow birds play a major role. Today, there is still a striking lack of knowledge on how rapidly the H5N1-virus is changing and how this affects the routes of transmission including geographic spread. Van Koeveringe and his team had to match shaky forecasts with known internal production and storage capacities. For years Tamiflu was used against seasonal influenza epidemics with considerably lower and short-term demands than in case of a pandemic. An issue was providing for another dimension of flexibility which meant expanding and shrinking procurement, production and stockpiling across rather broad ranges of volumes and times. Moreover, in the case of the avian flu at issue was providing a one-off demand for the drug in contrast to several years of use in the case of fighting more conventional influenzas. After treatment or prophylaxis the avian flu virus cannot harm you anymore as long as it does not change significantly.

### The Production Net

On 17 sites in Europe, the US, Asia and Latin America the company commands 5 chemical, 2 biotech and 14 galenical production units today (see chart). Investing in new facilities can be an option to increase production capacity and currently, amongst others, Roche is expanding facilities at Kaiseraugst (CH) and at Florence (US) getting into operations in 2008. These facilities are multi-purpose production units capable of manufacturing different products at a time. Equipment decisions have to be taken not just for a single product or production unit says van Koeveringe: «What technology do we need today, within the next 5 years and what will come our way at 10 years from now? And – is it the right equipment which covers the technology during this period? It is important that you are willing to change. At the moment, especially in the biotech area, traditional ways of development using fixed installed fermenters and downstreaming devices are step by step replaced by flexible disposable equipment.» Again, decisions often have to be taken at a time when forecasts lack required precision.

Another critical hurdle to overcome in the case of Tamiflu were political pressures to cede rights to the product. In China and India Roche provided sublicenses to local manufacturers. A smart move as governments can see for themselves how important sustainable co-operation with the originator is as knowledge of the substance does not entail knowledge of the manufacturing and formulation processes that turn an API into a drug.

In contrast to «conventional» influenzae the avian flu situation in 2004/05 lead to an unexpected upsurge in demand for Tamiflu. A backbone for Roche was its ability to network with contract manufacturers offering flexibility to increase production volume of Tamiflu without endangering the output of other drug products. Commissioning a CMO is far from just placing an order and transferring SOPs. For example, many of the evaluated galenical production companies were found to simply not being able to manufacture oseltamivir capsules as it requires a rather unusual granulation process and equipment. Once a supplier was found, the task of Roche's Technical Operations team was to start transfer of special process technology which by and large took another 12 to 15 months.

Roche, May 2007

**Roche Global Pharma Production Network: 17 production sites**

### Capacity for 400 million treatments per year– everything hunky-dory?

Today Roche has a production capacity of 400 oseltamivir treatments per year. But distribution issues persist as governments are reluctant to harness costs of stockpiling. The tug of war is about who should care and pay to have enough drugs in case of large infected populations? In the absence of a pandemic not to everybody understands that pharma manufacturers should not be pressured to heavily invest in R&D and manufacturing of efficient drugs, and then be punished for this. Exactly this would happen, if companies as Roche or GlaxoSmithKline would be required to stockpile these millions of drugs without some reasonable time frame where it can be sold. A liquid suspension of Tamiflu, which is perfect for administration to children, has a shelf life of 24 months. What if within this time span it is not needed? The company has to pay all cost. Capsules last 5 years, but stock rotation is not feasible with low volumes for traditional influenza epidemics. Moreover in the case of a pandemic quick response is mandated and this requires decentralized warehousing the cost of which simply cannot be bear by the manufacturer. Preparation for a pandemic is not just taking an influenza drug in advance, but also ensuring that the supply chain is organized within a division of work. Clearly, this requires more efforts than those of Jan van Koeveringe and his team. It is a good sign that responsible governments have taken preventive measures for their citizens and begun stockpiling oseltamivir. □

**Reference**

Viseshakul N et al (2004): The genome sequence analysis of H5N1 avian influenza A virus isolated from the outbreak among poultry populations in Thailand. Virology Oct 25, 328: 169-176.

# Do's and Don't's in Licencing Manufacturing

Jan van Koeveringe, Head Global Technical Pharma Operations.

INTERVIEW | **WOLF G. KRONER**

What are the do's and don't's of transferring production technology, process know how, not products?

First and foremost, you should not focus on cost alone. We have to meet worldwide requirements for quality, safety, health and environment, so other parameters to consider are: What is the compliance status of your potential partner? Is he approved for the large regulatory authorities, the FDA, the EMEA, the Australian Health Authorities, to name a few. Do they have a record on inspections to demonstrate that they do have that compliance status? That's one part of the equation. The other is: What is the experience of your potential partner in the particular area that you want him to do work for you? Is it demonstrated experience? Has he worked for Roche before? A third parameter is: What are the potential partner's proposed cost for doing that for you? And finally, you should be careful to ensure that he can do it with his equipment. Or does he need to invest? If that is the case, then this outsourcing becomes a risk-based partnership. Often potential partners want you to give a long-term commitment, 5, 10 years - depending on the level of the investment. This is legitimate, but on the other side you have much more uncertainties, whether the product he delivers is valid. Normally you get involved with such an agreement when your product is in some kind of a new mode, a newly launched product, a new indication.

Wolf G. Kroner

**Jan van Koeveringe**

What if the third party assure you of his capacities?

Pitfall is if you don't check and calculate whether the capacity is really there. If you start registration and then the capacity is not met, then you are in big, big trouble. You have to check it on site doing audits, looking into the equipment and get transparency as much as possible. And then you hopefully find a solution which is a win-win for both.

You also sublicenced manufacturing of oseltamivir. This is more risky than employing a CMO. What is your strategy there?

We expect our partners to acknowledge intellectual property wherever we do have that protection. If we give such a licence it is clearly defined for which countries that licence is valid. If the partner then would export to countries where the licence is not valid, we would have to seek our rights through the different possibilities which are available to us. But we expect a partner to stick to what we negotiated.

If you think about anti HIV drug saquinavir…?

This is a different situation. What we are trying to achieve here – especially in the Sub-Saharan countries – is to educate companies and governments to produce the drug themselves. They buy the active ingredient, a generic. We then help them with our expertise and teach them to formulate it. That's the more important part – they learn how to make a drug, for example capsules out of the API. So, we entertain contacts with different governments and different companies in Africa and educate how to produce the antiviral drug. We are fairly proud of what we have achieved so far, because it really starts to materialize. □

**INFO**

Jan van Koeveringe (1950) obtained a Ph.D. in Organic Chemistry form Universiteit Leiden in 1981. He joined Roche in 1978 as plant manager in chemical manufacturing of vitamins. In 1988 he was promoted Head of Chemical Manufacturing Pharmaceuticals and later on Head of Material Management Pharma Division. In 1995 he became Head of Global Chemical Manufacturing and Biotechnology. Since 2001 he is responsible for Global Technical Operations of Roche's Pharma business and member of Roche's Pharma Executive Committee. He is a member of the Royal Dutch Chemical Society and IUPAC.

# The Asian Biofuels Industry

Despite a biofuels hype in 2006, Asian countries have experienced a failure to take-off. Why? Will this continue? What role could and should Asia play, and what can it do make this industry flourish and be a success? What will Asia's impact on the regional and global biofuels industry be, and how will it contribute?

CHRIS DE LAVIGNE | TEXT

Asia compared to Europe and the US has been much slower to take off than Europe or the US concerning biofuels development, but has been looking to catch up fast since early to mid 2006. Driven by increased usage of crude oil for transportation (especially in China and India) and high oil prices, there has been a shifting focus towards alternative sources of energy, and with an abundance of potentially suitable feedstocks, Asia appears to be ideally placed to jump onto the biofuels bandwagon. Indeed, biofuels had become somewhat of a frenzy in 2006 with parties from all quarters trying to jump onto what was perceived as a gravy train, in a market with relatively low barriers to entry, further encouraged by some Governments that have granted production licences as fast as and as freely as the US seems to print money.

One or so year later the Asian Biofuels Industry has not really taken off as expected and hoped. The market seems to be suffering from a hangover and facing a murky future. But why has it failed to take off, and will this continue to be the situation in the future? What role could and should Asia play, and what can it do make this industry flourish and be a success? What will Asia's impact on the regional and global biofuels industry be, and how will it contribute? These are some of the very questions that this article will attempt to debate.

### Failure to take off

*Feedstock prices*

Currently very little biodiesel or biofuels have been sold either locally or for export. The main reason behind this has been the sharp increase in raw material prices, especially for biodiesel feedstocks. Just over a year ago when palm oil was trading at about US$400 per metric tonne, prospective biodiesel producers were wringing their hands in potential anticipation of rather hefty profits. One year on, and with palm oil trading around the US$750 per metric tonne level, biodiesel production in s.e Asia is very low, and most projects have come to a standstill.

