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Proposal for a new COST Action

COST B28

ARRAY TECHNOLOGIES FOR BSL3 AND BSL4 PATHOGENS¹

Action Proposer:

Dr Patrick BUTAYE
Veterinary and Agrochemical Research Centre
Department of Bacteriology and Immunology
Groeselenberg 99
B-1180 Brussels
Tel : +32 2 379 04 28
Fax : +32 2 379 04 01
E-mail : pabut@var.fgov.be

COST National Coordinator:

Mr. Hendrik Monard
Politique Scientifique Fédérale
Rue de la Science, 8
1000 Brussels
BELGIUM
Tel:+32 2 2383518
Fax:+32 2 2305912
monh@belspo.be

Rapporteur:

Prof. Marieta COSTACHE (TC – MH member)
University of Bucharest - Faculty of Biology
Department of Biochemistry - Molecular Biology Centre
Splaiul Independentei 91 - 95
RO-76208, Bucharest 5
Tel : (4021)4115207
Tel/Fax : (4021)4119901/102
E-mail : marietacostache@yahoo.com

¹ Bio-Safety Level

DRAFT

MEMORANDUM OF UNDERSTANDING

For the implementation of a European Concerted Research Action designated as

COST B28

ARRAY TECHNOLOGIES FOR BSL3 AND BSL4 PATHOGENS

The signatories to this 'Memorandum of Understanding', declaring their common intention to participate in the concerted Action referred to above and described in the 'Technical Annex to the Memorandum', have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 400/01 'Rules and Procedures for Implementing COST Actions' the contents of which the Signatories are fully aware of.
2. The main objective of the COST Action B28 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.
3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EURO 22.6 Million in 2004 prices.
4. The Memorandum of Understanding will take effect on being signed by at least five Signatories.
5. The Memorandum of Understanding will remain in force for a period of four years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter 6 of the document referred to in Point 1 above.

COST ACTION B28

ARRAY TECHNOLOGIES FOR BSL3 AND BSL4 PATHOGENS

A. Background

Resources for the study of highly pathogenic micro-organisms are sparsely spread over Europe and even over the world. Also culture collections containing large quantities of these highly pathogenic strains are sparsely distributed, mainly because they are highly demanding to handle. Moreover, most of these pathogens have been identified as agents that have potential to be used as biological weapons. Many emerging questions have been raised after the Anthrax attack in the United States. Other states were not at all prepared to handle either cases or the large quantities of analyses that were required. In Europe, many analyses have been performed, and most of the laboratories involved were not prepared to handle such large quantities or the specific nature of the materials involved. Fortunately nothing infectious was found in Europe. One major conclusion was that there were no ready diagnostics available for the detection of such pathogens, and since these agents are very infrequent, general knowledge on these organisms is sparse.

Diagnostic possibilities for detecting these highly pathogenic microorganisms are generally focused at one, or possibly a limited number of species. It never passed the border of, for example, Prokaryotes. However, in the case of an undefined sample, such as a “white powder” sample, it would be very useful to have a test that is capable of detecting viruses, prokaryotes and eukaryotes (fungi). Therefore a close collaboration between different microbiologists is necessary. Moreover, such a diagnostic test needs the full characterisation of the microorganisms. Therefore a large number of specialists in different fields are necessary and the information should be compiled consistently.

Such investigations will also bring a lot more information on several characteristics of these microorganisms related to immunology, antibiotic resistance and virulence.

Through the COST Action B28, possibilities will be created for collaborations between laboratories capable of handling dangerous pathogens and laboratories, specialised in the technical aspects of working with the inactivated fractions of the pathogens. This Action also involves veterinary, public health and defence laboratories, all with their specific tasks within the study of these pathogens.

B. Objectives and Benefits

The main objective of the COST Action B28 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 the epidemiology of these highly pathogenic micro-organisms that can be potentially used as biological weapons.

In the United States, several research laboratories are dealing with fast identification and characterisation of micro-organisms that can be used as biological weapons. They receive massive support from their government to develop tests that will help politicians in decision-

making in the event that the organisms, described in this COST Action, 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 Union. This means that when something happens with these microorganisms, Europe would be completely dependent on the co-operation with the United States. Moreover, some live microorganisms are only available outside of the EU (smallpox). Therefore the COST Action B28 will also collaborate with these institutes, to obtain the necessary information on these organisms, enabling the network to include them with research.

An investment into this subject, having this magnitude would be ineffective if the individual members of the EU had to do this individually. Moreover, not all technical knowledge and equipment are covered by the individual states, especially for the smaller member states and the newcomers in the EU, where such an investment would be very difficult to cope with financially. However, in Europe, there are several laboratories capable of making a major contribution in 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 which is the objective of this COST Action.

Several culture collections and laboratory facilities are also scattered over Europe, so it is absolutely necessary to unite the different laboratories to ensure that the 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. In addition a strong connection with a group of technologists will be established. This, together with additional help from people specialised in certain research items such as genomics, proteomics, glycomics and antigenicity, make this COST Action B28 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 thorough study of these rare but highly pathogenic and emerging microorganisms.

Several European laboratories that have dealt with the “anthrax threat” in 2001 were not sufficiently prepared to handle the demand. Different laboratories 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 supported by several of these laboratories and by networking these laboratories the Action gives access to new means for fast diagnosis and intervention. Many of these laboratories have experience in fields other than the biological weapons, for instance in taxonomy, antibiotic resistance, vaccine development and virulence; there are possibilities to enlarge the detection and diagnosis of these agents. The Action will create an overall comprehensive and applicable knowledge base concerning these particular microorganisms.

C. Scientific Programme

C.1. Identification of target genes or gene products for the development of specific diagnostic and characterisation tools

A. Genomic approach

In the genomic approach, the COST Action B28 will enlarge identification of unique fragments of DNA of specific microorganisms. In order to identify specific sequences to be used for the development of specific diagnostic tests, several genetic markers will be sought by “differential array”. These fragments, when identified, will also help to define

unique fragments for the development of antimicrobial and vaccine targets. Genomes of two different organisms, although closely related, will be hybridised one to another and to themselves. Whole genome libraries will be constructed and the inserted fragments of the library clones will be amplified by PCR and spotted on micro-array slides. Slides will be hybridised with a randomly labelled DNA probe prepared from fragmented genomic DNA, isolated from the organism from which the slides were prepared, and with a probe prepared from a closely related organism. Spotted library fragments that hybridise only with the homologue probe, and not with the probe prepared from the closely related organism. These fragments will be identified in the library and sequenced. The sequences will be compared to known sequences in publicly accessible databases (BLAST search). This will prove the specificity of the fragments. It is expected that several of the fragments obtained will not be present in other species, and likewise will prove their specificity. Random priming can be performed on the organism to have the fragment labelled for detection on the array. In the first instance an inventory of the actual data will be generated, and the participants in the network will fill gaps within this.

