

**Project Title:**

Providing milk supply chain with a rapid, portable and cost effective biosensor for multi-pathogen detection in milk

**Project Acronym:**

PATHOMILK

**Contract Number:**

COLL-CT-2006-30392

**Subject:**

**Publishable Final Activity Report**

**Prepared by:**

CRIC

**Dissemination Level:**

Confidential

**Project Coordinator:**

CRIC

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Final	December 2009	1M-39M	1/09/2006	39 months

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## 1. PROJECT EXECUTION

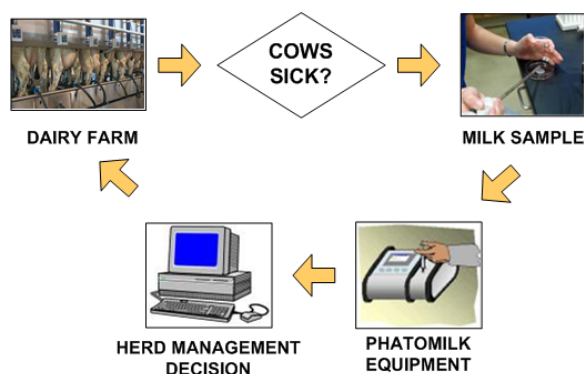
### PROJECT OBJECTIVES

Our goal is to improve the competitiveness of the European dairy sector through the reduction of losses associated to herd health problems. Animal diseases cause losses to producers in terms of reduced output, increased replacement costs, increased veterinary costs and increased labour requirements. They also affect milk quality and, in certain cases, might pose health risk for the consumers.

This goal will be attained through:

- the development of a new veterinary surveillance system
- the dissemination of its use among the European dairy farmers
- the establishment of a frame for the future marketing of the diagnostic test

Enabling simple, affordable on-farm diagnosis of pathogen presence would empower dairy farmers to reduce economic losses resulting from delayed disease detection. Moreover, these controls will help to keep disease outbreaks under control by early detection of their origin, thus safeguarding the health and welfare of the European herd. Farmers could produce more and better milk and certify quality, improving thus their competitiveness.



Functional diagram of the Pathomilk system

Most diagnostic techniques only detect one pathogen and many rely on stressing blood sampling. Moreover, the farmer may have to wait for several days, even weeks, before he gets the results of the tests. The objective of this project is to develop a rapid multi-pathogen analyser for detecting the most common pathogens in milk, avoiding thus blood sampling. Fast pathogen detection from milk samples will be possible using an innovative biosensor based on a DNA/RNA-hybridisation method and using Surface Plasmon Resonance as detection technique. Moreover, the system will be designed in an open way to enable easy expansion of the number of detectable pathogens.

## CO-ORDINATOR CONTACT DETAILS

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## CONTRACTORS INVOLVED IN THE PROJECT

Type	Nº	Name	Short name	Country
RTD	1	Centre de Recerca i Investigació de Catalunya	CRIC	ES
IAG	2	Associazione Italiana Coltivatori	AIC	IT
IAG	3	Leicar Associação Produtores Leite e Carne	LEICAR	PT
IAG	4	Lleters de Catalunya	LLET	ES
IAG	5	Royal Association of British Dairy Farmers	RABDF	UK
SME	6*	Biosurfit	BIOSURFIT	PT
SME	7*	Embio Diagnostics	EMBIO	CY
SME	8	Vitaltech ibérica S.L.	VITALTECH	ES
SME	9	Applied Research using OMIC Sciences S.L.	AROMICS	ES
SME	10	Výskumný ústav mliekarenský	VUM	SK
SME	11	MILCOM a.s.	MILCOM	CZ
SME	12	National Milk Records plc	NMR	UK
SME	13	Sociedade Agropecuária Vilamorim LDA	VILAMOR	PT
SME	14	Torre Sant'Angelo	TORRE	IT
SME	15	Figli Bigaran	BIGA	IT
SME	16	Cooperativa dos Produtores de Leite CRL	LECOOP	PT
SME	17*	Mas Pijoan	SOLIUS	ES
SME	18	Industrias del CADI	CADI	ES
SME	19	KE & LRC Edwards	KELR	UK
RTD	20	Feltalálói és Kutató Központ Kft	FKK	HU
RTD	21	Institut de Recerca de Tecnologies Alimentaries	IRTA	ES
RTD	22	Institute of Photonics and Electronics	IPE	CZ