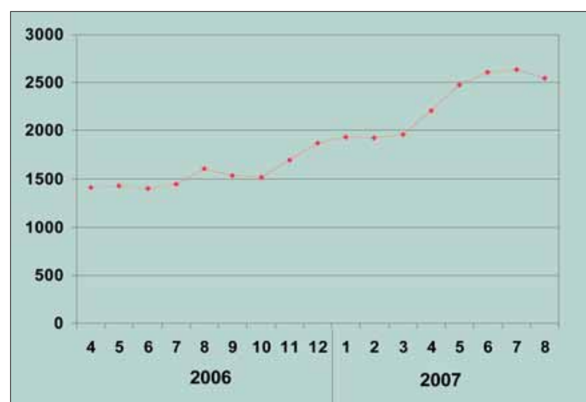



**Fig. 1: Average monthly palm oil prices 2006 - 2007.**

**INFO**

*Chris de Lavigne* (1969) received a BA (Hons) Management Studies from the University of Bradford Management Centre, UK in 1991 Thereafter he worked in the capacity of a Headhunter for Blue Chip banks, based in London. In 1996 he joined Frost & Sullivan where he worked for six years in the Paris and London offices as Head of Consulting for the Industrial Group covering the Chemicals and Materials industry. Subsequntly, he worked in Paris for three years for a major mangement consultancy, specializing in corporate strategy. Since 2003 he has rejoined Frost & Sullivan, based in its Singapore office in the function of Global VP Consulting, and has a strong focus on Renewable Energy projects, notably biofuels.

Although technologies have been getting less expensive in a low priced focused market, this has had little impact on helping with the ROI of projects as feedstock prices account for the 90% of operational expenses, and at prices above US$600 for palm oil, biodiesel production becomes not so economical.Lower methanol prices and higher glycerine prices have offered some temporary light at the end of the tunnel, but running a business based on a by-product that is only about ten per cent of the production output does not seem like a tenable business model.

Bioethanol has, conversely, not developed as fast, as feedstocks for it (sugar cane, cassava, corn), are not as prevalent as vegetable oils, or are still predominantly used in the food industry, and during the mad rush towards biodiesel that took the majority of the headlines, was almost overlooked.

*Legislation - «Roaring Tigers and Sleeping Giants»*

Legislation, or the lack of it has also been a major hindrance to the development of biofuels in Asia. The European and US biofuels industries have been largely supported by mandates and subsidies, but these have sorely been lacking in Asia to date. Asian petrol and diesel prices are heavily subsidized by Governments who have been gradually trying to do

away with these over time. The ideal situation for Governments would have been for biofuels to stand on their own two legs without the need for subsidies, but this seems unrealistic due to high feedstock prices. In order to stimulate these industries at a nascent stage, mandates and subsidies are required.

Quietly, some Governments in Asia have already mandated the use of biodiesel, albeit it low level to start with. There is a marked difference across Asia for the moment where some s.e. Asian countries have advanced more rapidly compared to their bigger North Asian counterparts. The Philippines, Thailand and Taiwan have already passed mandates in the 1-2% levels and have clear and well structured biofuels policies.

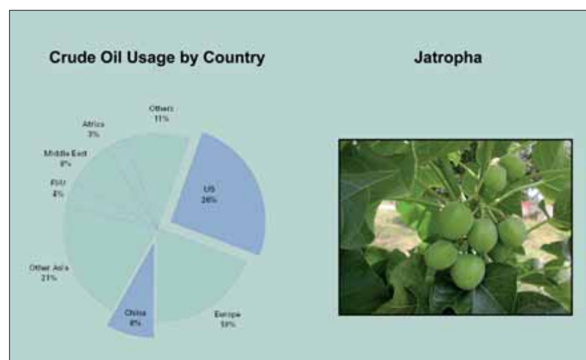



Figure 2: Usage of crude oil by country and growth rate (left). Picture of jatropha curcas (right).

On the other hand, China, Japan, and South Korea, have been slow to go down the biofuels path, in spite of their petrol and diesel prices being very high, especially the latter two counties. Japan and South Korea do have any local feedstocks for either bioethanol (though cellulosic bioethanol will hold potential) or biodiesel, and will have to either import feedstocks, or the finished biofuels, the later being the more likely. China would seem the perfect candidate for biofuels due to feedstock availability, and massive consumption of diesel and gasoline, but concerns over using food or feed crops for biofuels has hindered industry growth. Legislation for biofuels has not been fully structured either. Interestingly, China is also taking action against industrial processes that consume high levels of energy to process their goods, and emit high levels of $CO_2$. Biofuels could fall foul of such measures in the future.

The giant, that is an exception to the rule is India. Rumours on the grapevine (or should we say on the jatropha stem?) are that India is close to further structuring its biofuels legislation and providing financial support in the form of subsidies. This would make India a potentially exciting market due to its fast growing energy consumption, and relatively high diesel and petrol prices.

### Potential solutions

Although bioethanol is quietly gathering pace with more and more projects in Asia, biodiesel growth is fairly much at a standstill. So what can be done to further stimulate these industries. Firstly, solutions to the feedstock issues need to be found. On the biodiesel side, palm oil will not be the long term solution as it perceived as environmentally unfriendly, and clouds at +12 centigrade. Furthermore, palm oil prices are, in our assessment not likely to go down substantially if at all. Globally, and in the US in particular, more grains are being produced at the expense oilseeds to cater for the demand in bioethanol. Food demand for oils in increasing at a rapid rate, and with extra usage going into biodiesel, there appears to be an increasing gap between supply and demand in the global vegetable oil market. We thus see a baseline of US$600 per metric tonne for palm oil, with US$700 and above the likely level.

*Alternative Crops*

Clearly, alternative crops will need to be found. On the biodiesel side, jatropha has been making headlines for a while and is continuing to do so, and its attractiveness is undeniable. It produces a non-edible oil, can be grown on wasteland, turns out a decent yield of 1.5 tonnes of oil per hectare, and the biodiesel produced from it is of a very high quality. Currently though there is no large scale production of jatropha oil, although countries such as Burma, and India have planted quite a lot. Other countries with aggressive plans that will do well are the Philippines, Malaysia, Indonesia amongst other s.e. Asian nations. Jatropha will nevertheless be a little while off, and only when large quantities are in the market, will we see substantial growth in biodiesel.

Bioethanol is quite as problematic as biodiesel, but more sugarcane, cassava, and alternative crops such as sweet sorghum will need to be planted, ideally using a new breed that can increase the yields and extraction rates. Additionally, cellulosic ethanol should hold a lot of potential in Asia due to the availability of large quantities of biomass. Gasification technologies may be favoured over fermentation technologies though, as they could turn out to less expensive and have much lower levels of $CO_2$ emitted.

For the time being the outright winners in the current market seem to be the feedstock producers at the moment as increased food consumption and biofuels usage has increased raw material prices, and there is no sign of that changing either now or in the future.

*Rapid and effective legislation*

In order to stimulate growth in countries that have lagged behind, or where their biofuels industries are floundering, Governments need to provide them with support, and structure biofuels policies that are sustainable. Malaysian and Indonesian palm oil biodiesel producers are in dire need of such support. China needs to determine how it is to move ahead if it will not use food or feed crops, and Japan and South Korea need to structure legislation in order to allow imports into their markets. Once these three countries do come to the table, then the biofuels industry in Asia will begin to flourish, as these are the more logical markets for Asian biofuels users.

If crude oil prices continue to go up, as they may very well do, Asian Government may be forced to really sit up and

take notice of the bio-economy, and that it is very much a reality. The quicker they do so, the better for all concerned. Whether they subsidize this industry, is however, altogether another matter, as there is a distinct preference to let market forces operate.

*Requirements for the bio-economy to function*

However, even if and when, biofuels do finally really take off in s.e.Asia, a multitude of challenges will nevertheless have to be overcome. Infrastructure in the form of storage tanks are not readily present everywhere, retail and distribution facilities need to be addressed, trading platforms need to be put into place, clarity in import taxes will be required, as well as increased consumer awareness, to name but a few issues. A daunting but not impossible task, but the time and money required to achieve the above must not be underestimated.

### The potential role of Asian Biofuels in the Global Arena

So what of the role of Asian biofuels and biofuels crops in the future as part of the global market? As in any industry global trade flows are important for markets to function in an effective and efficient manner, and we have already seen this with Brazil exporting large quantities of its bioethanol production. We would expect Asia to play an important role both in terms of biofuels and crops production and in the global arena, but not without challenges.

Europe continues to makes aggressive announcements about biofuels legislation to 2010, and even 2020, with imports supposedly an important part of the mix, with an estimated 20% of usage coming from imports. Reality seems to dictate otherwise. Europe has shown a high level of protectionism, and little palm based biodiesel, for example, has found its way into Europe. Conversely, it seems to be more than open to receiving raw materials to make the biodiesel in its own backyard. Something will have to give in the future if manadated levels are to be met, albeit on a level playing field for all parties. Europe will be forced into reviewing changes in its biodiesel specifications.

