European Virus Archive

Reporting

Project Information

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**Final Report Summary - EVA (European Virus Archive)**

Executive Summary:
The aim of the European Virus Archive project (EVA) is to create and mobilise a European network of high calibre centres with the appropriate expertise, to collect, amplify, characterise, standardise, authenticate, distribute and track, mammalian and other exotic viruses.

The EVA project is establishing a web-based catalogue to advertise and distribute viruses in the collection as well as associated products. In addition the EVA network also produce associated reagents on demand, to laboratories throughout Europe and also worldwide.

EVA project reached its objective to develop a readily accessible virus reference library at the European level through the creation of its Virus Archive. Since it would create insurmountable problems to develop such a collection in a single laboratory, EVA uses the expertise and facilities of recognised centres of excellence in virology within Europe. EVA also exploits the high international reputations of these centres to obtain viruses currently held outside Europe.

EVA was initially conceived to develop its platform on the basis of selected European laboratories that have already accumulated specialised virus collections and the expertise to take these to the state-of-the-art level through integration into EVA. However, looking further ahead, EVA will ultimately be an open entity with the important objective of enhancing and extending the original archive of viruses to encompass all the major collections that exist globally. The successful initiation of the EVA project has attracted the attention of many of international laboratories with relevant virus collections.

Indeed, EVA has been a very active consortium, expending its network from 9 initial members to 27 nowadays, with the association of new partners from Europe (Italy, Germany, Netherlands), as well as international partners (Russia, South Africa, China, Turkey). Furthermore, EVA raised interest and has established associations with other network-based virus-associated programmes, as Global Outbreak Alert and Response Network (GOARN) form the World health organization (WHO), the project “European Network for Diagnostics of "Imported" Viral Diseases (ENIVD)” funded by the European Centre for Disease Prevention and Control (ECDC) and the Chinese department of Viral Encephalitis and Arbovirus from the Chinese Centre for Disease Control (CCDC). The EVA network continues to spread with futures collaborations foreseen with Australia and the USA.
The EVA web-based catalogue contains 972 well-characterized viruses and can supply the agents or derived products to the scientific community. This catalogue is functional, user-friendly and the number of products (1485) in the database increases continuously. The management structure of EVA ensures the highest standards of quality assurance, security, traceability and dissemination for the benefit of science, medicine, education and global information. The EVA website receives a significant quantity of visitors, with an average of 200 distinct visitors per day for a total of 131 492 visitors since the EVA website was made available online. EVA appears as a major provider of high quality products with over 2000 products provided over 4 years and the figure has risen steadily since the project began.

EVA is conceived ultimately to be an open entity aiming at developing synergies and complementarity capabilities by providing improved access to researchers. Following successful initiation of the project, EVA will continue to exploit its knowledge and scientific networks to attract other international laboratories with relevant virus collections and will continue to expand its collection.

Project Context and Objectives:

THE CONTEXT

Following the pioneering studies on smallpox and rabies virus by Edward Jenner and Louis Pasteur, the subsequent laboratory isolation and characterisation of viruses from field samples, in the early twentieth century heralded an important era in the development of medical and veterinary microbiology. Within years of these first experiments, diagnostic and research centres were established worldwide to investigate a wide range of virus diseases that had hitherto escaped all attempts to be cultured under laboratory conditions.

During the past 70 years or more, an extremely large number of mammalian viruses have been isolated and are now routinely used for diagnostic and research purposes. Inevitably, new viruses will continue to be discovered. As our knowledge of these pathogenic viruses has accumulated so too has the number of viruses that needed to be stored as reference reagents for diagnosis, research tools with which to begin to understand the underpinning basis of virus pathogenesis and disease control through the development of vaccines and antivirals, and also for education purposes.

Some research laboratories have accumulated collections of viruses that are primarily dependent on the particular speciality of the laboratory. For example, the American Type Culture Collection (ATCC) and the Centre for Disease Control and Prevention (CDC) (USA) maintain wide collections of mammalian virus pathogens (including Biosafety level 4 agents). Relatively large collections of arboviruses are currently held in Texas, in the UK, in Marseille, the Czech Republic and Slovakia and also in Scandinavia. A UK National Collection of Pathogenic Viruses (NCPV) has been prepared, and European collections of rabies and influenza viruses are maintained in the UK (Veterinary Laboratories Agency, Health Protection Agency) and France (Pasteur Institute). Biosafety level 4 (BSL4) viruses such as Ebola, Nipah etc., are maintained in specialised facilities in France, Germany, and the UK. Many other assorted viruses of medical and veterinary importance (coronaviruses, herpesviruses, retroviruses, adenoviruses, enteroviruses, etc) are held in laboratories around the world. Laboratories in Russia, China, India and south East Asia, hold their own collections many of which, to date, have been relatively inaccessible to
Western laboratories. Inevitably, the process of virus standardisation, characterisation, preservation and distribution, has been relatively arbitrary and largely dependent on the particular speciality of each laboratory.

Despite the efforts of these independent research laboratories, there is currently no centre in the world that systematically coordinates these collections for the benefit of science. Thus, there is no current coordinated, quality controlled laboratory facility that can supply authenticated viruses to research and/or diagnostic laboratories, teaching centres, industries involved in the production of diagnostic reagents, pharmaceuticals, and vaccines.

For a variety of reasons, it would be virtually impossible, to establish a single laboratory, to maintain supplies of all recognised mammalian viruses. Moreover, since the establishment of the “self propagating 911 script of the NIPC ” we can no longer rely on laboratories in the USA to provide Europe with pathogenic viruses. Consequently, from the European standpoint, and indeed for the benefit of the rest of the world, a quality assured virus collection will be of paramount importance if it can serve science, the environmental and public health authorities and the needs of the pharmaceutical industry in developing new technologies for disease control, and provide material for teaching and training purposes. EVA was conceived as a means by which all of these problems could be addressed.

OVERALL OBJECTIVE

The overall objective will be to create and mobilise a European network of high calibre centres with the appropriate expertise, to collect, amplify, characterise, standardise, authenticate, distribute and track, mammalian and other exotic viruses. The network will also produce associated reagents on demand, to laboratories throughout Europe and also worldwide.

1. Provide access to a unique resource

Current taxonomic data define 15 virus families, containing an estimated 500 recognised mammalian virus species. Since many distinct strains have been isolated from each virus species, we estimate that more than 10,000 viruses are distributed globally in small laboratory collections. Many of these could be lost to science if they are not brought together in a co-ordinated high quality collection. The network of EVA laboratories represents an extensive range of virological disciplines and currently holds approximately 50% of the 500 recognised species within the EVA collection. Moreover, through their own local networks they can access the collections of viruses held in small laboratories. The ultimate aim of EVA is to coordinate these collections to produce the largest library of authenticated, quality controlled and available viruses in the world. EVA will therefore “provide a wider and more efficient access to and use of” the virus collections.

Partner laboratories have been selected according to the following criteria: extent and relevance of virus collections, capacity to isolate new viruses; recognised high calibre; high quality of associated research; record of achievement in meeting targets; experience of working to standard protocols. EVA as a quality assured virus collection will have the important mission to provide this unique resource, resulting from the
merging of well established infrastructures existing in Europe, to service science, the environmental and public health authorities, the needs of the pharmaceutical industry in developing new technologies for disease control, and provide material for teaching and training purposes.

2. Implement common strategies

Common strategies used by EVA partners will “structure better and integrate on a European scale, the way the research infrastructures operate”.

The nine laboratories associated with EVA will merge their different specific collections to create a single web-based catalogue to distribute viruses. EVA will establish a web-based catalogue to advertise and distribute viruses in the collection. Standardised protocols will be produced for all virus production, assessment, storage and distribution procedures. These will be used uniformly throughout the network. In exceptional circumstances, modifications to these protocols may be made.

3. Enhance and extend the original archive of viruses to encompass all the major collections that exist globally

EVA will initially develop its platform on the basis of selected European laboratories that have already accumulated specialised virus collections and the expertise to take these to the state-of-the-art level through integration into EVA. However, looking further ahead, EVA was conceived ultimately to be an open entity aiming at “developing synergies and complementarity capabilities in such a way as to offer an improved access to researchers” In this context the consortium will have two important objectives:

Firstly, it is anticipated that following successful initiation of the project EVA will exploit the knowledge and scientific networks of the Scientific Advisory Board members (which will have an impressive list of members), to attract the attention of all other international laboratories with relevant virus collections. Thus, through the links formed with the assistance of SAB members and using an additional linked intranet (developed within the EVA programme), that will be accessible to international laboratories with large virus collections. EVA will expand its collection to encompass all the most important globally representative laboratories.

