COST ACTION B28
«ARRAY TECHNOLOGIES FOR BSL3 AND BSL4 PATHOGENS»

2th Management Committee and WG1, WG2, WG3, WG4, WG5 Meetings
November 21 & 22, 2005

Meeting Venue:

Institute of Virology,
Slovak Academy of Sciences
Dubravska cesta 9
845 05 Bratislava
Slovakia

Local Organiser:

Rudolf TOMAN
Laboratory for Diagnosis and Prevention
of Rickettsial and Chlamydial Infections
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WORKING GROUP Meetings
Monday 21 November

8.00 - 9.30

Registration, Welcome and Opening of the WG meetings

9.30 – 10.30

Working Group 1: Array technologies

Presentation of the partners in WG1:

- U. Nübel, E. Ehrentreich-Förster, F. Bier, W. Witte: DNA microarray for parallel detection of 14 bacterial biothreat agents.
- H. Nordstrom: DNA microarray technique for detection and identification of viruses causing encephalitis and hemorrharic fever.
- Y. Charbonnier, P. François, M. Bento, A. Huyghe, J. Hibbs, J. Schrenzel: Non-cognate hybridization systems (NCHS): an open strategy to identify the genetic background of the analyzed strains.
- G.W. Griffith, J.P. Day, H.M. Davey: Use of flow cytometry for the detection of airborne fungal spores.

10.30 – 11.00

Coffee break

11.00 - 12.30

- E. Pişkin: Biosensors/biochips for detection of pathogenic bacteria: focusing on QCM and SPR.
- M. Broekhuijsen, P. Larsson: Bacterial DNA microarray-comparative genomics and multi-agent identification.
- K. Kempsell, J.E. Burton, O.J. Oshota, N.J. Silman: Development of multipathogen DNA microarrays.

WG1 Participants to reimburse:

1.	Karl WALRAVENS	Belgium	To be reimbursed as WG1member
2.	Ulrich NÜBEL	Germany	To be reimbursed as WG1member
3.	Åke LUNDKVIST	Sweden	To be reimbursed as MC member
4.	Henrik NORDSTROM	Sweden	To be reimbursed as WG1member
5.	Gareth Wyn GRIFFITH	UK	To be reimbursed as WG1member
6.	Joachim FREY	Switzerland	To be reimbursed as MC member
7.	Erhan PIŞKIN	Turkey	To be reimbursed as external invited expert
8.	Jacques SCHRENZEL	Switzerland	To be reimbursed as MC member
9.	Michael MULVEY	Canada	Not to be reimbursed

10.	Martien BROEKHUIJSEN	The Netherlands	To be reimbursed as WG1member
11.	Dimitrios FRANGOULIDIS	Germany	To be reimbursed as MC member
12	Nigel J. SILMAN	UK	To be reimbursed as WG1member

Working group 2: Antigenicity

Presentations of the partners in WG2:

- P. Renesto, D. Raoult: Post–genomic analysis of intracellular pathogens.
- M. Sakarellos-Daitsiotis, E. Panou-Pomonis, D. Krikorian, C. Sakarellos: Sequential Oligopeptide Carriers (SOCn) for anchoring multiple copies of antigenic/immunogenic epitopes for immunoassays and vaccines.
- S. De Buck, C. Ensch, M. Pütz, F. Bouche, F. Fack, A. Steinmetz, C.P. Muller: Vaccines based on recombinant polyepitopes, peptide- and hapten- conjugates.

WG2 Participants to reimburse:

1.	Maria SAKARELLOS-DAITSIOTIS	Greece	To be reimbursed as MC member
2.	Claude L. MULLER	Luxemburg	To be reimbursed as MC member
3.	Patricia RENESTO-AUDIFFREN	France	To be reimbursed as MC member
4.	Bernd APPEL	Germany	To be reimbursed as MC member
5.	Axel CLOECKAERT	France	To be reimbursed as WG2 member

12.30 - 13.30

Lunch

13.30 - 15.00

Working group 3: Proteomics and glycomics

Presentations of the partners in WG 3:

- V. Veljkovic: Identification of conserved information encoded by primary structure of the surface glycoproteins of H5 influenza viruses: application in development of diagnostic tests and vaccine for H5N1 virus.
- R. Toman, P. Vadovič, K. Slabá, M. Fodorová, L. Skultety: Structure and function of lipopolysaccharide antigens of *Coxiella burnetii*, the causative agent of Q fever.
- L. Skultety, L. Hernychová, K. Slabá, E. Bereghazyová, P. Vadovič, M. Fodorová, R. Toman: Identification of *Coxiella burnetii* proteins using MALDI and ESI MS/MS techniques.
- M. Hubálek, I. Pávková, L. Hernychová, J. Lenčo, S. Janovská, A. Macela, J. Stulík: Proteome study of *Francisella tularensis*.
- M. Ducatez, J. Kremer, D. Revets, W. Ammerlaan, F. Fack, C.P. Muller: Virus host cell proteome studies: differencial proteomic approach to identify biomarkers of specific viral infections in human and animals.

- N.H. Beyer, N. Heegaard: Immunochemical and mass spectrometric identification of polypeptide and protein biotoxins.

WG3 Participants to reimburse:

1.	Jiři STULÍK	Czech Republic	To be reimbursed as MC member
2.	Aleš MACELA	Czech Republic	To be reimbursed as WG3 member
3.	N. Helena BEYER	Denmark	To be reimbursed as WG3 member
4.	Rudolf TOMAN	Slovakia	Not to be reimbursed
5.	Ludovit SKULTETY	Slovakia	Not to be reimbursed
6.	Fred FACK	Luxemburg	To be reimbursed as WG3 member
7.	Veljko VELJKOVIC	Serbia and Montenegro	To be reimbursed as MC member
8.	Pedro ANDA	Spain	To be reimbursed as WG3 member
9.	Constantinos SAKARELLOS	Greece	To be reimbursed as WG3 member

15.00 - 15.30

Coffee break

15.30 - 16.45

Working Group 4: Genomics

Presentations of the partners in WG 4:

- P. Wattiau, D. Fretin, P. Butaye: Development of a multi-species detection strategy.
- J. Frey, V. Perreten: Micro-array based detection of antibiotic resistance genes in *Bacillus anthracis*.
- A. Alderborn, M. Nilsson, U. Landegren: Tools to study genomes and their products.
- L. Moreno-Hagelsieb, J.L. Gala, D. Fladre: DNA hybridisation electrical detection for concentrations lower than 1 nM target ssDNA, based on interdigitated Al/Al_2O_3 capacitors.
- H. Tomaso, H.C. Scholz, S. Al Dahouk, H. Neubauer: Real-time PCR assays for the rapid identification of bacterial agents.