Asian biodiesel may find an easier home in the US driven by its hunger for energy security, though Europe is most likely to jump on jatropha biodiesel when available, with bioethanol mainly being targeted at countries such as Japan and South Korea. Once mandates come into play in Asia, it will nonetheless be very interesting to see what percentage of biofuels are slated for export compared to that for domestic consumption.  It will also be worth watching just how much jatropha oil gets exported to Europe and the US in the future for biodiesel usage, or whether this will be kept for local production, thus stimulating local downstream activities and investments. A healthy mix of domestic consumption and exports is likely to be the case, but this could be dictated by the price of crude oil, the higher it goes the more production of biofuels will be used domestically.

Asian biofuels crops will have the biggest impact on the markets. The tropical conditions are favourable to rapid, year long growth of crops, and at prices that are significantly cheaper than in the northern hemisphere. This is the area that US and European companies should consider making investments in. Whether this happens or not remains to be seen, and it is felt that in the future local Asian agri houses will become even more powerful, and it may give rise to new ones.

**Figure 3: Size of Asian biofuels industry: Asia never got to take off – prices of CPO above $600 per metric tonne makes it difficult to operate in the market. Most plants at a standstill. Very little is hence coming out of s.e. Asia in 2006; only a few players are active.**

### Conclusion

In conclusion, the Asian biofuels industry has struggled to take off, but holds much potential, and there is no continued lack of interest in it from Governments, industry players, and financial institutions to find solutions and establish this industry. However, the industry will need to switch to new business models and receive far more Government support for it to succeed, but there is visibly the will and a concerted push to make both the above happen. Nonetheless it will take to time develop, and although it is still early days to be able to determine exactly how the Asian biofuels industry will evolve, it will one day in the not too distant future be a viable and buoyant industry due to low, alternative feedstock prices. But just not quite yet. □

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# Lab Work in Flux – An enquiry into today's activity clusters and changes in lab work

How is lab work changing and what is the role of infrastructure and layout? A report on the «Trend-survey – Lab Work» – a European survey with researchers in life science laboratories.

TEXT | JÖRG CASTOR

**INFO**

*Jörg Castor* (1972) obtained a Spatial Engineering degree (Dipl.-Ing.) from the University of Dortmund in 2000 and started work at a consulting company where he was concerned with organizational real estate planning. In 2001 he joined the Fraunhofer-Institut für Arbeitswirtschaft und Organisation IAO (Fraunhofer Institute for Industrial Engineering). He is initiator and project leader of the conjoint research project »Lab 2020« – an innovation network that is concerned with the development of future-proof, innovative solutions for laboratory work and design. In conjunction with »Lab 2020« he has also initiated the »Lab Innovation Center« (LIC) in Stuttgart – a life-science based test bed and demo center for laboratory work and design.

The following article presents selected outcomes of the «Trendsurvey – Lab Work» – a European user survey with researchers in life science laboratories. The survey has been done in the framework of the Fraunhofer research project «Lab 2020» – a project that is concerned with improving the quality of use and efficiency of lab environments.

The survey gives insight into today's activity clusters in lab work and what researchers think are important topics for innovation and change in their lab environments. Out of the 103 respondents about half are researchers (including PhD students and post docs). The other half consists of lab managers and technicians (e.g. medical-technical assistants). About two thirds of the respondents are working in biotech with the rest working in chemical, medical and pharmaceutical businesses. The majority of respondents mentioned molecular biology, cell biology and analytics as their scope of research.

Author

Fig. 2a and b: Modular and mobile equipment rack at Merck Serono, Geneva (left); Mobile bioreactor at the Lab Innovation Center, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB, Stuttgart (right)

## Networking, Tech-Space and Adaptability – need for change according to the users

The idea behind inquiring about design issues on a five year scale was to identify focal points needing short-term change according to researchers. The responses evidence an increasing demand for issues of technology and networking in designing and organising lab work. This reflects the ongoing proliferation of technology and the comparatively little attention that has been paid to these issues in overall lab design. It also reflects in a way the age of a lot of labs today. About 30% percent of the respondents mentioned lab ages 20 years +. Especially in older laboratories the increased use of equipment with unsatis-

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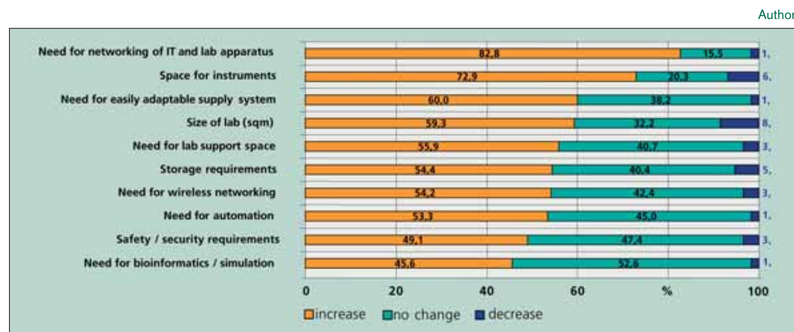


Fig.1: Need for change in lab design (Top 10; focus: 5 years)

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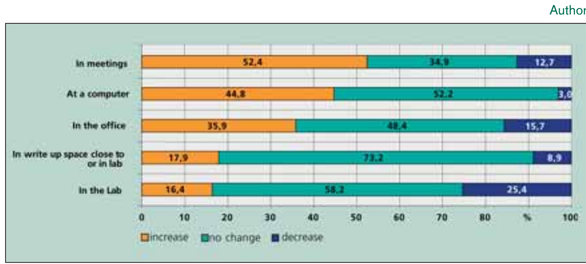


**Fig.3: Shifting work patterns (focus: 5 years)**

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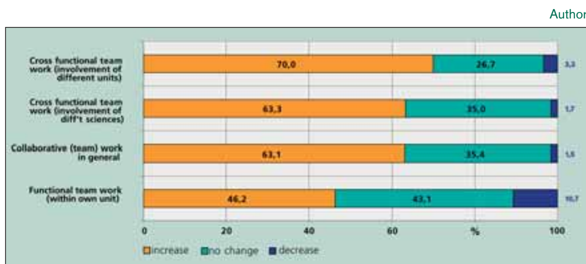


**Fig.4: Changes in teamwork (focus: 5 years)**

factory layouts and furnishings lead to constricted work environments with little space for scientists to work.

Infrastructure and space-layout are determining factors for integrating the increasing amount of equipment in laboratories. Lab infrastructure (e.g. supply systems – see Fig.1), lab layouts and furnishings need to be adaptable as well for that matter. A modular and adaptable design can support space efficiency and technology integration without sacrificing on bench and write up space. It also can support synergies in using equipment and thus further support space efficiency. Below two detailed examples of modular and mobile equipment are shown.

### Team work on the rise

Scientists forecast a further shift in their activity patterns at work within the next years. Whereas work in the lab and in write up space is considered as mostly not changing, work at computers is relatively on the rise going in line with changing work focuses to more documentation and «dry»lab work. Strikingly enough, meetings are considered to be the highest increasing activity at work. Interaction – as in other work environments – is an important paradigm for modern work styles. For scientists the specific aspect of teamwork is in particular considered to be cross-functional as the figures below indicate. The integration of different units and scientific disciplines (cross-functional team work) is increasing according to the users surveyed.

Interaction requires space – especially in constricted work environments that are defined by a high level of shared work (see also Fig. 5). As an earlier study with researchers indicated a high level of disturbance from equipment and speaking noise is felt by the users in laboratories (not shown). So, even though space for interaction is necessary it needs to be integrated in a non-disturbing way leaving space for concentrated work as well.

### Shared work and differentiated places of non-bench work in laboratories

The figures below indicate the present share of work in

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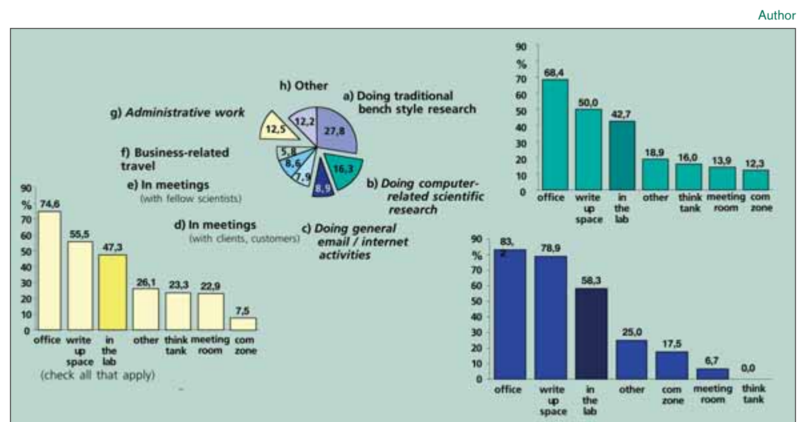


**Fig.5: Time patterns and places of work in lab environments today.**

laboratories. It is significant that researchers spend only one third of their time doing traditional bench-style research. Administrative and computer related work consumes another third. This obviously is in line with the changes related above and further underlines the trend to more «dry»and in silico work processes in life science laboratories.