## WORK PERFORMED AND RESULTS ACHIEVED

### First Period

During first steps of the project, a market survey was carried out. The purpose of the present market survey was to analyse the farmers' point of view of this tool. More specifically, the goal was to ascertain (i) the farmer's needs in terms of detection of the most important diseases and (ii) the farmer's perception on the usefulness and (iii) price sensibility of the new diagnostic tool. A questionnaire was developed and translated into three languages, i.e. Catalanian, Italian and Portuguese. The questionnaire was sent to the different associations for its dissemination through phone interviews among their members. Another questionnaire covering very similar aspects was also developed for the British market and sent to the British association. Although both questionnaires covered the similar issues of veterinary surveillance in dairy farms, the UK questionnaire was designed taking into account the specificities of the British market. 356 phone and personal interviews with members of partner IAGs were carried out. From that analysis it was concluded that Mastitis appeared as the most important disease by far in all the surveyed countries. If the project focuses in mastitis, the development of the project should take into account that low detection limits are required to detect subclinical mastitis. Moreover, the technology should be able to detect species from both environmental and infectious mastitis cases. Lameness, respiratory diseases and bovine viral diarrhoea were also identified as important veterinary problems. The potential market was also studied. Most farmers considered the tool very useful. These results were encouraging and suggest that farmers would consider purchasing such a system. The price they considered for the equipment was lower than the expected price of PATHOMILK. The project had to regard cautiously the price of the components of the prototype to decrease the final price of the equipment. The survey concluded that the system may have an added commercial interest in the farm market if focused in mastitis. Besides the farm market, other markets as the veterinarians and the laboratories should also be surveyed to assess their interest on PATHOMILK.

The next step in the project was to define the technical and biological requirements of the proposed system. Details on the probe design (bacterial species to be detected, target sequence and features required for the immobilization) were discussed among the RTDs performers with the collaboration of some SMEs. Besides the immobilization approaches and the test for their characterization were also described. The SPR equipment was specified including details on its parts (sensor cartridge, SPR reader optics and microfluidics system), as well as the experiments to be done to verify DNA/RNA hybridisation detection. Finally, some requirements on the sample treatment were also specified.

Afterwards, the sensor chip development was achieved. Two DNA probes for each microorganism (*Brucella abortus*, *Staphylococcus aureus*, *Mycobacterium avium* subspecies *paratuberculosis* and a model organism) were selected and validated. The DNA probes selected were immobilised onto SPR sensor surfaces and the protocols for the immobilization process were defined (in this project the combination of alkylthiol carboxy-terminated SAM with streptavidin covalent attachment was used.). The immobilised DNA probes were tested and characterised under various test conditions. The specificity, the selectivity, the possibility of the surface regeneration and the limited of detection were studied, obtaining promising results.

The development of the SPR equipment started in the first period and the design and implementation of the sensor cartridge was completed. The chips consisted in an active metal layer formed onto a grating structure made of polymer. In this case, the SPR device is based on coupling of light to a surface plasmon via a diffraction grating. This approach does not require high quality optical interface which allows separating the sensor chip cartridge from the measuring optics without introducing any physical contact between the part directly handled by the user (chip) and the reader optics itself. Diffraction grating is a low-cost approach and permits to be used with mass-production technologies. Finally, a simple sensor cartridge was developed for the characterization of the SPR sensor system using the polymeric lamination approach. The cartridge was interconnected with the fluidic system using commercial microfluidic connectors. The development of the SPR reader optics and the microfluidic system still was on-going task.

A suitable protocol of RNA extraction was defined and validated for the requirements of the PATHOMILK system. The final sample treatment system will thus use the RNeasy Protect Bacteria Kit (Qiagen) and would require of two devices, a minicentrifuge and a bead-beater.

It was expected that during the next twelve months, the SPR equipment would be ready and the software to analyse the data and to control the system would be implemented. The final goal of the project would be the PATHOMILK system integration and validation.

Finally, some dissemination activities were carried out, where the IAGs and some of the SMEs have actively participated on these tasks. Besides, the Innovation Related Activities have started. In this context, the Exploitation Manager was elected, a report containing the results of the technology watch of milk microbiological detection techniques was done, and a first draft of the Dissemination and Use Plan was defined.

