

COST

Domain Committee "BMBS"

COST Action B28

Title "array technologies for BSL3 and BSL4 agents"

MONITORING PROGRESS REPORT

*Period: from (27/5/2005)
to (December 2008)*

This Report is presented to the relevant Domain Committee and contains two parts:

- I. **Management Report** prepared by the COST Office/Grant Holder*
- II. **Scientific Report** prepared by the Chair of the Management Committee of the Action*

The report is a "cumulative" report, i.e. it is updated annually and covers the entire period of the Action.

Confidentiality: the documents will be made available to the public via the COST Action web page except for chapter *II.C. Self evaluation*.

Based on the monitoring results, the COST Office will decide on the following year's budget allocation.

I. Management Report prepared by the COST Office



I.A. COST Action Fact Sheet

- **COST Action** *number - title*
- **Domain** *name*

- **Action details:**

CSO Approval: *(day/month/year)*

End date: *(day/month/year)*

Entry into force: *(day/month/year)*

Extension: *(day/month/year)*

- **Objectives** *(from DB as in About COST)*
- **Parties:** *list of countries and date of acceptance*

Austria <i>(date)</i>	Greece <i>(date)</i>	Poland <i>(date)</i>
Belgium <i>(date)</i>	Hungary <i>(date)</i>	Portugal <i>(date)</i>
Bulgaria <i>(date)</i>	Iceland <i>(date)</i>	Romania <i>(date)</i>
Croatia <i>(date)</i>	Ireland <i>(date)</i>	Serbia <i>(date)</i>
Cyprus <i>(date)</i>	Israel <i>(date)</i>	Slovakia <i>(date)</i>
Czech Rep. <i>(date)</i>	Italy <i>(date)</i>	Slovenia <i>(date)</i>
Denmark <i>(date)</i>	Latvia <i>(date)</i>	Spain <i>(date)</i>
Estonia <i>(date)</i>	Lithuania <i>(date)</i>	Sweden <i>(date)</i>
Finland <i>(date)</i>	Luxembourg <i>(date)</i>	Switzerland <i>(date)</i>
FYR of Macedonia <i>(date)</i>	Malta <i>(date)</i>	Turkey <i>(date)</i>
France <i>(date)</i>	Netherlands <i>(date)</i>	United Kingdom <i>(date)</i>
Germany <i>(date)</i>	Norway <i>(date)</i>	

- **Intentions to accept:** *list of countries and date*

- **Other participants:**

(Institution Name, Country, Town)

Chair: *(name, institution, address, phone, e-mail)*

DC Rapporteur: *(name, institution, address, phone, e-mail)*

Science Officer: *(name, e-mail)*

Administrative Officer: *(name, e-mail)*

- **Action Web site:** <http://www.cost-b28.be/>
pabut@var.fgov.be)

Grant Holder *(Patrick Butaye,*

- **Working Groups** *(list of WGs and name)*



I.B. Management Committee member list

Name	Country	E-mail



I.C. Overview activities and expenditure

(year) Budget

Total Action Budget:

Remaining Action Commitment:

Meetings

Meeting Type	Date	Place							Cost	Total
										0

STSM

Beneficiary	Date	Place							Cost	Total
										0

Workshops

Title	Date		Place						Cost	Total
	From	To	From	To						
										0

General Support Grants

Beneficiary	Date								Cost	Total
										0

Schools

Title	Date	Place							Cost	Total
										0

Others

Action Total : 0

II. Scientific Report prepared by the Chair of the Management Committee of the Action

II.A. Results achieved during the period 27/5/2005 to December 2008

In the **WG1**, dealing with array technologies progress has been made in developing and validation of microarray technologies for the detection of BSL3 and BSL 4 agents. Several pathways have been searched for to develop appropriate probes and introduced new technologies for it. Specifically the use of padlock probes has been developed for the very specific detection of BSL3 bacteria. In connection with the MolTools FP6 project, the proximity ligation assay has also been introduced in our COST Action. Another pathway followed in this working group is based in surface plasmon resonance. Specific studies on different surfaces are executed. On this subject several proposals for funding have been submitted, however for the time being, no funding has been obtained to further elaborate this challenging topic into a more applicable diagnostic platform. Other platforms aimed at a more pan-pathogen array, including bacteria and viruses. These platforms used other labelling and detection methods than the padlock probes. Another platform, based on sequence specific end labelling of oligonucleotides, aimed at a cheaper, faster and more specific and sensitive micro-array for selected pathogens. Meanwhile there is still an evolution ongoing with the development of new types of arrays, aiming not only at the detection of the pathogen but also for the typing of the pathogen. Finally we come at a phase for validating these new tools. The latter is however dealing with some unexpected problems of reproducibility. Also in the isolation of the DNA from the clinical material, new problems have been encountered and are tackled at the moment. A FP7 proposal has been submitted by several members of this COST Action in order to make the micro-arrays suitable for applications on clinical material.