A second approach to DNA arrays will be specific for bacteria and fungi. In bacteria, several conserved regions exist to which (degenerate) primers can be constructed. By applying more or less stringent PCR outwards from these genes, fragments of different lengths (intergenic regions) can be produced. Intergenic spacers between the tRNA encoding genes, have been shown to be variable in length. Another possible target is the spacer region between 16S and 23SrRNA genes. For fungi, a similar method can be applied but for these organisms Internally Transcribed Spacer regions ITS1 (between 18S and 5.8S rRNA) and ITS2 (between 5.8 and 25S rRNA) can be used. Based on new and existing data on the length of the fragments, fragments will be chosen and a simple reverse line blot hybridisation will be performed for their possible cross hybridisations. To be able to encompass the discriminatory power of the obtained fragments, several fragments will need to be sequenced to determine their specificity. These sequences will generate further data for post-genomics. This development will need a close collaboration between microbiologists, genomics and groups specialised in technological developments. The major output will directly serve a detection test and will give study material for post-genomics.

B. Post-genomics: antigenic, proteomic and glycomic approach

Post genomics will enlarge knowledge on the proteins and lipopolysaccharides/glycoproteins in targeted microorganisms as genetic research alone cannot define unique regions encoding proteins, but protein analysis techniques can define such unique proteins. Proteomics aspires to know more than just to identity of proteins; its ultimate pursuit is to understand the functions of proteins and cell's metabolism. After the gene-to-amino acid process produces a protein, it is altered in a number of ways (by posttranslational modifications, glycosylations, phosphorylations, sulphatations, etc.) prior to it being used by the cell.

Besides whole cell lysates, purified integral membrane proteins and secreted proteins will be analysed. Using 2D gel electrophoresis combined with mass spectrometry the bacterial antigens that are differentially expressed in strains can be identified. Monoclonal antibodies against selected proteins will be raised in order to define the specificity of these proteins for a microorganism. Characterisation and sequencing of the selected peptides, proteins or protein fragments by MALDI-TOF- and LC-ESI-MS/MS will open the field for the preparation of the target peptides, proteins or protein fragments by conventional recombinant techniques. The proteomic results in combination with the immuno-approach

will be the basis for the utilisation of recombinant techniques for the construction of immune-based prophylactic and/or therapeutic tools exploiting both broad-spectrum and pathogen-specific microbial components. Moreover they can be used in the multiplexed detection assay using flow cytometry.

LPSs of pathogenic microorganisms have been considered to be major determinants of virulence expression and infection. They play an important role in interaction of the microbes with hosts, their pathogenicity, immunogenicity, and are capable of inducing antibody responses. Like proteins, the localization and characterisation of immunoreactive epitopes will be investigated in the LPSs by a combination of chemical and immunological methods. The next step includes characterisation and sequencing of the selected, poly- and oligomeric glycoses and their fragments, obtained from the native LPSs, by MALDI-TOF- and EIS-MS techniques.

C.2. Technology development

The technological developments in this COST Action enable further characterisation of the microorganisms covered by this Action.

Two technological platforms will be used for the diagnostic assay development:

a. DNA-microarray

Specific capture probes designed on the basis of the genomic research described above will be spotted on chips. After an amplification step (or preferably not) the sample material hybridised to this probe will be revealed by a labelled DNA probe. Different kinds of sample preparation, and signal amplification methods will be tested in order to achieve maximum analytical sensitivity. The final goal is to have as few as possible preparation protocols for the different types of sample that could be analysed.

b. Suspension microarray (DNA/Antibody/protein/LPS, etc.)

Suspension arrays of microspheres, analysed using flow cytometry, offer a new approach to multiplexed assays for large-scale screening applications. By optically encoding micron-sized polymer particles, suspension microarrays can be created to enable highly multiplexed analysis of complex samples. These multiple fluorescent microspheres, conjugated to different probes (DNA, Lipid, proteins, LPS or their fragments), constitute the solid phase for detecting the presence of a biological agent's gene, antigen or protein in samples.

C.3. Microbiology

The bacterial pathogens that will be studied are *Bacillus anthracis*, *Brucella* spp., *Burkholderia* spp. (*B.mallei* & *B.pseudomallei*), *Coxiella burnetti* (and closely related *Rickettsiae*), *Yersinia pestis* (plus *Y. pseudotuberculosis*), *Francisella tularensis*, and *Clostridium botulinum*. The yeasts and filamentous fungi under investigation will be *Aspergillus fumigatus* and *A. flavus*, *Blastomyces dermatitidis*, *Candida albicans*, *Cladophialophora bantiana* and *C. devriesii*, *Coccidioides immitis* and *C. posadasii*, *Cryptococcus gatti* and *C. neoformans*, *Exophiala dermatitidis*, *Fusarium verticillioides*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffei*, *Pseudallescheria boydii*, and *Ramichloridium mackenziei*. Viruses from the RNA virus families *Bunyaviridae*, *Filoviridae*, *Arenaviridae*, *Togaviridae*, *Paramyxoviridae*, *Orthomyxoviridae* and from the DNA virus family of the *Poxviridae* are prime candidates. All these microorganisms need special culture and growth conditions. They all have to be

treated in high containment environments like BSL3 and BSL4 conditions. Highly specialised skills are necessary to safely treat these microorganisms. Several laboratories that have high containment facilities and who are used to working with these highly pathogenic microorganisms have subscribed to this COST Action.

In order to evaluate the usefulness of the developed techniques, validation is necessary. Evaluation of the analytical test characteristics of each individual system will be done with the help of antigen or DNA extracts prepared from pure cultures or protein production at known quantity/concentration and with a panel of relevant biological agents that are known to interfere in other types of diagnostic assays. Once these steps of the analytical evaluation have been shown to be successful, the test characteristics of the different multiplexed detection systems will be evaluated and compared with more conventional approaches, brought by microbiologists, who are used working with the microorganisms in this study.

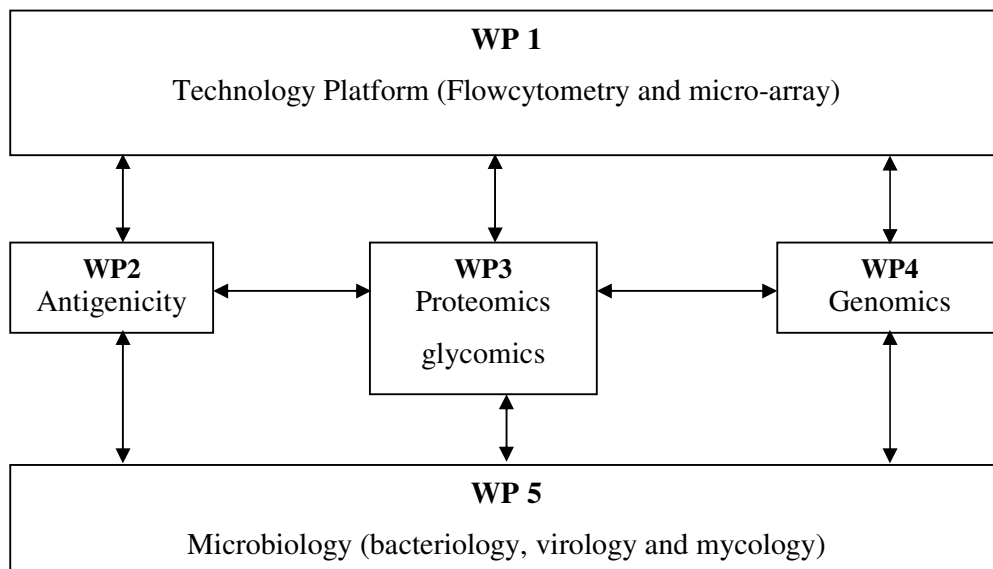
D. Organisation

The Management Committee (MC) of the COST Action B28 will coordinate all complementary research initiatives to attain the final research goals. Within the 5 Working Groups (WGs), described underneath, the work should be equally and fairly divided among its members. WG leaders will be in charge of this and will be part of MC. MC meetings will be organised at least twice a year normally together with the WG meetings. In collaboration with the respective WG leader, MC can decide to have additional separate WG meeting.