We also enquired on where specific tasks are done. It is striking how varied non-bench related work in lab environments is today. As shown in the figure below a very high percentage of such tasks is carried out in the lab itself. This points towards the need for more «dry»workspace in laboratories – again, taking into

Author

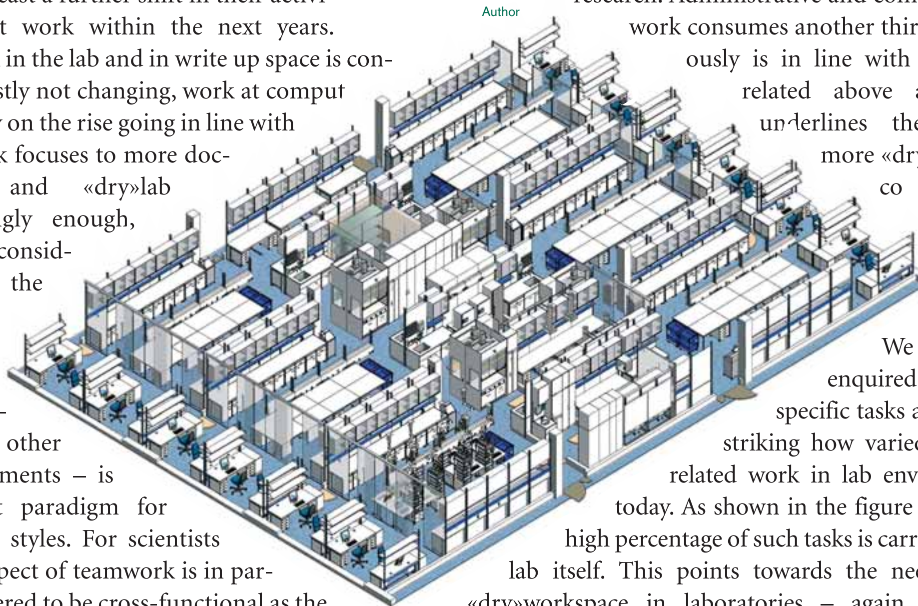

**Fig.6: Multi-space laboratory with integrated lab functions (Source: dr. heinekamp Labor- und Institutsplanung GmbH, Karlsfeld). On the outside write up space is located adjacent to lab benches and analyzers. The glazed doors can be closed. The middle zone incorporates lab support functions and sharable equipment such as fume hoods.**

account the age of many of today's laboratories. It is also an indicator for the process related integration of lab equipment and information and communication technologies (e.g. computers).

### Integrated research environments for diversified tasks

The results from the survey reported in this article document an increasing change in work styles and technology use. This is impacting on infrastructure and layout of laboratories. Research is becoming more collaborative than ever – the lab environment needs to mirror these changes in use. Adaptability and modularity – including furnishing, technology and supply systems – are important factors in providing researchers with enough space as well as diversified places to work. Yet these design features can support an integration of wet and dry tasks without drawing back on safety and the need for concentrated work.

Below is an example of a so-called «multi-space laboratory». It shows a transparent lab space fostering interaction as well as the integration of wet and dry processes. Lab space for research and lab support space are immediately adjacent to write up space for reports and computer related work. The laboratory brings together equipment and work spaces that are needed on a daily basis – in contrast to more «traditional»laboratory layouts that have a strict detachment of lab benches and office related work spaces. In addition to the incorporation of diversified research tasks synergies in the use of lab support space and equipment can reduce investment and operating costs. The spatial lay-out supports avoiding unnecessary equipment duplications and space utilisation.

*The full survey will be published in November.*

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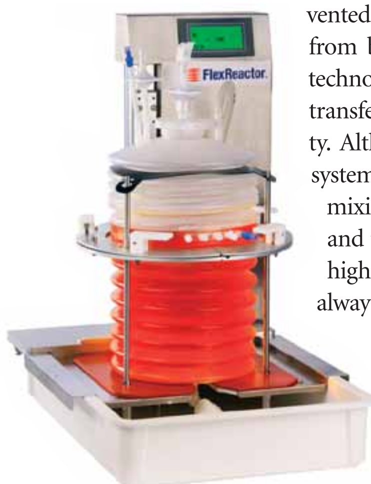
# The Wave FlexReactor™

## A Novel Disposable Platform for Bacterial Fermentation

TEXT **RICHARD FERRARO, DIRECTOR, NEW BUSINESS DEVELOPMENT**

To date there has not been a disposable fermentor specifically designed for bacterial fermentation. Typically there are three challenges that have prevented any one platform from being the true leading technology; mixing, oxygen transfer and cooling capability. Although most disposable systems do provide sufficient mixing, oxygen transfer and temperature control at high metabolism has always been a challenge. The Wave FlexReactor is the first bioreactor specifically designed to meet these challenges while providing the advantages of single use. The system is ideally suited for high oxygen demanding applications such as Yeast and *E. Coli*.




### Mixing

Most single-use systems employ some form of impeller or magnetic stir bar. The FlexReactor uses a unique jet mixing mechanism created by a perforated dish, also called a septum, located in the center of the bag. The septum can be moved up and down by an external actuator (See Figure 1). Each hole in the mixing septum is a jet mixer. A highly efficient jet of fluid going through each hole entrains and mixes the surrounding fluid. The septum motive power is supplied by an external non-invasive mechanical actuator.

**Figure 1: Jet Mixing Mechanism**

### Oxygen Transfer

The same jet mixing mechanism described above also provides superior oxygen mass transfer from the head space to the bulk fluid. As the septum moves through the fluid and approaches the liquid level, a vacuum is created pulling the oxygen rich gas from the head space into the liquid. An oxygen volumetric mass transfer coefficient kLa of $500 hr^{-1}$ is attainable even at moderate mixing speeds, this is comparable to a traditional stirred tank with bottom sparging (Figure 2)!

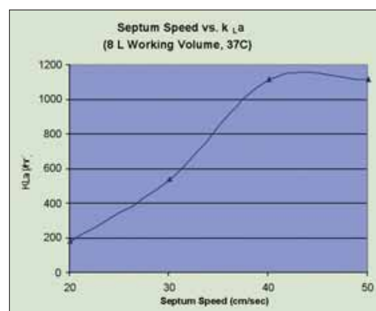



**Figure 2. Oxygen Transfer Study**

### Cooling Capability

During microbial fermentation there can be a significant heat load as the cells metabolize. Also it may be desirable to induce at lower temperatures (18-25 °C) to prevent inclusion bodies. For this reason a patented cooling septum has been developed which can handle a heat load 2x that of the theoretical load of a *Pichia Pastoris* fermentation. Chilled water fed to a connection on the bag flows through the 3D septum which acts as a heat exchanger cooling the bulk fluid; the water then flows through an outlet connection back to a client supplied chiller. A 10L FlexReactor can cool from 37°C to 20°C in less than 30 minutes (See Fig. 3).

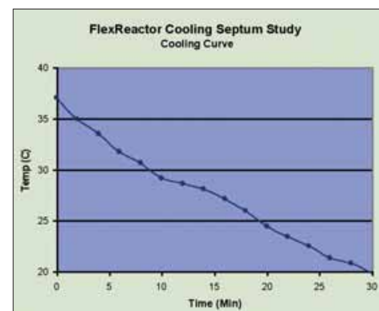



**Figure 3. Cooling Septum Study**

### Conclusion

The FlexReactor utilizes unique patented technologies to address the common challenges of bacterial fermentation and has been tested to match cell densities typical of a stainless steel fermentor. Currently the FlexReactor is available in bench top 10L working volume system ideally suited for a research or process development lab where 2-3 runs per week is common. Larger working volume systems (100-500L) are in final development. □

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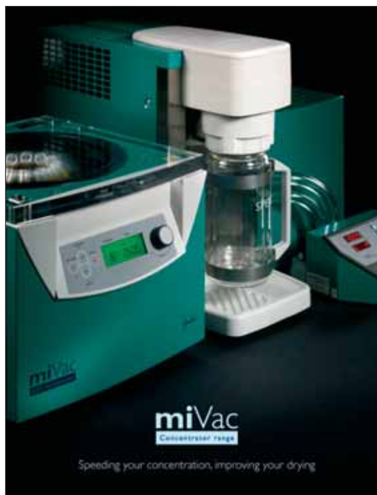
# One stop shop' for biological sample concentration




Genevac has announced a new 12-page brochure introducing its miVac concentrator range. The new brochure is available either in hard copy format or as a convenient downloadable pdf document from http://www.genevac.co.uk/miVac/applications/downloads1.html
Affordably priced the miVac range sets the standard for careful and speedy biological sample concentration and drying from a variety of sample formats including tubes, microplates and vials. Compact in design - miVac concentrators feature built-in special methods for working with alcohols, water and water mixtures to improve performance and optimise concentration times. Comprising three models of varying capacity each miVac concentrator features full LCD display with digital control and programming of time and temperature to minimise the chances of heat damage to your samples. The miVac pressure controller helps further to optimise concentration through smoothing the onset of concentration and preventing sample splashing. The miVac SpeedTrap is an optional refrigerated solvent condenser that offers more than twice the condensing power of other cold traps as well as being simple to use and empty. A range of medium and high vacuum pumps is also available. Offering unmatched performance, sample safety, versatility and ease-of-use - miVac is the first biological sample concentrator system with modules designed to work together and also look good.
The brochure provides an introduction to 'what is a centrifugal vacuum concentrator' and detailed information on all products in the miVac concentrator range. A selection guide helps you to select the most appropriate miVac system for your application. Further information is provided on Genevac's wide range of miVac sample holders, intuitive yet powerful software and range of optional accessories.