WG4 Participants to reimburse:

1.	Pierre WATTIAU	Belgium	To be reimbursed as WG4 member
2.	Jean-Luc GALA	Belgium	To be reimbursed as MC member
3.	Dragan ALAVANTIC	Serbia and Montenegro	To be reimbursed as WG4 member
4.	Anders ALDERBORN	Sweden	To be reimbursed as WG4 member
5.	Stefan PANAIOTOV	Bulgaria	To be reimbursed as WG4 member
6.	Ivan IVANOV	Bulgaria	To be reimbursed as WG4 member
7.	Bart van ROTTERDAM	The Netherlands	To be reimbursed as WG4 member
8.	Michel S. ZYGMUNT	France	To be reimbursed as WG4 member
9.	Herbert TOMASO	Germany	To be reimbursed as WG4 member
10	David ALBERT	France	To be reimbursed as WG4 member

WORKING GROUP Meetings
Tuesday 22 November

9.00 - 10.00

Working group 5: Microbiology (Bacteriology, Mycology and Virology)

Presentations of the partners in WG 5:

- I. Ivanov, P. Padeshki, T. Kantardjiev, S. Panaiotov: A seronegative case of oculoglandular tularemia confirmed with PCR.
- I. Ivanov, T. Kantardijev, R. Nenova, S. Panaiotov, P. Padeshki: Detection of human brucellosis with PCR directly in serum samples.
- M. Elschner, A. Rassbach, F. Melzer**:** The German reference laboratories for anthrax, glanders and brucellosis.
- P.A. Fonteyne: Molecular markers for identification of BSL3 fungi.

10.00 - 10.30

Coffee break

10.30 - 11.15

- D. Albert, N. Madani, B. Garin-Bastuji: The French reference laboratories for brucellosis and tularemia missions, activities and research objectives.
- R. Escudero, I. Jado, I. Rodrìguez-Moreno, M.I. Jimènes-Alonso, P. Anda: A molecular method for the identification of *Rickettsia* species in clinical and environmental samples.
- M. Weidmann, F.T. Hufert: Molecular detection of arboviruses.

WG5 Participants to reimburse:

1.	Frank VANDENBUSSCHE	Belgium	To be reimbursed as WG5 member
2.	Todor KANTARDIJEV	Bulgaria	To be reimbursed as MC member
3.	Plamen PADESHKI	Bulgaria	To be reimbursed as WG5 member
4.	Mandy ELSCHNER	Germany	To be reimbursed as MC member
5.	Richard SUMMERBELL	The Netherlands	To be reimbursed as WG5 member
6.	Mats FORSMAN	Sweden	To be reimbursed as WG5 member
7.	Patrick BUTAYE	Belgium	To be reimbursed as MC Chair
8.	Isabel JADO	Spain	To be reimbursed as WG5 member
9.	Raquel ESCUDERO	Spain	To be reimbursed as WG5 member
10.	Manfred WEIDMANN	Germany	To be reimbursed as MC member
12.	Pierre-Alain FONTEYNE	Belgium	To be reimbursed as WG5 member
13.	Nora MADANI	France	To be reimbursed as WG5 member

11.30 - 12.30

Lunch

12.30 - 17.00

2th Management Committee Meeting

1. Welcome to participants : P. Butaye

2. Adoption of the agenda : P. Butaye

3. Approval of the Minutes of the 1st M.C. meeting (Brussels): P. Butaye

4. Information from the Chair: P. Butaye
   a. STSM: election of the selection committee P. Butaye
   b. Web site installation P. Butaye
   c. Annual report

5. Information from the Scientific Officer M. Pascu

6. Election of the Chair of the Working Group 1

7. Election of the Chair of the Working Group 2

8. Election of the Chair of the Working Group 3

9. Election of the Chair of the Working Group 4

10. Election of the Chair of the Working Group 5

11. Working plans for the future P. Butaye

12. Place and date of the next meetings P. Butaye

13. Any other business

DNA MICROARRAY FOR PARALLEL DETECTION OF 14 BACTERIAL BIOTHREAT AGENTS

U. Nübel[1], E. Ehrentreich-Förster[2], F. Bier[2], W. Witte[1]

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[2]*Fraunhofer Institut für Biomedizintechnik, Potsdam, Germany*

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The rapid detection and identification of highly pathogenic bacteria is a prerequisite for their efficient surveillance and containment. We currently develop DNA microarrays for the characterization of genotypic features from a number of bacterial biothreat agents, including diagnostic markers for taxonomic affiliation, virulence genes, and antibiotic resistance determinants. To explore the suitability of DNA microarrays as highly parallel diagnostic tools, we have compared different types of arrays and alternative procedures for DNA labelling and detection with respect to multiplexing potential, sequence specificity, and detection sensitivity.

DNA MICROARRAY TECHNIQUE FOR DETECTION AND IDENTIFICATION OF VIRUSES CAUSING ENCEPHALITIS AND HEMORRHAGIC FEVER

H. Nordstrom

Abstract not delivered

NON-COGNATE HYBRIDIZATION SYSTEMS (NCHS): AN OPEN STRATEGY TO IDENTIFY THE GENETIC BACKGROUND OF THE ANALYZED STRAINS

Y. Charbonnier[1], P. François[1], M. Bento[1], A. Huyghe[1], J. Hibbs[1], J. Schrenzel[1,2]

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Bioterrorism and biocrimes are among the major threats in today's world security. Decisions should be taken rapidly, but major concerns lie in the rapid and appropriate detection, speciation and identification of toxins harbored by these bioagents. Our contribution will consist in two separate but self-complementary projects (with the group of Prof Joachim Frey, Bern) benefiting from the combined knowledge and expertise of the Geneva and Bern research groups.

We propose here the development of an efficient and rapid microarray-based detection method for assessing the presence of known pathogens, but also for identifying and characterizing new/unexpected or genetically engineered ones. This "non-cognate hybridization system" (NCHS) consists of novel microarrays and analytical tools designed to provide enough discrimination for bacterial identification, even for organisms that are not even considered at the time of array design. Using our experience in oligoarray design [1], this open strategy relies on a set of capture probes selected by an original bioinformatics strategy [2]. The strategy represents a balance between array complexity and the likelihood to reliably obtain discriminative biological signatures (i.e. a balance between cost and information). Such arrays have been validated *in silico* against published genomes for their capacity to: (i) identify the genetic background of the analyzed sample, (ii) compare biological signatures against reference database, (iii) discriminate samples at the species level, and (iv) provide enough discrimination power for strain genotyping and discrimination.