A website with a restricted access area will be created in order to improve data exchange between the different partners of the COST Action.

Great attention will be put on STSM, since techniques applied in this Action are highly specialised. Appraisal of such techniques requires specialised training. An STSM panel deciding on the STSM candidates that will be selected will be chosen from the MC. After STSM beneficiaries have finished their missions, their results will be orally presented to a MC/WG meeting, respectively. The COST Action B28 will organise training schools using the STSM financing scheme to create common expertise in the specific area of the Action.

A diagram is representing the interdependencies of the different WGs.



1. **WG1 Technology platform**

(a) Flowcytometric method with cytometric beads.

Objective: Development of a multiplexed flow array for the simultaneous detection of different pathogenic agents and/or pathogen products.

Description: Suspension arrays of microspheres analysed using flow cytometry offer a new approach to multiplexed assays for large-scale screening applications. By optically encoding micron-sized polymer particles, suspension microarrays can be created to enable highly multiplexed analysis of complex samples. Each element in the array is comprised of a subpopulation of particles with distinct optical properties and each array element bears a different surface receptor; these may be oligonucleotides, antigens or antibodies. These multiple fluorescent microspheres, conjugated to different probes, constitute the solid phase for detecting the presence of a biological agent's gene or antigen in samples. These assays have proven to have at least the same sensitivity as traditional immunoassays; in addition, they have a high throughput capacity, and provide a wide analytical dynamic range. Additionally, they have multiplexing ability and can be used simultaneously with different types of probes (DNA, Lipid, proteins etc.). Therefore, a major advantage for the diagnosis of biological threat agents would be to detect the presence of specific DNA sequences, antigens and/or toxins simultaneously in a suspect sample. The project contains two approaches that will be developed simultaneously: a DNA microarray and an antibody microarray. A suspension microarray based on the detection of specific nucleic acid sequences will be developed using nucleic captured/revelation probes. This will be compared to a suspension microarray using antibodies directed against antigens specific for the different biological agents. Changing the nature of targets by using antibodies as probes, will allow us to detect a wider range of agents' products such as toxins.

(b) Micro-array.

Objective: To use DNA microarray technology for characterisation of microorganisms and to evaluate a new diagnostic test for the broad-range diagnosis/detection and characterisation of specific pathogens.

Description: Genomic sequencing, although very popular for the genome-wide analysis of microorganisms and very successful in terms of generated knowledge, is not suitable for the study of large quantities of strains. To discover and use new and suitable genetic markers for broad-range diagnostics of microorganisms, many strains need to be examined by comparative genomics (DNA-DNA hybridisation experiments). Microarray technology is very suitable for a genome-wide analysis of many strains in parallel. It will also define unique fragments within these organisms that will allow the microorganisms under investigation to be fully characterised in all aspects of their genome. These fragments may encode virulence, which upon further investigations may lead to targets for vaccines and antimicrobial compounds. They can also be important for immunological investigations.

Microarray technology is technically very demanding and requires specialised and skilled staff. Moreover, it is a substantial investment for a laboratory.

High-throughput-PCR will be applied on genomic libraries from the micro-organisms under investigation, or alternatively, oligo-probes will be designed from strains that have been fully sequenced, whereafter the PCR products or oligo-probes will be spotted onto a solid phase. DNA

from comparative strains will be labelled and hybridised to the probes on the slide.

Microarray technology relies heavily on bio-informatics. There is a great need for specialised software methods for the design of microarrays, normalisation of array data, analysis of array data, and handling of the huge resulting databases. This will involve a significant part of the effort. Array design has been a neglected area. However, with designed arrays, data quality control will be much more efficient than today and in addition array design has the potential to improve analysis methods and in particular normalisation methods. Focus will be put on developing strategies for array design and developing analysis methods that take full advantage of the design. In particular three areas will be considered: data quality methods, normalisation methods and methods for estimating distributions of test statistics. In addition, the ambition is to develop a general statistical model that can describe DNA microarray data.

To convert the data into a common standard for transforming the existing data from participants, so that the synergies coming from common representation of data may be obtained, shall require a lot of effort. A User Manual will be specifically designed for the members of the COST Action B28 Network. Courses for the users will be organised in order to create a common expertise for mining expression data.

2. **WG2 Antigenicity.**

Objective: Preparation of antibodies against relevant biological targets including microorganisms, biotoxins and proteins. This will allow full characterisation of the microorganisms' antigenic properties.

Description: This work package will bring together experts in the field of antigenicity, diagnostics and prophylaxis. Polyclonal antibodies, specific antibody fragments from phage display libraries, and mainly monoclonal antibodies (MAbs) will be developed/produced for a rapid, specific, sensitive and differentiating detection of bacteria, viruses and fungi, and bacterial and fungal toxins. Antibodies will be produced against bacteria, viruses and fungi, and derived products that fall within the scope of the project. In virology, special attention will be put on related microorganisms from the human and veterinary fields, with a special emphasis on poxviridae and BSL4 viruses. In addition, antibodies will be generated against peptides, proteins, glycoproteins, and protein-conjugated glycooligo- and polymers. This will allow the full antigenic properties of the microorganisms to be defined, which may also lead to a better understanding of the immunological reactions to infections.

Antibodies will be produced against the biological targets using classical polyclonal antibody production in rabbits, classical hybridoma technology for the production of MAbs, and new technology for the production of specific antibody fragments by phage display libraries. The latter methodology is also called antibody engineering technology and is a new area in the field of molecular immunology for production of libraries of Fab fragments by bacterial expression of the genes coding for the VH and the VL domain and display of fragments on the surface of filamentous phage. Such phage display methodology permits a rapid construction of large combinatorial antibody libraries. The antibody genes can be recovered from lymphocytes mRNA by RT-PCR and then cloned.

3. **WG3 Proteomics and glycomics**

Objective: To identify unique proteins as well as glycans from microorganisms as candidate molecules for use in detection/diagnosis, therapy and prophylaxis.

Description: This WG will provide information on unique proteins and glycans that can be used for unambiguous determination of microorganisms and for the identification of targets for diagnosis, antimicrobial therapy and/or vaccine development. Immunogenicity and uniqueness are key factors in determining suitability of candidate molecules towards exclusive recognition of a microbial, viral or fungal species. In addition, this knowledge is indispensable for new leads in antimicrobial therapy and vaccine development. To meet these goals, both proteomic and glycomic approaches will be used and the main focus will be on identification of immunogenic, species and/or strain specific proteins as well as lipopolysaccharides (LPSs).

Immunogenic proteins derived from the various pathogens will be detected by performing separation of the proteins of a microorganism by means of 2D-PAGE, followed by western blotting with available human or animal sera that contain antibodies that were produced in the course of natural infection. Differential analysis using classical and shotgun proteomics tools followed by image analysis for comparison of protein maps will discover species and/or strain specific proteins. There will be a focus on membrane proteins, cytosolic (sub)fractions and secreted proteins. As glycosylation of bacterial proteins may also play a role in creating uniqueness, attention will also be paid to this post-translational modification.