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# Q-Scan: Quantitative and Mobile Lateral Flow Reader

Easy to use, portable, inexpensive, and decision-oriented visual read out - that is why lateral flow tests belong to the most important classes of
diagnostic tests today. While these tests are established as routine tools, many do not offer the user electronic data documentation. It is known that
traditional lateral flow tests entail an increased number of false-positive and false-negative results, lack documented quantification and show limited sensitivity. ESE GmbH has addressed these shortcomings with Q-Scan, the portable lateral flow reader. The instrument can be customized for gold
beads as well as for fluorescence read out. It offers evident reproducibility of scans in the 99.95% range. The Q-Scan can be configured for any cassette format and any test - a feature unique compared to other lateral flow test devices. Positioning errors of strips and test or control lines is no longer an issue. The instrument scans the entire test strip and detects peaks wherever they are located. Therefore the Q-Scan reader is ideal for point-of-care diagnostics, field-based tests, research and quality
control testing by manufacturers. The single button operation ensures simplicity of use. The reader can be equipped with add on features such as barcode reader, printer and wireless data transfer.

*ESE Embedded System Engineering GmbH*
*Ludwigshafener Str. 29*
*DE – 78333 Stockach*
*Tel +49 7771 91 6624*
*Fax +49 7771 91 6618*

*Northern Europe Representative Office*
*Tel +44 1642 769823*

# Targeted identification and quantitative monitoring of phosphorylation changes of focal adhesion kinase protein

We have developed a MS-based method to quantitatively monitor specific phosphorylation events on focal adhesion kinase. We were able to examine 17 potential S, T, or Y phosphorylation sites on 11 tryptic peptides from FAK and to assess the role that Src plays in phosphorylating these sites.

TEXT **EUGENE CICCIMARO[1], JOHN HEVKO[2], AND IAN A BLAIR[1]**

Mass spectrometry (MS)-based methodologies are being used increasingly to analyse phosphorylation sites on proteins, because of the high specificity of these approaches. Determining the temporal sequence of phosphorylation at multiple sites within a single protein remains a significant analytical challenge, but is key to understanding the biological process. Focal adhesion kinase (FAK), a protein with 1,052 amino acid residues, has thirty-eight Y, eighty-one S, and fifty-one T residues, all of which can be phosphorylated. Molecular and biochemical techniques have so far detected phosphorylation on only eleven of the S and six of the Y residues (Fig 1). Focal adhesions consist of integrin adhesion receptors and a focal complex, a multi-protein plaque associated with the cytoplasmic tail of integrins. Phosphorylation of this multi-protein plaque is dependent upon non-receptor protein tyrosine kinases (PTKs), including c-Src and FAK, which are key regulators of integrin signalling at focal adhesions as well as of a number of growth factor signalling pathways.

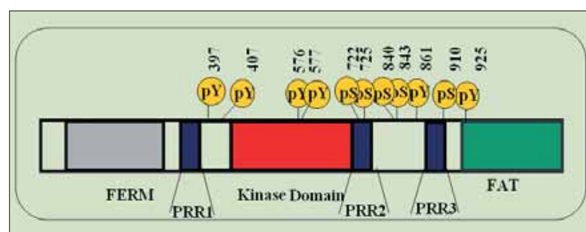



**Figure 1. Schematic of FAK protein. Phosphorylation sites indicated have previously been reported and experimentally verified to play important roles in regulating FAK kinase activity and guiding protein-protein interaction.**

We have developed a MS-based method to quantitatively monitor specific phosphorylation events on FAK. The MIDAS™ (multiple reaction monitoring (MRM) initiated detection and sequencing strategy) Workflow was used to identify and monitor 29 peptides resulting from the tryptic digestion of FAK that contained all of the known sites of phosphorylation together with a number of other potential phosphorylation sites.

All MS was conducted using a 4000 Q TRAP® system (Applied Biosystems/MDS SCIEX) operated in the positive mode using the Nanospray® source and heated interface. The MIDAS Workflow was developed for phosphopeptides determined from both full scan MS-based experiments and phosphotyrosine (pY) precursor ion scanning experiments. MRM transitions were also included to monitor a peptide in both its unmodified and phosphorylated forms. This technique could qualitatively differentiate between autocatalytic and Src-induced phosphorylation events1. MS/MS analysis and database searching were performed using ProteinPilot™ software (Applied Biosystems/MDS SCIEX) and the SwissProt FASTA file (uniprot_sprot20051220.fasta).

Our LC/MRM-based method[1] made it possible to examine seventeen potential S, T, or Y phosphorylation sites on eleven tryptic peptides from FAK and to assess the role that Src plays in phosphorylating these sites. The new method has identified six novel phosphorylation sites and allowed detailed mechanistic studies to be conducted on four additional sites[1]. We have demonstrated that Src is able to phosphorylate Y$^{861}$ at 37 ºC, regardless of the phosphorylated S residues (pS$^{843}$pS$^{850}$) that are present in the FAK. Two additional tryptic peptides, T$^{386}$HAVS$^{390-}$VS$^{392}$ET$^{394}$DDY$^{397}$AEIDEEDTY$^{407}$TMPSTR$^{413}$ and Y$^{570}$MEDSTY$^{576}$Y$^{577}$K$^{578}$ were also monitored and shown to be phosphorylated at different sites under the conditions studied[1]. Finally, the newly identified S and T phosphorylated residues on FAK were not altered by interactions with Src, in keeping with its known tyrosine kinase activity. The characterisation of these phosphory-

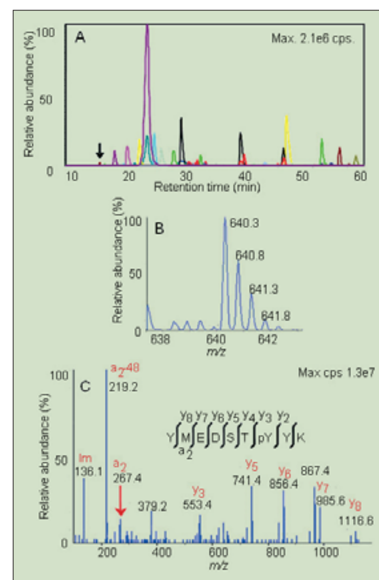



**Figure 2. Experimental Design. Chromatogram from a typical nanospray LC-MRM/MS/MS experiment on FAK tryptic digest in which 29 transitions were monitored (A). The arrow denotes the retention time of the phosphopeptide$^{570}$ YMEDSTpY$^{576}$YK$^{578}$. (B) An enhanced resolution scan of that ion was triggered when a parent ion eluted (m/z 640.3$^{2+}$ confirms parent ion mass of $^{570}$YMEDSTpY$^{576}$YK$^{578}$). (C) Product ion scan was then triggered which verified that the transition seen during the MRM experiment was the specific peptide targeted (Y$^{570}$MEDSTpY$^{576}$YK$^{578}$).**

lated residues suggests that there are as yet unknown S and T kinases that are involved in FAK-mediated cellular signal transduction; additional studies will be required to identify these kinases in cellular systems. The ability to quantitatively monitor the phosphorylation sites by LC-MRM will greatly facilitate such future studies and is key to understanding the underlying biology of phosphorylation. □

**Reference**

1. Ciccimaro EF, Hevko J, and Blair IA (2006). Analysis of phosphorylation sites on focal adhesion kinase using nanospray liquid chromatography/multiple reaction monitoring mass spectrometry. Rapid Commun Mass Spectrom **20**(24): 3681-3692

**Authors:**
1 Center for Cancer Pharmacology, Department of Pharmacology, University of Pennsylvania, Philadelphia, PA and
2 Applied Biosystems, Foster City, CA.

