



**SIXTH FRAMEWORK PROGRAMME OF THE EUROPEAN
COMMUNITY FOR CO-OPERATIVE RESEARCH ACTIVITIES
INVOLVING SMEs**

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Project acronym: BIOTPHEX

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Project full name: Multiplex Bioassay Using The Two-Photon Excitation Method

“BIOTPHEX”

Final Activity Report of Co-operative Research

Final Report 6.1

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Project coordinator name: Prof. Erkki Soini

Project coordinator organisation name: Arctic Diagnostics Oy – ArcDia Ltd

erkki.soini@arcdia.com

1. Project execution

BIOTPHEX-project was a collaborative between three RDT partners, University of Turku (UTU), Finland, University of Jyväskylä (JYU), Finland and University College of Cork (UCC) Ireland, and four SME partners: Arctic Diagnostics Oy (ArcDia), Finland, Standa Optomechanical Products, Lithuania, Merlin Technologies GmbH, Germany, New Optics Ltd, UK and Luxcel Biosciences Ltd, Ireland. ArcDia was acting as Coordinator.

The main objective of the project was development of applications and technology and components for ArcDia's and Luxcel's diagnostics and biotechnology products. Other SME partners had the role of developing special components for the related applications.

Besides many results of BIOTPHEX project that may have commercial potential, two major business opportunities have been identified in the field of biotechnology and diagnostics. Related business plans have been made or are under preparation:

- Distributed in vitro Diagnostics Concept (Arctic Diagnostics Oy – ArcDia Ltd, Finland)
- A new concept for rapid assay of infection diseases (ArcDia)
- A new methodology for MRSA detection (ArcDia)
- R&D tools and solutions to the research community (Luxcel Biosciences Ltd, Ireland)

In addition other SME-partners have developed new components, which outside bioscience applications may have potential in the optical component market.

- Microcip lasers (Standa Optomechanical Products)
- Certain fibre lasers (NewOptics/Fianium Ltd, UK)
- Microfluidics chips (Merlin Technologies GmbH, Germany)

2. Dissemination and use

Distributed Laboratory Diagnostics Concept (ArcDia)

Distributed Laboratory Diagnostics Concept allows for the first time to perform e.g. 30 most frequently requested near patient laboratory tests in the primary health care. These tests cover all necessary tests in clinical chemistry, immunology and microbiology, as one-step, separation-free assays, within 10 – 30 minutes, by using one and same inexpensive reader device. This new and unique technology performs chemistry and immunology laboratory testing rapidly during the first doctor's visit. There is no need to send the samples to any central laboratories. The quality assurance of the diagnostics is based on the use of Internet and the expertise available in central laboratories. The technology is suited also for large hospital departments, but in particular for local health centres in the areas, where the sample transportation normally requires a long time. The fully automated analyser is very compact and can be modified also for ambulance use. For this purpose Arctic Diagnostics Oy has **incorporated a new subsidiary ArcDia International Oy Ltd**, which will turn the Distributed Laboratory Diagnostics Concept to a product line and will organise international marketing activities. The laboratory tests could also include rapid testing of infectious diseases in primary health care. The test could be made always before possible prescription of antibiotics. This is expected to lead to considerable savings in needless use of antibiotics and – what most important – avoiding development of new multi-resistance pathogen strains, such as those causing generation of hospital infections (e.g. MRSA).

A new concept for rapid assay of infection diseases (ArcDia)

BIOTPHEX project has resulted a new concept for rapid assay methods for most common pathogens causing respiratory infection. These pathogens include streptococcus A, streptococcus B, influenza A, influenza B, adeno virus, and respiratory syncytial virus (RSV). These six pathogens are responsible in 90% of respiratory infection cases. A multianalyte diagnostic product for rapid point-of-care testing of these pathogens would be valuable tool in the primary health care. The use of such diagnostic product would thus allow pathogen specific medication, and would lead to elimination of unnecessary antibiotics prescription.

During the BIOTPHEX project period 2, we studied the applicability of the TPX technique for the detection of pathogens in respiratory infections. This study included method development for influenza A and B virus antigens in nasopharyngeal specimens. Furthermore, we studied compatibility of dry-chemistry reagents with the nanoparticulate approach, and found that these were compatible. The use of dry-chemistry markedly simplifies assay protocols. The results show that TPX assay technique can be used for rapid (15-30 min) point-of-care testing of respiratory infections with comparable performance to TR-FIA technique. The results have been reported in detail in a scientific manuscript entitled as “Rapid one-step assay method for influenza A and B using fluorescent nanoparticulate tracer and dry-chemistry reagents”. #57

Based on the results of this project, the researchers of UTU and ArcDia consider that the detection of pathogens causing respiratory infections is one the most potential application area for TPX detection technique. Rapid multianalyte testing of such pathogens in the doctor's office would allow differentiation between viral and bacterial infection, and prescription of specific medication against the identified pathogen. According to the current practise, a cotton swab sample is taken from the patient for microbiological analysis, or alternatively, the patient is prescribed directly with wide spectrum antibiotics. Microbiological analyses are expensive and time consuming providing results no faster than 24 hours, while direct prescription of wide spectrum antibiotics is harmful for the normal flora of the patient, and tends to lead formation of new resistant bacterial strains.

A new methodology for MRSA detection (ArcDia)

BIOTPHEX project has resulted a new rapid methodology for bacterial antibiotic sensitivity testing allows for the first time antibiotic sensitivity testing of MRSA-samples (Multi Resistant Staphylococcus Aureus = hospital infections) during a few hours time. The methodology eliminates the need of pre-culturing (takes normally 2 days) the patient samples.

