

# **COST Action B28**

## ***Array Technologies for BSL3 and BSL4 Agents***

### **Annual Report**

*Period: from (27/5/2005) to (12/2006)*

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# 1. OVERVIEW: ACTION IDENTIFICATION DATA

## Action Identification Data

**COST Action (number) Title:** *B28 array technologies for BSL3 and BSL4 pathogens*

**TC Recommendation:** *(day/month/year)*

**First MC meeting:** 27/5/2005

**CSO Approval:** 15/03/2005

**Last MC meeting:** 22/11/2005

**Start date:** 27/5/2005

**Final Report:** *(day/month/year)* (2)

**Duration:** 60

**Evaluation Report:** *(day/month/year)* (2)

**Extension:** *months*

**TC Evaluation:** *(day/month/year)*

**End date:** 27/5/2010

**Number of signatories:** 17

**Signatories and date of signature:** *(day/month/year)*

Austria 06/07/2005

Greece 19/08/2005

Poland

Belgium 01/06/2005

Hungary

Portugal

Bulgaria 20/05/2005

Iceland

Romania 4/11/2005

Serbia & Montenegro 27/05/2005

Croatia

Ireland

Israel

Slovakia 06/07/2005

Cyprus

Italy

Slovenia

Czech Rep. 30/06/2005

Latvia

Spain 27/05/2005

Denmark 26/05/2005

Lithuania

Sweden 06/09/2005

Estonia

Luxembourg 06/07/2005

Switzerland 06/07/2005

Finland

Malta

Turkey 05/01/2006

fYR of Macedonia

France 26/05/2005

Netherlands 01/06/2005

United Kingdom 21/06/2005

Germany 25/05/2005

Norway

**Institutes of non-COST countries:** *Canada*

**Area:** **Medicine and Health**

**Action Web site:** <http://www>. (under construction)

**Chair:** **Butaye Patrick**

<i>Title, name:</i> Dr. Butaye Patrick	<i>Tel.</i> +32 2 379 0415
<i>Affiliation</i> Veterinary and agrochemical research center	<i>Fax.</i> +32 2 379 0670
<i>Postal Address</i> Groeselenberg 99	<i>E-Mail</i> <a href="mailto:pabut@var.fgov.be">pabut@var.fgov.be</a>
<i>P.O. code, City</i> 1180 Brussels	<a href="http://www.var.fgov.be">http://www.var.fgov.be</a>
<i>Country</i> Belgium	

**TC Rapporteur:** *Title, name, country*

**External Evaluator:** *Title, name, affiliation, country*

**External Evaluator:** *Title, name, affiliation, country*

(1) Date of the first MC meeting.

(2) When the report is received by TC Secretariat

## 2. OBJECTIVES

The main objective of the Action is to increase knowledge on BSL3 and BSL4 agents in order to support the development of more accurate diagnostics, vaccines and therapeutics, and to better understand epidemiology of these highly pathogenic micro-organisms that potentially can be used as biological weapons.

In the United States, several research laboratories are dealing with fast identification and characterization of micro-organisms that can be used as biological weapons. They receive massive support from their government to develop tests that will aid politicians in decision-making in the event that the organisms, described in this project, would be misused. They are also heavily involved in development of further treatments and vaccines for these microorganisms. The outcome of all this research is, however, mainly covered by secrecy and is of little help to the European Community. This means that when something happens with these microorganisms, Europe would completely depend on the good will of the United States. Moreover, some live microorganisms are only available outside of the EU (smallpox). Therefore we will also collaborate with these institutes, to obtain the necessary information on these organisms, enabling the network to also have an output on these.

An investment into this subject of this magnitude would be very ineffective if the individual members of the EU had to do this individually. Moreover, not all technical and equipment possibilities are covered by the individual states, especially for the smaller member states and the newcomers into the EU, such an investment would be financially very difficult to cope with. However, in Europe, there are several laboratories capable of giving a major contribution into developing such research, though they are scattered over the different member states. These factors point to the need for a strategic collaboration of groups throughout Europe as proposed in this COST Action in order to cover the possibilities of studying these human pathogens.

Several culture collections and laboratory facilities are also scattered over Europe, so it is absolutely necessary to unite the different laboratories to ensure that all means for success are brought together. The existing research capacities of the different laboratories, active in the field of human, animal medicine and defence, who are dealing with the same microorganisms will be united in this COST Action. Also a firm connection with a group of technologists will be established. This together with additional help of people specialised in certain research items like genomics, proteomics, glycomics and antigenicity will make this COST Action unique in its capabilities. Moreover additional expertise of the several researchers in this COST Action in the field of antibiotic resistance, vaccine development and immunology will make this project unique in its kind and enabling the through study of these rare but highly pathogenic and emerging microorganisms.

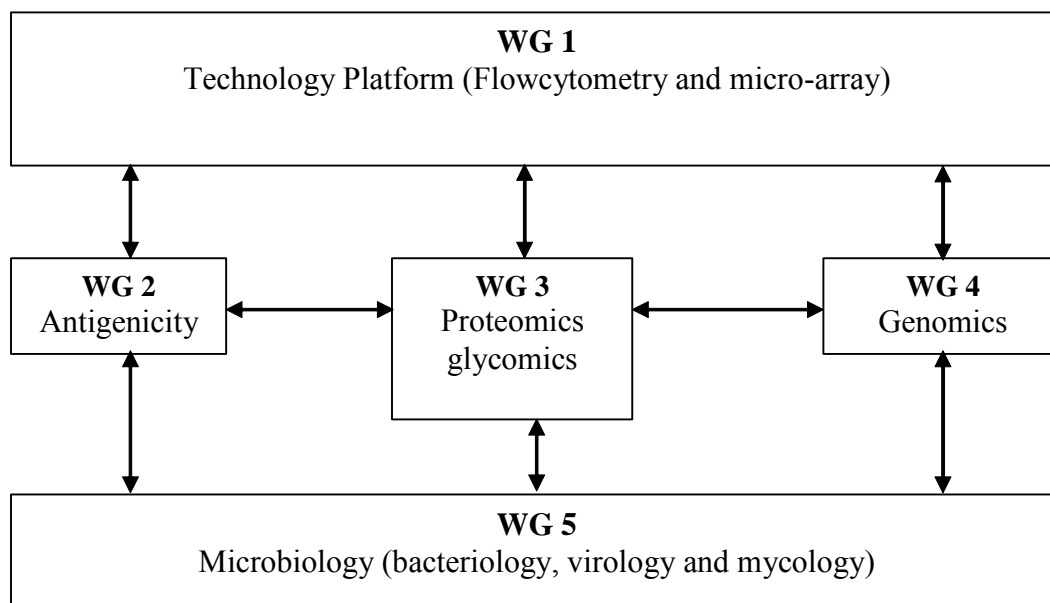
Several European-laboratories that have dealt with the “anthrax threat” that started in 2001 were not sufficiently prepared to handle the demand. Several laboratories also had different backgrounds, ranging from veterinary institutions through human medical institutions to defence institutions. Not all laboratories were able to perform fast and accurate detection/diagnosis of the agent. This COST Action is already supported by several of these laboratories and by networking them we would gather the experience of the past and offer them, in collaboration with other partners, several new means to enable fast diagnosis and intervention. Many of these laboratories also have other experiences than working on the biological weapons aspect. Experience concerning taxonomy, antibiotic resistance, vaccine development and virulence is also present, enlarging the possibilities behind the detection and diagnosis of these agents. Therefore the tools developed in COST Action will also allow their use in this diverse range of research applications. This Action will create an overall comprehensive and applicable knowledge base concerning these particular microorganisms. It

is also important that this network, once created can easily extend to other microorganisms specified; this is already present for some of the partners, who are dealing with several organisms important for public health.

### 3. TECHNICAL DESCRIPTION AND IMPLEMENTATION

Describe the items of technical work, the mode of operation, possible subdivision in Working Groups, and how the secretarial services were organised (no more than 2 pages).

The technical work has been subdivided as shown in figure 1. 5 work packages have been created in order to cover all items of importance for the study of BSL 3 and BSL 4 agents.



Working group chairs have been identified during the first WG and second MC meeting. They will be in charge of the coordination of the work within the Working groups.

A subcommittee of 3 persons on the selection of STSM has been established during the first WG and second MC meeting.

### 4. PARTICIPATION AND COORDINATION

#### 4.1 Management Committee

Chair:

**Patrick BUTAYE**

Veterinary and Agrochemical Research Center

Dept of Bacteriology and Immunology

Groeselenberg 99

B-1180 Brussels  
Tel : +32 2 379 04 15  
Fax : +32 2 379 04 01  
E-mail : [pabut@var.fgov.be](mailto:pabut@var.fgov.be)

Vice Chair:

**Dr. Rudolf TOMAN**

Laboratory for Diagnosis and Prevention  
of Rickettsial and Chlamidial Infections

Institute of Virology

Slovak Academy of Sciences

Dubravska cesta 9

SK – 845 05 Bratislava

Slovakia

Tel: +421 2 593 02418

Fax: +421 2 547 4284

E-mail: [Virutoma@savba.sk](mailto:Virutoma@savba.sk)

List: Members by country: only Title, name and affiliation

The actual MC list is not completed since some countries haven't assigned persons to the MC.

