



EUROPEAN
COMMISSION

Community Research

EU-FUNDED RESEARCH PROJECT

New and emerging technologies: Improved laboratory and on-site detection of OIE List A viruses in animals and animal products (Lab-on-Site)

Time of action: LAB-ON-SITE started in November 2004 and is scheduled to end in January 2008

EU budget (funding): €1.5 million

Abstract

Without reliable molecular methods it is impossible or extremely difficult to identify viral strains and to determine their genetic variability. Novel, powerful molecular methods are required, in order to track the spread of diseases and to implement effective control measures. Accurate diagnosis is the first, and arguably most important, step in controlling epidemic diseases in animals.

But the value of even the best diagnostic techniques is limited unless results can be obtained quickly. Highly pathogenic viruses can spread through entire flocks in a matter of days, so it is essential that diagnoses are made early, in time for preventative and protective strategies to be put in place.

The LAB-ON-SITE project combines the expertise of three universities, five veterinary research institutes and a Swedish biotechnology company. Together these partners will develop, test and distribute a range of new, improved diagnostic systems for virus detection. These will include on-site systems that will allow breeders, government agents and other people on the 'front line' to obtain immediate results. The group will also develop laboratory-based procedures that can be used to confirm the quick-and-easy pen-side approaches. The partners are focusing on nine diseases including foot-and-mouth disease, classical swine fever, highly pathogenic avian influenza and swine influenza.

For avian influenza the diagnostic tests will make use of several basic technologies. PCR assays can amplify specific viral sequences to detectable levels in just a few hours. The idea is then to build portable PCR devices that can be used for on-site testing. The project will also look at other novel nucleic-acid detection technologies, some developed in prior EU projects, that can detect viral sequences without the need for the repetitive heating and cooling of samples that is necessary with PCR.

ELISA assays, meanwhile, use antibodies to detect specific viral proteins and will be used in laboratories for the universal detection of influenza virus and for the specific identification of H5 and H7 subtypes. The project is also developing a dipstick technology for rapid pen-side detection of viruses.

The work of LAB-ON-SITE complements the investigations of the extended AVIFLU project and it is hoped that the two will coordinate their work together. The combination of improved and standardized diagnostics and AVIFLU's insights into avian influenza transmission should strengthen both Europe's position in animal welfare in general, and its protection from the immediate risk of avian influenza in particular.

Status (January 2006)

The work program is focused on new and emerging technologies specifically the development, validation and dissemination of robust, specific and sensitive diagnostic tests for nine OIE List A diseases.

Establishment of a sample collection: A virtual sample bank has been established containing 283 samples of viruses used in the project. This database will evolve and expand during the lifetime of the project to record details of material that is available to the partners for the validation of the assays generated by the other LOS work packages.

Application of rapid, simplified PCR techniques: Genomic sequences of Highly pathogen avian influenza virus (HPAIV, M-gene), Newcastle disease virus (NDV, F-gene), African horse sickness virus (AHSV, VP7-gene) and Bluetongue virus (BTV, NS1-gene) were collected from the Genbank. Alignments were performed and primers/probes for rapid detection assays (real-time PCR, capillary-based PCR) were selected and tested on virus isolates and clinically related pathogens. The work on setting the conditions for AHSV real-time PCR is ongoing.

Application of nucleic acid detection techniques without thermocycling: Two assays are used to fulfill the task: *Invader* and *NASBA*. An ASFV *Invader* assay has been partly evaluated on clinical material from pigs infected with the ASFV strain E75. Four CSFV specific *Invader* assays have been designed against the Ns5b and 3'noncoding regions, and manufactured by *Third Wave Technologies*. Optimisation of the assays is ongoing. Oligonucleotides for the *NASBA* detection assays against HPAIV and NDV were selected by using the Beacon Designer and Lasergene softwares. The best candidates have been purchased. The primers and probes have been designed for the subsequent *NASBA* assays against HPAIV and NDV.

Development of novel nucleic acids detection methods, including padlock probes and rapid readout formats: The cornerstone of this approach lies in the ability of padlock probes in conjunction with tag microarray to simultaneously detect hundreds of viruses. A preliminary set of padlock probes for the detection of Foot-and-mouth disease virus (FMDV), Vesicular stomatitis virus (VSV) and Swine vesicular disease virus (SVDV) has been designed and synthesized. A protocol has been established for ligation and amplification of the padlock probes. All padlock probes can be amplified using the same PCR primer pair, and each is equipped with a unique tag-sequence that allows the amplification products to be sorted on an oligonucleotide array. A detected signal (from a fluorescently labelled PCR primer) will reveal what (if any) virus was present in the sample. The method has capacity even for the determination of various serotypes of the viruses.