To identify proteins of interest that emerge from these investigations, equipment such as MALDI-TOF with PSD/LIFT and LC-ESI-MS/MS, and access to bioinformatics for sequence databases will be necessary. For analysis of functional domains and antigenic determinants the informational spectrum method, a virtual spectroscopy method for structure-function analysis of proteins is available. This information will be used for the design and synthesis of peptides with immunological reactivity.

As LPSs of pathogenic microorganisms have been considered to be major determinants of virulence expression and infection, and one of the main targets of the antibodies of the host immune system, attention will be paid to structural characterisation of LPSs and the subsequent identification, localization, and characterisation of their immunoreactive and/or species specific epitopes by combination of chemical and immunological methods. The next step will include characterisation and sequencing of the selected, potential diagnostic poly- and oligosaccharides and their modified derivatives, obtained from native LPSs by a number of separation techniques, in GLC-, MALDI-TOF- and EIS-MS.

The output of these studies should provide complete information on protein topology, amino acid sequence, post-translational modification, presence of functional domains, structural features of LPSs, and composition of immunoreactive epitopes in both proteins and LPSs.

4. **WG4 Genomics.**

Objective: Preparation of DNA fragments representing whole genomes and specific parts of genomes from microorganisms for use in micro-arrays. Analysis DNA fragments by sequence analysis. Cloning ORFs of interest in expression vectors.

Description: First an inventory of actual data on differential DNA markers and genomic libraries will be constructed for the microorganisms that need further investigation.

DNA will be sheared by sonication or partially digested by a restriction endonuclease, providing fragments of 1 to 2 kb. Fragments will be cloned into a conventional *E. coli* cloning vector and libraries will be propagated. Care will be taken to cover the complete genome of a microorganism. Libraries will be prepared from several strains of a species and from several strains of closely related species. Differences in virulence and antimicrobial resistance will be accounted for.

The results of the array analysis will lead to the identification of certain clones having unique fragments. Participants will sequence and analyse these fragments for the presence of open reading frames. An annotation of the sequence function will be provided where possible. If ORFs of specific interest are incomplete, the full ORF will be looked for. A sequence databank will be made available. For certain microorganisms, the aim will be to construct DNA libraries from strains of the bacterium, which are found in Europe, USA and the former Soviet Union. Shotgun sequencing of these libraries will allow comparisons to be made with the genome sequences of strains isolated these different geographical regions. This will allow whole genome comparisons to be made, and regions which are conserved and unique to isolates from these different locations to be identified. In turn this will allow diagnostic PCR probes to be devised and tested for strains from these different geographical locations.

The selected fragments will be analysed for (sub) species specificity and relation with virulence using the reverse line blot technique hybridisation. To this end genomic DNA from a panel of closely related informative micro-organisms will be prepared and labelled by random priming. This DNA will be hybridised to the selected DNA probes obtained from the array information. Another task will be the search for potential diagnostic fragments in conserved areas of the genome using PCRs with universal primers. Generic PCRs based on allelic diversity of genes, having regions of universal conservation will be performed. Additionally generic PCRs will be exploited in the search for unique intergenic spacers present in microorganisms. Virulence associated genes are frequently found in these regions (as exemplified between the tRNA encoding genes).

The gene sequences of the fragments obtained from the generic PCR approaches will be determined. Sequences will be annotated. Focus will be on (sub) species specificity virulence, specific target genes involved in antimicrobial resistance, and possible new antimicrobial targets. Specificity of the fragments at (sub) species level, possible relation with virulence or antimicrobial resistance/action will be determined by reverse line blot hybridisation, as described above. When a fragment has been proven to be associated with a single species, it can be included into the micro array for possible inclusion as a diagnostic probe.

5. **WG5 Microbiology (bacteriology, mycology and virology).**

Objective: Selection of a range of pathogens, including multiple strains of each species. Preparation of inactivated material such as DNA/RNA (genomic research), cell-free extracts (antigenicity and proteomics). To define the taxonomical relations between the closely related microorganisms. To develop primers to allow random amplification of viral RNA from a mammalian RNA background.

Description: This Working Group will bring together experts on a range of pathogenic microorganisms. The bacterial pathogens that will be studied are mentioned at Chapter 3 – Microbiology above. Virus strains of emerging viruses are not easily available. Since the anthrax attacks in the US, virus collections independent of the US and former Soviet research establishment, have become very important to guarantee free research in Europe. The

coordination of culture tasks and growth protocols as well as quality control and storage of the extracts could be the focal point of a new European collection of emerging microorganisms. The acquisition of new strains from around the world is therefore also a necessary task. These viruses could be deposited and curated within the pre-existing National Collection of Pathogenic Viruses (NCPV) housed within the Health Protection Agency (Porton Down site) in the UK.

Cell-free extracts (DNA extracts, fractions: cell wall, cytoplasmic extract, cell filtrates) from each of the strains examined will be prepared for use by the participants. Such extracts need to be validated as free of infectious agents and suitable for use in BSL (ACDP) level 2 laboratories and below.

For use in antigenicity and immunogenicity studies, live, killed or fixed whole cells of each strain will also be prepared. These cells are potentially of use for raising polyclonal antisera or MAb, and also screening antisera produced from either cell-free extracts. Specific attention will be paid to the large genomes of these organisms and the attribution of these to the post-genomic activities.

Optimisation of protocols for the preparation of these deliverables will be done in close collaboration with the concerned WG's in order to obtain high quality products.

The strain collection will also be further characterised with emphasis upon defining unique regions allowing identification at the genus or species level and below. New developments in the taxonomy of these species will be followed in order to be prepared when new closely related species are described which might be of interest in the differentiation of the species under investigation. Multiple geographic isolates of microorganisms of interest will be needed. All of these strains will be examined using conventional microbiological techniques (morphology, staining, biochemistry, molecular techniques e.g. 16S rRNA gene sequence, RFLP, AFLP, PCR-Restriction, etc.) and all strains under investigation will be fully characterised, both microbiologically and genetically, and a genotype assigned (where an appropriate genotyping scheme exists).

Another approach with the aim of defining unique DNA regions within the genomes of interest will be to apply such techniques as fluorescent AFLP (Amplified Fragment Length Polymorphism) and other similar technologies (e.g. Random amplified polymorphic DNA (RAPD), tandem repeats, IS elements, SNP's, REA, etc.). It has been observed that within the profiles of different organisms unique genus, species or strain specific restriction fragments have been identified. The species-specific unique DNA fragments will be of major interest. The specificity of the fragments will be evaluated by reverse line blot hybridisation.

DNA preparations will also be amplified using PCR with consensus primer pairs. Examples of such primer pairs are those widely reported for amplification of 16S and 23S rRNA genes. Primers to amplify other genes, conserved within a particular species, will also be employed (e.g. *gyrA*, *sod*,...). Also intergenic spacers between conserved regions will be investigated. Focus will be set on the intergenic spacers between the tRNA genes (possible sites for insertion of virulence associated genes) and 16S-23S intergenic spacers and the internally transcribed spacer region 2 between the 5.8S and 28S rRNA genes for fungi.