The provisional list is given below.

## **AUSTRIA**

Hr. Dr. Levente Bodrossy

ARC Seibersdorf research GmbH, Abt. Biogenetics

## **BELGIUM**

Jean-Luc GALA

Université catholique de Louvain

Laboratoire de Technologies Moléculaires Appliquées

Pierre-Alain FONTEYNE (Substitute)

Scientific Institute of Public Health

Mycology Section

Patrick BUTAYE

Veterinary and Agrochemical Research Center

Dept of Bacteriology and Immunology

## **BULGARIA**

Mr. Todor Kantardjiev

National Center of Infections and Parasitic Diseases

Stefan Panaiotov, PhD

Bulgarian Association of Microbiologist

Department of Microbiology

## **CZECH REPUBLIC**

Dr. Jaroslav URBAN  
Transport Research Centre

Dr. Jiri STULIK  
University of Defence  
Faculty of Military Health Service

## **DENMARK**

Niels HEEGAARD  
Department for Auto Immunology  
Statens Serum Institut 81/536

## **FRANCE**

Dr. Patricia Renesto-Audiffren  
Unité des Rickettsies – CNRS UMR 6020  
Faculté de Médecine

Dr. Bruno GARIN-BASTUJI  
Unité Zoonoses Bactériennes  
Laboratoire d'Etudes et de Recherches en  
Pathologie animale et Zoonoses (Lerpaz)  
Agence Française de Sécurité Sanitaire des Aliments (AFSSA)

## **GERMANY**

Prof. Dr. Frank Hufert  
Institut für Virologie  
Universitätsklinikum Göttingen

Dr. Manfred Weidmann (Replacement)  
Universitätsklinikum  
Unit for Microbiological Preparedness and  
Emerging Infectious Diseases

Dr. Mandy Elschner  
Bundesforschungsinstitut für Tiergesundheit  
Institut für Bakterielle Infektionen und Zoonosen

## **GREECE**

Dr. Maria SAKARELLOS  
University of IOANNINA

## **LUXEMBOURG**

Claude P. MULLER  
Laboratoire National de Santé  
Département d'Immunologie

## **NETHERLANDS**

Dr. Ruud Busker  
TNO Defence, Security and Safety  
Business Unit Chemical & Biological Protection

Sybren DE HOOG  
Centraal Bureau voor Schimmelcultures

## **ROMANIA**

Dr. Iuliana Apostol  
Dr. Victor Babes Foundation

Dr. Maria NICA  
Hospital Dr. Victor Babes Bucharest

## **SERBIA AND MONTENEGRO**

Dr. Veljko Veljkovic  
Senior Scientist  
Center for Multidisciplinary Research and Engineering  
Institute of Nuclear Science VINCA

## **SLOVAKIA**

Dr. Rudolf TOMAN  
Laboratory for Diagnosis and Prevention  
of Rickettsial and Chlamidial Infections  
Institute of Virology  
Slovak Academy of Sciences

## **SPAIN**

Dr. Raquel ESCUDERO  
Lab. Espiroquetas y Patógenos Especiales  
Dept. Bacteriología  
Instituto de Salud Carlos III

## **SWEDEN**

Åke LUNDKVIST  
Karolinska institutet  
Mikrobiologiskt och tumörbiologiskt centrum

Anders SJÖSTEDT  
Umeå Universitet  
Universitetssjukhuset

## **SWITZERLAND**

Mr. Prof. Jacques SCHRENZEL  
Hôpitaux universitaires de Genève HUG  
Laboratoire de Génomique Fonctionnelle  
Division des Maladies infectieuses

Mr. Prof. Dr. Joachim FREY  
University of Berne  
Institute of Veterinary Bacteriology

## **UNITED KINGDOM**

## **TURKEY**

Dr. Erhan PISKIN  
Department of Chemical Engineering  
Faculty of Engineering  
Hacettepe University

## **NON-COST COUNTRIES**

## **CANADA**

Dr. Michal MULVEY  
Canadian Science Centre for Human and Animal Health  
National Microbiology Laboratory

## **4.2 Participating Institutes**

List: denomination and country without address

ARCS Seibersdorf	Austria
Veterinary and agrochemical research center	Belgium
Scientific institute for public health	Belgium
UCL	Belgium
National Center of Infections and Parasitic Diseases	Bulgaria
Bulgarian Association of Microbiologist	
Department of Microbiology	Bulgaria
Canadian Science Centre for Human and Animal Health	Canada
University of Defence	Czech Republic
Urban	Czech Republic
Statens Serum Institut	Denmark
INRA	France
AFSSA	France

Université de Marseille	France
Federal Institute for Risk Assessment (BFR)	Germany
Bundesforschungsinstitut für Tiergesundheit	Germany
Institut für Mikrobiologie der Bundeswehr	Germany
Universitätsklinikum Göttingen	Germany
Robert-Koch Institut	Germany
University of Ioannina	Greece
Laboratoire National de Santé	Luxemburg
Dr. Victor Babes Foundation	Romenia
Institute of Nuclear Science	Serbia and Montenegro
Slovak Academy of Sciences	Slovakia
Instituto de Salud Carlos III	Spain
Uppsala University	Sweden
Karolinska institutet	Sweden
Umea University	Sweden
University of Bern	Switzerland
University of Geneva	Switzerland
TNO	The Netherlands
Centraal Bureau voor Schimmelcultures	The Netherlands
RIVM	The Netherlands
Hacettepe University	Turkey
University of Wales, Aberystwyth	UK
HPA	UK

### ***4.3 Meetings of the Management Committee***

Brussels, Belgium, 27/05/2005:  
 Bratislava, Slovak Republic, 22/11/2005  
 Antalya, 3 October, 2006

### ***4.4 Meetings of the Working Groups***

Bratislava, Slovak Republic, 21-22/11/2005  
 Antalya, 1-3 October 2006

### ***4.5 Short-term scientific missions***

A core group of 3 persons has been assigned to evaluate the proposed STSMs. Actually there hasn't been any submission for STSM. To promote the STSMs, A renewed call to the MC and WG members was launched and a summer school is planned by one of the participants.

## **5. RESULTS**

## **5.1 Results of the MC meetings**

### First MC meeting

The first meeting of B28 was a MC meeting organised in Brussels at the COST secretariat on 30/5/2005. Ten MC members were present. An equal number of MC members was excused. At that time 12 countries assigned MC members and 5 signed the MoU. Information on COST was distributed. Patrick Butaye was elected as chair and Rudolf Toman as vice chair.

It was decided to have a first WG-MC meeting in Bratislava where the objectives and work programme will be better established and this at the WG level. A second aim of the meeting was to better get known with the activities of the participating institutions and universities in order to be able to establish collaborations.

### Second MC Meeting

Election of an STSM panel:

The MC agreed on the following composition of the STSM Panel consisting of 3 people :

Rudolf Toman - vice chair of the action

Patricia Renesto-Audriffen

Maria Sarakellos

All the applications should be sent to Rudolf Toman as the leader of the panel who will treat the applications according to the COST regulations for STSMs

A B28 website will be constructed.

The following WG leaders were elected:

- A. WG 1  
Jacques Schrenzel
- B. WG2  
Claude P. Müller
- C. WG3  
Jiri Stulik
- D. WG4  
Stefan Panaiotov
- E. WG5  
Mandy Elschner

The next WG-MC meeting was prepared and would be a combined WG-MC and International Conference meeting in Turkey. The conference subject would be innovative array technologies.

### First WG meeting

The First WG meeting was held on 21 and 22 november of 2005 in Bratislava, Slovak Republic. 56 participants were inscribed.

The aim of this meeting was to let the partners present their laboratory activities in the field of the work programme as described in the annex of the MoU.

Two invited speakers were asked: One speaker from Turkey, a country with the intention to sign the MoU and one speaker from Russia. The latter would also be included in the COST Action, since one partner from a near neighbour country can attend one meeting a year. This partner would be extremely valuable, since it is the only partner with experience in all pox viridae, an experience lacking in the EU and highly relevant to the programme.

Twenty nine presentations were given dealing with the different aspects of the studies to be performed. Seven presentations were given in WG1, three in WG2, six in WG3, five in WG4 and seven in WG5. Of nearly each presentation an abstract is available.

First exchanges of contacts were laid and collaboration plans were established between several partners. Collaborations are establishing and will be reflected in the next annual report.

### Third MC meeting

The attendance and organisation of the meeting was difficult therefore some actions were discussed and decided upon to improve that point:

The attendance rate of this meeting was not as high as last meeting and disappointed some partners of the COST action. Reasons for these low attendance rate were looked for and, the cost of the meeting, the combination with other meetings, and the recent bombing were possibly identified as causes.

Joachim Frey, who chaired a former COST Action had a similar experience, in which the second meeting was poorly attended. The action however evolved well and had a large attendance rate with many new participants during its evolution.