References:

[1]. Charbonnier, Y., B. M. Gettler, P. Francois, M. Bento, A. Renzoni, P. Vaudaux, W. Schlegel, and J. Schrenzel. A generic approach for the design of whole-genome oligoarrays, validated for genomotyping, deletion mapping and gene expression analysis on *Staphylococcus aureus*. BMC Genomics 2005 Jun 17;6(1):95.

[2]. Schrenzel, J., Hibbs, J. Non-cognate hybridization system (NCHS). 00/75377 A2 [Patent 6'544'777]. 2003

USE OF FLOW CYTOMETRY FOR THE DETECTION OF AIRBORNE FUNGAL SPORES

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We have used a Partec PAS-III flow cytometer to differentiate sporangia of the late-blight pathogen *Phytophthora infestans* from other airborne particles. Using the PAS-III, light scatter and intrinsic fluorescence parameters could be used to differentiate sporangia from conidia of *Alternaria* or *Botrytis*, rust urediniospores and pollen of grasses and plantain. Clear differentiation between *P. infestans* sporangia and *Erysiphe* conidia was only possible using data analysis rules evolved using the methods of Genetic Programming, following staining with the fluorescent brightener Calcofluor white M2R [1]. Initial field data are presented and the potential application of these techniques to the prediction of late-blight epiphytotics in the field is discussed [2].

References:

[1]. Day, J.P., D.B. Kell & Griffith, G.W. (2002). Differentiation of *Phytophthora infestans* sporangia from other airborne biological particles using flow cytometry. *Applied and Environmental Microbiology*. **68(1),** pp. 37-45.

[2]. Griffith, G.W., J.P. Day & D.B. Kell (2002). Use of flow cytometry in the detection of plant pathogenic spores. *Proceedings of the British Crop Protection Council conference, Brighton*, 18-21st November, pp. 417-424.

BIOSENSORS/BIOCHIPS FOR DETECTION OF PATHOGENIC BACTERIA: FOCUSING ON QCM AND SPR

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Pathogenic bacteria are considered as one of the most import and dangerous elements of bioterorism today. There are several laboratory techniques to determine the type and concentration of these bacteria and their toxins which show their existance in the environment. These conventional techniques for detecting microbiological contamination involves in time consuming enrichment steps and expensive and complicated instrumentation. Determination time of these recognition techniques is somewhat long and consumes a total assay time of up to 1 week in certain cases. Note that even few bacteria may cause important contaminations and therefore important and high risk which may be deadly. In many cases contaminations may include a number of bacteria which makes the analysis highly complex. Over the last decade, a great deal of research has focused on the development of biosensors using biological molecules as ligands for the detection of biological entities including microorganisms, cells and viruses. Quartz crystal microbalance (QCM) systems are quite simple and in expensive systems which measure the interaction between the immobilized ligand and its complementary target molecule (or even microorganism) on the crystal surface. This interaction causes the change of the mass on the crystal surface which in turn results a frequency shift that is measured and therefore the extent of the interaction can be monitored. Surface plasmon resonance (SPR) is a rather a new method for characterizing macromolecular interactions on the sensor surfaces. It is an optical technique that uses the evanescent wave phenomenon to measure changes in refractive index very close to a sensor surface. The binding reaction between the target in the solution and the ligand immobilized on the sensor surface results in a change in the refractive index. The interaction is monitored in real time and the amount of bound ligand and rates of association and dissociation can be measured with high precision. Both QCM and SPR techniques are label free, and portable systems can be design, which makes them much stronger among the available biosensors. Multi-channel SPR biosensors (maybe called as biochips) may allow recognizing and quantification of various pathogens simultaneously in one assay in a very short period with quite high sensitivity.

BACTERIAL DNA MICROARRAY-COMPARATIVE GENOMICS AND MULTI-AGENT IDENTIFICATION

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Using DNA microarray technology for comparative genomics, it is possible to discover new genetic markers for discrimination of species and subspecies. The approach is feasible when genomic sequence data, DNA preparations representative of the species, and a genomic clone library of one strain are all available. This has been shown for *Francisella tularensis* [1]. Even if a clone library is not available, the approach can be modified by using an oligonucleotide probe array, designed from genomic sequence data, as demonstrated for *Brucella* (this study). The results of such an approach are new insight in inter- or intra-species relatedness and new genetic markers that can be used for identification methods, e.g. PCR.

Additionally, when sufficient genetic markers are known, one can design a socalled "Multi-agent Identification Microarray", which contains several genetic markers for many microorganisms of interest, e.g. infectious agents. The purpose of such a multi-agent array is to use it as an identification method, with a single analysis sufficient to identify any relevant agent in the sample (provided that all relevant markers are on the array). The advantage compared to PCR is that there is no need for any previous knowledge about the agent that might be present in the sample, and no need to run a series of specific amplifications for a list of agents. To apply enough DNA from the sample to the array, it is necessary to pre-amplify the sample DNA. In order to maintain a generic approach, this is done using random PCR. This approach has already been described [2].

Ultimately, the multi-agent array can be incorporated into a fully integrated and automated identification device. The concept of such a device is provisionally termed IBI (Integrated Bio Identification). The design is aimed at including an automated sample preparation unit, a random amplification and labelling module, a disposable multi-agent microarray module, an integrated fluorescent scanner, and software for data analysis and interpretation. Users without specific expertise should be able to operated the device in a field environment. The development of such a device requires a high level of automation, integration, and miniaturisation.

References:

[1]. Broekhuijsen M and Larsson P et al. (2003): *J. Clin. Microbiol.* **41** (7) - 2924-2931.
[2]. Burton J E, Oshota O J, North E, Hudson M J, Polyanskaya N, Brehm J, Lloyd G & Silman N J (2005): *Molecular & Cellular Probes* **19**, 349-357.

DEVELOPMENT OF MULTIPATHOGEN DNA MICROARRAYS

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DNA microarrays are an attractive technology for the rapid and simultaneous detection and diagnosis of a range of bacterial and viral pathogens. We have developed a multi-pathogen oligonucleotide microarray, which currently contains approximately 2200 different gene probes. Bacterial and viral pathogens are detected on the same chip. 16S rRNA probes designed against the hyper-variable regions give genus level identification and specific virulence gene probes allow absolute identification[1]. For detection of viral pathogens, a slightly different approach has been taken. Since there is no equivalent consensus gene such as 16S or 23S rRNA in viruses, probes have been designed (between 5 and 10 per virus) which target the regions of high discrimination within the viral genome. All probes have been extensively checked for potential cross-hybridisation *in silico* by database searching. Much of the development work has been undertaken using *Bacillus anthracis* as a model system; however, probes which detect *Francisella tularensis, Yersinia pestis* and many other Australia Group pathogens have been incorporated. Similarly, probes have been included to detect the viral pathogens on the Australia Group list, as well as a large number of respiratory and enteroviruses, orthopox viruses and parapox viruses. Data obtained indicate that the microarray has very high specificity and is capable of routinely detecting as little as 28pg of bacterial DNA. In summary, a DNA microarray has been developed that uses DNA probes to allow rapid and simultaneous detection of both bacterial and viral pathogens.