The identification of viruses always has to occur against a mammalian background. It is very difficult to enrich viruses from patient material. An approach to identify viruses by hybridisation arrays from clinical or environmental sources can only work if viral material can be selectively amplified. Since RNA viruses are notoriously diverse, it seems impossible to define specific targets for each pathogenic RNA virus. Therefore the aim of the Action is to design and test random primers for RAPD that specifically match viral RNA. In order to be able to design primers for random amplification that specifically match to RNA viruses bioinformatic approaches that allow tetranucleotide analysis up to decanucleotide analysis need to be

investigated. The approach published by Yap et.al (2003) seems to be appropriate to define such RNA virus specific tenmers for RAPD. To use this approach computer facility for the calculation needs to be at least a Sun Fire server with 24 CPUs each running at 900 MHz.

The resulting primers will be distributed to test them on the viral RNA extracts of the viruses from their collections. PCR trials will follow to come up with universal protocols to amplify viral RNA from cell cultures. PCR bands generated by this approach will be ligated into plasmids and sequenced. This will happen in close collaboration with WG 4. The viral sequences obtained will then be spotted onto the arrays of the WG1.

The family of the *Poxviridae* is composed of dsDNA viruses with genomes ranging from 130-375Kb. In the genus *Orthropoxvirus* (OPV) camelpox virus (CMPV) is most closely related to variola virus VAR (Gubser and Smith, 2002). Both viruses are host specific. The high frequency of genomic recombination in OPVs or simple mutations in the CMPV genes coding for the virus virulence factors or for virus coat proteins interacting with the cell receptor-binding sites could cause a breakthrough in the host species barrier. These mutations could occur naturally or artificially. The CMPV antigens and genome will be analysed, looking for unique genes and proteins responsible for the genes mentioned above. Other OPVs are to be included for differential diagnosis such as monkeypox, cowpox and vaccinia virus as these are all infectious to humans. These segments could be used in the early diagnosis of potential dangerous mutants or hybrid viruses.

Genomic libraries of the viruses under investigation will be constructed within the COST Action B28. The libraries should not contain large fragments in order not to loose their differential capacities. Differences between these viruses under investigation could be missed when larger fragments would be investigated, since these would allow more non-specific hybridisation.

Furthermore, for test validation this group has all means for the validation of an eventual diagnostic test coming out of this research. Initial estimates of repeatability and of analytical sensitivity and specificity will be investigated. The application will delineate the minimum acceptable requirements for diagnostic sensitivity and specificity.

E. Timetable: 5 Years

The 5-year period of the Action is justified by the following:

- one of the basic support elements of the Action is the large geographical dimension of it which means that in order to involve a greater number of countries, a larger time interval is needed; the participation of different States is not formal so that significant contributions of each of the participating States are expected;
- the object of the COST Action is timely and complex and the set-up of the necessary links followed by common scientific activities by the Action member laboratories needs at least 5 years;
- during the Action, durable links should be established with National and International organisations (such as NATO), which requires a longer time interval, needing at least 5 years.

WG1: Technology platform	Year	Year	Year	Year	Year
	1	2	3	4	5
<u>a. Microarray</u>					
Comparative genomics (DNA-DNA hybridisation)	■	■	■	■	■
Design of oligo-probes			■	■	■
Software optimisation		■	■	■	■
Array design					■
Data conversion manual					■
<u>b. Flow cytometry</u>					
Installing bead array technology	■	■			
Preliminary tests on DNA		■	■		
Preliminary tests with proteins			■	■	
Preliminary tests with AG-AB			■	■	
Preliminary tests with different components				■	■
Composition of a multiplexed flow array				■	■
WG2: Antigenicity					
Production of polyclonal antibodies,	■	■	■	■	■
Production of specific antibody fragments from phage display libraries	■	■	■	■	■
Production of monoclonal antibodies	■	■	■	■	■
Testing of specificity and sensitivity		■	■	■	■
WG3: Proteomics and glycomics					
2D-Page and western blot	■	■	■	■	■
Differential analysis using classical and shotgun proteomics tools	■	■	■	■	■
Identification of proteins		■	■	■	■
Post-translational glycolysations		■	■	■	■
Characterisation of LPS		■	■	■	■

WG4: Genomics																				
Preparing genomic libraries																				
Sequencing and annotation of selected fragments																				
Completion of incomplete open reading frames (ORF)																				
Prevalence/specificity of selected ORFs in populations																				
WG5: Microbiology																				
a. Bacteriology																				
Selection and cultivation of specifically selected strains																				
Characterisation of the selected strains with conventional methods																				
Determination of the exact taxonomic position towards their close relatives																				
Completion of strain collection																				
Determination of specific fragments by conventional methods (AFLP, RAPD, specific gene analysis, intergenic spacer analysis)																				
b. Virology																				
Selection and cultivation of specifically selected strains																				
Characterisation of the selected strains with conventional methods																				
Determination of specific random primers for viruses																				
Construction of genomic libraries																				
Determinants of variability in host adaptation of poxviruses																				
C. Mycology																				
Selection and cultivation of specifically selected strains																				
Characterisation of the selected strains with conventional methods																				
Determination of the exact taxonomic position towards their close relatives																				
Completion of strain collection																				

Determination of specific fragments by conventional methods (AFLP, RAPD, specific gene analysis, intergenic spacer analysis)											

The COST ACTION will start with a first general meeting (MC and all WG's), shortly after approval. There is a need for a meeting at least every half a year, because different tasks are depending on the conclusions of common results of the concerning specialists. It may be expected that some WGs will need to have separate meetings in between the common meetings, depending on this need; separate WG meetings can be included.

F. Economic Dimension

The following 14 COST countries have actively participated in the preparation of the Action or have indicated their interest: Austria, Belgium, Bulgaria, Czech Republic, Denmark, France, Germany, Italy, Luxembourg, The Netherlands, Serbia and Montenegro, Slovak Republic, Sweden, United Kingdom

On the basis of national estimates provided by the representatives of these countries, the economic dimension of the activities to be carried out under the Action has been estimated, in 2004 prices, at roughly EURO 22.6 Million.

This estimate is valid under the assumption that all countries mentioned above, but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

G. Dissemination Plan

The target audience for the dissemination of the results is mainly microbiologists involved in the diagnosis of infections, veterinarian diagnosticians for the microorganisms also involved in animal diseases, researchers involved in microbiology, physicians and veterinarians and health officials involved in emerging disease control.

Participants belong to academic and non-academic research laboratories, each with their specificities: e.g. National reference laboratories will disseminate through their reference function. At one of the biannual MC meetings a half a day to one day plenary session will be included that will be open to everyone that is interested. At this occasion, proceedings of the plenary meeting and workshops will be provided. At the two-year stage and at the final stage, a larger, international conference will be organised.

Results will also be published in international peer-reviewed journals, as well as communicated at national and international scientific meetings. The MC will establish a website with a secured part for internal results sharing and a general web site to inform the scientific community including general information about the COST Action B28 and free PDF files of the publications of this Action.

COST B28

ARRAY TECHNOLOGIES FOR BSL3 AND BSL4
PATHOGENS

ADDITIONAL INFORMATION

NOT PART OF THE MOU

PART 2 : ADDITIONAL INFORMATION

2.1. BSL3, BSL4 definition

BSL means Bio-Safety Level

BSL3 & BSL4 mean, according to the DIRECTIVE 2000/54/EC of the European Parliament and of the Council of 18 September 2000:

- * BSL3: Highly pathogenic for humans and/or animals and danger of fast dispersion and difficulties in the treatment, elevated mortality eg. bacillus anthracis, swine fever, avian influenza, brucella, etc.
- * BSL4: Extremely high pathogenicity, non treatable, high mortality, danger of fast dispersion, high mortality rate eg. ebola and other hemorrhagi fevers.