### Second Period

During the second period of the project, further development was performed on the design and construction of the SPR reader optics. The task was concluded and a laboratory prototype of the sensor system was designed and fabricated. The optical system of PATHOMILK SPR sensor was designed (light source, optical lenses and detector) to meet the requirements such as sensor size and number of sensing channels (sensor design is compatible with up to 12 sensing channels, each 0.5 mm wide). The SPR signals obtained at the sensor chip diffraction grating are captured by a CCD that camera is connected to a personal computer for data acquisition and analysis. The bulk refractive index sensitivity of the sensor is about 130 deg/RIU. The observed baseline noise of the sensor was about 0.003 deg which translates to a refractive index resolution in the order of 10-5 RIU. The baseline noise is caused mainly by the limited mechanical stability of the laboratory prototype of the sensor device and can be improved by integrating all the key optical components into a single platform. Development of a more compact and mechanically robust version of the PATHOMILK SPR sensor device is in progress in the integration task.

The microfluidic system was concluded and it was designed in view of the development of disposable cartridges suitable for the detection platform being developed in the project framework. These cartridges consist of microfluidic chips containing a gold-coated diffractive grating present on a plastic substrate. Microfluidic chips containing different configurations allowing for simultaneous detection of 10 different channels (or recognition events) were developed. The chosen material for prototyping the cartridges was a PMMA, given its optical transparency, wetting behaviour and forming properties. Immobilization should be possible mainly by flow-through functionalization, but also by micro-spotting.

The SPR equipment was properly tested for DNA targets and its functionality and feasibility for this nucleic acid was assessed. A series of experiments commenced for the detection of 16S rRNA. The choice of the small subunit rRNA species as the target sequence can avoid amplification steps. The 16S rRNA is relatively abundant since a single cell contains between 10 000 and 60 000 ribosomes. The selected sequences have been tested for specificity, selectivity, stable secondary structure, thermodynamic properties and accessibility of the of the 16S rRNA. Based on the analysis of these parameters, 2 probes for each organism were selected to be tested by SPR and are currently under use for a Gram negative and a Gram-positive (E. coli and S aureus).

During the first period, a suitable protocol of RNA extraction was already defined and validated for the requirements of the PATHOMILK system. The final sample treatment system will thus use the RNeasy Protect Bacteria Kit (Qiagen) and requires two devices, a minicentrifuge and a bead-beater. During the second period, several extra tasks were defined considering the detection of 16S rRNA to be extracted and isolated from raw milk samples. These extra tasks are related to the extraction of RNA for later support to validation works and to the treatment of the molecule itself. In fact, after extraction there are other factors related to the molecule, which in a final analysis can contribute to improve the performance of the PATHOMILK equipment. The tasks developed seek to determine the minimum bacterial concentration required for successful RNA isolation, determine how metabolic state may affect bacterial RNA concentration and define RNA extraction stability on milk at 4°C, study of RNA fragmentation conditions and production of in vitro E. coli 16sRNA for SPR requirements, testing, validation and positive controlling.

Another extra task under course during these last 12 months was to check the possibility of developing centrifugal microfluidic systems, to carry out RNA extractions. Considering the PATHOMILK framework, the simplification of the RNA extraction step by such a device would largely enhance the global simplicity and effectiveness of the pathogen detection process. It has been clearly demonstrated that cell lysis is possible for gram negative and gram-positive bacteria, which are relevant in the project framework. Work is being conducted regarding RNA purification in view of the detection process. The results obtained in this stage show the real possibility of developing and constructing a device that will automatically perform the RNA extraction based on the optimized protocol and equipment already set in the project. This is an ambitious goal since if successful it will represent a major breakthrough, both technically and scientifically with an impact that crosses the boundaries of the PATHOMILK project.

The analysis algorithm software and the user interface software were developed simultaneously and have successfully reached the proposed objectives. Both softwares are compatible with two target platforms: PC/Laptop and PDA. The PC/Laptop solution provides less mobility but is the most developed option and offers greater capacity and compatibility. PDA has the advantage of mobility but has less connectivity options and less processing capability. The analysis algorithms retrieves a CCD camera image from the SPR device, via Microsoft.NET based Camera Software Development Kit (SDK). The acquired image is then pre-processed to a surface plasmon resonance spectrum where the maximums and minimums are localized. The software is then capable of following the wavelength changes of the SPR, thus analysing different signals for different molecular recognition processes occurring on the surface of the DNA chip. The signals generated and processed are delivered to the Graphical User Interface (GUI) using Windows Event Handling. The GUI has three basic tools: step-by-step wizard, report generating and report directing. The wizard has simple instructions for the user, with schematic figures. The report generator allows recording vital information concerning the identification of the sample, the results of the analysis and a date & time stamp. A XML file is created that then can be directed to a local database or remotely sent to a central database. More software tasks are scheduled which are expected to accompany the last developments of the integrated instrumentation.