# Automated preparation of filter assays for determination of total bacterial counts

MEDIAJET vario is a new automated Petri dish filler from INTEGRA Biosciences purpose designed to fulfil the media preparation requirements of quality control laboratories in the beverage and environmental / drinking water sectors.

Further expanding the functionality of the successful MEDIAJET platform, the new system offers the capability to quickly process large numbers of 60 or 90mm diameter dishes making it the ideal system for preparation of filter assays for determination of total bacterial counts.

The new MEDIAJET vario enables automated filling of up to 540 Petri dishes at the push of a button. MEDIAJET vario can be converted from filling 90mm diameter dishes to 60mm diameter dishes in less than 5 minutes, providing invaluable flexibility to your media preparation. Using a proven mechanical dish guidance system, monitored by a set of sensors throughout the filling process, the compact MEDIAJET vario provides truly reliable, walk-away operation. Operational downtime and media loss due to 'dish jams' resulting from variations in the diameter, shape and rim profile of the disposable plastic Petri dishes are completely eliminated.

All functions on the MEDIAJET vario are conveniently controlled via a large graphical user interface. Intuitive software ensures operation of the system is completely self-explanatory even to occasional users. The ability to store up to 20 programmable methods provides facility to further improve unattended productivity. Designed to incorporate seamlessly into IQ/OQ certified environments the MEDIAJET vario provides facility, via an optional inkjet printer module, for automated dish imprinting with all the relevant data to ensure complete dish traceability.

The MEDIAJET vario has a unique 'Agar Spread Function' capability that provides the most efficient use of agar through ensuring homogeneous distribution and an even surface. By minimising the agar level in each Petri dish the new system enables up to 30% savings on media use compared to some traditional systems.

For consistent agar plate quality, maintaining a clean environment during the dispensing process is essential. The surface of the MEDIAJET vario filling chamber is manufactured from a single piece of chemically resistant polyethylene facilitating convenient and efficient cleaning. A powerful UV lamp further provides optimal bactericidal efficiency over the full length of the rotor where the dishes are opened during the dispensing process.

For every lab involved in media production, the TUBEFILLER option is the perfect expansion to the functionality of MEDIAJET vario, as it allows conversion of the automated Petri dish pourer into a test tube filler (13 –30mm) in just a minute.

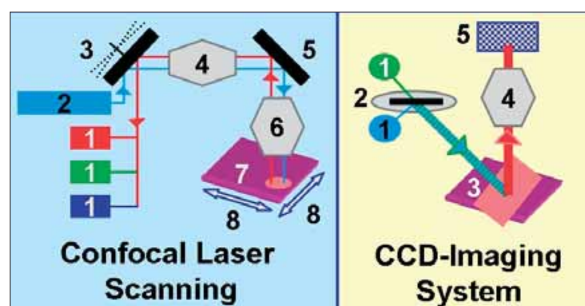
*INTEGRA Biosciences*
*phone: +41 (0)81 286 95 30*
*email: info@integra-biosciences.com*

# Microarray Measurements in Routine Applications

Developers of multiplexed assays and tests based on microarrays can now address a wider application base thanks to lower cost, robust instrumentation. A key and enabling factor is the optical detection, a crucial but often neglected part of microarray technology.

TEXT HANSWILLY MUELLER, STEFAN BICKERT, PAUL HING

Microarray technology is increasingly used in routine applications in many fields – ranging from diagnostics to food analysis to homeland security. These assays rely on optical detection, which mostly involves fluorescence measurement. Detection of microarrays demand a new generation of "microarray readers", and is currently performed using large and expensive laser scanner-based readers. Their high cost, complexity of handling and large footprint which consumes valuable lab space, however, limit their use to labs with full analytical infra-structure. This conflicts with the requirements of small and miniaturized biochip applications intended for de-centralized use in labs away from hospitals, in doctors' offices or at the point-of-care – at the patient's bedside. To overcome this barrier, an innovative, small, rugged and cost-efficient detection platform was developed for use in mobile and easy-to-use analytical instruments. This platform supports commercialization of routine microarray applications and, in the end, offer decisive benefits to users and theirs customers.




**Measurement principle of a confocal PMT-based scanning- and a CCD based System:** ***Laser Scanner for Research:*** **1-PMT; 2-Laser; 3-Scanner; 4-Scan Lens; 5-Mirror; 6-Objective Lens; 7-Sample; 8-X-Y Sample Movement.** ***CCD-System for Routine:*** **1-LED; 2-LED Optics; 3-Sample; 4-Microscope Lens; 5 CCD based Measurement.**

### The role of optical detection in microarray experiments

Most microarrays require an optical detection system, because the chemistry is based on fluorescent dyes. Thus any fluorescent microarray detection system has to excite these dyes of the sample and collect the emitted light in a quantitative fashion. Furthermore, because the size of a microarray spot typically is in the µm range, fluorescence images of the microarrays must be acquired with an appropriate magnification and high resolution. Along with efficient chemistry, the robustness of the analysis is dependent on high quality optical instruments for generating, archiving and reproducing reliable images. Technically speaking, fluorescence images are generated from a microarray with two techniques: Confocal Laser Scanning and CCD Imaging.

Confocal Laser Scanning uses a Photomultiplier Tube (PMT) as a sensor, and can only detect one signal at a time. Therefore the excitation of the sample must be done sequentially by a scanner. Here a single-wavelength laser beam is scanned back and forth (raster scanned) across the sample, exciting an area representing a single pixel at a time. The emitted light travels back through the objective lens and is collected by the PMT, which finally gives one single digital value which is proportional to the intensity of the emitted light. All PMT values combined together result in a 2-dimensional image of the sample.

The CCD Imaging System can produce a 2-dimensional image of the sample from one single exposure. For excitation a filtered LED-light source is used for homogenously illuminating the entire sample area. The emitted light from the entire field of view (i.e. microarray) then is captured through corresponding emission filters by a stationary CCD array. Thereafter, the signal intensity of each pixel on the CCD array is converted to a digital image.

One of the main differences between a Confocal Laser Scanning instrument and a CCD-based analytical system is the overall technical complexity of the former device. A PMT-based scanner requires expensive components such as laser sources and delicate scanning mechanics. A CCD-instrument operates with less moving parts, smaller components, and no light sources that may be hazardous to health.

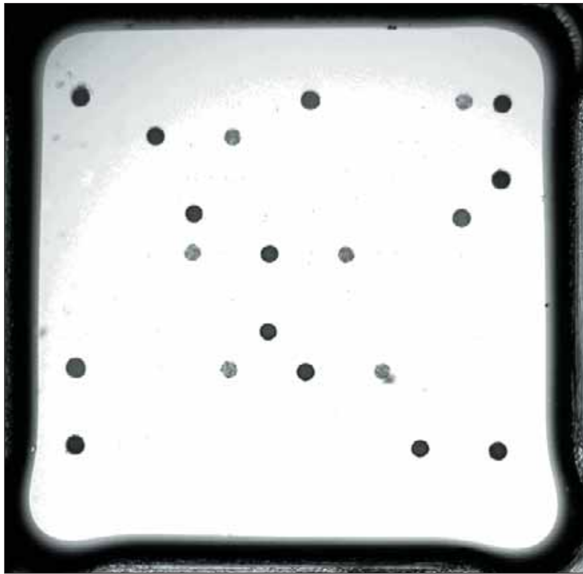


**Image obtained from a DNA Microarray for HPV Genotyping taken with Sensovation's LumiSens® Reader.**

Although Confocal Laser Scanners may offer high flexibility for well equipped research labs, the CCD Imaging systems are more suited for robust, smaller, portable and lower cost applications where ease of use is important.

### Routine microarray detection instruments

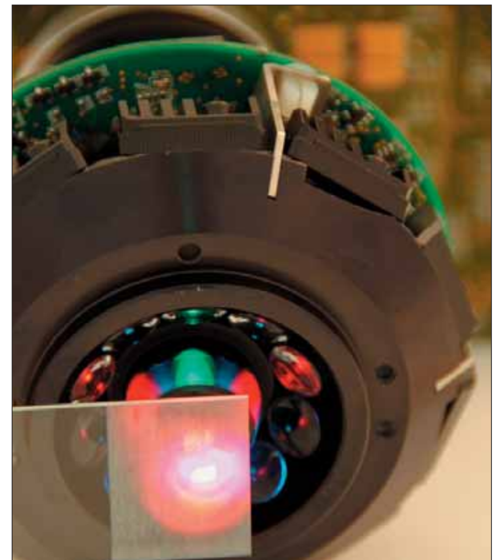
Current prices for Microarray readers range between 20,000 Euro up to several 100,000 Euro. There is no low-cost microarray detection instrumentation on the market for Point-of-Care diagnostic assays. This situation represents a barrier to the market penetration of many new microarray based assays, targeting affordable and routine diagnostic applications.