2.2. List of Experts

List of subscribing institutions:

1	VAR	Veterinary and Agrochemical research center	Patrick Butaye +32 02 379 0415 pabut@var.fgov.be	Groeselenberg 99 1180 Brussels Belgium
2	UU	University Umea	Anders Sjostedt +46 90 785 1120 anders.sjostedt@climi.umu.se	Umea University SE-901 85 Umea Sweden
3	ISS	Istituto Superiore di Sanità	Antonio Cassone + 390649387113 cassone@iss.it	Viale regina Elena 299 00161 Rome Italy
4	CMR	Institute of Nuclear Sciences VINCA	Veljko Veljkovic +381 11 2440 871 veljko01@hotmail.com	Mihjla Petrvica Alasa 24 11001 Belgrade Serbia and Montenegro
5	RIVM	National Institute of Public Health and the Environment	Wieger Homan +31 300 2742909 wieger.homan@rivm.nl	Antoni van Leeuwenhoeklaan 9 3721 MA Bilthoven The Netherlands
6	LNS-DI	National Public Health Laboratory	Claude P. Muller +352 490686 claudio.muller@lms.etat.lu	18, Rue Dicks L-1417 Luxembourg Luxemburg
7	HPA	Health Protection Agency	Nigel Silman +44 1980 612836	Porton Down SP4 0JG Salisbury

			Nigel.silman@hpa.org.uk	UK
8	UW	University of Wales, Aberystwyth	Hazel Davey +44 1970 621829 hhr@aber.ac.uk	Old College, King Street SY23 2AX, Aberystwyth UK
9	UM	University of Marseille	Didier Raoult +33 491 385517 didier.raoult@medecine.univ -mrs.fr	27 Boulevard Jean Moulin 13385 Marseille France
10	CBS	Centraalbureau voor Schimmelcultures	Sybren de Hoog +31 30 2122663 de.hoog@cbs.knaw.nl	Uppsala laan 8 3508 AD Utrecht The Netherlands
11	IPH	Institute of Public Health	Nicole Nolard +32 2 6425533 n.nolard@iph.fgov.be	Juliette Wytsmansstraat 14 1050 Brussels Belgium
12	TNO	Prins Maurits Laboratorium, CBB	Martien Broekhuijsen +31 152843512 leeuw@pml.tno.nl	Schoemakerstr. 97 2600 JA Delft The Netherlands
13	SAS	Slovak Academy of Sciences	Rudolf Toman +421 2 59302418 virtuoma@savba.sk	Dubravská cesta 9 845 05 Bratislava Slovak Republic
14	PMM A	Purkyne Military Medical Academy	Ales Macela +420 495 518 833 amacela@pmfhk.cz	Trebesska 1575 500 01 Hradec Kralove Czech Republic
15	SSI	Statens Serum Institut	Niels Heegaard + 45 3268 8118 NHE@ssi.dk	Artillerivej 5 2300 Copenhagen Denmark
16	DSTL	Defence Science and Technology Laboratory	Richard Titball + 1980 613301 rtitball@dstl.gov.uk	Porton Down SP4 0JQ Salisbury UK
17	AFSS	Agence Française de Sécurité	Bruno Garin Bastuji	22 Rue Pierre Curie

Additional Information

	A	Sanitaire des Aliments	+33 1 49 77 13 00 b.garin@alfort.afssa.fr	F 94703 Maisons Alfort France
18	ARCS	Seibersdorf research	Levente Bodrossy +43 50 550 3548 Levente.bodrossy@arcs.ac.at	Department of Biotechnology A-2444 Seibersdorf Austria
19	UCL	Université Catholique de Louvain	Jean-Luc Gala +32 2 7643165 gala@lbcm.ucl.ac.be	Clos Chapelle-aux- Champs 30 1200 Brussels Belgium
20	UF	University of Freiburg	Manfred Weidman +49 761 203 5436 Manfred.weidmann@uniklini k-freiburg.de	Stefan Meier str. 19 79104 Freiburg Germany
21	SMI	Swedish Institute for Infectious Disease Control	Åke Lundkvist +46 8 4572641 ake.lundkvist@smi.ki.se	Nobels väg 18 171 82 Solna Sweden
22	NCIP D	National Center of Infectious and Parasitic Diseases	Todor Kantardjiev +350 2 9446999 kantardj@ncipd.netbg.com	Janko Sakasov 26 1504 Sofia Bulgaria
23	NML	Canadian Science Centre for Human and Animal Health National Microbiology Laboratory	Michael Mulvey + (204) 789-2133 Michael_Mulvey@hc-sc.gc.ca	1015 Arlington St. Winnipeg, Manitoba R3E 3R2 Canada

2.3 Main implied institutions and recent publications of their participant members

1 Veterinary and Agrochemical Research centre (VAR), Brussels, Belgium

Scientific and technical personnel involved in the project:

- **Dr. Patrick Butaye:** Co-ordinator and Contract Manager for the CODA. Doctor in Veterinary Medicine. PhD in microbiology.
- **Dr. Karl Walravens:** Senior scientist. PhD in Immunology and microbiology.

Additional Information

- **Dr. Jacques Godfroid:** DVM, MSc, PhD. Senior scientist. Member of the Editorial Board of Vet. Immunol. & Immunopathol.
- **Dr. Kris De Clercq:** DVM, MSc in animal production. Head of the Section of Development of Diagnostic Tools for Epizootic Diseases. Chairman of the FAO Research group of the Standing Technical Committee of the FAO European Commission for the Control of FMD. Member of several EU and OIE Scientific working-groups. Member of the Board of the Society for Epidemiology and Economics. Co-ordinator of the EU project 503603 on the Improvement of FMD control.
- **Dr. Mik Staes:** Scientific Assistant. PhD in Biological Sciences.
- **Dr. Ir. Jan Mast:** PhD in Applied Biological Sciences (K.U.Leuven, 1998).
- **Dr. Frank Boelaert:** Doctor in Veterinary Medicine, MSc in Biostatistics, PhD in Veterinary Epidemiology.
- **Dr. Didier Verloo:** Doctor in Veterinary Medicine, Veterinary epidemiologist.

2 University of Umeå, Umeå, Sweden

Scientific and technical personnel involved in the project:

- **Anders Sjöstedt**, Professor, Head of Department. MD and PhD specialist in clinical bacteriology.
- **Laila Noppa**, Ph. D. Senior Scientist.
- **Patrik Ryden**, Ph.D. in mathematical statistics.
- **Jan Komorowski**, Senior Scientist.
- Molecular biologist **Linda Näslund**, Molecular biologist **Linda Stenman** Both represent technical staff with several years of experience working on DNA microarrays.

3 Istituto Superiore di Sanità -ISS, Rome, Italy

Scientific and technical personnel involved in the project:

- **Prof. Antonio Cassone:** Director of the Department of Infectious, Parasitic and Immunemediated diseases.
- **Dr. Alessandra Carattoli:** Senior Researcher. BSc in Biology; PhD in Molecular Biology.
- **Dr. Loredana Nicoletti:** Senior Researcher. BSc in Biology; Specialist in General Pathology.
- **Dr. Ida Luzzi:** Senior Researcher. BSc in Biology; PhD in Microbiology.
- **Dr. Giovanna Franciosa:** Senior Researcher. BSc in Biology; PhD in Biotechnology.