Secondly, EVA will also link up with other network-based virus-associated programmes, using an interface designed to facilitate the exchanges and integration (this will be the extranet or EVA network) between external/additional groups and EVA partners. In addition to the use of this extranet for the virus collections, it will also be designed and used to initiate training and other events organised in common with the training sessions. Thus, through the resulting exchanges between personnel from laboratories worldwide, the EVA network will drive the programme towards the second generation of EVA that will integrate a wider number and range of virus collections and associated reagents, together with international access to training and education courses. EVA will therefore provide science with the viruses and tools for fundamental and applied research; it will also feed improved scientific competence, knowledge and skills through its exploitation of the internet.

4. EVA as a tool to investigate new fields of virology
As indicated earlier, the scientific implications of EVA extend well beyond the creation of the largest, systematically developed quality controlled and validated virus collection in the world. For example, a wide variety of research infrastructures, including medicine, industry, academia, health agencies, other government agencies and other end-users could potentially benefit their research and development programmes by exploiting the EVA collection and its associated reagents, which, under contract, could be custom-designed (including the viruses). Thus, they could have the capacity to investigate and develop novel, and in some cases, unanticipated areas of research, hitherto inaccessible to their fields of interest, with a more coordinated approach. Additionally, by exploiting the knowledge and experience of the individual partners in EVA, they will gain access to Europe’s finest scientists in fundamental and applied research, public health, veterinary medicine, and specific training and education programmes.

5. EVA as a structure interacting with other fields of research to increase scientific knowledge and to drive industrial development

EVA has the potential to generate a new open attitude to offset the often observed “closed door philosophy” of scientists from different disciplines. The successful creation and subsequent maturation of EVA with an integrated extranet that can out-source the collection, and expertise of the partners, will generate closer interactions between scientists from many related disciplines. EVA will directly encourage these interactions by planning workshops, international conferences, educational and informational videos, and exploitation of the intranet information capabilities. Where possible, EVA will directly facilitate cross-disciplinary collaboration and a more open approach to the sharing of knowledge and technologies. EVA will look for opportunities to encourage direct interactions between academia and industry to the mutual benefit of both areas of science. Special attention will be given to facilitate interactions with the industrial community. Using a dedicated website interface, the EVA users platform, will promote EVA foreground to the Industry. The derivative products activities (JR8 workpackage) will contribute to the establishment of robust relationships with industrial groups in providing unique resources (antigens, viral proteins, viral enzymes) necessary, for instance, for antiviral drug development or for the production of diagnostic kits.

Project Results:
At a certain stage of the project each WP has generated a major progress in the practise usually found in classical collections. Seven WP are mostly concerned by those significant progresses.
1 - Implementation of a Quality Management System (WP2)
2 - Coordination with related scientific networks to expend EVA resources (WP4)
3 - Procedures for the users access to the collection (WP5)
4 - Networking and Database development (WP6)
5 - Tools for virus characterization(WP7&9)
6 - Animals models (WP10)
7 - Virus production and characterization P4 (WP13)

Conclusion and future of the EVA project
A Quality Manager was appointed immediately at the start of the project. He, then agreed a quality policy and guidelines with the EVA Steering Committee.

The quality standard position as broadly defined by the guidelines, if achieved would facilitate and support EVA partners in achieving certification to an internationally recognised Quality Standard (e.g. ISO9001:2008).

Quality Survey of all constituent virus collections was initiated and workshops were organized. During the initial project planning stage, a workshop was planned whereby all partners would meet together over two days to exchange best practice and receive guidance from the QM and Work Package Leader. Following this initial plan, fears were expressed over the effectiveness of this approach, not least was whether or not all representatives could be available over two days at the same time and place. The QM and N1 WP Leader agreed to change this task by carrying out targeted audits at each institute based upon the results of the self-assessment survey. These audits were completed within the timeframe of this second periodic report.

One of the aims of this work package is to provide advice and guidance to those institutes (including associated labs) wishing to become a recognised Biological Resource Centre (BRC) through the audit programme. Currently, four (AHVLA, HPA, UL & BNI) partner labs are recognised to an international quality standard (ISO9001:2008), two (IRD & UKB) have declared an intention to achieve certification, two (UNIGE & IVSAS) are possible candidates and one (UNIMED) will not seek external certification.

Notwithstanding this, it remains the objective of the N1 work package that all nine partner institutes will achieve a minimum 80% compliance with the EVA minimum standard for quality systems. Some examples of improvements made during the reporting period are:

The BNI (Bernhard Nocht Institute) has arranged for pipetters, freezers, fridges and water incubators operated in the virology research lab to be under a calibration and servicing contract. All virus stocks prepared for the EVA collection are now sequenced, quantified and checked for mycoplasma contamination, plus all historical virus acquisitions have been characterised in the same way. In some cases this has given rise to strain type corrections. Batch, accession and passage numbers have been fully documented. The recording of stock has progressed from hand-written notes to the interim position of a Master Index using Microsoft XL; the BNI is developing a web-based database meanwhile. Virus data (recommended storage instructions, resuscitation and growth directions) sheets are created and accompany all virus dispatches to customers. A Material Safety Data Sheet for EVA use is planned.

The UL (University of Ljubljana) has now carried out stock separation of new batches when prepared. Small numbers of vials from each batch are now stored within geographically different locations on site, served by separate power supplies. Also, paper records of virus isolations have been scanned electronically and stored on the institute’s server.

The VLA (now called APHA) has begun work on an improved database (ICAST) of which a working version is in use on site. This will greatly improve stock movement tracking and traceability. Full implementation of this system is currently on hold awaiting commissioning acceptance of a new CL3 facility by the UK Health & Safety Executive. Some other low level improvements have also been made.
The AMU/EPV (University of the Mediterranean) has implemented calibration and servicing contracts for all critical laboratory equipment, and now carries out reviews of the outcome of calibration exercises to identify out-of-range settings. Log books have now been created and assigned to critical lab equipment. Documented protocols have been centrally stored on a database, and authorised by a responsible scientist.

AMU/AFMB lab prepares lab reagents, viral cDNA and viral protein for EVA and now stores intermediate product at a separate location to allow for reconstitution if necessary at short notice. Data sheets already in use are now validated before dispatch. Unique numbers are now issued to track the progress of corrective actions arising from any non-conforming product.

The IVSAS (Institute of Virology, Slovakian Academy of Science) has reviewed and improved servicing and calibration of critical equipment such as CO2 incubators, freezers, safety cabinets and pipettes. Log books for these and other items of equipment have been introduced recording maintenance and servicing events.

For most of the partners,
- Ultra-low temperature freezers are now alarmed and connected to CO2 injectors.
- Virus master stocks are recorded on an electronic database, and critical reference samples divided between two buildings to mitigate against unforeseen disasters eg fire.
- Movement of virus stocks is recorded and documented.
- Lab staff hold weekly meetings to discuss management /lab event issues.
- Certificates of Analysis are included with all EVA dispatches and detail virus name, accession & batch number plus test results for viability, purity and identity.
- Mycoplasma testing now takes place as routine for all EVA products, and data sheets accompany each dispatch.
- Virus Data Sheets detailing recommended storage guidance, opening, resuscitation and growth instructions have been entered onto the EVA portal for supplied viruses in the web-based catalogue.

Current situation: at this stage of the project all EVA partners had satisfied at least 80% of the critical parameters, with six partners meeting all the critical requirements (the remaining three partners have training systems to). All business and scientifically important processes have been documented.

This ensures that all products supplied through EVA are characterized and released to a customer in accordance with a specification and with a certificate of analysis.

All products are stored securely in a monitored and secure storage with second site storage secured for all CL3 and 4 products, in the event of loss. Products are released to customers who have the facilities and expertise to handle the virus under the correct containment level and shipments are positively tracked. Five of the partners are now linked to an internationally accredited quality system ISO9001:2008 or ISO 15189:2007.

All partners have shown improvements in their quality systems and the work package can be deemed to
have achieved all of its objectives and EVA can now be viewed as a supplier of choice to its customers: among the main achievements can be noticed.

IRD: have installed a temperature monitoring and telemetry alarm system, implemented the provision of a Certificate of Analysis with all products despatched. They are routinely screening their products for mycoplasma contamination and have established a controlled documentation system. Critical parameters agreed for their improvement and compliance with the EVA guidelines have been completed.

PHE (PHE) (previous HPA): now provide certificates of analysis (virus, nucleic acids, cell lines) electronically for the supply of their products via the PHE Culture Collections website. An electronic QMS system has been implemented and a thorough training and competency assessment system has been established. All critical parameters have been satisfied.