Furthermore, the tools are tried out in the group also on other micro-organisms as salmonella and other food-borne pathogens. Specifically for salmonella, the method is dealing not only with the detection but also with the typing of the organism at subspecies level. Also the focus is changing towards more clinical arrays dealing not only with BSL3 and BSL4 agents but dealing with a clinical problem that is compatible with symptoms of agents of safety level 1 to 4.

In **WG2**, dealing with the antigenic properties of BSL3 and BSL4 agents, worked mainly in the field of virology and Rickettsiology. Two new members have joined the workgroup in 2007. One of the laboratories has inaugurated a state of the art BSL3 facility and has benefited from the BSL3/4 training course provided within the COSTB28 activities. In the group different approaches to identify pathogen-specific antigens using chemical approaches to develop new antigens and immunogens were evaluated. Antigens were also used to induce antigen-specific immune-responses in animals. Another focus was the genetic and antigenic variability of relevant pathogens. In the field of influenza, substantial progress has been made in understanding the highly pathogenic avian influenza virus H5N1, and due to its specific antigenic properties investigate the possibility of this virus to cause a pandemic. The evolution of the virus, especially in sub-saharan Africa has been investigated in detail. Specifically in the diagnostic field, a new mRT-PCR-FT-RDB (multiplex reverse transcription polymerase chain reaction combined with flow-through reverse dot blotting assay) and a new multiplex Real Time PCR has been developed for the detection, not only of influenza virus but also other respiratory viruses. Within this group, studies concerning Measels, hepatitis, chicken anemia virus and avian metapneumovirus. Within the group the persistence of the influenza virus under different environmental conditions have been investigated. The importance of the virus load in dead animals has been demonstrated. This is very important in order to be able to break the

infection cycle. As towards *Rickettsia*, the expression of genes, inducing an antigenic reaction have been further investigated. This has been done in several ways. First, in connection with WG1, transcriptomics on micro array have been investigated and new factors on how *Rickettsia* circumvent the immune reaction have been identified. Specific monoclonal antibodies, using recombinant protein technology, have been developed for further evaluating functions of proteins in *Rickettsia*. Antigens have also been isolated for the serodiagnosis of specific *Rickettsial* infections. Furthermore, two groups involved in peptide chemistry, have developed peptides, involved in interaction with the hosts immunity and investigated them for their use as immunomodulators.

In **WG3** dealing with proteomics and glycomics, is a rather small group. In this group further analysis on the proteins of bacteria and viruses is planned. Also, and in collaboration with WG2, a booklet has been prepared and is coordinated by the WG2 chair. This booklet is actually under revision, and soon a request for publication support will be introduced. Next to that a proteomics training school has been successfully organised. The group is also actively looking for funding of new research, this aided by the COST Action connections made, as shown in the commonly submitted research projects. Specific work on *Francisella tularensis* has led to the detection and identification of new virulence factors that are candidates to be included in diagnostic tests. Further more these virulence factors will lead to a better understanding of the host-pathogen interactions, especially with its immunological reaction. This may also lead to new subunit vaccines. Next to that whole cell proteins of *Coxiella burnetii* have been investigated for their possibility in the detection and identification of this pathogen. Several extraction procedures have been evaluation The lipopolysaccharides of *Coxiella* and *Chlamydiaceae*, considered as important virulence factors, have been further investigated in detail. The interaction with the hosts immune system have been evaluated in detail. Also in this group, bioinformatics platforms are developed in order to better handle the large amount of data that become available and to analyse these data for possible new targets for antivirals, antibacterials and vaccine candidates.

In **WG4**, dealing with genomics of the bacteria, focus has been laid on molecular typing of pathogens. The group participants worked on and collaborated in several topics: development of a SNP-based probe assay for multiplex detection and typing *Brucella* directly in clinical material; establishing molecular diagnostics for *Leptospira*; *Brucella*; *Coxiella* and *Rickettsia*; molecular typing to *Brucella* isolates from different epidemiological investigations; VNTR and AFLP analysis of *Coxiella* strains; developing high resolution melting curve analysis applied for discrimination of *B. anthracis* strains and detection of microorganisms by suspension microarrays. MVLA-VNTR and SNP analysis are typing methods that have been applied on *B. anthracis* epidemiological studies performed by several partners of the project. Further progress is made in the molecular typing of bacteria.