References:

[1]. J. E. Burton, O. J. Oshota, E North, M. J. Hudson, N. Polyanskaya, J. Brehm, G. Lloyd & N. J. Silman (2005). Development of a multipathogen oligonucleotide microarray for detection of *Bacillus anthracis. Molecular & Cellular Probes* **19,** 349-357.

POST-GENOMIC ANALYSIS OF INTRACELLULAR PATHOGENS

P. Renesto, D. Raoult

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Rickettsiae are fastidious obligate intracellular bacteria phylogenetically classified in 2 groups, the typhus group including both *R. prowazekii* (epidemic typhus) and *R. typhi* (endemic typhus) and the spotted fever group (*R. conorii, R. felis*, etc…) [1]. Although systemic approaches have provided substantial information about the biology of rickettsiae, the molecular basis underlying the invasive mechanism remains largely unknown. This results from the intrinsic difficulties in working with these strictly intracellular bacteria and the lack of adequate methods for their genetic manipulation [2]. Last few years, the sequencing of the whole genome of several rickettsiae was achieved to study the evolution of rickettsiae and the molecular basis of different life-styles and pathogenicity. Availability of these sequences now permits to develop modern experimental approaches such as transcriptomics and proteomics. Proteomics strategies were developed to identify rickettsial proteins through the access to facilities including MALDI-TOF, Ion-Trap mass spectrometer and nano-HPLC. This allowed to construct the first proteomic reference map of both *R. conorii* and *R. prowazekii* and to identify highly antigenic proteins. Overlay assay performed on rickettsial samples separated by 2D-gel electrophoresis also permit to characterize putative ligands recognized by endothelial cells. Another post-genomic application was the analysis of *R. conorii* transcriptome by using microarrays, an approach depending on the absence of eukaryotic nucleic acid contamination. Post-genomic analysis was also envisaged as a way to associate *in vivo* phenotypes of these bacteria to genomic features, most particularly for *R. felis* [3]. As concerning other rickettsiae, obligate intracellular nature of this microorganism hindered progress in the detailed characterization of its phenotypic diversity. Thus, analysis of the complete genome sequence of *R. felis* highlighted genes susceptible to be involved in pili biogenesis. Based on these findings, an electron microscopy study was undertaken and allowed to demonstrate that pili were really expressed.
The aim of our work is to further expand our knowledge about proteins really expressed by these microorganisms and encoded by virtual annotated genes and which would constitute new diagnostic ot therapeutic targets.

References:

[1]. D. Raoult and V. Roux: *Clin. Microbiol. Rev.* , **10** (1997) 694-719.
[2]. D.O. Wood and AF Azad. Infect. Immun., **68** (2000) 6091-9093.
[3]. H. Ogata, P. Renesto, S. Audic, C. Robert, G. Blanc, P.E. Fournier, J.M. Claverie et D. Raoult, PLoS Biology, (2005) e248

SEQUENTIAL OLIGOPEPTIDE CARRIERS (SOCn) FOR ANCHORING MULTIPLE COPIES OF ANTIGENIC/IMMUNOGENIC EPITOPES FOR IMMUNOASSAYS AND VACCINES

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A novel class of Sequential Oligopeptide Carriers, SOCn, for anchoring multiple copies of antigenic/immunogenic peptide epitopes is successfully applied in our laboratory. The carrier, formed by the repetitive Lys-Aib-Gly moiety displays a predetermined 3D structure (3_{10} helix), while the anchored epitopes, by the Lys-$N^{\varepsilon}H2$ groups, preserve their original "active" conformation. The helicoid structure of SOCn helps the reconstitution and/or mimicking of the native forms of the epitopes, so that potent immunogens can be generated. SOCn-constructed were used as antigenic substrates in developing specific, sensitive and reproducible solid phase immunoassays, and for the induction of high titers specific antibodies recognizing the priming construct and the cognate antigen. Selected examples will be presented, as well as a modified SOCn with a built-in vaccine adjuvant [1-3].

References:

[1]. C. Alexopoulos, M. Sakarellos-Daitsiotis and C. Sakarellos: Curr.Med.Chem., 12 (2005) 1469-1479.

[2]. D. Krikorian, E. Panou-Pomonis, C. Voitharou, C. Sakarellos and M. Sakarellos-Daitsiotis: Bioconjugate Chem., 16 (2005) 812-819.

[3]. M. Sakarellos-Daitsiotis, C. Alexopoulos and C. Sakarellos: J.Pharmaceut.Biomed., 34 (2004) 761-769.

VACCINES BASED ON RECOMBINANT POLYEPITOPES, PEPTIDE- AND HAPTEN- CONJUGATES

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[1]*Institute of Immunology, Laboratoire National de Santé, 20a rue Auguste Lumière, L-1011 Luxembourg.* [2]*Institut de Biologie Moléculaire des Plantes - CNRS, 12 rue du Général Zimmer F-67084 Strasbourg, France*

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In the following we describe two examples of conjugate vaccines developed in our Institute, the technology of which could applied also to pathogens of interest of the COST Action B28. One vaccine is based on linear neutralizing epitopes of the measles virus; the other one is based on derivatives of carcinogens to explore an immunoprophylactic approach.

A peptide-conjugate vaccine. Two B cell epitopes (BCE) of the measles virus (MV) hemagglutinin protein were identified, which can be mimicked by short peptides. Peptides derived from these epitopes induced virus neutralising and protective antibodies even in the presence of pre-existing anti-MV antibodies. The structure of both epitopes was investigated with mabs, substitution peptides libraries and phage display libraries. One epitope corresponds to a helix. The other one corresponds to a planar loop constrained by a disulfide bridge, and critical contact residues present in more than 90% of wild-type viruses. Binding data of peptides and mimotopes were compared with 3-D docking models using advanced algorithms and antigen binding domains of protective mabs (with distinct VDJ germline genes). Since chemical stabilisation of the peptide in the antibody-induced conformation was difficult, and resulted in low-level neutralising antibodies a recombinant approach was used to generate a small permutational library of multiple copies of the epitopes to high-molecular weight polyepitope constructs (<50 kDa). Proper conformation and antigenicity of the polyepitope was confirmed by antibodies and T cell proliferation assays in mammalian cells and in edible transgenic plants. Some of the permutational polyepitopes generated high titers of neutralizing antibodies even against all wild-type viruses with mutations in the BCE. Polyepitopes with random conformations are less susceptible to processing and digestion of edible food than viral proteins and have a potential as edible vaccines.