IVSAS: have implemented a temperature monitoring and telemetry system for their virus stocks as well as storing their stock as a reserve collection in a second location. A controlled documentation system has been established. Certificates of analysis are generated for all stocks describing the mycoplasma status, titre and sequence of the virus on release to the customer/recipient. Lyophilisation technology has been implemented for the preservation of virus stocks.

AHVLA: has a rigorous procedure for the release of virus with comprehensive certificates of analysis, tested to approve specifications. Suppliers are assessed and monitored for their provision of services. All critical parameters have been satisfied.

UNIGE: a training system for control of equipment in terms of calibration and greater monitoring and telemetry systems have been implemented. A training system with staff training records has been established and documented manual/systems of protocols are now controlled. Products (viruses, viral products and antibodies) are now released against a pre-determined specification and with a certificate of analysis.

UKB: the University is accredited to ISO9001. A temperature monitoring and telemetry system is in operations for CL3 virus stocks, a documentation system, specifications for virus stocks and PCR standards has been established and products are released against specifications with certificates of analysis.

AMU: a controlled documentation system and system for control of equipment has been established, assuring the storage conditions for intermediate and master stocks, second site storage has also been implemented. Specification and release criteria have been agreed for products (viral cDNA, viral proteins and reagents) which are released with certificates of analysis.

UL: have secured the storage of their stocks across two locations, have arranged a backup of their data and also now provides a Certificate of Analysis with all products despatched. Improved monitoring and telemetry systems have been implemented at their facilities with better notification systems. Procedures for order processing have been formalised. All critical parameters have been satisfied.
BNI: diagnostic lab is accredited to ISO 15189:2007. The collection is duplicate and stored at an offsite facility. A new high containment (CL4) facility has been brought into operations, and mouse models have been established for haemorrhagic viruses. Specifications are in place for characterization of viruses, certificates of analysis provided with all stocks. Training systems and records in place all critical parameters have been met.

EVA is currently in the situation to provide high quality products with certificate of authentification. This is the only infrastructure able to reach such a level for a full range of products covering RC2 to RC4 viruses. An efficient methodology has been implemented which will be applied in the context of the EVAg project

2-Coordination with related scientific networks to expend EVA resources (WP4)

EVA was initially conceived to develop its platform on the basis of selected European laboratories that have already accumulated specialised virus collections and the expertise to take these to the state-of-the-art level through integration into EVA. However, looking further ahead, EVA will ultimately have to be an open entity with the important objective of enhancing and extending the original archive of viruses to encompass all the major collections that exist globally.

The successful initiation of the EVA project has attracted the attention of many of international laboratories with relevant virus collections, resulting in

- Firstly, through the links with associated partners formed with the assistance of SAB members, EVA is expanding its collection.
- Secondly, EVA is also linked up with other network-based virus-associated programmes and the European Biobanking Consortium.

Following the recommendation given by one of the referees of the project, a South African collection, the Onderstepoort Veterinary Institute (ARC, Pretoria, South Africa), has been contacted to initiate a proposal of association with EVA aiming at increasing the virus collection with African viruses, and sharing information related to the EVA Quality Management System.

Those discussions have resulted in the signature of a Memorandum of Understanding in July 2009. The OVI is an EVA associated member, participating in all the Consortium activities.

During 2009 contacts have been initiated with six of the major Russian virus collections, namely:

- Chumakov Institute (Moscow, RF)
- Mechnikov Institute (Moscow RF)
- Ivanovsky Institute (Moscow RF)
- Influenza Institute (St Petersburg, RF)
- Biomedical Centre (St Petersburg, RF)
- Pasteur Institute (St Petersburg, RF)

Those collections represent an immense archive of viruses isolated from the environment and from clinical
samples. The long term preservation of some of the strains provides access to a unique historical resource, necessary to understand the actual viral diseases. With the assistance of the scientific networks of the Scientific Advisory Board members and following a very convincing workshop organised in April 2010 in Moscow by partner 2, the six Russian institutes have agreed to be associated with EVA, to share their knowledge and some of their resources, the exchange of viruses with foreign laboratories being prohibited by the Russian laws. The timing of this workshop coincides with the update of the preliminary task carried out by VLA concerning the establishment of a list of select agents. No unique rules in Europe exist concerning this classification. Each country applies its own set of guidelines. Russia has also a very specific set of guidelines. The workshop was an opportunity to discuss the similarities and the difference in those rules. The place of this workshop was chosen for strategic reasons: Russian collections were expecting a strong indication of interest from the European consortium before deciding to join it. At the end of the meeting, the 6 Russian collections decided to meet in September to formerly sign the Memorandum of Understanding, together in the presence of the EVA coordinator. Since that time an additional Russian partner, Dr German Shipulin director of the Center for Molecular Diagnostics (Moscow, Russia) has joined the EVA consortium. This laboratory is specialized in epidemiology studies and has developed a robust know-how in the development of new diagnostic tools notably for the diagnosis of viral diseases. His contribution will be important as this topic is becoming more and more important for the consortium due to its link with international Health agencies.

In March 2010 during the ESFRI meeting in Barcelona a proposal has been made by the coordinator of the European Research Infrastructure for High Security Laboratories (ERINHA) to associate EVA to this infrastructure. This proposal has been formally introduced in the “road map” document: “some contacts have been established from now with the European Viral Archives (EVA) project (FP7 CAPACITY, Research Infrastructures, GA 228292). Indeed, the overall objective of EVA is to create and mobilise a European network of high calibre centres with the appropriate expertise, to collect, amplify, characterise, standardise, authenticate, distribute and track, mammalian and other exotic viruses, including L4 pathogens. EVA develops its platform on the basis of selected European laboratories ….Some of them are full partners of ERINHA (BNI in Germany and HPA in UK). “” EVA would act as a provider of virus highly pathogen (BSL4 viruses) to produce derived viral products for the research and for industrial developments (vaccine, antiviral, diagnostic kit).

In June 2010, EVA has been invited to present its organization in the context of a meeting organized by the BBMRI Infrastructure. Following this presentation there was expression of a strong interest by this consortium to integrate EVA as a member, to participate in the Biobanking activity in the field of biological material of Infectious diseases, more specifically on tasks related to the definition of requirements for biobanks working with medium/high risk pathogens and to the design of an interface between biobanks of infectious diseases established in health care and in the up-coming ERINHA project.

Discussions concerning possible collaboration with EVA are ongoing with other collections in the world, for instance with the Robert Koch Institut, Berlin, Germany, the UOC, IstitutoNazionaleMalattieInfettiveRoma, Italia, the CSIRO in Geelong, Australia, and its director, professor Peter Daniels (also member of the EVA SAB), with CDC ‘s in Europe and in China.

In December 2011 during a meeting organized by the China-CDC in Beijing, its director, Professor Dexin
Li has agreed to sign a collaboration agreement between China-CDC and EVA, one of China CDCs Units, namely the Dpt of Viral Encephalitis and Arbovirus (dir: Prof Guo-Dong Liang) will become EVA associated partner. The same month a MoU has been discussed with Professor Gao Fu director of the Laboratory of Pathogenic Microbiology, Chinese Academy of Science, in Beijing. The signature of those protocols is ongoing.

Public health networks like the WHO Emerging and Dangerous Pathogens Laboratory Network (EDPLN), European Network for Highly Pathogenic Bacteria / Establishment of Quality Assurances for Detection of Highly Pathogenic Bacteria of Potential Bioterrorism Risk (ENHPB/EQADeBa), Quality Assurance Exercises and Networking on the Detection of Highly Infectious Pathogens (QUANDHIP), European Network of P4 Laboratories (EuroNetP4), or the European Network for Diagnostics of Imported Viral Diseases (ENIVD) depend on reference material for their successful work. Members of EVA are participating in some of these networks already and can establish stronger links between the culture and reference material collection of EVA and the public health networks. Following discussions with those organizations, EVA has been invited to join the WHO/GOARN. The E-CDC will integrate our consortium in the ENIVD.

Due to the increasing number of veterinary institutions links have been established with OIE (C Vallat)

In 2011, potential new collaborations are being pursued in Europe, MoU has been signed with 2 new associated partners (Italy and Germany). Today about 30 institutes are collaborating with EVA. Recently UTMB-CDC Galveston USA, FriedrichLoeffler Institute, the largest Veterinary Institute in Germany, Pasteur Institute in France together with its non-metropolitan network jointed the consortium. Exchanged of know-how and resource are organized within the consortium but also outside of the consortium.

EVA is becoming one of the leading virus collection organisations worldwide.