To address the increasing demand for routine microarray readers Sensovation has introduced LumiSens®, a fluorescence detection module. LumiSens® has full fluorescence microscope functionality, with a considerable smaller footprint and lower cost. LumiSens® is a module which is optimized for routine microarray detection and enables the use of Microarrays on a much broader base than before.

The design of the module became possible, because of the advances in CCD technology and in LED technology. Both technologies were driven by totally different markets. The advances in CCD technology were driven by digital photography. Over the past few years the pixel resolution and the overall quality of CCD sensors increased dramatically while in parallel the prices continuously dropped. The advance in LED technology was driven by the automotive and lighting industries which demand brighter and more powerful LED's with more colors.

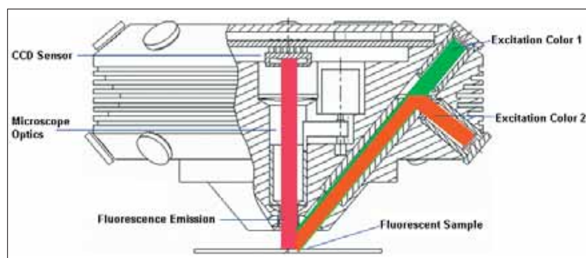
These two technological advances, together with an increasing demand for miniaturized fluorescence detection systems, contributed to the development of the innovative LumiSens® detection module, which incorporates 10 high power LED's and a high resolution, 8.3 MPixel CCD.

The LEDs are used as the sources of fluorescence excitation. A modular design allows measurement of 2 fluorescent dyes. The respective emitted light is captured with a microscope, including optimized emission filters. The 3.4x magnification allows visualisation of most common spot- and microarray sizes. Finally the image is captured by an 8.3 MPixel CCD sensor, equipped with microlenses for increased quantum efficiency.



**LumiSens® miniaturized fluorescence microscope for Biochip detection**

LumiSens® is used for imaging all sorts of microarrays, biochips and even microarrays in 96-well-plates (Multiplexing). LumiSens® permits operation in mobile, easy to use analytical instruments - in routine diagnostic testing, Point-of-Care and biotechnology. Due to its small size, its ruggedness (no moving parts), its compactness and cost effectiveness LumiSens® enables assay developers to enter new applications and markets in routine diagnostic testing, Point-of-Care testing and biochemical analysis. □




**LumiSens® light path schematics.**

**Correspondence:**

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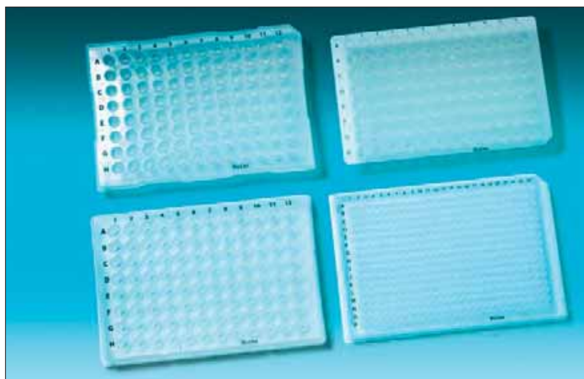
# Comprehensive range of 96- and 384-well PCR plates



Designed for high throughput Polymerase Chain Reaction assays, Bio-Dot PCR plates from Porvair Sciences (www.porvair-sciences.com) are compatible with the majority of 96-block and 384-block PCR and sequencing instruments.
Manufactured from ultra clean medical-grade polypropylene under Class 10,000 clean room conditions, Bio-Dot plates are free from DNase and RNase enzyme activity, enabling optimised PCR results. Available in 96-well and 384-well formats, plus a choice of full plate skirt, half plate skirt or no skirt designs - Bio-Dot PCR plates high rigidity minimises distortion before and after thermal cycling.
To ensure full compatibility with robotic systems all Bio-Dot PCR plates conform to SBS / ANSI dimensions. Available in packs of 25 and 50 individually wrapped plates and lids - 96-well Bio-Dot plates reduce the cost of PCR analysis without compromising performance. Available in packs of 50 individually wrapped plates and lids - 384-well Bio-Dot PCR plates have a working well volume of only 30 µL meaning that they are highly economical on valuable samples.
Porvair Sciences Ltd has specialised in the manufacture of microplates since its formation in 1992. Via its global distributor network, Porvair Sciences serves Life Sciences, Biotechnology, R&D and Molecular Biology with microplate solutions for all applications, from sample preparation to high throughput screening. Porvair Sciences is a subsidiary of Porvair plc, a world leader in microporous materials.

*Porvair Sciences Ltd.*
*phone +44-1932-240255*
*email int.sales@porvair-sciences.com.*

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# High cell density cultures in the CELL-tainer® single-use bioreactor.

With regard to bioreactors applied in the biopharmaceutical industry, the objectives are clear: cell densities should increase, productivity should increase, scalability is a must, costs should decrease. Single-use bioreactors offer clear advantages to this market segment and these have been described often. However disadvantages are clear too. The issues are:

- Scalability
- Limited mass transfer and mixing
- Only mammalian cell culture
- Mainly used in R&D environment

The patented technology of the CELL-tainer® offers high mass transfer including reliable measurement of pH and DO in the culture medium:

- Scalability by comparability (up-scaling / down-scaling)
- Improved motion (two dimensional rocking) for mixing and mass transfer
- Motion kept the same at all scales
- Bags can be linear increased in volume for inoculation using clamping principle integrated in the disposable: reduces handling / operational risks
- In-line measurements of critical process parameters selected (fluorescence pH, DO)

Experiments with mammalian cells proved that the mass transfer (oxygen and CO2) in the CELL-tainer® is superior to the currently available wave-type bioreactors and even superior to the traditional stirred tank bioreactors as applied with cell cultures.
The CELL-tainer® 15L (working volume) can achieve a $k_La$ up to 300 $hr^{-1}$ which is far above the mass transfer of a Wave bioreactor (kLa = 4–8 $hr^{-1}$). A traditional stirred tank reaches a value of 10-20 $hr^{-1}$.
The high mass transfer in the CELL-tainer® is sufficient to support oxygen consumption of high density cell cultures and certainly of most microbial applications.

*CELLution Biotech B.V.*
*Hans van den Berg, Director M&S*
*Cereslaan 2, 9641 MJ Veendam*
*The Netherlands*
*Tel. +31 (0) 598 631921*
*hans@cellutionbiotech.com*
*www.cellutionbiotech.com*

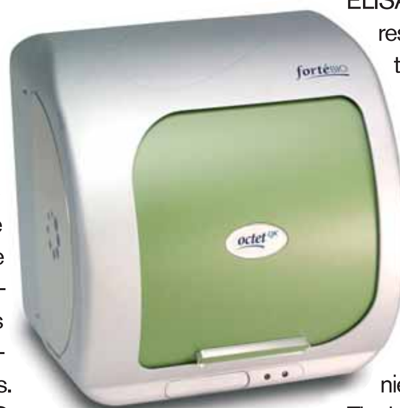
# The FortéBio Octet System

ForteBio's Octet family of instruments and related consumables provides scientists with real-time information about biomolecular interactions. Life Science Research, Drug Discovery and Process Development applications include:

**Quantitation** – what is the sample concentration?

**Kinetics** – how fast and tight are the interactions?

- Measure association rates
- Measure dissociation rates
- Determine affinity constants
- Determine specificity



**Key Benefits**

Label-Free Detection: The Octet System does not require the use of detection labels to measure the binding interaction at the biosensor surface. There are no labeling steps before the analysis, and there is no interference from fluorescent or chromogenic tags.

Real-Time Results: The Octet System continuously measures the protein binding at the biosensor surface throughout the interaction. Raw data are displayed in real time and the rapid analysis fits with process workflow.

Minimal Interference: Because the Octet System only detects binding at the sensor surface, there is minimal interference from biological sample media. Proteins can be assayed in cell culture media or crude lysates without interference.

Automated: The Octet System is automated to perform multi-step experimental pro-to-cols and complete data analysis. The system runs up to eight samples in parallel, and up to 96 samples in unattended operation.

The Octet System is comprised of the Octet instrument, Octet software, and biosensors. The system uses Bio-Layer Interferometry (BLI) technology to enable real-time analysis of biomolecular interactions in recoverable small sample sizes.

This new approach provides greater value where existing methods such as HPLC, ELISA or surface plasmon resonance have limitations in throughput, performance and cost. Ease-of-use, fast processing times and minimal sample preparation are hallmarks of the Octet system. Up to 96 samples are analyzed in real-time and label- free in convenient microplate formats. The benchtop analyzer brings back your projects to your own lab and workflow.

**Please visit us at our booth during Ilmac!**

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*www.bucher.ch*

NeurODrug
2007
23 - 25 October 2007
The Royal Garden Hotel, London, United Kingdom

## CNS disorders – what the future holds for drugs and therapies

Advances in the understanding of underlying disease mechanisms of neurological disorders means that novel therapeutic targets can be identified and drugs can be targeted to the cause of a disease rather than the effect. New classes and formulations of drugs for psychiatric disorders are also in development which will make better use of healthcare resources. There is a huge unmet need for drugs and therapies with improved safety, tolerance and efficacy.