A hapten conjugate vaccine against benzo[a]pyrene. Our goal is to develop an immunoprophylactic strategy for cancer prevention via carcinogen-specific antibodies that can alter the process of carcinogenesis. Monoclonal antibodies were generated by immunisation with a benzo[a]pyrene (B[a]P)-carrier conjugate. Our data indicated that the antibodies directed towards the ubiquitous carcinogen benzo[a]pyrene may protect cells in different ways based on crossreactivity with metabolites of both the activation pathway (7,8-diol-B[a]P) and detoxification pathway (phenols): (i) by decreasing cellular uptake and metabolic activation of B[a]P as demonstrated by decreased formation of phenol metabolites and higher recovery of unmetabolized B[a]P; (ii) by sequestration of 7,8-diol-B[*a*]P in the extracellular space. The biological relevance of B[*a*]P and 7,8-diol-B[*a*]P redistribution by antibodies was demonstrated by reversion of induced immunosuppression and by inhibition of CYP 1A1 induction in HepG2 cells. The results of this study provide a basis for an immunoprophylactic activity of antibodies against B[a]P induced immunotoxicity and carcinogenesis.

IDENTIFICATION OF CONSERVED INFORMATION ENCODED BY PRIMARY STRUCTURE OF THE SURFACE GLYCOPROTEINS OF H5 INFLUENZA VIRUSES: APPLICATION IN DEVELOPMENT OF DIAGNOSTIC TESTS AND VACCINE FOR H5N1 VIRUS

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The variability of antigenic determinants of the H5N1 avian influenza virus represents the main obstacle in development of a safe and effective vaccine against this highly pathogenic virus. We previously demonstrated that primary structures of variable proteins encode specific information which is responsible for their biological function [1]. This information, which is insensitive on mutations, also allows recognition of these proteins by the host immune system [2]. The bioinformatics analysis performed by the informational spectrum method, a virtual spectroscopy method for analysis of nucleotide and protein sequences, revealed the highly conserved information encoded by primary structure of the hemagglutinin HA1 subunit of H5N1 virus. Possible application of this information in development of diagnostic tests and an effective protective vaccine against H5 influenza viruses has been considered.

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STRUCTURE AND FUNCTION OF LIPOPOLYSACCHARIDE ANTIGENS OF *COXIELLA BURNETII*, THE CAUSATIVE AGENT OF Q FEVER

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Coxiella burnetii, the causative agent of Q fever, is found world-wide and is responsible for an acute illness with fever, chills and muscle pains similar to severe influenza. Pneumonitis, hepatitis, and a significant incidence of neurologic complications may also accompany the disease. Persistent infections may lead to chronic form of the disease, which may be associated with endocarditis. The microbe is extremely resistant to harsh environmental conditions due to spore formation, it readily becomes airborne, and it is highly infectious for humans. For these properties, it is at the list of biological warfare agents in "Category B". Diagnostic assays for *C. burnetii* are not straightforward. At present, serological diagnosis is the most commonly employed diagnostic method for Q fever.

Upon serial laboratory passages in embryonated hen eggs, the bacterium undergoes a virulent (phase I) to low-virulent (phase II) variation, which is accompanied by noticeable modifications in both composition and structure of the cell outer membrane components/antigens. During the acute Q fever, antibodies against phase II antigen are detected earlier and at higher titers than those against phase I antigen. In contrast, titers to phase I antigen are higher during the chronic form of the disease. We have found that the phase I antibodies are mostly directed against the O-specific chain of the *C. burnetii* lipopolysaccharide (LPS I) that is considered to be the major phase I antigen. Because of important biological properties of the LPS, its structure/function relationship studies are of potential interest. From virulent phase I cells, the LPS I and from low-virulent phase II cells the LPS II were isolated. Both LPSs were subjected to systematic structural studies. Based on the combined results of methylation-linkage analysis, FAB- and ESI-MS, and most recently of MALDI-ToF-MS, a structural model for the LPS II could be proposed. The LPS II is of rough (R) type in contrast to the LPS I, which is phenotypically smooth (S) and contains chemically distinct populations of the O-polysaccharide chains differing in their antigenic reactivities. The LPS I contains a noticeable amount of virenose (Vir) and dihydrohydroxystreptose (Strep) that have not been found in other LPSs and can be considered as important chemotaxonomic markers. A remarkable decrease in the serological activity of LPS I was observed when these two sugars were selectively removed from its O-specific chain. This indicates that most phase I antibodies are directed against the epitopes containing terminal Vir and Strep. Thus, our results may suggest that Vir and Strep are involved in the immunopathobiology of the disease.

IDENTIFICATION OF *COXIELLA BURNETII* PROTEINS USING MALDI AND ESI MS/MS TECHNIQUES

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Fast and easy detection and identification of biological warfare agent as *Coxiella burnetii* is extremely important in the whole world. We have prepared whole cell lysate as well as several extracts from this bacterium and subjected them to MS and/or MS/MS analyses in order to find unique peaks of protein biomarkers. Fingerprints of whole linear MS spectra and m/z values of characteristic peaks were exploited for creation of an integrated database as an approach for the tentative identification of biological agents. Furthermore, we have used MS/MS approach for detailed protein characterization. Thus, more reliable identification of the bacteria might be achieved. The presented results might serve for screening of immunodominant and/or immunoprotective bacterial proteins that could be used in diagnosing or vaccine development against coxiella infection. In addition they would be utilized for both rapid detection and identification of the microbial taxons.