- **Dr Annalisa Pantosti**, Research Director, MD and Specialist in Infectious Diseases.

4 Institute of Nuclear Sciences VINCA (CMR), Belgrade, Serbia and Montenegro

Scientific and technical personnel involved in the project:

- **Dr. Veljko Veljkovic**: Head of CMR. PhD in chemical engineering, University of Belgrade.
- **Dr. Jelena Prljic**: PhD in molecular biology, University of Belgrade.
- **Dr. Dragan Alavantic**: PhD in molecular biology, University of Belgrade.
- **Dr. Miroslav Demajo**: PhD in biology, University of Belgrade.
- **Sanja Glisic**: M.Sc. in molecular biology, University of Belgrade.
- **Tatjana Veljkovic**: B.Sc. in biology, University of Belgrade.

5 National Institute of Public Health and the Environment (RIVM) Bilthoven, The Netherlands.

Scientific and technical personnel involved in the project:

- **Dr W.L. Homan**: Senior scientist .
- **Dr F Reubsaet**: Senior scientist.
- **Dr L Schouls**: Senior Scientist.
- **Ing. S. Svraka**: Laboratory technician.
- **Dr. K.Wernars**: senior scientist.
- **Dr. B.J. van Rotterdam**: Senior scientist.

6 Institute of Immunology, National Public Health Laboratory/ Centre de Recherche Public-Santé, (LNS-DI), Luxembourg, Luxembourg

Scientific and technical personnel involved in the project:

- **Prof. CP. Muller, MD, M.S.**: Head of the Institute of Immunology, director of WHO Collaborating Center and WHO European Reference Laboratory for Measles/Rubella. Professor of Immunology at the University of Trier (GER), Associate Professor of the U. of Homburg (GER), and Ibadan (Nigeria), Member of WHO Steering Committee for measles, Board of Fraunhofer Center for Molec
- **MN Mulders Ph.D.**, virologist: (currently seconded to WHO-EURO).
- **F. Fack, Ph.D.**, molecular biology.
- **S. Mahot, Ph.D.**, virology.

- **F. Bouche**, Ph.D., molecular biology.
- **C. Enschede**, Ph.D., organic chemistry: Post-doc, specialist in synthetic medicinal chemistry.
- **W. Ammerlaan**, Senior Technician.
- **MF. Henriette**, Ph.D., biology.

7 Health Protection Agency, Porton Down, Salisbury, UK

Scientific and technical personnel involved in the project:

- **Dr. Nigel Silman**: Head of Novel & Emerging Diseases Group (NED). BSc In Microbiology & Genetics; PhD in Microbial Biochemistry
- **Prof. Richard Sharp**: Scientific Leader, Bacteriology.
- **Dr. Mark Outlaw**: Head, National Collection of Pathogenic Viruses (NCPV).
- **Dr Valerie Mioulet**: Senior Virologist NCPV.
- **Dr Jane Burton**: Senior Bacteriologist NED, DNA multipathogen microarray design and analysis.
- **Mr James Oshota**: Technologist NED.
- **Dr Roger Hewson**: Senior Virologist NED.
- **Ms. Angela Sweed**: Technologist NED. PhD Student.

8 University of Wales, Aberystwyth (UWA), Wales, UK

Scientific and technical personnel involved in the project:

- **Dr. Hazel Davey**: Research scientist. BSc in Microbiology and Zoology; PhD on flow cytometry of microorganisms.
- **Dr Gareth Griffith**: Lecturer in Mycology. BSc in Microbiology; PhD Fungal Ecology.
- **Dr. Michael Winson**: Lecturer in Microbiology; BSc in Microbiology/Zoology, PhD in the molecular Biology of catabolic plasmids.

9. University of Marseille Unité des Rickettsies, WHO reference center for Rickettsia and Rickettsial diseases – Marseille, France

Scientific and technical personnel involved in the project:

- **Didier Raoult**: Scientific leader of the project.
- **Jean-Marc Rolain** : scientific collaborator, specialist of antibiotics.

- **Bernard La Scola** : scientific collaborator, culture and molecular biology, genome sequencing.
- **Florence Fenollar**: scientific collaborator, proteomics and serology.
- **Patricia Renesto**: scientific collaborator, genomics, proteomics, and vaccine research.
- **Pierre-Edouard Fournier** : scientific collaborator, molecular biology, diagnostic tools.
- **Jean-Louis.Mege** : scientific collaborator, immunology of intracellular bacteria.
- **Hubert Lepidi**: scientific collaborator, anatomopathologist. MD-PhD

10 CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Scientific and technical personnel involved in the project:

- **Prof. Dr. G.S. de Hoog**: senior researcher.
- **M. Sudhadham**, BSc: researcher.
- **Ing. A.H.G. Gerrits van den Ende**: Laboratory assistant .

11 Institute of Public Health, section Mycology, Brussels Belgium

Scientific and technical personnel involved in the project:

- **Dr. Nicole Nolard, Ph D**, head of section Mycology at the IPH Brussels.
- **Prof. Danielle Swinne, Ph D**.
- **Dr. Pierre-Alain Fonteyne, Ph D**, research assistant.
- **Mr. Frédéric Fauche** Lab tech, is responsible for DNA.

12 TNO, The Netherlands

Scientific and technical personnel involved in the project:

- **Ir. Martien Broekhuijsen**: graduated 1982 (c.l.) "Molecular Sciences" at the Agricultural University in Wageningen. At TNO he is senior scientist on genetic identification of biowarfare agents.
- **Dr. Netty Zegers**: senior/staff scientist, PhD in Immunochemistry.
- **Dr. Ben van Baar**: senior scientist, Ph.D. in Chemistry (mass spectrometry).
- **Dr. Marijke Mol**, PhD in biology.
- **Dr. Agnes Tan** PhD in Cellular Biochemistry.
- **Dr. Ir. Arthur Wolterink**: M.Sc. in Bioprocess technology; Ph.D. in Microbiology.
- **Dr. Jos van der Vossen**: Senior scientist working in the risk management.
- **Drs. Jeroen Carol**, graduated in 2001 at the Free University of Amsterdam in analytical chemistry.

13 Department of Rickettsiology and Chlamydiology, Institute of Virology, Slovak Academy of Sciences, Bratislava, Slovak Republic

Scientific and technical personnel involved in the project:

- **Dr. Rudolf Toman:** Doctor in Natural Sciences (DSc in Microbiology). PhD in Organic Chemistry, Slovak Academy of Sciences in Bratislava.
- **Dr. Ludovit Skultety:** PhD in Biochemistry, Slovak Technical University in Bratislava.
- **Ing. Marcela Fodorova:** PhD student in Microbiology, specialization in glycomics, and in GC and GC/MS techniques.
- **Ing. Katarina Slaba :** PhD student in Microbiology, specialization in cultivation of *C. burnetii* and other *Rickettsiae*.
- **Ing. Pavol Vadovic :** PhD student in Microbiology, specialization in glycomics and partially in proteomics, and in GC and GC/MS techniques.