*NeuroDrug 2007* focuses on drug discovery and development across the CNS sector. Our agenda focuses on novel therapeutic targets for neurological, neurodegenerative and psychiatric disorders. Key learnings include case studies on cutting edge drugs and therapies, lead optimisation, novel biomarkers and imaging, latest approaches to drug delivery and crossing the blood brain barrier.

“*NeuroDrug 2007* offers an excellent insight into multiple aspects of CNS drug discovery. The meeting has topics that relate to most CNS disorders such as new technology, drug delivery, biomarkers... this should make an interesting meeting with opportunity to discuss and network”

**Dr Michael J. O'Neill,**
Research Advisor, Neurodegeneration Drug Team,
**Eli Lilly**

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Les Diablerets (CH)
**Biology of Aging**
www.grc.org

Sept. 24– 26
Stockholm (SE)
**Biotech Forum + Scanlab 2007**
www.biotechforum.org

Sept. 25– 26
Milano (IT)
**bioforum 2007**
www.bioforum.it

Sept. 25– 27
Frankfurt (DE)
**Generic Medicines Europe 2007**
www.LifeScienceWorld.com/2007/gmed%5Feu

Sept. 25– 28
Basel (CH)
**ilmac 2007**
www.ilmac.ch

Sept. 26
Frankfurt a.M. (DE)
**Patents**
http://kwi.dechema.de

Sept. 26– 28
Lille (FR)
**EuroBIO 2007**
www.eurobio-event.com

Sept. 26– 28
Sao Paulo (BR)
**Analytica Latin America**
www.analyticanet.com/br

Sept. 27– 28
Zurich (CH)
**Clinical Trials - Beyond Borders**
www.mondialresearch-group.com

Sept. 30– Oct. 3
Napoli (IT)
**Innovation in Drug Delivery: From Biomaterials to Devices**
www.apgi.org

## 2007 I OCTOBER

Oct. 2 – 4
Milano (IT)
**CPhI Worldwide 2007**
www.cphi.com

Oct. 2 – 4
London (UK)
**Trade fair and conference for analysis, bio and laboratory equipment ExCeL London**
www.lab-uk.co. uk

Oct. 2 – 5
Québec (CA)
**BioContact Québec**
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Oct. 3 – 4
Göttingen (DE)
**International Symposium on «Life inside cells»**
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Oct. 3 – 5
Milano (IT)
**RichMac 2007**
www.fieramilanotech.it

Oct. 3 – 5
Québec (CA)
**BioContact Québec**
www.biocontact.ca

Oct. 4 – 6
Würzburg (DE)
**2nd Congress of the German Society for Stem Cell Research**
www.stammzellforschung.de

Oct. 7 – 10.
Göttingen (DE)
**Prokagenomics 2007 - 3rd European Conference on Prokaryotic Genomics**
http://events.dechema.de/prokagenomics2007.html

Oct. 8 – 10
Düsseldorf (DE)
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www.kongress.stamm-zellen.nrw.de

Oct. 8 – 10
Lyon (FR)
**World Vaccine Congress 2007**
www.LifeScienceWorld.com/2007/wvcl

Oct. 9 – 11
Hannover (DE)
**2nd VPM Vaccine Development Days**
www.vakzine-manager.de

Oct. 9 – 11
Hannover (DE)
**Biotechnica 2007**
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Oct. 10
Basel (CH)
**LSMW Symposium: «How to realize drug manufacture»**
www.lsmw.co

Oct. 15 – 16
Toronto (CA)
**Innovations in Clinical Trials: Implementation of Biomarkers**
www.healthtech.com/2007/ctd/index.asp

Oct. 15 – 17
Providence, RI (USA)
**Quantitative PCR, Microarrays, and Biological Validation**
www.healthtech.com

Oct 16 – 17
Toronto (CA)
**Adaptive Clinical Trial Design**
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Oct 16 – 18
Aachen (DE)
**ProcessNet**
www.dechema.de/jt2007

Oct. 17
Basle (CH)
**2. Swiss Innovation Forum**
www.ch-innovation.ch

Oct. 17
Santa Barbara, CA (USA)
**Eclipse FFF-MALS Focus Meeting**
www.wyatt.com/events/fff-malsfocusmeeting

Oct 17
Kobenhavn (DK)
**LSMW Symposium**
www.lsmw.com

Oct 18 – Oct. 20
Leipzig (DE)
**3rd World Congress on Regenerative Medicine: New Products for Diagnostics and Therapy**
www.regmed.org

Oct. 23 – 25
London (UK)
**NeuroDrug 2007**
www.terrapinn.com/2007/neuro

Oct. 24 – 26
Kuala Lumpur
**EU-Malaysia Biotechnology Business Partnership 2007**
www.eumbio.org

Oct 29 – Nov. 2
Warsaw (PL)
**ICRS World Congress**
www.cartilage.org

Oct. 31 – Nov. 2
Hyderabad, India
**analytica Anacon India 2007**
www.analytica-world.com

## 2007 I NOVEMBER

Nov. 5 – 6
Vienna (AT)
**Biomarkers Europe**
www.BiomarkerSeries.com/Europe

Nov. 5 – 7
Lyon (FR)
**European Antibody Congress 2007**
www.lifescienceworld.com/2007/antibody

Nov. 12 – 14
Hamburg (DE)
**BioEurope 2007**
www.edbgroup.com

Nov. 21 – 22
Prague (CZ)
**Audits and Inspections for Clinical Trials and Pharmacovigilance**
www.informa-ls.com/inspections

## 2007 I DECEMBER

Dec. 2 – 4
Prague (CZ)
**European Electronic Document Management Conference**
www.diahome.org

Dec. 10 – 11
Amsterdam (NL)
**Laboratory Analysis in Clinical Trials**
www.cfpa.com/200712102137-18a/2137/f/2137/0/CourseSearch.aspx

## 2008 I JANUARY

Jan. 22 – 23
Geneva (CH)
**BioData 2008**
www.biodataforum.com

Jan. 31 – Feb. 1
Frankfurt (DE)
**Chipstechnologies**
www.dechema.de/chips

## 2008 I FEBRUARY

Feb. 26 – 28
Genf (CH)
**BioBusiness 2008**
www.wbr.co.uk/BioBusiness08

## 2008 I MARCH

March 10 – 11
San Diego (USA)
**Outsourcing Manufacturing of Biopharmaceuticals**
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March 12 – 114
San Diego (USA)
**Antibody Development & Producion**
www.ibclifesciences.com

## 2008 I APRIL

April 1 – 4
Munich (DE)
**analytica 2008**
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April 7 – 10
Barcelona (ES)
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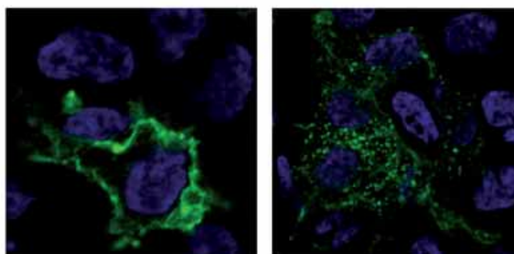

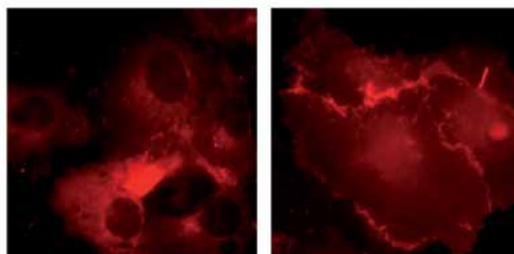

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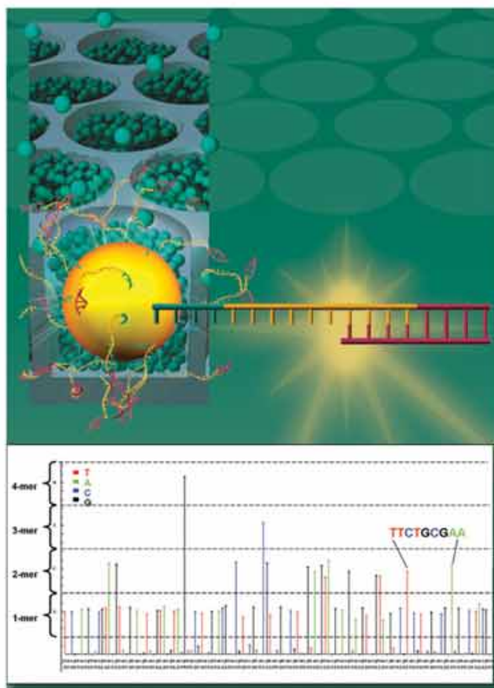


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