Up to date the list of those associated partners is:
- INMI (BSL4 lab) (Dr Maria Capobianchi), Roma, Italy
- Robert Koch Institut, (Prof Matthias Niedrig), Berlin, Germany
- RIVM, (Prof Marion Koopmans), Rotterdam, The Nederlands
- ARC- Ondersteypoort Veterinary Institute, (Dr C Sabeta), Pretoria, South Africa
- Chumakov Institute, (Dr A Lukashev), Moscow, Russia
- Ivanovsky Institute,( Prof D Deryabin), Moscow, Russia
- Mechnikov Institute, (Prof V Zverev), Moscow, Russia
- The Biomedical Center, (Prof A Koslov),St Petersburg, Russia
- The Influenza Institute, (Prof M Eropkin), St Petersburg, Russia
- Pasteur Institute, (Prof A Zhebrun), St Petersburg, Russia
- CRI Epidemiology, (Prof G Shipulin), Moscow, Russia
- Laboratory of Pathogenic Microbiology, (Prof G Fu Gao), CAS, Beijing, China
- Dpt of Viral Encephalitis and Arbovirus, (Prof G D Liang), China-CDC Beijing, China
- Institute of Virology (BSL4 lab), (Prof Zhi-Hong Hu), CAS, Wuhan, China
- Hacettepe University, Biology Department, Virology unit (Prof K Ergunay) Ankara, Turkey
- Center for Biodefense & Emerging Infectious Diseases (BSL4 lab), (Prof R Tesh) Galveston, Texas,
3-Procedures for the users access to the collection (WP5)

The complexity of this work-package was relying on two main issues, the first was consisting in the implementation of an efficient interface for the partners to promote the resource included in their collections, and the second issue was to develop a logistical approach to facilitate the access to the resource for the end-users.

A set of Guidelines, and advice, for the partners to assist them to establish the basic control systems that are needed for shipping virus strains and derive products, were defined.

With the use of the EVA platform as the unique access for the users, a supply procedure has been set up which consists in “Ordering Guidelines” which maintains the operating efficiency of the Network.

Additionally, a comprehensive set of guidelines was developed to guide the EVA partners in the transnational access activities. This document highlights some basic definitions and rules (eligibility conditions, selection panel evaluation, and definition of an access) and gathers the documentation and procedure required for sponsored and non-sponsored access. This guidance document described the regulations for transport of infectious substance in terms of biosafety issues, packaging and labelling.

From the end-user point of view, simplification of the access to the information through the portal has been carried out. The search bar has been improved and a notice has been made to all the partners for the clarification of the title of the products. The EVA web site has implemented modalities of access into the web based catalogue so that the users are clearly identified and the scientific projects are defined regarding the products of interest (see WP N5).

Since June 2010 the online access procedure was supplemented by requests for further details regarding the users’ projects and the list of scientific publications in the field of research.

Another aspect of the access provision was related to the biosafety and the biosecurity of the procedure: face to the biosafety risk related to the access to potential pathogenic resources, the documentation required to the users needed to be performed. These review procedure are continuously updated according to experts comments or cases encountered.

A specific access to an hot line has been designed:

An ‘Incident Response Plan’ has been agreed by the consortium (see Deliverable D11.4). The objective of this deliverable was to provide details for directly contacting experts within the EVA consortium in the case of a sanitary crisis (within or outside of the consortium) as a biosecurity web-based ‘hotline service’. All Biological Resource Centres can refer to in order to determine how to report investigations in breaches of security. To facilitate BRC in the event of a biosecurity breach, a web-based ‘hot-line’ service has been established on the EVA website:

http://www.european-virus-archive.com/contact.html
The biosecurity web-based ‘hotline service’ provides a point-of-contact for any institution within the EU that requires guidance on the actions that should be taken for recording, reporting and investigating security breaches in the event of a major biological incident and to ensure the safety of all staff.

Advice can be obtained following any incident:
- For tropical (exotic) viruses and Biosafety level 4 viruses: BNI [guenther@bni.uni-hamburg.de]
- For other human viruses of medical importance: University of Bonn [drosten@virology-bonn.de]
- For veterinary / animal pathogens of economic importance: APHA [virology.office@apha.gsi.gov.uk] - +44 1932 357840

The biosecurity web-based ‘hotline service’ is live on the EVA website on the contact page.

All the users requesting access to the resource will have to submit an end-user qualification form to manipulate the products according to their corresponding ethical/safety issues. Some of the received requests require the decision of the Scientific Advisory Board. They have the responsibility to select the suitability of the end user for his request. For all incoming enquiries requiring a free of charge access to the EVA collection, the eligibility of the request is evaluated through the Selection panel to comply with Article III.3.5 of the Annex III accompanying the Grant Agreement. The use as main criteria for selecting or rejecting Access applications are the criteria stipulated under Article III.3.6 of the Annex III accompanying the Grant Agreement (i.e. first the scientific merit of the application as well as the relevance of the scientific project).

A special interface has been developed to allow a perfect transparency in the evaluation process. Each member of the selection panel has to log in to a dedicated and restricted website area gathering all the free of charge application received. Each enquiry received need to be evaluated to be “closed” and forwarded to the providing partner. An automatic email is sent both to the partner and the end-user to give the status of the evaluation. The non-advisory partners can access the table to see the status of the incoming enquiries; however they have no access to the vote. During the last period the EVA Portal has integrated the Research agreement form into the online request procedure to facilitate the storage of the information and its evaluation by the scientific panel.

The review procedure for free of charge access was facilitated by the development of a vote interface for the Scientific Panel. Each request for free of charge access is reviewed on this new interface, by each member of the scientific panel, taking into account information stored such as the Research agreement, the scientific project, the user identification, the scientific background and several other points.

Last but not least, an attractive website has been conceived to give an easy access to all kind of information related to virology. The number of daily visits can peak up to 250. The secure web facility is centralizing all the communication and management tools related to the EVA consortium and its supply activity. The developed EVA portal was made available online during the first period to any external user. Several product descriptions were added to the catalogue during the current period so that our web based catalogue contained 1464 distinct products at the end of the project. This web based catalogue constitutes a user friendly format for the access to the EVA resource. During the last period the EVA interface has
integrated a tool to sort enquiries of interest. It allows a better management of the enquiries, the stage of the ordering process, and a better discrimination between free of charge enquiries and regular access to the collection.

In order to increase the EVA recognition in the public health landscape and in order to diversify the type of users requiring the EVA products or services, a slant had been put on the promotion activities in various ways: promotional document, meetings with the different targeted market players, attendance to nongovernmental organization or ESFRI meetings, business meeting with pharma, biotech, or foundations. The frequentation of the website is continuously increasing as well as the number of enquiries and end-users.

As a direct consequence, the TNA have been consistently increasing too. Additionally representatives of EVA have already been attending meetings with other consortia, institutions and industries.

Promotional activities to raise EVA’s profile and the efficient thematic collaboration existing between the partners have led the project to be a major contributor to virology biobanking, research and diagnostic applications. The increasing number of products distributed from the EVA portal, more than 2500 during the last four years clearly demonstrates the efficiency of the actions related to this WP

4-Networking and Database development (WP6)

During the first 18 months of the project, the first database structure was completed by:
• 2 new tables representing new categories of products. These new categories were required by consortium members to describe new categories of products in our web based catalogue and are now available from our website under the labels “Diagnostic reagents” and “Other Derived Products”.
• New fields declaration so that the database structure was also adapted to handle subcategories of products: this capability was required by consortium members to help to distinguish different sub-types of products among the diagnostic reagents category (i.e. IVT, Plasmids and Other Control Reagents).
• A structure was created to manage information related to the enquiries passed through the website, allowing our members to follow the progress status.
• A further table to store the vote of our scientific panel regarding the requests for free of charge accesses
• Another structure was added to store the list of scientific publications related to the EVA project.

The EVA database is the core of the bioinformatics aspects of the EVA project and needs permanent cares in terms of maintenance, checking for the reliability of the data entered and also uniformity of the information.