The rapid methodology for bacterial antibiotic sensitivity determination has been verified with *Staphylococcus Aureus* and *Streptococcus pyogenes* by various samples with high non-specific bacterial contamination.

The results on microbiology have been reported in a scientific manuscript entitled as “A new rapid approach for antimicrobial susceptibility testing” #56.

Luxcel Biosciences

The mission of Luxcel Biosciences is to become a leading provider of new enabling R&D tools and solutions to the research community, particularly for drug discovery, biomedical, environmental and food sectors. This is achieved by developing and commercializing a spectrum of proprietary platform technologies.

Luxcel Platform Technologies Include:

- Phosphorescent Label Technology - a wide range of bioaffinity assays with time-resolved fluorescence detection (TR-F)
- Respirometric Screening Technology (RST) - measurement of oxygen uptake in biological samples by phosphorescence quenching
- Luxcel Microplates - low-volume high-sensitivity bioassays in RST, TR-F, TPX formats
- Multi-Functional Detection Instrumentation - TR-F/RST/TPX plate readers ArcDia™
- Phase-Fluorometric Oxygen Sensor System – for process control and packaging applications
- Two-Photon Excitation Fluorescence Technology (TPX) - micro-volume, separation-free, multi-analyte detection methodology, instrumentation, chemistry, accessory tools and applications

These technologies complement each other, exploit the advantages of Luxcel new label and probe chemistries, assay formats and detection principles. They provide a full pipeline of highly competitive products and applications with unsurpassed sensitivity and selectivity, enhanced performance, and high information content. Luxcel technologies cover a broad range of core bioanalytical applications, they also bridge the entire preclinical process of drug development.

R&D and commercial agreements have been signed with several leading drug discovery and pharma companies including Pfizer (US).

ArcDia and Luxcel are planning to exploit the results in the field of biotechnology and diagnostics market. For this purpose both companies are seeking for considerable venture capital investments.

Peer reviewed publications Period 1

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- 2) Juhani T. Soini, Matti E. Waris, Pekka E. Hänninen, *Detection methods of microsphere based single-step bioaffinity and in vitro diagnostic assays*. Journal of Pharmaceutical and Biomedical Analysis 34 (2004) 753-760.
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- 4) Niko J. Meltola, Alekski E. Soini and Pekka E. Hänninen, *Syntheses of Novel Dipyrromethene-BF₂ Dyes and Their Performance as Labels in Two-Photon Excited Fluoroimmunoassay*, Journal of Fluorescence 14(2), (2004) 129-138.
- 5) Janne O. Koskinen, Jonne Vaarno, Niko J. Meltola, Juhani T. Soini, Pekka E. Hänninen, Juhani Luotola, Matti E. Waris and Alekski E. Soini. *Fluorescent nanoparticles as labels for immunometric assay of C-reactive protein using two-photon excitation assay technology*. Anal. Biochem. 328 (2):210-218 (2004).
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- 7) Jonne Vaarno, Emmi Ylikoski, Niko J. Meltola, Juhani T. Soini, Pekka Hänninen, Riitta Lahesmaa, Alekski E. Soini. *New separation-free assay technique for SNPs by two-photon excitation fluorometry*. Nucleic Acids Research, 32:e108 (2004).
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- 11) Meltola NJ, Kettunen MJ and Soini AE. Dipyrrometheneboron difluorides as labels in two-photon excited fluorometry. Part I –Immunometric assays. *Journal of Fluorescence* (2005). 15(3) 231-242.
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- 13) Marko E. Tirri, Roope J. Huttunen, Juha Toivonen, Pirkko L. Härkönen, Juhani T. Soini and Pekka E. Hänninen. *Two-Photon Excitation in Fluorescence Polarisation Receptor-Ligand Binding Assay*. Submitted to *Journal of Biomolecular Screening*.
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- 52) Rina Wahlroos, Juha Toivonen, Marko E Tirri, Pekka Hänninen, *Two-photon Excited Fluorescence Energy Transfer. A Study Based on Oligonucleotide rulers*. *Journal of Fluorescence* (2006) accepted
- 53) Burke M., O'Shea D., Yashunski D., Soini A.E., Papkovsky D.B. Design of new fluorogenic and phosphorogenic oligosaccharide-based substrates for amylase enzyme – (*Anal. Biochem.* in preparation)
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- 57) Koskinen JO, Vainionpää R, Meltola NJ, Hänninen PE, Soini AE. Rapid separation-free assay method for influenza A and B using fluorescent nanoparticulate tracer and dry-chemistry reagents. Submitted for publication in *Journal of Clinical Microbiology*.

List of monographies

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- 62) Martina Burke, Phosphorescent metalloporphyrin labels in oligonucleotide probes for nucleic acid detection’ Doctoral dissertation, UCC, March 2005.
- 63) Niko Meltola, Dipyrrometheneboron difluorides as labels in two-photon excited fluorometry, Doctoral dissertation, UTU, December 2005.
- 64) Kaisa Luotonen, Implementation of fluorogenic ELISA technique in ArcDia TPX platform., in finnish, Batchelor thesis, August 2005.
- 65) Jonne Vaarno, UTU, Applications of two-photon excitation fluorometry in bioaffinity assays, PhD thesis manuscript in preparation.
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- 68) Virva Lepomäki, Optical cell reader (transl.). Masters Thesis, University of Turku 2005 (in finnish)