In terms of system integration, the optical system, the microfluidic chip system and the software would compose a new, small-size optical platform for SPR sensing. The setup consists of a laser diode (light source), a polarizer, a CCD camera, a set of lenses, a chip holder, a microfluidic system with pumping equipment and an interface. This platform is based on spectroscopy of surface plasmons on diffractive gratings and allows simultaneous measurements in multiple sensing and referencing channels provided by the microfluidic assembly. This way the optical system has the ability to detect multiple targets in real-time time and possesses a robust internal referencing, suppressing interfering effects, which allows a higher sensitivity for the detection of low concentrations of analytes.

Regarding validation tasks, a validation study was performed for the biosensing platform for the detection of *Staphylococcus* spp in milk and dairy products. This study involved the selection and introduction of an anchor method for *S. aureus*, building a collection of reference strains, the study of matrix influence, the study of *S.aureus* occurrence in dairy products and evaluation of standard analytical methods, the study of growth characteristics of the organism and establishing a methodology for the final validation of the PATHOMILK prototype.

The planification of training session at European level started in the second period. The IAGs would commence to mobilize their resources and contacts to build an efficient network of training sessions with their associates and other companies. The SMEs are also getting involved and are searching for opportunities to expand the possible audience for the PATHOMILK training actions.

Several dissemination activities were carried out during the last 12 months of this project. The consortium as a whole disseminated the project in journals, meetings and web sites. The web site of the project was constantly updated with all the information concerning the project. This web site contains general information on the project. The site also includes a private section where only partners have access. In this private section, the partners can find the deliverables and meeting minutes among other information. They can also have discussions through a forum.

New courses of action were planned for the dissemination of the project, particularly the identification of critical points, needs and the creation of a pool of dissemination. The pool has been created with material dissemination created by partners that the entire consortium can use in its own dissemination activities identified as adequate.

Examples of materials already available in the pool were a PATHOMILK logo, a trademark, posters, flyers, Power Point presentations for different audiences and generic articles adaptable for different audiences and journals within Europe. The consortium took further steps in the development of the dissemination planning, particularly in the definition of the media to be used, identification of relevant events, creation of a database of target audience for dissemination purposes and the definition of an action framework.

All the efforts allocated to innovation related activities lead to several achievements. The Exploitation Manager, the IAGs and the Technical SMEs were the driving force behind the exploitation of the results of this project. More progress was done towards the creation of the second draft of the Exploitation Agreement. With the new knowledge acquired during the activity of technology watch, the Exploitation Committee could more clearly define the exploitation processes, the protection of intellectual property rights and the dissemination and use plan for establishing and developing a co-operation agreement. Moreover, new structured planning of the detailed role of each member of the consortium has been set for the coherent execution of immediate, mid-term and long-term exploitation actions. A study on socio-economic aspects was concluded and it demonstrated the impact of the main achievable specifications of the proposed technology: biosensor dedicated to rapidly identify multiple pathogens directly in milk, allowing the precocious detection of contaminated animals and to increase the quality and commercial value of the produced milk. This study was supported by a survey in the UK market and a technology review in agro-food analysis.

### Third Period

The results achieved during the first and second periods, already shown that the designed SPR optical system is capable of detecting DNA at low concentrations. Several evaluations were made on detection limits, specificity and sensitivity.

The goal for the third period of the PATHOMILK project was the application of the previously optimized SPR biosensor, for the detection of bacterial RNA extracted from real food samples and validation of the sample preparation and detection protocol. For that purpose bacterial RNA samples and synthesized model RNA molecules were investigated.

The fact that we are detecting RNA increases the potential commercial competitiveness of a future product. However the detection limits of 16S rRNA molecules from pathogenic cells must still be improved. It has been proven that the issue does not lie in the molecule itself, since synthetic RNA fragments quantitatively respond in the same manner as DNA fragments. The issue relies either in a given interferent that hinders the detection limits or in the fragmentation treatment developed, that needs to be optimized. Nevertheless the results achieved clearly point the way to the feasibility of this new pre-competitive milk analytical tool, as well as the specific technical challenges required to take the prototype from a pre-competitive state into to a competitive format.

The final prototype was completely integrated and is functional. It's a compact device with small dimensions (29x22x12 cm) and is light weighted (2,4 kg) in which the entire optical system is assembled, properly isolated from ambient light and insulated thermally.