PROTEOME STUDY OF *FRANCISELLA TULARENSIS*

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Francisella tularensis is a facultative intracellular bacterial pathogen capable of causing disease, tularemia, in many mammalian species including humans. Naturally, it can initiate human infection following ingestion of contaminated food or water, through dermal microabrasions when dressing infected animal carcasses, via bites from various hematophagous arthropods, and by inhalation of contaminated air. Two subspecies of *F. tularensis*, subspecies *tularensis* (type A), and subspecies *holarctica* (type B), exist and both are highly infectious for humans. However, only type A strains of *F. tularensis* routinely cause lethal infection in people especially following exposure to infectious aerosols of the pathogen; inhalation of as few as 10 cfu of virulent type A bacilli is sufficient to initiate severe disease. Very little is known about the bacterial virulence factors needed for infection, although it is clear that intracellular growth, especially in macrophages is essential to the virulence of *F. tularensis.* As with the virulence factors of *F. tularensis* there is also paucity of information about *F. tularensis* immunodominant antigens that hampers the development of new tularemia vaccine. Two years ago we launched extensive comparative proteomic studies of *F. tularensis* strains exhibiting differential virulence in order to find candidates for virulence factors (1,2,3). From the same reason we have also searched for bacterial proteins produced by *F. tularensis* microbes during cultivation under stressful stimuli (4). Finally, we performed 2-DE immunoblotting studies with patient`s sera to identify new immunoreactive bacterial antigens (5). The results from these studies will be presented.

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VIRUS HOST CELL PROTEOME STUDIES: DIFFERENTIAL PROTEOMIC APPROACH TO IDENTIFY BIOMARKERS OF SPECIFIC VIRAL INFECTIONS IN HUMAN AND ANIMALS

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In order to identify biomarkers for the detection of specific pathogens we need to gain further insight in the protein pattern characteristics, including post-translational modifications, of the infectious agent and of the host cell following infections by specific BSL 3 and 4 agents. For this purpose we use a gel based comparative proteomics approach to investigate the proteomes of human and avian cells after viral infection.

Two model systems of infection are studied using cultured cells: infection of human lymphocyte (ThP1) cells by measles virus (MV501) and the infection of chicken T lymphocytes (MSB1) by Chicken Anemia Virus 1(CAV1).

Culture medium and infection protocols (multiplicity of infection) of these host cells have been adapted for proteomic studies and protein extraction protocols have been optimised to prepare cytoplasmic, nuclear and membrane proteins. To identify an optimal time-point for protein extraction, we monitored the infection kinetics using FACS. An in-house developed monoclonal antibody directed against measles virus hemagglutinine has been used to detect cells expressing viral antigens on the host cell surface in the measles virus model. A time point presenting an optimal compromise between high infection rate and still reduced cell mortality was chosen for the first proteome analysis.

These different protein fractions have been analysed on 2D gels after post electrophoretic staining with Coomassie or Silver. Landmark proteins have been picked and identified using MALDI–TOF mass spectrometry on an Ultraflex I TOF/TOF instrument. Protein identifications based on peptide mass fingerprints (PMF) and MS/MS data were realized for both host organisms. The identification of chicken proteins appeared sometimes less straight forward, partially because of the reduced data library available for this organism, compared to the human cell line.

Differential proteomics studies using a 2D-DIGE approach are currently undertaken to identify modifications in the proteome after infection. In this approach the proteins before and after infection are labelled with specific fluorescence dyes and co-migrate in a same 2D gel, reducing experimental variation and ensuring an efficient identification of proteome variations.

IMMUNOCHEMICAL AND MASS SPECTROMETRIC IDENTIFICATION OF POLYPEPTIDE AND PROTEIN BIOTOXINS

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Some of the most lethal biological toxins are of proteinaceous nature and thus belong to a group of mid-spectrum agents (MSA) that are not readily assayed neither by chemical agents methods nor by assays for microorganisms. Our goal is to develop assays for the specific high priority agents ricin and Botulinum toxin as well as to devise generic methods for identification the protein content of unknown samples. The generic assays are based on mass spectrometric (MS) peptide mass fingerprinting of bands excised from sodium dodecyl sulfate-poylacrylamide gel electrophoresis (SDS-PAGE). Subsequent protein identification based on database matches usually offers a very high specificity but the approach is considerably less sensitive and less compatible with complex matrices than specific immunochemical assays for toxins. The immunochemical assays depend on specific antibodies raised against the biotoxins and are used in ELISA (enzyme-linked immunosorbent assay) formats. We here characterize the polyclonal antibodies against botulinum and ricin toxins, i.e. their use in ELISA and performance characteristics of the developed assays in model systems as well as in the analysis of environmental samples from actual cases. We also illustrate the performance of the generic SDS-PAGE-MS approach in connection with unknown substances. The availability of both immunochemical and mass spectrometric methods makes both the confirmation and exclusion of the presence of specific biotoxins in suspicious samples very reliable.

DEVELOPMENT OF A MULTI-SPECIES DETECTION STRATEGY

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MICRO-ARRAY BASED DETECTION OF ANTIBIOTIC RESISTANCE GENES IN *BACILLUS ANTHRACIS*

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A disposable microarray in form of array-tubes was developed for detection of 90 antibiotic resistance genes in Gram-positive bacteria by hybridization. Each antibiotic resistance gene is represented by two specific oligonucleotides chosen from consensus sequences of gene families, except for nine genes to which only one specific oligonucleotide could be developed. The Oligonucleotides of 26 to 33 nt in length with similar physicochemical parameters were spotted onto the microarray. For the validation of the sensitivity and the specificity of the different oligonucleotides we analyzed 36 strains of Gram-positive bacterial species carrying specific antibiotic resistance genes on the microarrays [1]. Among these strains we used well-characterized multidrug resistant strains of *Enterococcus faecalis* and *Enterococcus faecium*, *Lactococcus lactis*. In order to assess the performance of the microchip for analyses of antibiotic resistance genes in *Bacillus anthracis*, we constructed an avirulent strain of *Bacillus anthracis* harboring the broad-host range resistance plasmid pRE25. For this purpose, we conjugated the 50 kb conjugative plasmid pRE25 which carries 5 resistances genes including *erm(B), catpIP501, aph(3')-III, sat4* and *aadK* from *E. faecalis* to an avirulent *B. anthracis* lacking the toxin plasmid pXO1. Microarray analysis of the transconjugant *B. anthracis* strain revealed all the antibiotic resistance genes encoded on plasmid pRE25, as well as the two chromosomally encoded β-lactamase genes bla1 and bla2 which are not expressed in *B. anthracis*. We have shown that *B. anthracis* can easily acquire antibiotic resistance genes from other gram-positive bacteria by conjugation. These acquired resistance genes can be be detected accurately in *B. anthracis* with the microarray developed for Gram-positive bacterial antibiotic resistance genes. The array-tube platform presents the advantage of rapidly screening bacteria for the presence of antibiotic resistance genes known in Gram-positive bacteria. This technology has a large potential for applications in rapid detection of antibiotic genes in severe Gram-positive pathogens, as well as for basic research, food safety surveillance programs for antimicrobial resistance.