The discussion on attendances was ended with a positive note that the MC members and WG chairs will activate the WG members to participate to the meetings.

Patrick Butaye stressed on the fact that MC members should take their responsibilities and should attend the MC meetings as much as possible. If they are frequently unable, they can look for a replacing member for the MC (as there is for Belgium). Some countries should check with the website and their national representatives for their membership. Also, there is seemingly some degree of confusion cause by bad communication between COST and the national representatives. Also the website is not complete, compared to the list from the COST secretariat.

The Next meeting would be sooner following the last meeting to improve contact and to allow the participants that were absent in Antalya not to loose contact with the other participants. It was decided to organize a meeting in April 2007 in Bulgaria. Bulgarian participants and personally Dr. Panaiotov assumed the responsibility to contact, coordinate and organize the meeting.

## **5.2 Results of the WG meetings**

## 5.2.1 Results of WG1 meetings

1) Name: Levente BODROSSY and Tanja Kostić

### Contribution:

Design, validation and application of microbial diagnostic microarrays. Development and adaptation of novel techniques to improve sensitivity and specificity of detection via microbial diagnostic microarrays. Development of specific microarrays for *Salmonella* serotyping and for waterborne pathogens.

### Publications:

T.Kostić, A.Weilharter, A.Sessitsch and **L.Bodrossy**, High sensitivity, PCR-free detection of microorganisms and their functional genes using 70mer oligonucleotide diagnostic microarray. *Analytical Biochemistry*.346:333-335. 2005.

A.Loy and **L.Bodrossy**, Highly parallel microbial diagnostics using oligonucleotide microarrays. *Clinical Chimica Acta* 363(1-2):106-119. 2006.

**L.Bodrossy** and A.Loy, Oligonucleotide microarrays in microbial diagnostics. In: *Encyclopedia of Medical Genomics and Proteomics* (Editors: J. Fuchs and M. Podda). Online edition. DOI: 10.1081/E-EDGP-120041475. Taylor & Francis. 2006.

**L.Bodrossy**, N.Stralis-Pavese, M.Konrad-Köszler, A.Weilharter, T.Reichenauer, D.Schöfer, and A.Sessitsch. mRNA-based parallel detection of active methanotroph populations using a diagnostic microarray. *Applied and Environmental Microbiology*, 72 (2): 1672-1676. 2006.

A.Loy, M.W. Taylor, **L.Bodrossy** and M.Wagner, Applications of Nucleic Acid Microarrays in Soil Microbial Ecology. pp. 18-41. In: *Molecular Techniques for Soil and Rhizosphere Microorganisms* (Editors: JE Cooper, JR Rao). CABI Publishing, Wallingford, Oxfordshire, UK. 2006.

A. Sessitsch, E. Hackl, P. Wenzl, A. Kilian, T. Kostic, N. Stralis-Pavese, B. Tankouo Sandjong and **L. Bodrossy**. Diagnostic microbial microarrays in soil ecology. *New Phytologist*, 171:719-736. 2006.

T.Kostić, A.Weilharter, S.Rubino, G.Delogou, S.Uzzau, K.Rudi, A.Sessitsch and **L.Bodrossy**, A microbial diagnostic microarray technique for the sensitive detection and identification of pathogenic bacteria in a background of non-pathogens. *Analytical Biochemistry*. 2006. In Press.

### Collaborations: in the field of pathogen detection:

Knut Rudi, Matforsk, DK; Martin Wagner, Veterinary University, Vienna, Austria; Salvatore Rubino, University of Sassari, Italy; Alexander Loy, University of Vienna, Austria.

2) Name: Jacques SCHRENZEL ([Jacques.schrenzel@genomic.ch](mailto:Jacques.schrenzel@genomic.ch))

### Contribution:

Design, development and validation of innovative microarray formats for detecting unknown or unusual pathogens. Two formats are currently under development: a non-cognate hybridization system (NCHS), i.e. an array with >8'000 probes to encompass all combinations of two nucleotides on 13mers. The second format uses a hierarchical classification of 25mer probes to cover the 16S rDNA diversity. Each probe is then mapped to the node of the tree that it can determine.

Publications: in preparation

Collaborations: with the group of Patricia RENESTO (Marseille, France) for pathogen annotation and microarray analyses.

3) Name: Karl Walravens ([Karl.walravens@var.fgov.be](mailto:Karl.walravens@var.fgov.be))

Contribution:

Development and validation of a bead array for the multiplex detection of zoonotic pathogens by the help of a FACS. Validation fo bacterial strain typing by the help of Monoclonal antibodies and FACS analysis.

Publications: too early

Collaborations: Martien Broekhuizen, members of the former COST action 845 (Brucella typing work).

4) Name: Martien BROEKHUIJSEN ([martien.broekhuijsen@tno.nl](mailto:martien.broekhuijsen@tno.nl))

Contribution: Design and development of microarrays for 1) genomic comparison of Brucella strains, 2) multipathogen identification.

Publications: in preparation

Collaborations: with the group of Pierre WATTIAU (CODA-CERVA, Brussels, Belgium) for microarray analysis of Brucella; with Manfred WEIDMANN (university Göttingen, Germany) and Nigel SILMAN (HPA Porton Down, UK) for development of multipathogen microarray analysis.

5) Name: Ulrich Nübel ([nuebelu@rki.de](mailto:nuebelu@rki.de))

Contribution:

Bacterial genotyping on the basis of DNA microarrays: diagnostic detection of taxonomic markers, virulence associated genes, and antibiotic resistance determinants; single nucleotide polymorphisms; comparative genome hybridizations

Publications:

Antwerpen et al., DNA microarray for detection of antibiotic resistance determinants in Bacillus anthracis and closely related Bacillus cereus, Mol. Cell. Probes, in press (page proofs attached to this email); additional manuscripts in preparation

Collaborations: Patricia RENESTO / Didier RAOULT (Marseille, France) --  
microarray diagnostics of Rickettsia spp.  
Rudolf TOMAN (Bratislava, Slovakia) –  
microarray diagnostics of Rickettsia spp.

6) Name: Gareth W GRIFFITH and Hazel M. DAVEY  
([gwg@aber.ac.uk](mailto:gwg@aber.ac.uk); [hlr@aber.ac.uk](mailto:hlr@aber.ac.uk))

Contribution:

Use of flow cytometry for the detection of airborne bioaerosols, including biowarfare agents and other pathogenic microbes. Use of fluorescently-tagged antibodies allows the specific detection of target propagules in a background of other bioaerosol materials, in association with evolutionary computing methods for defining gating parameters. The system is being tested with spores of / *Aspergillus fumigatus* in the first instance but it is generic in its capability for detection. Developing methods for rapid determination of microbial viability and monitoring the effectiveness of antibiotic therapy.

Publications:

Assunção, P., Antunes, N.T., de la Fe, C., Rosales, R.S., Poveda J.B and Davey, H.M. (2006) Flow cytometric determination of the effect of antibacterial agents on *M. agalactiae*, *M. putrefaciens*, *M. capricolum* subsp. *capricolum* and *M. mycoides* subsp. *mycoides* large colony-type. *Antimicrobial Agents and Chemotherapy* 50: 2845-2849.

Collaborations: With the group of Erhan Piskin (Ankara, Turkey) for the development of specific antibodies.

7) Name: Michael MULVEY (Michael\_Mulvey@phac-aspc.gc.ca)

Contribution: Development of standardized molecular forensic methodologies for the typing of *B. anthracis*, *F. tularensis*, and *Y. pestis*. Development of improved methodologies, such as single nucleotide polymorphisms (SNPs) and single nucleotide repeats (SNRs) for typing the above listed organisms. The use of DNA microarrays for comparative genomic and expression analysis of *B. anthracis* genomes.

Publications:

Stratilo CW, C. Lewis, L. Bryden, M. R. Mulvey, D. Bader. 2006. Identification of *Bacillus anthracis* isolates using single nucleotide repeat analysis (SNRA). *Journal of Clinical Microbiology*, 44:777-782.

Collaborations: Dr. Silman (United Kingdom) and Mats Forsman (Sweden) for standardizing molecular typing methodologies.

8) Name: Ulf LANDEGREN (ulf.landegren@genpat.uu.se).

Contribution: Participation in the COST Action B28, “Array Technologies for BSL3 and BSL4 Pathogens” as representative of the EU Integrated Project MolTools that is coordinated by Ulf Landegren. The MolTools project aims to develop array-based tools for analysis at levels of DNA, RNA, protein, and also viable cells. We are pleased to interact with this COST Action to disseminate information arising in our project that could be relevant for pathogen detection. Several recent developments in MolTools could be of interest for the COST Action, and three are mentioned here:

The group of Jörg Hoheisel in Heidelberg have established arrays with mirror-image L-form oligonucleotides, capable of hybridizing to corresponding tags on probes, but exhibiting no tendency to hybridize to DNA sequences representing biological sequences (Hauser et al. *Nucl Acids Res* 34: 5101-5111, 2006).