The sensor system is enclosed in a special housing to suppress the influence of the environmental conditions (stray light, temperature fluctuations and vibrations). The polystyrene temperature insulation and outer metal casing are surface finished to yield chemical resistance to solvents.

It possesses a chamber where the cartridges can be easily installed for each analysis. The special cartridge-handling system enables convenient insertion/removal of the cartridge by an operator. The developed cartridge holder assures reproducible position of the cartridge with respect to the sensor optics. The sensor microfluidics was tested and found reliable for flow rates: 5 – 150 µl/min.

The sensor cartridge consists of sensing chip (diffraction grating) and a microfluidic cover window with ten different channels. These cartridges permit the use of the prototype in two distinct ways:

- 1) One cartridge can detect one pathogen in ten different milk samples;
- 2) One cartridge can detect ten different pathogens in the same milk sample.

The performance of the sensor was studied:

- ✓ Sensitivity: 95 deg/RIU
- ✓ Dynamic range: 0.025 RIU
- ✓ Channel-channel reproducibility: > 90%
- ✓ Temporal drift: <  $2 \times 10^{-6}$  RIU/min
- ✓ Resolution:  $6 \times 10^{-7}$  RIU
- ✓ Detectable surface coverage: 0.6 pg/mm<sup>2</sup>

The prototype is able to detect molecular fragments of DNA and RNA. This means that the prototype is able to inform the dairy farmers, in the same day that the sample was collected, which are the microorganisms that are infecting each cow. This allows a better approach when it comes to proceed with the proper treatment of each diseased animal, without having to wait several days for the results and paying expensive laboratory services. This also means that with the same technological platform we can take pathogen analysis to the next level. In the future there will be the need to distinguish not only between species but also between strains of a given species. In fact the individual treatment to each animal is more successful if the strain is identified since the resistance to different treatments poses now a great challenge which will become more and more difficult in the future.

The PATHOMILK device was tested in order to characterize its capability to detect nucleic acids of specific pathogens responsible for provoking known diseases in dairy herds.

The PATHOMILK biosensor was shown to be able to detect nucleic acids related to selected bacterial pathogens at levels below 0.1 nM.

The prototype showed the following performance:

- ✓ Reproducibility:  $\geq 90\%$
- ✓ Regenerability: at least 10 cycles
- ✓ Specificity: up to 3-mismatches
- ✓ Limit of detection <100 pM

A single user's guide was developed, comprising all protocols developed for the use of the PATHOMILK prototype. The guide is basically divided into three sections, each related to a different component: i) Guide for milk sample treatment; ii) Guide for SPR use ; iii) Guide for software.

Each guide shows the instructions necessary for the correct use of the prototype in the detection of pathogenic bacteria present in milk.-

Using the integrated prototype and the user's guide, the consortium proceeded with the industrial validation of the device and a validation protocol for validation the PATHOMILK biosensor was established. CRIC lead all validation efforts and designed the validation procedure with the collaborations of the other partners. For international recognition the validation procedure implemented in PATHOMILK was based on the European standard dealing with validation protocol for alternative microbiological methods – EN ISO 16140:2003 "Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods".

According to the project consortium decision, two target microorganisms were selected: *Staphylococcus aureus* and *Escherichia coli*. The matrix involved in the procedure was UHT milk and raw cow milk directly originated from dairy farms.

The anchor methods, the PCR method for detection of *E. coli* and cultivation method according to EN ISO 6888-2 for detection of *S. aureus*, were chosen

Calibration curves of the Pathomilk sensor response for *S. aureus* and *E. coli* detection using the anchor methods, were obtained. The results showed a relevant spread of measured data regarding the set of calibration samples, mainly due to issues related to the yield for the extraction of nucleic acids. Sensor response at highly concentrated samples seemed not to be proportional to bacteria concentration, leading to a normal saturation curve, normally expected in the chemical study of molecular interactions and reaction kinetics.

Nevertheless the results for the analysis of milk from healthy and diseased animals showed that the prototype performance was satisfactory. It was proven the concept of using a detection strategy based on 16S rRNA molecules, instead of DNA. The true challenge lies in the improvement of the sample treatment protocol. Anyway the issue of nucleic acids extraction in Gram positive cells is a current challenge that the scientific community is trying to solve. The future developments in this area will benefit the PATHOMILK technology and contribute to its future as a credible and feasible methodology for fast microbiology in milk samples.

A training committee was set up to take responsibility for and coordinate all training actions and tasks and to mobilise the active engagement of the rest of the consortium. The training committee was lead by RABDF and CRIC.