At month 18, it contained 18 sub-collections, for which 196 viruses were declared and related to 158 distinct products among which:
- 127 Viruses
- 14 cDNAs
- 9 Proteins
- 8 Hybridomas
At month 36, it contains 31 sub-collections, for which 408 viruses were declared, and related to 456 distinct products among which:
- 280 Viruses
- 45 cDNAs
- 82 Viral Nucleic Acids & Diagnostic Reagents
- 11 Proteins
- 12 Hybridomas
- 7 Antibodies
- 2 Mice
- 5 Services
- 12 Other Derived Products

At month 60, it contains 53 sub-collections, for which 885 viruses were declared, and related to 1459 distinct products among which:
- 754 Viruses
- 8 Recombinant Viruses
- 356 cDNAs
- 204 Viral Nucleic Acids & Diagnostic Reagents
- 69 Proteins
- 20 Hybridomas
- 14 Antibodies
- 2 Mice
- 6 Services
- 26 Other Derived Products

At month 72, it contains 53 sub-collections, for which 972 viruses were declared, and related to 1485 distinct products among which:
- 771 Viruses
- 8 Recombinant Viruses
- 361 cDNAs
- 207 Viral Nucleic Acids & Diagnostic Reagents
- 69 Proteins
- 20 Hybridomas
- 14 Antibodies
- 2 Mice
- 6 Services
- 27 Other Derived Products

In addition to this work, a physical maintenance of the servers that hosts the database, programs and the website had to be performed: the AFMB laboratory has invested in a new server system that we have installed and configured. The EVA database was then transferred into this new cluster of servers. The advantages of this new system are the higher reliability, a system of high availability and responsiveness of the resource thanks to the software subsystem (VMware Vsphere) that handles redundant resources.
and virtualization.

A tool for online products comparison was developed. It allows the users to easily determine what the differences between selected products are (up to 4 products can be selected). It is possible to add a product to the comparator from several locations of the EVA portal, including the detailed information sheet, the list of products in each category or either from a search result.

Edition interfaces and online product information sheets where adapted to handle and display the new fields stored in the database.

The interface dedicated to the Selection Panel allowing to vote for free of charge accesses was improved by adding search functionalities by columns and by providing the Selection panel with complementary information so that it helps in retrieving former enquiries and is fastening the final decision.

The interface dedicated to EVA partners that lists online enquiries was improved to allow EVA members to update online the enquiries status, turn enquiries into orders, set item prices and shipping fees online. In addition search capabilities similar to the ones developed for the selection panel interface, were also implemented here.

In addition to all of the developments, all programs had to be maintained, corrected for bugs, and a special care was brought in live help of the project members (e.g. via email, presentations, videos...).

5-Tools for virus characterization (WP7&9)

PCR controls library

A preliminary work has consisted in the construction of PCR controls to detect quantitatively high-profile RNA and DNA viruses. This has been completed for the viruses identified as such high-profile targets in the previous report, thereby enabling sustainability of UKB as an EVA functional member by guaranteeing high TNA after the end of the funding period. Controls have also been generated for a number of additional viruses (see list below) and yet more viruses are currently incorporated.

In-vitro RNA transcripts (IVT):

1. Sapovirus
2. Astrovirus
3. Norovirus Genogroup 1
4. Norovirus Genogroup 2
5. Rotavirus
6. Hepatitis A virus
7. Hepatitis C virus X-tail genomic region
8. Hepatitis C virus 5’-ncr genomic region
9. Measles virus
10. Mumps virus

11. Rhinovirus
12. Influenza A virus
13. Influenza B virus
14. Enterovirus
15. Human Parechovirus
16. Human Coronavirus OC43
17. Human Coronavirus 229E
18. Human SARS Coronavirus
19. Respiratory Syncytial virus
20. Human metapneumovirus
21. Parainfluenzavirus 1
22. Parainfluenzavirus 2
23. Parainfluenzavirus 3
24. Parainfluenzavirus 4

25. Human Immunodeficiency virus 5’-LTR region
26. Rift Valley fever virus
27. Yellow fever virus
28. Dengue virus
29. Hantavirus
30. Vesicular stomatitis SV IVT
31. Newcastle disease virus

DNA plasmids:

1. Sapovirus
2. Astrovirus
3. Rotavirus
4. Norovirus genogroup 1
5. Norovirus genogroup 2
6. Human adenovirus

7. Human coronavirus OC43
8. Human coronavirus 229E
9. Influenza A virus
10. Rhinovirus
11. Enterovirus
12. Human parechovirus

13. Yellow fever virus
14. Hantavirus
15. West Nile virus
16. Measles virus
17. Mumps virus

18. Hepatitis A virus
19. Hepatitis B Virus
20. Human Immunodeficiency virus Internal Control Plasmid
21. Human Immunodeficiency virus 5’-LTR
22. Hepatitis C virus X-tail
23. Hepatitis C virus 5’-ncr
24. Hepatitis C virus Internal Control

25. Herpes Simplex virus I (HSV-I)
26. Cytomegalovirus (CMV)
27. Epstein-Barr virus (EBV)
28. Varizella-zoster virus (VZV)
29. Human herpesvirus 6
30. Human herpesvirus 8
31. JC-virus
32. BK-virus
33. Bocavirus 1
34. Parvovirus B19

Wholevirus RNA / DNA

1. Dengue virus 1
2. Dengue virus 2
3. Dengue virus 3
4. Dengue virus 4
5. West Nile virus, African strain
6. West Nile virus, New York strain

For these additional viruses, controls reagents were generated based on the demand at our university hospital diagnostics (= controls which we believe to be interesting for a broad public, thereby generating high access).

Each protocols has been validated before its release

An important aspect of this task is the characterisation of virus quantity of stocks in the strain collections, taking in account the potential demand of viruses.
This task has been shifted towards the generation of materials enabling quantification of the most relevant human viral pathogens in order to guarantee a product of potential high demand by customers.
Almost all viruses submitted by individual EVA partners are submitted the database accompanied by an individual quantification. This applies specifically to more exotic viruses, for which assays are only available in the specialized laboratories submitting the viruses. In agreement with the EVA partners, efforts have therefore shifted towards the generation of controls for the above referenced high profile viruses that are attractive to customers involved in diagnostics and which, furthermore, are frequently dealt with in laboratories worldwide.

Both medical diagnostics and frequent usage of these high profile viruses are likely to generate higher TNA demand and guarantee EVA sustainability at the partner site.

Associated to this production activity have been developed tools and protocols to improve the production of viral genome sequence and their analysis. The concept followed is the set up of sequencing capacity available for all the partners.

Protocols have been established in order to allow reliable identification and characterisation of all strains added to the EVA database thanks to high throughput sequencing. All viral strains available from EVA have been at least partially sequenced. Each partner established and used protocols suitable for a given virus family or genus. Some strains, considered most important, have already been fully sequenced by classic viral genomic, especially relevant strains such as type-species.

The use of high capacity pyrosequencing methods for the fully characterization of virus strains is ongoing in several labs of the consortium. Partner involved in building up sequencing capacities are purchasing specific equipment. Partner 1 has a complete pyrosequencing facility (Ion Torrent, life technologies) and full access to 454 machines is possible for other EVA partners.

Partners aim to provide the complete sequence of a large number of viruses in the EVA collection using next generation sequencing plate-forms. At the moment, one hundred and forty viruses included in EVA collection (Partner 1) have been completely sequenced. The objective is to provide approximately two hundred complete genomic sequences before the end of the project.

Partner 5 has recently produced 60 new virus stocks. These have been through the QC process and have been titred. All strains have been authenticated by partial genomesequencing. It is in the process of developing a whole genomesequencing programme. The aim is to fully sequence all the viruses that are listed in the catalogue over the next 3-5 years.

Partner 2 has substantially increased the number of lyssaviruses successfully sequenced from 3 at the start of the project to 28 to at its end, including representatives from the species RABV, LBV, MOKV, EBLV-1, EBLV-2, BBLV, and a newly discovered lyssavirus IKOV, EVA was acknowledged in the publication.

A sequencing capacity has been set up at Partner 2: the Central Sequencing Unit (CSU) run by a dedicated team of sequencing experts. Equipment consist in both 454 and Illumina machines and the capacity to sequence samples from external sources. In addition there is a bioinformatics department with expertise to utilise available bioinformatics programs and developing bespoke programs for our specific needs. The close relationship existing between the CSU and the scientists has enabled, with support of...
the EVA project, to develop protocols and test the boundaries and limits of NGS technologies with excellent results whilst understanding the importance of the initial sample quality.

As a consequence of this existing infrastructure have been developed protocols to obtain full genome consensus sequence from cultured viruses using PEG precipitation on 5-50ml of tissue culture supernatant (TCSN) and extracting directly from cell pellets. Both methods have reliably resulted in not just consensus sequence, but also good depth of coverage enabling investigation into viral heterogeneity. This has resulted in the publication of these techniques.