Another tag-array approach developed in Uppsala is in preparation, and involves a dual-tag recognition step on arrays, which ensures both high specificity and sensitivity because of inbuilt amplification steps (Ericsson et al. in preparation). Tag array approaches could be

valuable for detection of pathogens since they allow solution-phase probes to detect and amplify signals, before sorting on arrays.

Finally, also in Uppsala, the so-called proximity ligation technique has been applied for detection of viral particles and bacteria (Gustafsdottir et al. Clin Chem 52: 1152-1160, 2006). The detection sensitivities observed were far superior to that of ELISA and similar to real-time PCR of the nucleic acids of the infectious agents, and sample preparation steps were minimal.

More information about the project can be found at [www.moltools.org](http://www.moltools.org).

#### Publications:

Gustafsdottir SM, Nordengrahn A, Fredriksson S, Wallgren P, Rivera E, Schallmeiner E, Merza M, Landegren U. Detection of individual microbial pathogens by proximity ligation. Clinical Chemistry 52: 1152-1160 (2006).

9) Name: Jean-Luc gala ([gala@lbcm.ucl.ac.be](mailto:gala@lbcm.ucl.ac.be))

Contribution: design, development and validation of microarray for typing bacteria. Low high density microarray have been used to identify bacterial species. Beads technology is now being developed for same purposes. Alternatively, the CMOS chip are being investigated for the detection of SNP.

#### Publications on microarray:

(a) S. Hamels, J.L. Gala, S. Dufour, P. Vannuffel, N. Zammateo, J. Remacle. Consensus PCR and microarray for diagnosis of the genus Staphylococcus, species, and methicillin resistance. Biotechniques 31 (2001) 1364-1366,1368,1370-1372.

(b) F. Lecouvet, L.M. Irengé, B. Vandercam, A. Nzeusseu, S. Hamels, J.L. Gala\*. The etiologic diagnosis of infectious discitis is improved by amplification-based DNA analysis. Arthritis & Rheumatism, 2004; 9: 2985-94

(c) Mycobacteria typing : in preparation

Collaborations: with Eppendorf Array Technology for microarray analyses.

10) Name: Erhan Pişkin ([piskin@hacettepe.edu.tr](mailto:piskin@hacettepe.edu.tr))

Contribution: design, development and validation of innovative microarray formats based on surface plasmon resonance and elipsometer for detecting different type of pathogenic bacteria. These studies were initiated by using different species of mycobacterium which causes tuberculosis. This is based on hybridization on array surfaces.

Publications: in preparation

Collaborations: not started yet but we are hoping to collaborate with the partners who are looking array platforms for their target pathogens. Submission of FP6 programme on SPR for mycobacteria with partners from COST Action B28

Name: Dimitrios FRANGOULIDIS ([DimitriosFrangoulidis@Bundeswehr.org](mailto:DimitriosFrangoulidis@Bundeswehr.org))

Contribution: design, development and validation of a novel Low-Cost-and-Density (LCD)-Microarray for the detecting of *Coxiella burnetii* and other unusual pathogens. One prototype is finished and the sensitivity was determined to be up to 10 genomic copies/ $\mu$ l template for IS1111 and 100 copies for *adaA*. Array modifications according to the design of the internal control are just planned.

Publications: in preparation

Collaborations (planned): with the group of Rudolf TOMAN (Bratislava, Slovak Republic) for further evaluation with different materials and strains.

### 5.2.1 Results of WG2 meetings

**1. Introduction.** The workgroup antigenicity includes 7 laboratories from 6 countries. The WG is coordinated by the Institute of Immunology in Luxembourg. First exchanges and contacts were established during and after the WG meeting in Bratislava. Ongoing research from different partner laboratories was presented as oral communications and provided the basis for new collaborations. The presentations in Bratislava and Antalya covered antigenicity issues of several pathogens and different approaches to identify pathogen-specific antigens. Some of these antigens were displayed on synthetic carrier molecules or were expressed as recombinant constructs used as diagnostic antigens or used to induce antigen-specific immune-responses in animals. Results of different groups will be presented later in a COST B28 booklet.

**2. The Unité des Rickettsies CNRS UMR 6020 - IFR 48 - Faculté de Médecine, Marseille, FR** (P. Renesto; [patricia.renesto@medecine.univ-mrs.fr](mailto:patricia.renesto@medecine.univ-mrs.fr)) investigated antigenic properties of Rickettsiae. New transcriptomic and proteomic studies of these intracellular bacteria responsible for epidemic and endemic typhus (*R. conorii* and *R. prowazekii*, respectively), and for spotted fever have been initiated since the complete genome of these pathogens is available. Proteomics strategies were developed to identify rickettsial proteins by accessing facilities including MALDI-TOF, Ion-Trap mass spectrometer and nano-HPLC. This allowed the construction of the first proteomic reference map of both *R. conorii* and *R. prowazekii* and to identify highly antigenic proteins. Putative ligands recognized by endothelial cells could be identified. Another post-genomic application was the analysis of *R. conorii* transcriptome by microarrays. This research aims at further expanding our knowledge about the proteins (actually) expressed by these microorganisms and encoded by virtual annotated genes and which would constitute new diagnostic or therapeutic targets. The group has further characterised of what so far are only putative genes, termed ORFans, of Mimivirus, the largest known virus in particle size and genome complexity (1.2Mb genome) and a possible pathogen responsible for pneumonia.

**3. The Laboratory of Peptide Chemistry, Department of Chemistry, University of Ioannina, GR** (M. Sakarellos-Daitsiotis; [msakarel@cc.uoi.gr](mailto:msakarel@cc.uoi.gr)) developed sequential oligopeptide carriers (SOCn) for anchoring multiple copies of antigenic/immunogenic peptide epitopes. Carriers formed by the repetitive Lys-Aib-Gly moiety display a predetermined 3D structure (310 helix), while the epitopes anchored by the Lys-N $\epsilon$ H<sub>2</sub> groups, preserve their original conformation. The carrier conformation ensures the presentation of antigenic epitopes as potent immunogens. Different SOC constructs were used to induce high titers of specific antibodies recognizing the priming construct and the target antigen. These molecules can also be used for sensitive and specific immunoassays (Artificial Carriers: A Strategy for Constructing Antigenic/Immunogenic Conjugates. Maria Sakarellos-Daitsiotis\*, Dimitrios

Krikorian, Eugenia Panou-Pomonis and Constantinos Sakarellos *Current Topics in Medicinal Chemistry*, 2006, 6, 1715-1735).

Based on the Bioinformatic Analysis developed by Veljko Veljkovic and co-workers the core sequence of a peptide that belongs to the N-terminal sequence of H5 – VIN1, which represents the prototype antigen for the universal anti-H5N1 vaccine, was coupled in two copies on a synthetic carrier (SOC<sub>4</sub>) to use it in immunoassays and vaccine preparations.

Maria Sakarellos-Daitsiotis, Dimitrios Krikorian, Eugenia Panou-Pomonis and Constantinos Sakarellos Artificial Carriers: A Strategy for Constructing Antigenic/Immunogenic Conjugates *Current Topics in Medicinal Chemistry*, 2006, 6, 1715-1735.

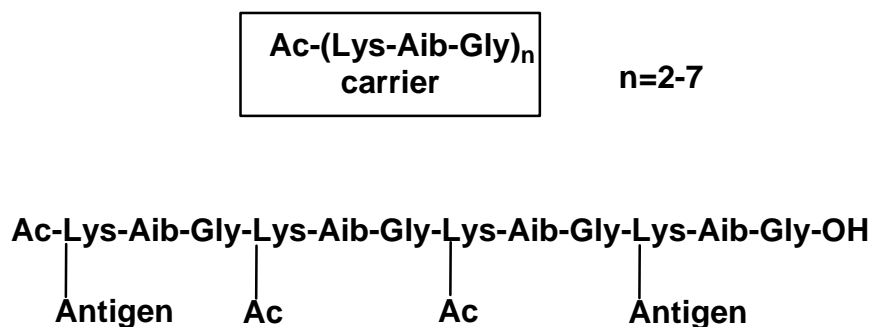


Figure 1. Sequential Oligopeptide Carriers, SOC<sub>n</sub>. Antigen: sequence 54-69 of HA1.

**4. The Institute of Immunology, Laboratoire National de Santé, LU** (C.P. Muller; [claude.muller@LNS.ETAT.LU](mailto:claude.muller@LNS.ETAT.LU)) work on conjugate vaccines to protect against viruses and environmental risk compound. The technology could also apply to pathogens of interest of the COST Action B28. One vaccine is based on linear neutralizing epitopes of the measles virus; the other one on derivatives of carcinogen benzo[a]pyrene to explore an immunoprophylactic approach.

**4.1 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. 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 polypeptide constructs (<50 kDa). Proper conformation and antigenicity of the polypeptide was confirmed by different methods. Some of the permutational polypeptides generated high titers of neutralizing antibodies even against all wild-type viruses with mutations in the BCE.