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TOOLS TO STUDY GENOMES AND THEIR PRODUCTS

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Total genome sequence of man and of infectious organisms will provide new insights in disease mechanisms, but improved research tools are required to take advantage of these new opportunities. MolTools is a 3½-year joint research project, funded by the European Union FP6 program, with the goal to establish a next-generation tool box with procedures and reagents to specifically analyze all the macromolecules that are being identified in the course of genome projects (www.moltools.org). The project joins 12 leading European academic groups, four biotech SMEs and one US laboratory working in the area of postgenomic technology development. The MolTools project is expected to provide resources for the research community, but also to strengthen the biotech-industry in Europe. It is aimed at developing the following technologies:

- DNA resequencing to detect unknown sequence alterations
- DNA arrays for high-throughput SNP genotyping
- Array-based techniques for global transcriptome analyses
- Large scale protein measurements using protein microarrays
- Methods for single cell and single molecule analyses
- Functional cell microarray technologies

The MolTools project is coordinated by Professor Ulf Landegren, whose research group is developing a set of ligation-based molecular tools for highly specific analyses of nucleic acids and proteins in microbes, eukaryotic cells and tissues. These tools include padlock probes, which are linear oligonucleotide probes capable of reacting to the presence of specific target nucleic acid sequences by being converted to DNA circles. Large sets of circles can be amplified and identified, for example by hybridization to universal tag microarrays. In proximity ligation, antibodies or other suitable target binders are equipped with DNA strands capable of being joined by ligation when pairs of reagents bind the same target molecule(s). The process effectively translates target molecules to DNA molecules that can then be identified for highly sensitive, precise protein detection. This procedure has been applied in both homogenous and solid-phase assays, and parallel analyses of large sets of proteins with read-out on microarrays. Reacted padlock and proximity probes can be amplified by copying the circular molecules by so-called rolling-circle replication, an ideal method for precise amplification of large sets of reacted probes or for detection of even single target molecules, directly in cells and tissues, in microfabricated devises or on arrays.

The ligation-based tools have recently been applied on the analysis of microbial pathogens, such as ones that could be used in biological warfare and terrorism. Padlock probes have been used for highly specific identification of bacterial DNA, as demonstrated by the analysis of *V. cholerae*. Similarly, proximity ligation was successfully used for analysis of single or a few copies of pathogens of importance in veterinary medicine.

DNA HYBRIDIZATION ELECTRICAL DETECTION FOR CONCENTRATIONS LOWER THAN 1nM TARGET ssDNA, BASED ON INTERDIGITATED Al/Al_2O_3 CAPACITORS

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Several electrical methods have been studied for the detection of DNA hybridization on silicon chips, using capacitance or resistance changes of micro-arrays of electrode fingers. In this work, we study the possibility of detecting DNA hybridization using 1 micron thick aluminum interdigitated capacitors structures covered by metal oxides (obtained by anodization), i.e. Al2O3, fabricated on Si substrate. Silver precipitation over Au-nanoballs labeled target DNA is used for hybridization signal enhancement. Devices are prepared for low and high frequency measurements. Electrical variables under study are the resonance frequency shift, the capacitance between electrode fingers, and the effect on the MOS capacitance through the substrate. All methods show capability for detecting down to the 0.1 nM target ssDNA concentration, which makes it competitive with traditional fluorescent labeled target probes method but with potentially much lower cost under mass-volume production, as well as unique miniaturization and portability features. The ongoing project is to assess the detection B. anthracis specific DNA targets by use of this new nanodevice.

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REAL-TIME PCR ASSAYS FOR THE RAPID IDENTIFICATION OF BACTERIAL AGENTS

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Background: Real-time PCR assays are highly specific and sensitive tools that have the potential of automation and allow the presumptive identification of agents within a few hours. Approximately half of all microbiological laboratories in Germany and Austria use this technique routinely. Objective: Our aim is to evaluate real-time PCR assays that cover the spectrum of bio threat bacterial agents. This panel of assays should be applicable in all laboratories equipped with real-time PCR instruments. Results: We have evaluated previously published PCRs and developed new hybridization probes and 5´nuclease assays including internal amplification controls that reliably detect relevant bacterial agents. Several assays can be performed in parallel using identical cycling conditions. Conclusions: In a bio terrorist scenario clinical microbiology laboratories will have to be able to identify biological agents. In general, clinical laboratories lack strain collections to evaluate assays for the identification of biological agents. So far, we have published assays for *Yersinia pestis*, *Burkholderia mallei* and *Burkholderia pseudomallei*. Currently, we evaluate real-time PCR assays for *Brucella* spp. However, multi-centre studies will be necessary to prove the robustness of promising in-house assays and round robins will have to be performed on a regular basis as a tool for quality control.

A SERONEGATIVE CASE OF OCULOGLANDULAR TULAREMIA CONFIRMED WITH PCR

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During the last ten years several tularemia outbreaks occurred in the Balkan peninsula. In Bulgaria, the 1997-2005 outbreak affected 285 people [2]. Most of the clinical forms were presented as oropharyngeal but there were several oculoglandular cases. The infections were laboratory confirmed with several methods: serology, IFA, PCR and culture. We obtained a very good inter-method correlation. Ten strains were isolated from various sources and genotyped by AFLP. A new genotype of *F. tularensis* ssp. *holarctica* was identified as causative agent [1].
In certain cases, positive by culture and/or PCR, specific antibodies do not appear even after six months from the end of therapy. In these cases reliable methods other than serology should be used for confirmation of the diagnosis. In one such case, (oculoglandular form) we succeeded in the PCR amplification of *F. tularensis* specific DNA from ocular smear swab and from blood. The analysis was carried out with two primer sets and with inclusion of internal control. After literature search and to our knowledge this is the first tularemia case detected by ocular swab specimen.

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DETECTION OF HUMAN BRUCELLOSIS WITH PCR DIRECTLY IN SERUM SAMPLES

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Human brucellosis is still a public health problem in many developing countries. Although the overall mortality rate is as low as1-2%, the timely diagnosis represents a certain problem especially in the case of chronic disease.
The purpose of this study was to be developed a reliable PCR assay for confirming brucellosis diagnostic serology. It has been shown that serum is the preferred clinical specimen for diagnostic PCR (Zerva et al.[1]). We have optimized a PCR assay based on B4/B5 primers to work directly with serum without any DNA extraction. With suitable sample processing (microwave irradiation) it was possible to amplify *Brucella* specific DNA directly from 5µl serum. Bovine serum albumin was used to reduce the inhibitory effect of serum. The method was assessed on fifty Coombs and Wright positive serum samples from patients with clinical signs of brucellosis. The PCR assay showed very good correlation with serology results. Future investigations will be directed towards the applicability of this assay for analysis of patients with chronic brucellosis.