Major improvement in the genomic sequencing

For genomic characterization of viruses, a platform of NGS was developed in Partner 1 lab. The Ion Torrent™ semiconductor chip technology onto the Ion PGM Sequencer (Life Technologies) is at the heart of this platform. Ion Torrent™ uses a semiconductor technology based on a well-characterized biochemical process: in nature, when a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a by-product. If a nucleotide, for example a C, is added to a DNA template and is then incorporated into a strand of DNA, a hydrogen ion will be released. The charge from that ion will change the pH of the solution, which can be detected by the ion sensor. The sequencer will transfer data to the base, going directly from chemical information to digital information. The supernatant of virus cultures is directly used for NGS sequencing. A volume of 140 μl of supernatant is incubated during 7 hours in an enzyme, the Benzonase, in order to eliminate cellular DNA and RNA. After treatment, samples are processed for RNA/DNA extraction. Then reverse-transcription PCR using tagged random primers and tag specific PCR are performed, following a specific technical protocol (manuscript in preparation). The PCR products are finally purified and performed on the Ion Torrent™ for sequencing.

This methodology allowed, using a unique experimental protocol to provide complete sequences from EVA isolates such as Dengue virus, Chikungunya virus, Influenza viruses, enteroviruses, adenoviruses etc.. It also allowed to identify and sequence unknown viruses producing CPE in cell culture (e.g. new Paramyxoviridae isolates) and ultimately to identify a new flavivirus directly from an individual phlebotomine.

High capacity pyrosequencing is now used for routine sequencing. New experimental protocols to provide rapidly complete sequences from EVA isolates are being developed for different viruses (Dengue, Influenza, Respiratory syncitial virus, Enteroviruses), based on the use of specific amplification protocols.

Progress on sequencing unknown samples and other virus families

The success has also been achieved when sequencing samples with unknown virus present, or those samples with low viral RNA content. We have successfully obtained the full genome sequence of Louping Ill Virus (LIV) from the spinal cord of a sheep; resulting in many publications.

Full genome sequence of Rift Valley Fever Virus was obtained from 1 ml TCSN during an inter-agency simulation exercise to assess the UK’s ability to detect the introduction of an exotic virus.
Sequencing RNA from RT-PCR positive Hantavirus and WNV samples has identified viral specific sequence, and full genome sequencing is in progress. The low viral load in the samples provided is likely to be a confounding factor but EVA partners are collaborating to optimize techniques.

Reverse genetic for safe RNA virus shipment

The development of molecular methods allowing producing infectious viruses from DNA copies of their genomes has significantly improved our knowledge of RNA virus life cycle and pathogenesis, by allowing the development of "reverse genetics" studies. Viruses could now be obtained from the complete genome sequence.

Partner 1 is developing very simple reverse genetic method that allowed us to produce infectious RNA viruses. This procedure allowed the rescue of infectious RNA viruses from genomic DNA material with the transfection of overlapping DNA fragments.

This technique has the potential to allow in the future the design of large reverse genetics experiments for RNA viruses. Because DNA fragments can be obtained by PCR, error-prone PCR may be also be used to create artificial viral heterogeneity, e.g. for facilitating the selection of adapted viruses under various experimental selection conditions and, conversely, high-fidelity polymerases and clonal amplification templates may be used to control the degree of clonality of the viruses produced.

Finally, this technique may revolutionise in the next future the exchange of RNA viruses between scientific institutions, by allowing the separate shipment at room temperature of simple, non-infectious, DNA genomic fragments that, combined and transfected by the recipient would allow to recover the infectious viral strain.

6- Animals models (WP10)

The screening of drugs having antiviral properties the validation of diagnostic protocols targeting specifically a virus family, are the main rational for development of animal model in the EVA project. The combination of unique resource like recent viruses collections, the know how related to these viruses, the available facilities to manipulate them provide the basis for an animal model collection.

Partner 2 has refined and validated new inbred mouse models for rabies virus.

Partner 3 has developed animal models for Crimean Congo hemorrhagic fever (CCHF) as well as for Ebola virus, and has exploited these models and validated them by identifying new antiviral drugs to combat the respective diseases (manuscript in revision).

Partner 3 and Partner 6 jointly have developed a humanized mouse model for Lassa fever, and have delineated governing principles of the disease’s pathogenesis (Flatz et al. PLoSPathog. 2010).

Partner 6 further has standardized animal models for lymphocytic choriomeningitis virus, vaccinia virus,
vesicular stomatitis virus, murine gammaherpesvirus 68 and mouse cytomegalovirus infections, and has demonstrated the utility of the novel and refined methodology for investigating basic mechanisms of viral persistence and immunosuppression (Bergthaler et al. Proc. Natl. Acad. Sci. 2010), and for the discovery of fundamental principles in antiviral immune defence (Bonilla et al. Science 2012), respectively. A total of twenty-four protocols for exploiting these various animal infection models have been published on the EVA website. They comprise the detailed methodology how to produce the respective viral stocks, to inoculate animals, to process samples for determination of infectivity in organs as well as for measuring protective antibody and T cell responses in a reliable and standardized manner. In summary, the work conducted in this WP provides a new and refined basis for investigating viral disease and immune defence under reproducible, standardized and relevant in vivo conditions.

Preliminary standard protocols based on data mining and amended upon completion of our proof-of-principle experiments are in the publication process to upload on the EVA database. Thus, they are made available to the scientific community and to industry on the EVA website, except for contents awaiting peer-reviewed publication.

7- Virus production and characterization P4 (WP13)

Emergence of high risk pathogen becomes a major concern for the international Health agencies. A priority has been given to the completion of a consistent database related to those virus in the EVA catalogue.

In 2014, in Western Africa a new emerging Ebola virus called Zaire Ebola virus variant Makona was isolated, characterized and added to the EVA Catalogue. By the end of 2014, approximately 90% of the RG4 virus part of the BNI collection are accessible via the EVA portal (see below). All viruses were grown in cell culture in the BSL4 facility and characterised after sequencing. They fulfil the criteria for dissemination via the EVA website. From all of them genomic RNA can be provided on request.

• 29 Liberian Lassa virus strains (primary patient isolates)
• 4 Guinean Lassa virus strains (primary rodent isolates)
• 13 Nigerian Lassa virus strains (primary patient isolates)
• 1 Ivory Coast Lassa virus (primary patient isolate)
• 2 Sierra-Leone Lassa virus strains (classicallyaboratory strain “Josiah”, and primary patient isolate)
• Guanarito virus (classical lab strain)
• Junin virus (classical lab strain)
• Sabia virus (classical lab strain)
• Machupo virus (classical lab strain)
• Marburg virus Leiden (primary patient isolate)
• Marburg virus Musoke (classical lab strain)
• Marburg virus Popp (classical lab strain)
• Crimean-Congo hemorrhagic fever virus (CCHFV) Afg09-2990 (primary patient isolate)
• Sudanebolavirus Gulu
• Zaire ebolavirus Mayinga (classical lab strain)
• Zaire ebolavirus Gabon 2003 (primary patient isolate)
• 3 Zaire ebolavirus Makona 2014 (primary patient isolate from Guinea)

Conclusion

Recognized virus collection and provider of high quality products and know how

It took four years for this consortium to be viewed and accepted as an important provider of high quality resources. During the past 4 years, EVA has distributed more than 2500 accesses (products) worldwide to both industrial customers and to the scientific community at large. Those deliveries concern very high quality products which are not available anywhere else. They exceed the initial objective of this project.

Every day the EVA website is visited by more than 200 different potential customers, for different purposes such as collecting scientific and educational information, obtaining updates on catalogue content, placing enquiries for different supplies...

EVA is a key player for the preparedness and control emerging viral diseases

a-MERS-CoV outbreak in Saudi Arabia:

Two cases of rapidly progressive acute respiratory infection in adults associated with a novel coronavirus have generated an international public health response. The two infections were acquired three months apart, probably in Saudi Arabia and Qatar. An interim case definition has been elaborated and was published on the World Health Organization website on 25 September 2012. In a collaborative activity coordinated by major European and national epidemic response networks UKB has developed diagnostic real-time reverse-transcription polymerase chain reaction (RT-PCR) assays suitable for qualitative and quantitative detection of the new agent. In December 2012, WHO, in its interim guidelines entitled “Laboratory testing for novel coronavirus”, recommended PCR assays as the method to detect the virus in blood sample. The European Virus Archive was mandated for the delivery of the assay reagents (positive controls): up to date more than 300 kits distributed worldwide

During 2013, EVA has been providing worldwide more than 200 PCR reagents kits for the diagnostic of the MERS-CoV infection, under the WHO umbrella. We has been the only one to be able to do it because we had a logistical platform fully operational, and we had the scientists knowing the virus and being able to design the best probes to allow an high level of sensitivity and of selectivity of the assay.