**4.2 Hapten conjugate vaccine.** The second example was a hapten conjugate vaccine against benzo[a]pyrene. Monoclonal antibodies were generated by immunisation with a benzo[a]pyrene (B[a]P)-carrier conjugate. The results of this study provide the basis for an immunoprophylactic approach against B[a]P induced immunotoxicity and carcinogenesis.

**4.3 2D-Western detection of viral protein.** In order to improve the detection of antigenic proteins on 2D gels and their subsequent identification, a method based on the combination of 2D-DIGE and multiplex western blot was developed. Fluorescently labelled proteins of virus infected cells were separated on 2D gels, blotted on PVDF membranes and viral antigens are

detected by monoclonal or polyclonal antibodies and fluorescence-labelled secondary antibodies. The use of 3 different fluorescence labels allows for multiplexing on a same 2D Western blot (ECL-Plex) and for an unambiguous spot overlay prior to the protein identification by MALDI TOF/TOF analysis. Since small 2D-gels with a high density of protein spots can be used for unambiguous spot identification, this approach allows to detect antigenic proteins from microgram amounts of total cell extract. This method provides a very sensitive tool to detect and characterize post-translationally modified species of pathogen- and host cell proteins. Considering the functional importance of such protein modifications as determinants for viral virulence and modulators of mechanisms of the innate immune response, this method is of great interest for the investigation of BSL3/4 pathogens.

**4.4 HPAI Avian influenza virus.** During the recent outbreak of avian influenza in Africa the Institute of Immunology established the first laboratory in Nigeria and most of W-Africa with the capacity to diagnose H5N1. The mission has been reported in **Nature 440, 726-727, 2006**. Phylogenetic analysis based on full length genome analysis of the viruses isolated on farms in the South-West of the country and in the Northern States represented three lineages and showed evidence for multiple introductory events in Nigeria (**Nature 442, 37, 2006**). The viral haemagglutinin cleavage-site sequence PQGERRRKKRG was identical to that of the highly pathogenic strains already found in Europe, Russia and Central Asia.

Ducatez MF, Olinger CM, Owoade AA, De Landtsheer S, Ammerlaan W, Niesters HG, Osterhaus AD, Fouchier RA, Muller CP., *Nature*, 442 :37 (2006)

**5. The Yıldız Technical University, Bioengineering Dept., Istanbul, TK** (Sevil Dinçer) used phage display technology based on filamentous phages presenting peptides or proteins to produce affinity reagents such as scaffold proteins, antibodies, or inhibitors. Displayed proteins can be used in protein arrays, separation, drug development, epitope mapping and protein-protein interactions. The technology will be applied to the selection of peptides specific to mycobacterium tuberculosis and use these peptides as probes in the detection of the related bacteria.

**6. The Statens Serum Institut, Denmark,** (N. Helena Beyer/N. Heegaard, [www.ssi.dk](http://www.ssi.dk)) produces monoclonal antibodies and polyclonal sera for the development of sensitive detection tests for several neuro- and cytotoxins.

**6.1 Botulinum neurotoxin.** Several antibody-producing clones against botulinum neurotoxin of serotype A (BoNT/A) have been obtained and the mAb will be available soon. Mice have immunized with BoNT B and BoNT E peptides to generate mAbs against neurotoxins of other serotypes. Polyclonal rabbit antibodies against BoNTs A, B, C, D, E, F have been produced and are available. An ELISA for detection of BoNT A and BoNT B with L.O. D. of 625 pg is ready.

**6.2 Ricin.** Rabbit polyclonal antibodies against ricin A- and B-chain have been produced and these antibodies are available. An ELISA for the detection of ricin with a L.O.D. of 1.6 ng is in place. Furthermore, experiments are ongoing to determine whether polyclonal antibodies against ricin A- and B-chain have any protective effect in case of intoxication.

**7. The SVB-Dr Victor Babes Hospital for Infectious and Tropical Diseases and the FVB-Dr. Victor Babes Foundation, Bucharest, Romania** (Maria Nica, [centru@cdt-babes.ro](mailto:centru@cdt-babes.ro)) is developing a clinical & epidemiological surveillance program for infectious diseases with

bioterrorist impact. The first pilot study related to bioterrorism agents and surveillance systems for unusual situations, in Bucharest area, ran between 15 Nov 2004 - 15 Oct 2005. Two Romanian participant institutions (FVB and SVB) with 7 „work stations” (2 General Practitioner offices, 4 emergency rooms and 1 microbiology laboratory).

The study aimed the evaluation and connection of some national hospitals for infectious diseases, in order to obtain an organized response in case of unusual situations in order to improve the recognition of critical biological agents.

A randomised selection of 200 cases of acute infectious diseases were presented at FVB or SVB and the patients were classified according to syndromes using the high risk sub-syndroms definition for agents of group A, B and C (CDC classification for critical biological agents). Clinical and epidemiological analysis was based on biostatistical parameter analysis. Using our software algorithm we identified: 2 cases of group A agents (botulism); 51 cases of group B agents (A paratyphoid fever, salmonellosis, shigellosis); 0-group C cases.

The meetings in Bratislava and Antalya were a first important step for the partners in the workgroup Antigenicity to discuss results and complementarity of their research competences in areas important for COST Action B28.

### **5.2.1 Results of WG3 meetings**

Introduction: The scientific activities of members of workgroup 3 in the year 2006 concerned the application of advanced proteomic and glycomic technologies for the identification of viral or bacterial structures possessing immunostimulatory properties or molecules with unique expression for particular microbial subspecies. The concrete achievements in individual laboratories are as follows:

University of Defence, Czech Republic: The facultative intracellular pathogen *Francisella tularensis* is the causative agent of the serious infectious disease tularemia. Despite intensive research, the virulence factors and pathogenetic mechanisms remain largely unknown. In order to identify novel putative virulence factors we have carried out a comparative proteome analysis of fractions enriched for membrane-associated proteins isolated from the highly virulent subspecies *tularensis* strain SCHU S4 and three representatives of subspecies *holarctica* of different virulence including the live vaccine strain. We identified six proteins uniquely expressed and four proteins expressed at significantly higher levels by SCHU S4 compared to the *ssp. holarctica* strains. Four other protein spots represented mass and charge variants and seven spots were charge variants of proteins occurring in the *ssp. holarctica* strains. The genes encoding proteins of particular interest were examined by sequencing in order to confirm and explain the findings of the proteome analysis.

In order to identify new immunogenic proteins of *Francisella tularensis* the sub-immunoproteome analysis of membrane-enriched fractions was applied. Furthermore bacteria cultivated under normal and stressful condition were used for membrane collection. By this approach 35 immunoreactive antigens were identified, 15 of them showed to be completely novel immunogens.

Institute of Virology, Slovakia :*Coxiella burnetii* is the causative agent of Q fever. The bacterium is highly infectious and is classified as a category B biological weapon. A rapid and unambiguous identification of *C. burnetii* is of utmost importance in the localization of naturally occurring Q fever outbreaks or in cases of a deliberate release of the infectious agent. We have developed a Multiple Locus Variable Number Tandem Repeats (VNTR)

analysis (MLVA) for an unambiguous identification of *C. burnetii*. The used VNTR markers have revealed many polymorphisms resulting in nine unique MLVA types that cluster into five different clusters. This proves that the MLVA system is highly discriminatory. The developed MLVA method is a promising tool for the characterization of *C. burnetii* isolates and their epidemiological studies.

A new diagnostic preparation for in vitro use has been developed for a rapid and economical serological diagnosis of Q fever using an immunofluorescent (IF) test. Simultaneously, a procedure for its application in the IF test has been developed and standardized. The diagnostic preparation for in vitro use has found a wide application mainly in screening large numbers of sera from human and animals having Q fever.

Institute of Nuclear Sciences VINCA, Serbia and Montenegro: The scientific activity was focused on the development of the EIIP/AQVN bioinformatics platform for structure/function analysis of protein and nucleotide sequences of viral and bacterial pathogens for identification of therapeutic, diagnostic and vaccine targets and selection of drug candidates. This bioinformatics platform was used for (i) identification of conserved determinants of HIV-1 envelope glycoprotein gp120 which are essential for interaction between HIV and the CCR5 receptor, and (ii) identification and valorization of compounds and peptides representing candidate HIV entry inhibitors.

Plan of work for the year 2007:

University of Defence, Czech Republic: Development and testing of mutants strains *Francisella tularensis* with selected deletion of genes encoding new potential virulence factors. Comparative proteome analyses of wild and mutant strains. Structural analysis of selected virulence factors using proteomic and mass spectrometric approaches

Institute of Virology, Slovakia:

Proteomics: In collaboration with a proteomic group at the Faculty of Military Health Sciences, Hradec Kralove, Czech Republic, a systematic mapping of the *C. burnetii* immunoreactive peptides and proteins recognized by the specific anti-*C. burnetii* human and animal antibodies developed in the course of natural infection will be accomplished.

Glycomics: A more detailed chemical composition and structure of O-polysaccharide antigen of *Rickettsia typhi*, the etiological agent of murine typhus will be established.