Detection of OIE List A viruses with real-time PCR assays, including portable PCR and automated procedures: Primers and probes for the detection of HPAIV, ASFV, CSFV, NDV, SVDV, and FMDV using real-time PCR assays are designed. Among the systems used are PriProET (Primer-Probe Energy Transfer) assay, MGB (Minor Groove Binding) probes and LUX (Light Upon extension) primers. By development of one-step PriProET RT-PCR assays for CSFV and FMDV the assay set-up is markedly simplified, which makes such assays more suited for automation and also for adaptation to portable PCR machines. At the same time two-step SVDV PriProET real-time was developed, and optimised. The manuscript was recently submitted for publication to the Archives of Virology.

Light Upon Extension (LUX) quantitative real-time PCR assays were developed to detect HPAIV and NDV. Several primers and two dyes (JOE and FAM) were tested in various combinations and a reaction mixture was established allowing effective virus detection. The assays proved to be specific that can be conveniently confirmed by melting point analysis upon amplification. An MGB probe assay was designed for AFSV as an alternative to the LUX primers. This assay is fully optimised and only requires further testing on alternative strains and specificity testing. An FMDV MGB assay has also been designed. This assay works well on DNA target and is currently being optimised for RNA. The design process for assays to detect CSFV is well underway.

The ability of two automated robots (BR9604, Qiagen and MagNA PURE LC, Roche) to extract

nucleic acids from different biological matrices were compared. A Qiagen Biorobot 3000 for automated extraction of nucleic acids from various biological materials was installed and calibrated as well.

Development and optimisation of simple and sensitive monoclonal antibody-based antigen-antibody assays: Sandwich ELISA using MAbs as coating and conjugated antibody detection was developed. MAbs combinations for the specific typing of six out of seven FMDV serotypes were evaluated. Competitive ELISAs for the specific detection of antibodies to four FMDV types (O, A, C and Asia 1) was developed and evaluated as well.

Development of rapid methods for the on-site detection of viral antigens (Dip-stick tests): Selection of MAbs and preparation of dip-stick devices was performed on FMDV and VSV Indiana. The best MAb candidates were selected as they react with all or most of the different FMDV strains in capture ELISA. Dip-stick devices have been put together and the best combinations of MAbs have been chosen. The work is continued using three promising MAbs, which are going to be tested on FMDV field samples. The work is ongoing with AHSV, BTV, Classical swine fever virus (CSFV), Swine influenza virus (SIV), NDV, HPAIV and VSV New Jersey.

Transfer of knowledge to EU member and candidate countries, as well as to the third countries. The involvement of nine partner laboratories, one third party and two subcontractors, representing diagnostic institutes, universities, one SME and international organizations of the United Nations (IAEA, FAO) provide guarantee that the gained knowledge will be directly applied in routine diagnostic laboratories, in undergraduate and postgraduate teaching, in industries, as well as in national and in international networks of biotechnology and health sciences. Contact with Animal Health Staff in different countries of Central and South America - Costa Rica, Panama, Belize, Nicaragua and Argentina - as well as with OIRSA organization (Organismo Internacional Regional de Sanidad Agropecuaria) present in all Central America countries, Africa (Morocco and Algeria), Asia (China) and Middle East (Iran) was established. All these countries will be included in LAB-ON-SITE information transfer network.

Project coordinator

Prof. Sándor Belák
Department of Virology
The National Veterinary Institute (SVA)
Ulls väg 2B
SE-751 89 Uppsala

List of partners (listed countrywise). Coordinator will only give out names and other contacts upon request

SE – National Veterinary Institute, Uppsala
UK – Queen's University of Belfast, Virology Laboratory, Northern Ireland
DK – Danish Institute for Food and Veterinary Research, Lindholm
ES – Universidad Complutense de Madrid, Facultad de Veterinaria, Madrid
HU – Veterinary Institute of Debrecen, Debrecen
SE – SVANOVA Biotech AB, Uppsala
IT – Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia
BE – Ghent University, Merelbeke
UK – Institute for Animal Health, Pirbright

Website

www.labonsite.com