Following this first experience we have been approached by industrial companies of the diagnostic field to discuss their participation to our activity.

b-Ebola outbreak in Western Africa

One of the main contributions of specialised EVA project partners is associated with the deployment of mobile laboratory units in African countries where the virus emerged. EVA Partners or Associated partners
have been sending teams of scientists and mobile laboratories to support local authorities in the diagnosis of suspect cases. The project "Establishment of Mobile Laboratories for Pathogens up to Risk Group 4 in Combination with CBRN Capacity Building in Sub-Saharan Africa" is funded by the European Commission and builds on the capacity to bring state-of-the-art technology, diagnostics and highly trained scientists from Europe and Sub-Saharan African countries to the field in the context of the Ebola disease outbreak:

Bernhard-Nocht-Institute (Prof S. Gunther) is the coordinator of this consortium.

The EMlab deployment is organized in Guinea, Nigeria and more recently in Liberia: the European Mobile Laboratory Consortium sent teams to Guéckédou, Guinea, that have been performing diagnostics for Ebola Virus Disease since the start of the outbreak. The teams present on site are regularly replaced by new teams. This continuous turn-over of scientists guarantees efficient operation of the facilities which continuously provide fast molecular diagnostics for patients being admitted to treatment centres in the region.

Colleagues from the Univerza v Ljubljani from Slovenia, the Robert-Koch-Institute from Germany and the Heinrich-Pette-Institute from Germany, and L. Spallanzani national Institute for Infectious Diseases (INMI) from Italy have sent scientists to the different locations. A very significant contribution of Public-Health-England from UK has generated an important number of data from local biobank which will supply essential information on the virus evolution/adaptation, immune correlates of protection and biomarkers of disease outcome. The project is continuing its support in the affected regions, the country of Guinea, Médecins Sans Frontières, and the WHO in a major effort to help mitigate the current crisis caused by the Ebola Virus epidemic.

A team from BNI has joined a Nigerian unit to ensure that the European Mobile Laboratory Project succeeds in the deployment of the lab. The unit is investigating Ebola suspected patient samples in Enugu and Port Harcourt in Nigeria.

Following the request by the WHO, the EMLab Project urgently deployed a third mobile laboratory unit to Liberia. Liberia is one of the countries in West Africa that has most severely been hit by the Ebola Virus outbreak. Health care workers are faced with the enormous challenge of caring for a high number of patients presenting with symptoms of Ebola Virus Disease. Patients need to be diagnosed rapidly.

Other EVA partners are associated with this project: Laboratoire P4 Inserm Jean Merieux, Lyon, France and the Universitaetsklinikum Bonn, Bonn, Germany

A similar initiative is coordinated by the Centre for emerging and Zoonotic Disease of the National Institute for Communicable Diseases (NICD), Johannesburg, an associated partner of the EVA consortium. The deployment of mobile laboratories (MLU) is organized in Freetown-Lakka in Sierra Leone under the WHO/GOARN umbrella. A team of highly trained scientists from NICD led by Prof. J Paweska is in the country since mid of August to conduct on-site field qPCR diagnostic of the infection in suspected patients.

EVA partners are also contributing to this capacity building in different fields and different countries where the disease emerged: University of Texas Medical Branch – Centre biodefense & Emerging Infectious
Senior scientist has been sent on-site field in Sierra Leone to manage a team with the objectives to identify and to isolate infected Ebola patients and associated contacts. Several other investigators at UTMB have projects testing vaccine candidates and anti-Ebola agents in vitro and in non-human primates in our BSL-4 facility.

China-CDC: Staffs of the Department of Viral Encephalitis and Arbovirus, National Institute for Viral Disease Control and Prevention, China CDC are sent to Sierra Leone since September 2014 to perform diagnostic tests (qPCR) on samples collected from local Ebola patients. Others teams are prepared to replace this first group during November.

These activities are generating a mass of information and many clinical isolates from which EBOLA virus strains have been extracted, purified and authenticated. The access to most of those products is given to the Scientific Community at large, through the EVA website.

For instance, Human serum samples from blood donors in the Republic of the Congo have been screened at Unité des Virus Emergents in Marseille for Ebola virus seropositivity and the results have been analysed in the context of a questionnaire relating to human exposure to birds and bats (manuscript submitted for publication).

From its website, EVA is distributing PCR reagents to be used for the identification of the virus in different media, or for the diagnostic of the disease. So far more than 50 products have been shipped by BNI to more than 12 different countries in Europe, South East Asia and Middle East.

EVA is an active component of the European-CDC/ENIVD.

Because of our updated virus collection, we can provide current clinical isolates to ENIVD to carry out diagnostic EQA in Europe and also outside Europe.

This activity is KEY to guarantee that the test results obtained by the laboratories in hospital are valid. The most recent demand concerned the supply of an inactivated Ebola strain (a high risk pathogen classified as a BSL4 special pathogen). Our partner BNI from Hamburg (BSL4 lab) has been organizing the supply of this strain.

BNI alone will not be able to provide this resource as the logistical organization put in place at the consortium level is needed to satisfy this demand and others that will come later.

Future for the European Virus Archive: European Virus Archive goes Global

The results generated during the EVA project have revealed that a virtual infrastructure collecting data of existing resource located at the consortium partner places is achievable. This proof of concept will be used to created an international organization called EVAg.

The major EVAg project objective is to meet the needs of scientists, worldwide, by generating a carefully authenticated human/animal virus collection that is larger than any existing repository, and readily available to all laboratories that meet approved ethical, safety and security standards.
By extensive exploitation of the web and incorporation of appropriate teaching/training courses, EVAg will provide a valuable resource for all human and animal health-related disciplines in academia, fundamental and applied research, the education sector, public and environmental health. To match these ambitious project objectives a carefully chosen core group of high calibre International laboratories has been selected. These consortium members have years of experience of working together on a wide variety of EU projects.

Consequently they will integrate their unique and overlapping skills and knowledge to produce a balanced consortium of resource holders. Conjointly, they hold wide ranging collections of different animal viruses and reagents with the knowledge (in some cases unique to the specific laboratory), experience and commitment to continue to extend their individual and distinct collections. These viruses range from non-pathogenic through the entire spectrum to the highest risk pathogens. The staffs in these laboratories also have experience of developing validated experimental protocols.

Thus, the EVAg consortium which will be led by the successful management team from EVA, has a high level of complementarity that embraces all the requirements of EVAg. Furthermore, by integrating their infrastructures, through EVAg, these individual laboratories will enhance their capacity to achieve their objectives.

During the EVA project, each laboratory has built its reputation on its ability to isolate, identify, catalogue, preserve and to characterise the viruses that will constitute the collection. They are therefore ideally suited and committed to the tasks that will be assigned to them.

One of the key features of this project will be the implementation of platforms dedicated to providing access to the EVAg resource, including virus discovery techniques, sequencing and derived material production for diagnostic applications.

In addition, EVAg was conceived to operate as an open entity by linking with other specialist laboratories that hold virus collections both within and outside Europe. This was initially achieved by involving additional experts with the previous EVA consortium either through their role as members of the Scientific Advisory Board or in some cases, by inclusion in EVA as associate partners. Many of these experts have been invited to become full partners of the EVAg project, thus using their expertise to provide invaluable advice to the successful operation of EVAg and secondly, to ensure its continued expansion and increasing importance globally. Whilst it is not planned to develop a specific commercial involvement in EVAg, many of the viruses and reagents will be of value to industrial/commercial companies involved in the preparation of diagnostics, drugs and vaccines. The level, to which such associations with these activities will evolve, will be discussed during appropriately convened committee meetings.

An international Virus Archive

The EVAg partner list includes 26 partners among them 10 laboratories are from the non-EU member states. The presence of the non-EU countries partners is an essential component for the success of this project. EVAg is conceived as an international virus collection collecting viruses all over the World with the
objective to reflect the infinite biodiversity of the material but also to support effectively the Response to any emerging viral disease. The main criteria to choose which institution to integrate in the consortium are first, its virus collection content and second, its location. For example, the Center for Biodefense and Emerging Infectious Diseases, Galveston, USA maintain wide collections of arbovirus and other virus pathogens (including Biosafety level 4 agents). Many other assorted viruses of medical and veterinary importance (coronaviruses, herpesviruses, retroviruses, adenoviruses, enteroviruses, etc) are held in laboratories around the world. Laboratories in Moscow, or Saint Petersburg, Russia, in Beijing or Wuhan in China, India, south East Asia, Australia, Japan, hold their own collections many of which, to date, have been relatively inaccessible to Western laboratories. The EVAg concept corresponds to an attempt to create bridges between those collections and to share the resource with the objective to both improve their quality and to guaranty a sustainable access to them. Large countries are well represented but also countries having a specific location corresponding to a unique source of material.