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THE GERMAN REFERENCE LABORATORIES FOR ANTHRAX, GLANDERS AND BRUCELLOSIS

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The aim of the presentation is to introduce the safety level 3 laboratories for anthrax, brucellosis and glanders of the Federal Research Institute for Animal Health in Germany. It will be informed about the task and research projects of the reference laboratories, the available diagnostic methods, and the facilities which can be useful for collaboration in connection with COST Action B28 activities.

MOLECULAR MARKERS FOR IDENTIFICATION OF BSL3 FUNGI

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Abstract not delivered

THE FRENCH REFERENCE LABORATORIES FOR BRUCELLOSIS AND TULARAEMIA MISSIONS, ACTIVITIES AND RESEARCH OBJECTIVES

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The AFSSA, (French Food Safety Agency) is a government institution reporting in three Ministries, Health, Agriculture and Consumers Affairs. Its missions comprise assessment, scientific and technical support and research. The agency ‘s objective is to ensure food safety from the production of raw material right through distribution to the consumer. AFSSA is composed of a risk evaluation department, itself organised in 10 different expert commissions, and of a network of 12 laboratories. Within these laboratories, the Animal Pathology and Zoonoses Research Laboratory comprises, among 4 units; the Bacterial Zoonoses Unit.

This unit is the OIE/FAO and Veterinary National Reference Laboratory for Animal Brucellosis, Tuberculosis, Paratuberculosis, as well as the Veterinary National Reference Laboratory for Tularaemia, Avian Chlamydiosis and Anthrax and National Reference Centre for human Brucellosis.

Brucellosis and Tularaemia reference laboratory’s activities include scientific and technical support, mainly in reference diagnosis as identification of bacterial strains using classical and molecular tools, control of diagnostic reagents and vaccines, confirmation of cases or outbreaks, epidemiological studies in wildlife for the sanitary surveillance. This laboratory also maintains a representative collection of strains and organises specific training at regional and international levels.

The research activities are focused on: the development, assessment and validation of tools for diagnostic purpose and for studying the epidemiology of bacterial strains (conventional and Real-Time PCR, biochemical characterization, PFGE, VNTRs, RFLP-PCR) and their insertion in a sanitary decision system validated at the epidemiological level.

“The purpose of this communication is to present the Brucellosis and Tularaemia Reference Laboratories, their actions, their technical and research supports with the aim of initiating a fruitful cooperation with other laboratories in the COST ACTION B28.”

A MOLECULAR METHOD FOR THE IDENTIFICATION OF *RICKETTSIA* SPECIES IN CLINICAL AND ENVIRONMENTAL SAMPLES

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Objectives:
Rickettsia species are increasingly identified as human pathogens. However, the confirmation of a specific species in both, ticks and clinical samples, needs the sequencing of the PCR-product and accurate phylogenetic analyses, methods not available for all laboratories. The aim of this study was to design and validate a molecular method for the identification of *Rickettsia* species in clinical and environmental samples.

Methods:
23S-5S rRNA intergenic spacer was used to design generic primers and a series of genus- and species-specific probes for *reverse line blotting* (RLB) [1, 2, 3]. Tandem Repeat Finder and GeneQuest software were used to analyze repetitive elements found in the genome of the bacteria [4]. Fourteen rickettsia species were used to set up the method.

Results:
The PCR showed a high sensitivity, and species-specific identification by RLB was achieved for spotted fever and typhus groups. The use of the described PCR/RLB method was evaluated with clinical and environmental samples, allowing the simultaneous detection of multiple species in a single sample, and avoiding the need of sequencing.

Conclusions:
We are presenting a method that detects *Rickettsia* species with a high sensitivity and identifies most of the human pathogens at the species level.

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MOLECULAR DETECTION OF ARBOVIRUSES

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Our group specializes in the molecular detection of Arboviruses. We have developed 27 mobile real time assays for Bunyaviruses and Filoviruses. Currently we are working to expand our range of molecular detection assays to Flaviviruses and Togaviruses. Together with the Viral Genetic Diversity Network in Brasil we are planning to sequence the Pilaski-Collection of Arboviruses, a lifetime collection of arboviruses (120 strains of 42 virus species) collected by the late veterinarian Dr. Jürgen Pilaski. Our current focus on assay development and sequence data collection, are preliminary to the development of detection biochips. Especially a viral haemorrhagic fever chip and a viral encephalitis chip should be of value for focusing diagnostic efforts in the case of a infectious disease syndrome of unknown origin.
In another approach we are beginning to look at nanotechnology based point-of-care diagnostic systems. We are currently planning to use the Bio-disk platform developed in Freiburg, Germany to integrate diagnostic procedures such as molecular detection of nucleic acids and detection of antibodies.

COMPARATIVE GENOMICS OF *BACILLUS ANTHRACIS* USING DNA MICROARRAYS

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The purpose of this report is to describe some of the National Microbiology Laboratory activities related to *B. anthracis*, *Y. pestis* and *F. tularensis*. We have typed these isolates in our national collection using multilocus variable number tandem repeat analysis (MLVA) and are working with the Department of National Defence, Canada to harmonize the protocols to allow rapid electronic exchange of this information. We are also interested in establishing an international database of patterns to be used in bioforensic investigations.

In addition to this work, we are utilizing DNA microarrays to compare various *B. anthracis* strains in our national collection. The *B. anthracis* microarray oligo set is based on the 5.09-Mb genome of *B. anthracis* strain A2012, sequenced by TIGR, (GenBank Accession number NC_003995) is a representative of the organism's entire genome as well as the 182kb pX01 plasmid (GenBank Accession number NC_003980) and the 95kb pX02 plasmid (GenBank Accession number NC_003981). A set of 5716, 70mer oligonucleotides (5544 genome ORF sequences, 116 ORFs from plasmid pX01 and 56 ORFs from plasmid pX02) were designed and prepared by Qiagen Operon.

We performed comparative genomic hybridization of 13 *B. anthracis* isolates against *B. anthracis* Ames strain. Seven strains showed no differences in gene content. Four other strains differed by either: two, four, six or eight genes. One strain lost 22 genes compared to the Ames strain as detected by microarray analysis. The absence of these genetic elements will have to be verified by PCR.

These studies may identify potential genetic areas of variability in these strains and may be useful in microbial forensic and evolutionary studies related to *B. anthracis.*