In **WG5**, dealing with the microbiology of bacteria and viruses, major achievements have been made through the collections, and the experience of working with these agents. This knowledge has been given to the other participants of the COST Action through the micro-organism database, and a training school on working with BSL3 and BSL4 agents. The group has studied the rapid detection methods for several bacteria and viruses. It as investigated the epidemiology of *Bacillus anthracis* in detail and over a long period. Also the prevalence of *B. anthracis* in imported wool, has been investigated and it has been shown that cashmere, originating from endemic regions, was most likely to be contaminated. Furtheron the use of Raman spectroscopy for the detection of biothreat

agents is under investigations. The members of his group also frequently report on infections with BSL3 and 4 agents and take care of quality assurance programmes in the detection of these highly pathogenic agents. Several surveillances on the prevalence of BSL3 and BSL4 agents in Europe, but also outside of Europe are ongoing. The diagnostic tools used are ranging from microbiological methods (culture) over serology and molecular methods.

Towards the biosafety of working with these dangerous pathogens, the group is preparing a response on the EU green paper on bio-preparedness.

As for the **STSMs**, the number remains low as already mentioned in the former reports. While in 2007 2 STSMs were accomplished, 3 STSMs have been executed in 2008. This low numbers are due to the fact that working in a BSL3 and BSL4 environment remains problematic for the laboratories (administration to be fulfilled is enormous) and frequently it is impossible to acquire a non-skilled person in such a laboratory. Therefore, within the COST Action, we organised training courses on working in a BSL3/BSL4 environment in 2007 and 2008, which were very successful. The courses have been fully booked, making a total of 20 persons that have had the chance to follow the course (the number of attendances is limited to 10 for practical reasons: size of the lab). Also in 2008 a course on proteomics has been organised and 6 people attended this course.

Involvement of non-COST participants. In this Action, we have scientists included from Canada, the US and South Africa. Though they cannot attend every meeting (due to relative high cost to attend) they continue to be involved and are kept update of our activities. They are part of our network and as such, collaborations are established. As to promote our COST Action outside the EU, we are inviting external experts from outside of Europe for giving an extended lecture as invited speaker.

Synergistic activities. There are synergisms with the FP activities. Projects are introduced and some are accepted (see underneath). Several members of B28 are also involved in reviewing proposals.

II.B. Dissemination of results

- *Action related Publications and Reports (list)*

In this COST Action, we have decided to publish our work under the format of booklets. A first booklet dealt with microarray and is in press now. A second booklet by the WG2 and WG3 is near finishing and can be submitted for financing by COST in the near future. WG4 is composing the booklet actually. WG 5 will have two booklets, one dealing with biosafety, and this booklet will be finished by June this year. A second booklet dealing with the micro-organisms is planned to be finished by the end of the year, beginning of 2010.

The list of publications dealing with the topics of our COST Action is listed in the annex of the report. A total of 133 publications have been in 2008. as a grand total for this COST Action, the number of publications is 292. These do not include the publications for the booklets discussed above.

- *Conferences, Workshops and Training Schools (list and programme)*

In this COST Action the following meetings have been organised:

Bratislava, Slovak Republic, 21-22/11/2005 (All WGs)

Antalya, 1-3 October 2006 (All WGs)

Plovdiv, April 20-22, 2007 (All WGs)

Vienna, December 10-12, 2007 (All WGs)

Bucharest, Romania, September 10 – 12, 2007 (All WGs)

Ghent, Belgium December, 9-10, 2008, WG5 Meeting on Biosafety and Biosafety regulations

The next meeting is planned April 22-24, Belgrade, Serbia.

In this COST Action, 3 training schools have been organised to date:

1. may 22 to may 24, 2007: BSL3 / BSL4 Training School
2. 28.05.08-29.05.08: BSL3 / BSL4 Training School
3. 7 October – 10 October 2008: Proteomics Training Course:

In the BSL3/BSL4 training school, students are taught about handling dangerous pathogens. This is both theoretical and practical. In the practical courses, students are learned about the dangers of the micro-organisms through a lecture on the micro-organisms and on the reported lab accidents. Further on, they are learned about the organisation of such labs and the way how to keep it “clean” through manipulations and disinfection. The practical course (which limits the number of participants to a maximum of 10) is done in the lab itself with mock agents. Several possible manipulations are executed and controls are built in to detect eventual contaminations followed the actions of the students.