Institute of Nuclear Sciences VINCA, Serbia and Montenegro: The EIIP/AQVN bioinformatics platform will be used for:

(i) Identification of domains of the hemagglutinin subunit 1 (HA1) determining specific recognition and targeting of influenza A viruses (subtypes H1-H16, including 1918 H1 pandemic strain) to the host-cell receptor (RTM molecular marker).

(ii) Characterization of peptides mimicking the H5 RTM molecular marker, representing candidate viral entry inhibitors.

(iii) Selection by virtual screening of chemical libraries and molecular modeling studies of small molecules representing candidates for the multi-resistant H5 entry inhibitors targeting the H5 RTM molecular marker.

Lectures and publications

Four lectures covering the data obtained from the study of above-mentioned topics were given by representatives of University of Defence and Institute of Virology in the frame of the 3rd Management Committee and WG1, WG2, WG3, WG4, WG5 Meetings, held Antalya, Turkey.

The list of publications released from the beginning of the project:

2005:

Havlasová, J., Hernychová, L., Brychta, M., Hubálek, M., Lenco, J., Larsson, P., Lundqvist, M., Forsman, M., Krocova, Z., Stulik, J., Macela, A.. Proteomic analysis of anti-Francisella tularensis LVS antibody response in murine model of tularemia. *Proteomics*, 2005, vol. 5, no. 8, p. 2090-2103

Hrstka, R., Stulík, J., Vojtěšek, B. The role of MAPK signal pathways during Francisella tularensis LVS infection-induced apoptosis in murine macrophages. *Microbes and Infection*. 2005, vol. 7, no. 4, p. 619-625.

Lenčo, J., Pávková, I., Hubálek, M., Stulík, J. Insights into the oxidative stress response in Francisella tularensis LVS and its mutant DeltaiglC1+2 by proteomics analysis. *FEMS Microbiology Letters*, 2005, vol. 246, no. 1, p.47-54.

Pávková, I., Hubálek, M., Zechovská,, J., Lenčo, J., Stulik J. Francisella tularensis live vaccine strain: proteomic analysis of membrane proteins enriched fraction. *Proteomics*, 2005, vol. 5, no. 9, p. 2460-2467.

Vadovic, P., Slaba, K., Fodorova, M., Skultety, L., TOMAN, R.:  
Structural and functional characterization of the glycan antigens involved in immunobiology of Q fever.  
*Ann. NY Acad. Sci.*, 1063 (2005) 149-153.

Fodorova, M., Vadovic, P., Skultety, L., Slaba, K., TOMAN, R.:  
Structural features of lipopolysaccharide from Rickettsia typhi, the causative agent of endemic typhus.  
*Ann. NY Acad. Sci.*, 1063 (2005) 259-260.

Skultety, L., Hernychova, L., TOMAN, R., Hubalek, M., Slaba, K., Zechovska, J., Stofanikova, V., Lenco, J., Stulik, J., Macela, A.:  
Coxiella burnetii whole cell lysate protein identification by mass spectrometry and tandem mass spectrometry.  
*Ann. NY Acad. Sci.*, 1063 (2005) 115-122.

Kováčová, E., Cmarko, D., Mucha, V., TOMAN, R., Čiampor, F., Kosma, P.  
Tyczka, J., Sekeyová, Z.: Analysis of specificity of interaction between synthetic key saccharide components of lipopolysaccharides and monoclonal antibodies to Coxiella burnetii.  
*Acta Virol.*, 49 (2005) 261-270.

2006

Skultety L, Hernychova L, Toman R, Hubalek M, Slaba K, Zechovska J, Stofanikova V, Lenco J, Stulik J, Macela A. Coxiella burnetii whole cell lysate protein identification by mass spectrometry and tandem mass spectrometry. *Ann N Y Acad Sci*. 2005 Dec;1063:115-22.

Svraka, S., Toman, R., Skultety, L., Slaba, K., Homan, W.L.: Establishment of genotyping scheme of *Coxiella burnetii*. FEMS Microbiol. Lett., 254 (2006) 268-274.

Kubeš, M., Kuzmová, Z., Gajdošová, E., Ihnatko, R., Mucha, V., Toman, R., Kováčová, E.: Induction of TNF- $\alpha$  in murine macrophages by various strains of *Coxiella burnetii* and their lipopolysaccharides. Acta Virol., 50 (2006) 93-100.

Pavkova I, Reichelova M, Larsson P, Hubalek M, Vackova J, Forsberg A, Stulik J. Comparative Proteome Analysis of Fractions Enriched for Membrane-Associated proteins from *Francisella tularensis* Subsp. *tularensis* and *F. tularensis* Subsp. *holarctica* Strains. J Proteome Res. 2006 Nov 3;5(11):3125-3134.

Veljkovic V., J.F. Mouscadet, Veljkovic N., Glisic S., Debyser Z. Simple criterion for selection of flavonoid compounds with anti-HIV activity. Bioorganic & Medicinal Chemistry Letters, 2006, (u stampi) [referenca elektronskog izdanja na sajtu ScienceDirect: 10.1016/j.bmcl.2006.12.029, 2-12.

### **To be published in 2007:**

Lenčo, J., Hubálek, M., Fučíková, A., Brychta, M., Macela, A., Stulík, J. Proteome analysis of the *Francisella tularensis* response to iron restriction: induction of the *F. tularensis* pathogenicity island proteins encoded in *igl* operon. FEMS Microbiology Letters, 2007, in press

Janovská, S., Pávková, I., Hubálek, M., Lenčo, J., Macela, A., Stulík, J. Identification of new immunogenic proteins of *Francisella tularensis* LVS by sub-immunoproteome analysis. Immunology Letters, 2007, in press.

Skultety, L., Hernychova, L., Bereghazyova, L., Slaba, K., Toman, R: Detection of characteristic spectral markers of *Coxiella burnetii* isolates by MALDI-TOF mass spectrometry. Acta Virol., submitted.

Vadovic, P., Fodorova, M., Skultety, L., Toman, R: Structural characterization of lipid A, the endotoxic center of *Piscirickettsia salmonis*. Acta Virol., submitted.

Fodorova, M., Vadovic, P., Skultety, L., Toman, R: Chemical composition and structural features of lipid A from *Rickettsia typhi*. Acta Virol., submitted.

Veljkovic V., Veljkovic N., Este J., Huther A., Dietrich U. Application of the EIIP/ISM bioinformatics concept in development of new drugs. Current Medicinal Chemistry, 2007; 14: 133-155.

Metlas R., Srdic T., Veljkovic V. Anti-IgG antibodies from sera of healthy individuals neutralize HIV-1 primary isolates. Current HIV Research, 2007; 5: 169-174.

The booklet reviewing the current proteomic and glycomic technologies and their applications in molecular microbiology and virology is under preparation.

### **5.2.1 Results of WG4 meetings**

In Antalya meeting annual results were presented by Pierre Wattiau – Belgium, Prof. Joachim Frey – Switzerland and Stefan Panaiotov from Bulgaria. Dr. Wattiau presented a lecture on ‘Single nucleotide polymorphisms as targets for real-time PCR and DNA-array based

identification of Bacteria'. He presented his research focused on SNPs as targets for the molecular identification of bacteria at subspecies level. He underlined the fact that often discrimination at the sub-species level rely on SNPs variability. With a few number of properly chosen SNPs, it should be possible to discriminate bacteria belonging to different biovars, serovars and genomovars. He presented description and validation of a group of SNPs for the identification of some biosafety level 3 (BSL3) bacteria. With the methodology presented he is able successfully to discriminate 4 out of the 5 different biovars of *Brucella suis*. He validated these SNPs on a collection of strains by DNA sequencing and Real-Time PCR.

Another presentation was given by Prof. Joachime Frey from the University of Bern on 'Molecular diversity and antibiotic susceptibility of *Bacillus anthracis* strains causing animal death in Chad: detection of new phylogenetic groups'. In his presentation he described the fruitful collaboration they have with African colleagues. Fifteen *Bacillus anthracis* were isolated from carcasses of cattle in different regions of Chad and were analyzed by use of various markers. Multiple-locus variable-number of tandem repeat analysis (MVLA-VNTR) of eight markers was used to genotype the strains. The analyzed strains formed a novel genetic lineage designated A-beta. Significantly the Chadian anthrax strains were susceptible to 11 tested antibiotics. All tested strains were resistant to ceftiofur, which is a 3rd generation cephalosporin restricted to animal use. The microarray-based analysis of the DNA revealed the presence of the beta-lactamase genes *bla1* and *bla2* which are endogenous to *B. anthracis*, but not expressed. Besides these two beta-lactamase genes, the strains were shown to be free of all tested antibiotic resistance genes. Prof. Frey concluded that the low diversity of Chadian *B. anthracis* genotypes and the absence of geographic clustering of the two genotypes is likely a reflection of extensive long distance transhumance in the country. The molecular analysis of Chadian isolates suggested that this region contains a unique lineage of *B. anthracis*.