In addition to the full partner list there is a group of laboratories ready to share their resource and their know-how on the basis of an association with the consortium. Currently, this second list includes 20 partners in Russia, Greece, Turkey, Jordan Sweden and partners associated to Foundation networks:
- GABRIEL network (5 institutes from Asia, South America, Africa)
- Pasteur Institutes network (5 institutes from south East Asia, Africa)

Potential Impact:

POTENTIAL IMPACT AND MAIN DISSEMINATION ACTIVITIES

The objectives of the European Virus Archive project (EVA) are to develop a readily accessible European virus reference library. Since it would create insurmountable problems to develop such a collection in a single laboratory, EVA will utilise the expertise and facilities of recognised centres of excellence in virology both within and outside Europe. The EVA laboratory network represents an extensive range of virological disciplines currently holding approximately 50% of the 500 recognised human pathogens within the collection. The ultimate aim of EVA is to coordinate these collections and to extend its network to other European and non-European collection to produce the largest library of available quality controlled viruses in the world. Formally the project ended in December 2012, but because of the activity of the consortium, amendments have been submitted to the EC to extend the project duration.

The two project extensions which have been obtained from the EC have as ultimate goal to maintain the service provided by the infrastructure to the scientific community but also to prepare a future for the consortium.

Main achievements

Quality Management: An EVA project quality manager was appointed commencing 2009. A quality policy was drafted and ratified by the EVA scientific committee together with key principles and objectives for the project. Best practice guidelines were drafted, ratified and issued during the first quarter. This became the quality management model for the consortium. A self-assessment survey was circulated to member laboratories. It was completed and analysed to coordinate the operational quality systems and the best practice guidelines.
By July 2010, each partner had been audited and a personal set of SMART objectives agreed for each group. This enhanced the speed of progress in improving the quality of EVA products through sharing of best practice methods across the consortium.

A new audit has been carried during 2013 to finalize the deliverable related to this topic. Clearly the progresses made by each partner have been impressive both at the level of the practices but also at the level of the product quality. Today EVA can supply a Bio-ressource recognized by the end-users as unique. The figures related to the number of accesses provided so far clearly demonstrate that the project has been beyond its initial objective on the matter.

EVA Website: Priority was given to the developments that will constitute the basic structure for further developments. The website is the main tool for advertising the project, and also serves as an access to the different EVA database interfaces.

This work resulted in an operational database containing 18 sub-collections, for which 196 viruses were declared and related to 158 distinct products.

Access to the restricted area (dealing with distinct “disclosure levels’) was opened to "Associated Members", ie scientists included indirectly in the project thus extending the EVA collection via worldwide collaboration. The Website is a efficient feature used by the partners but also by visitors. The number of visit may peak to more that 200 per days. It contributes to give a large visibility to the consortium and to the EU FP7.

EVA Catalogue, access procedure and logistical platform: The web-based catalogue developed through the first stage of the project provides a unique entry point to EVA resources. This catalogue is functional, user-friendly and the number of products in the database increases continuously.

Faced with biosafety issues related to access to pathogens, and the need for high quality safety procedures, EVA has developed Guidelines for the partners to implement control procedures needed for a safe shipping of viruses and derivative products.

The delivery of viruses is being promoted through fliers, posters, visiting cards, and a logo, but also meetings with targeted markets (pharmaceutical industries, biotechnology/diagnostic companies, research institutes).

A new activity has emerged linked to viral diseases outbreaks in the World.

During the MERS-CoV crisis in the Saudi Arabia the EVA logistical platform based on this Web-based catalogue has demonstrated its efficiency. Under the WHO umbrella more than 300 PCR products for the diagnostic of the infection has been sent to more than 60 countries worldwide, in less than four months, making of this organization a unique feature for the preparedness and control against viral emerging diseases.

Since the start of the Ebola epidemic in Western Africa, EVA partners and associated partners have contributed to the actions aiming at controlling the disease spreading. One of the main contributions of specialised EVA project partners is associated with the deployment of mobile laboratory units in African countries where the virus emerged. EVA Partners or Associated partners are sending teams of scientists and mobile laboratories to support local authorities in the diagnosis of suspect cases. They are also contributing to this capacity building in different fields and different countries where the disease emerged.

As a side effect of this intense activity can be noticed: firstly, the generation of a mass of information and many clinical isolates from which EBOLA virus strains have been extracted, purified and authenticated. Secondly, the access to most of those products which is provided to the Scientific Community at large.
through the EVA website as well as the distribution of PCR reagents for the diagnostic of the disease. From its website, EVA is distributing PCR reagents to be used for the identification of the virus in different media, or for the diagnostic of the disease. So far more than 50 products have been shipped to more than 12 different countries in Europe, South East Asia and Middle East.

**Network extension:** EVA is conceived ultimately to be an open entity aiming at developing synergies and complementarity capabilities by providing improved access to researchers. Following successful initiation of the project, EVA has exploited its knowledge and scientific networks to attract other international laboratories with relevant virus collections. EVA has expanded its collection to encompass globally representative laboratories. Conventions are already signed with 12 institutes (including South Africa, China, USA, Australia, Turkey and Russia). In 2011, potential new collaborations are being pursued in Europe, MoU has been signed with 2 new associated partners (Italy and Germany). Today about 30 institutes are collaborating with EVA. This extension will proceed through the collaboration with existing networks active in "South Countries. Pasteur Institute in France together with its non-metropolitan network present in Africa and South East Asia has joined the consortium. Discussions with the GABRIEL network active in South America, in central Africa and in Asia are on going to prepare a collaboration agreement. EVA is becoming one of the Virus collection organisation worldwide.

EVA links up with other network-based virus programmes. In 2012, EVA has integrated the WHO/GOARN organisation and is member of the EDPLN network for the control of viral disease in developing countries. A associated member of EVA is coordinating ENIVD, part of the E-CDC in charge of the validation of the diagnostic methods used in European hospitals.

Due to the increasing number of veterinary institutions links have been established with OIE.

**Association with related ESFRI:** During the ESFRI meeting in Barcelona (March 2010) the coordinator of the European Research Infrastructure for High Security Laboratories (ERINHA) proposed an association with EVA to provide access to highly pathogenic BSL4 viruses and derived viral products. The presence of nine BSL4 laboratories in the EVA consortium will facilitate its association with ERINHA, the objective being to development researches and services opened to both academic and industrial end-users.

In June 2010, EVA presented its organization during a meeting organized by BBMRI-ERIC which indicated its strong interest to integrate EVA as a service unit dedicated to pathogens. The integration process of EVA both in BBMRI and in ERINHA will take time but is very satisfactorily progressing. In november 2014 a MoU has been signed between BBMRI and EVA to specify fields of interest and means to be implemented to proceed to the association of both entities.

EVA has made outstanding progress during its first six years of existence, A clear proof of concept has been established that an effective European organization can substitute the lack of high quality resource available for the scientific community at large. The concept of a single, centralized web-based catalogue that links laboratories from all over the world is simple but robust. The most common difficulty encountered in maintaining a service to the scientific community is to generate a continuous funding source. By spreading itself so widely and using well established laboratories, with well-organized infrastructures, that in many cases provide backup for other partner laboratories, EVA should generate long-term stability and sustainability.

Within six years, EVA has developed from a consortium of 9 European partners to encompass 30 partners in Europe, Africa, Russia, China, USA, Australia associated around the same concept and the same
objective. EVA will continue to mature and exist as a globally networked, non-profit making, quality controlled virus archive for the benefit of Science.

DESCRIPTION OF THE EXPECTED FINAL RESULTS AND THEIR POTENTIAL IMPACTS AND USE

The current EVA infrastructure provides:

- A wide ranging and efficient access to virus collections held in laboratories worldwide.
- The potential for access to recently isolated viruses from clinical, veterinary and field samples.
- A continually expanding collection of viruses as the number of contributing laboratories increases.
- Quality control through standardized protocols for all virus production, assessment, storage methods and distribution procedures.
- Potential access to EVA isolates of specific interest following analysis of sequence databases through relevant web-links; to be established via the EVA catalogue.
- Service to science, the environmental and public health authorities, the needs of the pharmaceutical industry in developing new technologies for disease control, and providing material for diagnosis, teaching and training purposes. Improved access to an expanding network of globally representative virological laboratories.
- Access to the combined experience and knowledge of high calibre associated research laboratories members of the EVA consortium
- A concept that will enable laboratories in developing countries to contribute to the expanding pool of quality-controlled viruses and reagents.
- The capacity for contributors – including those from developing countries - to accommodate local ethical priorities through retention of ownership of their viruses and reagents.
- The opportunity for closer interactions between scientists from many related disciplines.

List of Websites:
http://www.european-virus-archive.com/

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