The proteomic course was conceived for beginners. There were six participants from Spain, Belgium, UK and Turkey. The program of the course covered all aspects of current proteomic technologies i.e. classical gel-based approaches exploiting two dimensional gel electrophoresis as well as non gel-based approaches designated like shotgun proteomics. The course was divided into two parts theoretical one and practical classes. The theoretical part encompassed four lectures focused on general introduction into proteomics, description of basic protocols of gel separation techniques, including two-dimensional gel electrophoresis (2-DE) and native PAGE separation techniques for the analysis of protein complexes, furthermore, description of current mass spectrometric approaches utilized for protein identification and, finally, the current possibilities of quantitative shotgun analyses. Another theoretical part covered bioinformatics procedures used for mass spectra processing, database searching, protein quantification and application of different prediction tools in order to get information about protein biological function, cell topology and structure including possible posttranslational modification. The practical part then covered sample preparation followed by protein separation on small 2-DE gels, staining of the gels with colloidal coomassie blue stain and, finally scanning of destained gels. Next practical classes were focused on preparation of samples for mass spectrometry analyses. In this part the participants carried out spot excision and in gel digestion for LC-MALDI. Then they continued with spotting on MALDI target and

measurement using MALDI TOF/TOF equipment. The practical part was finished with practical training in data processing and bioinformatics using own experimental data. The whole course was then completed by the round table with all tutors where the all aspects of the course were discussed with the aim to evaluate its usefulness and to make improvements of the possible weak points. Finally all participants received the certificates confirming they successfully passed the training in proteomics.

- *Web site (description)*

The VAR, Brussels, Belgium has established the website displaying information on all WGs. It is mainly dealing with the aims of the WGs. Partners are listed and WG chairs have administrator rights to update their workgroup at website. Other documents can be added if wanted.

The part on the management committee and the part on meetings is updated by the chair. The website is also a communication tool for the meetings (forms, agenda, practical information). It gathers also all abstract booklets.

It contains also information on and links to the participating institutions.

- *Scientific and Technical Cooperation*

Firm contacts have been established between different partners and collaborative plans have been made. Specific details of collaborations between COST Action B28 partners are listed in the results section of the additional information (annex) of each WG.

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”.

FP7 project ‘TM-REST’ – ‘A new platform for fast molecular detection of MDR and XDR resistant strains of M. tuberculosis and drug resistant malaria’ was financially supported by the EU Commission. Project Coordinator is Daniela Cirillo – Milano Italy and partner in the project is S. Panaiotov, NCIPD, Sofia, Bulgaria. The platform includes development and evaluation of microchip for MDR-TB markers. The three year project starts in 02.2008 and ends in 2011.

New project proposals have been introduced by several members of the consortium, however, not all are as successful. The group nevertheless continues in submitting new proposals for FP7 and other funding organisations (as NIAID in the USA).

- *Transfer of results*

Transfer of results do not yet have a practical application. Most diagnostic tests common for our research proposal are experiencing validation problems, once these are overcome commercialisation may be a possibility. Of course, the individual partners have their own contacts and contracts with private industry for some applications.

II.C. Self evaluation

This group of researchers is a heterogeneous group of researchers active in different fields. They also have quite different backgrounds, for biologists, molecular biologist, medical doctors, veterinarians, military,...

This has certainly many advantages seen the different backgrounds, and these have also been experienced in the group, though it has also disadvantages, as being unfamiliar towards each others practices and this has caused the slow start of the collaborations. However, now firm contacts between the partners have been established. Collaborations in different fields are ongoing as demonstrated in the annex to this report

Another difficulty experienced in this project is the difficulties to exchange students. Working in BSL3 and especially in BSL4 conditions are not things that you are doing just like that. This is also the reason why in this project, not so many STSMs have been executed till now. However, we could overcome some things by organising training schools. At the moment, 20 persons have had the chance to experience what it is working under BSL3 conditions. This course is so popular and has gained international acknowledgement beyond the borders of our COST Action.

The main success if this COST Action is that such different group of people can now work together. The group got also acknowledged for its training courses on BSL3/BSL4 agents, a course that is now appealed on, not only by scientists but also by governments who want to know more about these specific working conditions.