S. Panaiotov presented their experience with the management of an outbreak of Q-fever occurred in Bulgaria in 2004. Bulgarian colleagues reported that the outbreak occurred in a small town 100 km North of Sofia. Clinical signs of patients suggested atypical pneumonia. Initial analysis (microbiological, serological and molecular) did not confirmed infection due to *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, influenza and other respiratory viruses. The outbreak involved 220 hospitalized patients and was the biggest since 30 years. Serological tests confirmed *Coxiella burnetii* as etiological agent of the pneumonia. Postfactum PCR tests confirmed *C. burnetii* in swab samples. Epidemiological investigations confirmed that the outbreak originated and spread from infected animals. Serum samples from 270 sheep, goats and cattle were tested. 74 of them were positive for *C. burnetii*. The group intends to investigate Rolling Cycle Amplification and Proximity Ligation amplification techniques with the aim to develop specific and sensitive method for *Coxiella burnetii* detection in clinical samples.

### Contributions to the Action

#### Contribution:

Anders Sjöstedt - Sweden

Design, validation and application of eukaryotic and microbial diagnostic microarrays.

Development of bioinformatic tools for the microarray analysis.

S. Panaiotov (BG), R. Toman (SK), E. Piskin (TR), J. Frey (S) – development of projects for FP6 and bilateral collaborations

### **Established collaborations:**

During the meetings the group leader Dr. Panaiotov underlined the collaborations that were established between the COST B28 partners.

**Dr. Panaiotov** coordinated the preparation of a project under the call ‘Marie Curie Host Fellowships for the Transfer of Knowledge (ToK) Development Host Scheme’ The project was entitled ‘**Transfer of knowledge for molecular identification, drug susceptibility testing and genotyping of tuberculosis in Bulgaria**’. **J. Frey, E. Piskin and P. Butaye, members of B28, participated in preparing the section - Biosensors and microarray technologies for drug resistance markers and identification of mycobacteria.**

**Prof. Erhan Piskin** from Turkey coordinated and in collaboration with S. Panaiotov and Marc Govaerts from VAR-Belgium developed a project entitled ‘**Design of Novel Miniaturized-Portable-Multichannel Sensors Carrying Aptamers for Detection of Mycobacteria Based on Surface Plasmon Resonance (SPR)**’. The project was under the FP6 scheme: Development of fast tests for diagnosis of poverty related diseases suitable for use in resource-poor settings – STREPs dedicated to SME. Project aim concerned detection of genus Mycobacterium and identification of three most common pathogenic mycobacteria species *M. tuberculosis*, *M. bovis* and *M. avium* subsp. *paratuberculosis* in one test.

**Stefan Panaiotov and Rudolf Toman** proposed to the Ministry of Education of the Slovak Republic, Division of Science and Technology, Department of Bilateral Cooperation and International Organizations Slovak and to the Bulgarian Science and Technology Co-operation Department for years 2007 – 2009 the project ‘**Rolling cycle amplification and proximity ligation techniques for sensitive and specific detection of *Coxiella burnetii*, the ethiological agent of Q-fever**’. The two years project (2007-2009) was approved by the bilateral Bulgarian-Slovak Science and Technology Commission. Contract will be signed in Feb 2007. Unfortunately for the first two projects experts’ evaluation results were very positive, but due to lack of funding or ‘very innovative proposed ideas’ the projects failed for funding.

### CODA, Pierre Wattiau

Contribution: identification of genetic markers and single nucleotide polymorphisms (SNPs) suitable for the identification and typing of bacteria belonging to the BSL3 containment level. The methodologies used to validate the identified markers and SNPs are : PCR, real-time PCR, Multiplex Ligation-dependent Probe Amplification (MLPA) and low-density microarray screening. The focus is mainly on *Brucella*, *Brucella mallei* / *pseudomallei* and *B. anthracis*. The identification of relevant markers for the development of "thematic arrays" assessing specific subsets of human / animal pathologies is currently investigated.

### **Publications:**

1. Ivanov, I. Advanced methods for detection of biological weapons. 2006. Medical Report, 42:2
2. Kantardjiev T, Ivanov I, Velinov T, et al. Tularemia outbreak, Bulgaria, 1997-2005. Emerg Infect Dis 2006;12(4):678-80

3. **T. Kantardjiev, N. Brankova, E. Dobрева, S. Panaiotov, V.** 2006. Advanced diagnosis of community acquired atypical pneumonia caused by *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. *Med. Rev.*, 42. 2:58-60
4. **N. Brankova, S. Panaiotov, V. Levterova, T. Kantardjiev.** 2006. Asymptomatic patients infected with *C. trachomatis*, Screening study. *Akush. Ginekol.* 2:25-28.
5. **T. Kantardjiev, V. Levterova, S. Panaiotov, I. Ivanov, E. Hristozova.** 2006. Molecular taxonomy of *Cryptococcus neoformans* varieties displaying phenotypic similarities. *Biotechnol. & Biotechnol. Eq.*, vol.2 p.101-103
6. **T. Kantardjiev, V. Levterova, N. Brankova, I. Ivanov, P. Angelov, S. Panaiotov.** 2006. Role of fluorescent amplified fragment length polymorphism analysis in taxonomy, identification and epidemiological examinations of yeast pathogens. *Biotechnol. & Biotechnol. Eq.*, 1:103-106
7. Henrik Andersson, Blanka Hartmanová, Rhonda KuoLee, Patrik Rydén, Wayne Conlan, Wangxue Chen, Anders **Sjöstedt**. (2006) Transcriptional profiling of host responses in mouse lungs following aerosol infection with type A *Francisella tularensis*. *J. Med. Microbiol.* 55:263-271.
8. Henrik Andersson, Blanka Hartmanová, Erik Bäck, Henrik Eliasson, Mattias Landfors, Linda Näslund, Patrik Rydén, Anders **Sjöstedt**. (2006) Transcriptional profiling of the peripheral blood response during tularemia. *Genes Immun.* 7:503-513.
9. Henrik Andersson, Blanka Hartmanová, Patrik Rydén, Laila Noppa, Linda Näslund, Anders **Sjöstedt**. (2006) A microarray analysis of the murine macrophage response to infection with *Francisella tularensis* LVS. *J. Med. Microbiol.* 55:1023-1033.
10. Patrik Rydén, Henrik Andersson, Mattias Landfors, Linda Näslund, Blanka Hartmanová, Laila Noppa and Anders **Sjöstedt**. (2006) Evaluation of microarray data analysis methods using spike-in experiments. *BMC Bioinformatics.* 7:300-317.
11. P. Wattiau and D. Fretin. 2006. Real-time PCR typing of single nucleotide polymorphism in DNA containing inverted repeats *BioTechniques* 41:544-546.

### Communications:

1. Ivanov IN, Nenova R, Kantardjiev T. Direct Serum PCR as a Rapid Tool for Detection of Brucellosis. – 1st International Meeting on the Treatment of Human Brucellosis, 7-10 November, Ioannina, Greece
2. Nenova R, Ivanov I, Tomova I, Kantardjiev T, Tcherveniakova T, Popov B. Imported Cases of Brucellosis in Bulgaria- Clinical Management, Diagnosis, and Treatment- 1st International Meeting on the Treatment of Human Brucellosis, 7-10 November, Ioannina, Greece
3. R. Nenova, T. Kantardjiev, I. Ivanov, I. Tomova, B. Popov, Vl. Novkirishki. Microbiological investigation of imported Brucella infection among Bulgarian citizens- XII congress of the bulgarian microbiologists, Varna, X.2006

### 5.2.1 Results of WG5 meetings

The activities of WG 5 have focussed on exchange of strains For facilitating these exchanges, a discussion on a common culture collection has been started. The collection would be decentralised, each laboratory would keep its own collection but information on it would be

collected in an excel or access database. It will also include the person to contact and the characteristics of the strains. During the next year this topic will be further worked out and implemented.

Another specific point on which the WG group is working is the extension of the current collections. This is especially true for the more exotic viruses and bacteria, like strains of Viral haemorrhagic fever viruses. Plans are made for the invitation of researchers from areas where the diseases are present and who have a strain collection they want to share. Problems for sharing the collections may be of financial (in general the strains come from developing countries).

Working with live BSL3 and BSL4 agents is not easy. Since the opportunities to work under such conditions within an EU context are rather limited, and since the different countries have different legislations and likewise ways of working in these laboratories, it was judged that a training school, offering an intense training on working under BSL3 and BSL4 agents would be given. The international character of the COST Action B28 will offer the possibilities for the young students to come into contact with the different ways of working in the different countries and will offer them a comparative basis for improving the safety regulations in their laboratories. The organisation of this will be further discussed in the April meeting in Bulgaria, where it will be decided whether a separate WG meeting would be necessary to further organise things.

During the meetings, main accent of the presentations was on the epidemiology of the BSL3 and BSL4 agents. In depth studies diagnostic limitations of current tests were highlighted. Limitations and possibilities of strain typing were demonstrated by practical studies.

**Name:** Dimitrios Frangoulidis

**Contribution:** design, development and validation of a novel Low-Cost-and-Density (LCD)-Microarray for the detecting of *Coxiella burnetii* and other unusual pathogens. One prototype is finished and the sensitivity was determined to be up to 10 genomic copies/ $\mu$ l template for IS1111 and 100 copies for *adaA*. Array modifications according to the design of the internal control are just planned.