14 Purkyne Military Medical Academy, Hradec Kralove, Czech Republic

Scientific and technical personnel involved in the project:

- **Dr. Ales Macela:** Doctor in Natural Sciences. PhD in immunology.
- **Dr. Jiri Stulik:** Ph.D in medical biochemistry
- **Ing. Lenka Hernychova:** Ph.D. in immunology.
- **Mgr. Martin Hubalek :** PhD student – biochemistry.
- **Mgr. Juraj Lenco:** PhD student – molecular biology.
- **Mgr. Sylva Szkanderova:** PhD student- radiobiology.
- **Mgr. Ivona Klimtova:** specialization – cell fractionation.

15 Statens Serum Institut, Copenhagen, Denmark.

Scientific and technical personnel involved in the project

- **Niels H. H. Heegaard,** M.D., D.Sc.. Head of Research and Development, Department of Autoimmunology and Hybridoma Laboratory, Statens Serum Institut.
- **Leif Bruun,** Ph.D.: Head of unit for antibody production.
- **Christian Schou,** Ph.D.: Head of unit for advanced analytical.

16 Defence Science and Technology Laboratory (Dstl), Porton Down, UK.

Scientific and technical personnel involved in the project

Additional Information

- **Prof. Richard Titball.** Is a group leader at Dstl Porton Down and has a PhD and DSc in bacteriology.
- **Dr Martin Pearce.** Is a group leader at Dstl Porton Down and has a PhD in Forensic Science.
- **Ms Helen Diaper.** Has a BSc in Biological .
- **Dr Karen Isherwood.** Has a BSc in Biomedical Sciences and a PhD in bacteriology
- **Ms Gail Middlemass.** Has a BSc in Microbiology.

17 Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Maisons-Alfort, France.

Scientific and technical personnel involved in the project:

- **Dr. Bruno GARIN-BASTUJI:** Doctor in Veterinary Medicine, PhD in Life Sciences Head of the Bacterial Zoonoses Unit, Head of the National Reference Centre (NRC) for *Brucella* and of the National and OIE reference laboratory/FAO collaborating centre for animal brucellosis.
- **Dr. Josée VAISSAIRE:** Doctor in Veterinary Medicine.
- **Dr. Maria-Laura BOSCHIROLI:** PhD in microbiology.
- **Ms. Sylvie HÉNAULT:** MSc in microbiology, Research Assistant.
- **Mr. David ALBERT:** BSc in Biology, MSc in Animal production, Research Assistant.

18 ARC Seibersdorf research GmbH (Seibersdorf research), Seibersdorf, Austria

Scientific and technical personnel involved in the project:

- **Dr. Levente Bodrossy:** Research scientist.
- **Dr. Angela Sessitsch:** Head of the microbiology group.
- **Dr. Evelyn Hackl:** Post-doctoral fellow.
- **Nancy Stralis-Pavese:** Ph.D. on the development and validation of the microbial diagnostic microarray platform.
- **Dr. Christa Nöhammer:** Research scientist.
- **Herbert Wiesinger:** Ph.D. student working on human pathogen and antibiotic resistance microarrays.
- **Bertrand Sandjong :** Ph.D. student working on *Salmonella* detection microarray.

19 Université Catholique de Louvain Laboratory of Applied Molecular Technologies (AMT); Brussels, Belgium

Scientific and technical personnel involved in the project

Additional Information

- **Dr. Jean-Luc Gala:** doctor in medicine and specialist in internal medicine, PhD in biomedical sciences.
- **Dr. Leonid Ireng:** doctor in medicine, PhD in biomedical sciences.
- **Dr. Michel Heusterspreute:** PhD in biomedical sciences.

20 Abteilung Virologie, Institut für medizinische Mikrobiologie und Hygiene, University of Freiburg

Scientific and technical personnel involved in the project

- **Frank T. Hufert, M.D., D.T.M.& H.** Associate Professor, Head of the Group specialist in Arboviruses and Herpesviruses.
- **Dr. Manfred Weidmann,** PhD in Microbiology.

21 Swedish Institute for Infectious Disease Control (SMI), Stockholm, Sweden

Scientific and technical personal involved in the project

- **Åke Lundkvist, PhD,** Senior Microbiologist, Associate Professor, Head of Viral Zoonoses Group and of BSL-4 Laboratory, Swedish Institute for Infectious Disease Control (SMI).
- **Alexander Plyusnin, PhD,** has major experience in Viral genetics and is Part-time guest professor.
- **Sirkka Vene,** med-lic, skilled in the development of diagnostics, and basic virology.
- **Ali Mirazimi, PhD** specialised in Molecular virology.
- **Mikael Nilsson, PhD.**
- **Kirill Nemirov,** post-doc, specialised in molecular virology.
- **Elisabeth Gustafsson,** assists to the diagnostics, recombinant antigens, PCR.
- **Jonas Hardestam,** PhD student in immunology and pathogenesis.
- **Jonas Klingström,** PhD student in molecular virology and animal models.
- **Helen Karlberg, Gunnel Lindgren,** are skilled technicians.

22 National Center of Infectious and Parasitic Diseases (NCIPD), Sofia Bulgaria.

Scientific and technical personnel involved in the project:

- **Dr. Todor Kantardjiev:** Assoc. Prof., M.D., Ph.D. in Microbiology, Head of Microbiology Department, National Consultant in Microbiology by the Ministry of Health.

- **Dr. Stefan Panaiotov:** Research Scientist, M.Sc., Ph.D., Head of Ref. Lab. of Molecular Microbiology.
- **Victoria Levterova:** Research Scientist, M.Sc. in Biology. Ph.D. student.
- **Ivan Ivanov:** M.Sc. in Molecular Biology, Ph.D. student.
- **Dr. Plamen Padeshki:** Research scientist, M.D.
- **Dr. Rumiana Nenova:** Research scientist, M.D.
- **Dr. Tzvetan Velinov:** Research scientist, M.D.
- **Dr. Boris Popov:** Assoc. Prof. M.D. .
- **Dr. Iskra Tomova:** Research scientist, M.D..

23 National Microbiology Laboratory (NML), Health Canada, Winnipeg, Manitoba Canada.

Scientific and technical personnel involved in the project:

- **Dr. Michael Mulvey:** Chief, Nosocomial Infections and Antimicrobial Resistance. BSc and PhD In Microbiology.

2.4. History of the proposal

Shortly after it was known that there were letters containing spores of *Bacillus anthracis* sent around in the US, people in Europe started to get afraid and saw everywhere possible anthrax contaminated letters. These had to be analysed, frequently by laboratories that were not ready to perform such analysis in a highly specific, sensitive and quick way. Locally some meetings (eg. the Belgian, Dutch and Luxemburg laboratories met) were organised to see what could be done together to improve the situation. However, many problems could not be solved. Therefore the idea came to construct a collaboration network between many European laboratories involved. To construct a tool to fast, sensitive and specific detection of bacteria and viruses is a very demanding task, including highly specialised laboratories in technical topics, and laboratories having high containment possibilities. These two things are very different and few laboratories (if any) can span the whole of the research needed.

The implied laboratories became also aware that already several laboratories were active in one of the subtopics of research in this area. Therefore the laboratories decided that it would be good to bring together competences in the multitude of scientific fields and needs cited in the scientific programme in part one. COST Action B28 would be a good tool to get started to collaborate.