**Publications:** in preparation

Collaborations (planned): with the group of Rudolf TOMAN (Bratislava, Slovak Republic) for further evaluation with different materials and strains.

**According to publications:**

Frangoulidis D, Schröpfer E, Al Dahouk S, Tomaso H, Meyer H (2006) "Comparison of Four Commercially Available Assays for the Detection of IgM Phase II Antibodies to *Coxiella burnetii* in the Diagnosis of Acute Q Fever. In: "Century of Rickettsiology: Emerging, Reemerging Rickettsioses, Molecular Diagnostics, and Emerging Veterinary Rickettsioses", Annals of the New York Academy of Sciences, Volume 1078, Oct 2006.

**Name:** Anders Sjöstedt

**Contribution:** Design, validation and application of eukaryotic and microbial diagnostic microarrays. Development of bioinformatic tools for the microarray analysis.

**Publications:**

Henrik Andersson, Blanka Hartmanová, Rhonda KuoLee, Patrik Rydén, Wayne Conlan, Wangxue Chen, Anders Sjöstedt. (2006) Transcriptional profiling of host responses in mouse lungs following aerosol infection with type A *Francisella tularensis*. *J. Med. Microbiol.* 55:263-271.

Henrik Andersson, Blanka Hartmanová, Erik Bäck, Henrik Eliasson, Mattias Landfors, Linda Näslund, Patrik Rydén, Anders Sjöstedt. (2006) Transcriptional profiling of the peripheral blood response during tularemia. *Genes Immun.* 7:503-513.

Henrik Andersson, Blanka Hartmanová, Patrik Rydén, Laila Noppa, Linda Näslund, Anders Sjöstedt. (2006) A microarray analysis of the murine macrophage response to infection with *Francisella tularensis* LVS. *J. Med. Microbiol.* 55:1023-1033.

Patrik Rydén, Henrik Andersson, Mattias Landfors, Linda Näslund, Blanka Hartmanová, Laila Noppa and Anders Sjöstedt. (2006) Evaluation of microarray data analysis methods using spike-in experiments. *BMC Bioinformatics.* 7:300-317.

**Name:** Raquel Escudero; Pedro Anda

**Contribution:** A molecular method for the identification of *Rickettsia* species in clinical and environmental samples (Presented in the meeting of 2005, Bratislava); A molecular method for the discrimination between *Francisella tularensis* subspecies and *Francisella*-like endosymbionts (Presented in the meeting of 2006, Antalya).

**Publications:**

Isabel Jado, Raquel Escudero, Horacio Gil, María Isabel Jiménez-Alonso, Rita Sousa, Ana L. García-Pérez, Manuela Rodríguez-Vargas, Bruno Lobo, Pedro Anda. A molecular method for the identification of *Rickettsia* species in clinical and environmental samples; *J Clin Microbiol* 2006; 44:4572-4576.

Isabel Jado, José A. Oteo, Mikel Aldámiz, Horacio Gil, Raquel Escudero, Valvanera Ibarra, Joseba Portu, Aranzazu Portillo, María J. Lezaun, Cristina García-Amil, Isabel Rodríguez-Moreno, Pedro Anda. *Rickettsia monacensis*, a new rickettsia species causing human disease. *Emerg Infect Dis* (in press).

Rosa de los Ríos Martín, Juan Carlos Sanz Moreno, Fernando Martín Martínez, M<sup>a</sup> Ángeles Tébar Betegón, Marta Cortés García y Raquel Escudero Nieto. Q fever outbreak in an urban area following a school-farm visit. *Med Clin (Barc)* 2006; 162:573-575.

Sanz JC, de los Rios R, Martin F, Tebar MA, Jado I, Anda P. Application of four ELISA techniques (two for IgM and two for IgG) for serological diagnosis of an outbreak of Q fever. *Enferm Infecc Microbiol Clin.* 2006 24:178-81

**Contributions to other meetings:**

R Escudero, H Gil, JF Barandika, A Toledo, K Kováčsová, M Rodríguez-Vargas, C García-Amil, SA Olmeda, AL García-Pérez, P Anda. Description of two PCR methods for *Francisella* detection and its comparison with available methodologies. 5th International Conference on Tularemia. Woods Hole, MA, Estados Unidos, 1 al 4 de noviembre de 2006.

Jado I., Bolaños M., García Pérez A., Quevedo M., Téllez A., Escudero R., Martín Sánchez A.M., Oporto B., García Amil C., Santana Rodríguez E., Rodríguez Vargas M., Anda P. Analysis of *Coxiella burnetii* from humans and reservoirs in Spain. 4th International Conference on Rickettsiae and Rickettsial Diseases. Logroño, La Rioja, 18-21 de junio, 2005.

Jado I., Escudero R., Portillo A., Barandika J.F., Márquez F.J., Pérez A., Rodríguez-Moreno I., Oteo J.A., García-Pérez A., Jiménez S., Ibarra V., Jiménez-Alonso M.I., Anda P. A molecular method for the identification of *Rickettsia* species in clinical and environmental samples. 4th International Conference on Rickettsiae and Rickettsial Diseases. Logroño, La Rioja, 18-21 de junio, 2005.

Collaborations: With Pierre Wattiau Veterinary & Agrochemical Research Center (VAR - CODA - CERVA), Brussels, for the exchange of strains of *Francisella*.

**Name:** N.J. Silman

**Publications:**

J.E. Burton, O.J. Oshota and N.J. Silman :Differential identification of *Bacillus anthracis* from environmental *Bacillus* species using microarray analysis. *Journal of Applied Microbiology* 101 (2006) 754–763

Jane E. Burton, O. James Oshota, Emma North, Michael J. Hudson, Natasha Polyanskaya, John Brehm, Graham Lloyd, Nigel J. Silman\* (2005) Development of a multipathogen oligonucleotide microarray for detection of *Bacillus anthracis*. *Molecular and Cellular Probes* 19, 349–357

**Name:** G. Schmoock, M. Elschner

**Started project:** Development a diagnostic DNA-microarray for the rapid and simultaneous identification of the BSL3 agents *Burkholderia mallei*, *Burkholderia pseudomallei*, *Bacillus anthracis* and *Brucella* spp.

**Name:** M. Weidmann

**Publications:**

Weidmann M, Schmidt P, Vackova M, Krivanec K, Munclinger P, Hufert FT (2005) Genetic evidence for Dobrava virus spillover in rodents identified by nested RT-PCR and TaqMan-RT-PCR *J Clin Microbiol* 43(2):808-812

Spiegel M, Schneider K, Weber F, Weidmann M, Hufert FT (2006) Interaction of SARS Coronavirus with Dendritic Cells *General Virology* 2006 87(7): 1953-60.

Weidmann M, Schmidt P, Hufert FT, Krivanec K, and Meyer H (2006) Tick borne encephalitis virus in *Clethrionomys glareolus* in the Czech Republic *Vector-Borne and Zoonotic Diseases* (accepted)

Weidmann M, Hufert FT, Sall AA (2006) Viral load among patients infected with Marburgvirus in Angola *J Clin Virol* (accepted)

## **6. DISSEMINATION OF RESULTS**

### ***6.1 Publications and Reports***

Two abstract books of the WG meetings are be available.

A WG booklet is planned by the WG1 and scheduled to be ready next year. The preparation on a second booklet by WG3 is ongoing.

The scientific publications are cited in the WG reports. A total of 46 publications were published by the group partially in 2005 and in 2006. Quite some publications are in

### ***6.2 Conferences and Workshops***

A new WG and MC meeting is scheduled for April in Bulgaria

### ***6.3 Web site***

The VAR, Brussels, Belgium has nearly established the website containing information on and links to the participating institutions. CV of the participants will be added.

Abstracts of the presentations will be available on the website.

### ***6.4 Scientific and Technical Co-operation***

Firm contacts have been established between different partners and collaborative plans have been made.

A firm collaboration with the FP6 funded IP “moltools” has been established. This group is specifically working on high technological array systems. Actually their applications are foreseen for Eukaryotes. Collaboration for working on Prokaryotes has been scheduled.

There is a connection by several partners with the FP6 funded preparatory action “IMPACT”

Co-operations are limited to different partners and some seem to have difficulties to find a common way. One of the major problems seems financing of projects that may allow firm collaboration.

### ***6.5 Transfer of results***

By means of the website, results will be disseminated. It was also agreed that each work package will produce a booklet reviewing the current state of the art of research activities and results within their field.

### ***6.6 Contacts in the ERA***

## **7. ECONOMIC DIMENSION**

List funds received from the COST budget for each year and for the entire duration of the Action utilised for Secretariat, Publications, Workshops and Seminars, MC meetings, Short-Term scientific missions, other and total.

## **8. SELF EVALUATION (only in the last annual progress report)**

Indicate, in no more than 1 page, what were, in the opinion of the MC, the main successes, the drawbacks (if any) and the key difficulties encountered (if any).