The training committee decided to develop a single Training CD, that compiles all documents deemed necessary for this action. The creation of a single Training CD was decided due to the possibility of creating a portable media, with all the relevant information digitalized, printable and ready-to-use in properly equipped meeting rooms.

The training material on PATHOMILK mainly consisted on different sets of documents: a Power Point presentation, video presentations and tutorial technical documents, printable for the audience.

The presentation was planned to contain a comprehensive overview of the project itself, its objectives, a presentation of the team involved, the evolution of knowledge generated by the consortium, relevant actions taken (like dissemination activities, protection of IPR, etc.).

Three main studies performed during the project would be presented to the audience. The first one is the market survey done at European level and also the one done at the UK level. The second is the study on socio – economic aspects. Finally the third is the technology watch and technology transfer focused on the milk analysis area.

The technical part of the tutorial on the PATHOMILK system was decided to be divided in to two main scenarios: sample collection and processing followed by the biosensor detection phase. To this purpose, the presentation would include a technical tutorial on the use of the PATHOMILK system, according to the updated state of the art on the three main modules: sample collection and treatment, use of Surface Plasmon Resonance transducer device and use of software.

The PATHOMILK technical tutorial would show the different steps in the use of the developed pre-competitive prototype, in a straightforward and easy to understand way. The presentation was destined to a broader audience (dairy farmers, veterinarians, laboratory technicians), and so the idea was to have it as universal as possible. So, in order to aid the training audience, the PATHOMILK tutorial would also have videos of the main steps in the use of the system.

The training committee (RABDF and CRIC with the support of the IAGs ) responsible for all actions and tasks in Workpackage 7, implemented a series of training events according to a specific plan. This plan was used as a road map for the training of IAG staff and respective members (end-user SMEs) in their respective countries.

All training materials created and compiled in a single CD were successfully used in the training courses. The training CD as comprehensive PATHOMILK tutorial, clearly transmitted the correct message about PATHOMILK through the Powerpoint Presentation, technical documents, and the PATHOMILK video.

The in-consortium training to the staff of the IAGs and the core group of SMEs on the PATHOMILK methodology was performed in two events during 2009: March in Prague and June in Barcelona. This “train the trainer” action provided the IAG technical staff the necessary tools and autonomy to later on develop their own training sessions with their associated SMEs in Portugal, Spain, United Kingdom and Italy during the months of July, September and October.

The presentation made to the IAG members contained a comprehensive overview of the project itself, its objectives, a presentation of the team involved, the evolution of knowledge generated by the consortium, relevant actions taken (like dissemination activities, protection of IPR, etc.).

The receptivity of the IAG members to the Training sessions was very positive, and several professionals with different backgrounds (dairy farmers, veterinarian, agronomic engineers, and governmental officers) were quite interested in the final results of PATHOMILK. One of the most common questions was the time predicted to have

a commercial version in the market, since the potential shown by the PATHOMILK technology is in tune with the expectation of the milk sector regarding solutions for a more efficient herd health management.

In total, hundreds of participants attended all training sessions performed for staff and members of all four associations:

During the third period the PATHOMILK web page was updated, with a new design, contents and with the PATHOMILK video which works as a multimedia publishable summary report. The video shows every step of the PATHOMILK process, from milk sampling in dairy farms and the work in sample treatment in laboratory, to the preparation of the microfluidic chips and the consequent detection procedure using the prototype. The link is <http://pathomilk.cric-projects.com>.

Besides the maintenance of the website portal, all members of the consortium were actively involved in several dissemination activities. During the third period these activities included several articles published by the IAGs and SMEs in journals and web sites, interviews to newspapers, magazines and TV, participation in national and international conferences and seminars, and the organization of workshops.

The final version of the Exploitation agreement was created with the participation of the IAGs and the technical SMEs. This action was lead by the Royal Association of British Dairy Farmers (RABDF) in the role of Exploitation Manager, with the assistance of CRIC and EMBIO acting as internal technical consultants. The document updates the generated knowledge after three years of work and defines the position of all the possible intervenients for further negotiations.

The owners of the intellectual property, the IAGs recognize that indeed the PATHOMILK project generated results with a relevant exploitation potential that has not only to be protected but also used in an effective way. Different results were indentified and classified according to its technical nature, potential use or application, the target market and the route of protection.

Meanwhile an application to register "Pathfinder" as the trademark for PATHOMILK, was submitted to the EC Office for Harmonisation in the Internal Market. This trademark to be jointly owned by the four IAG partners.

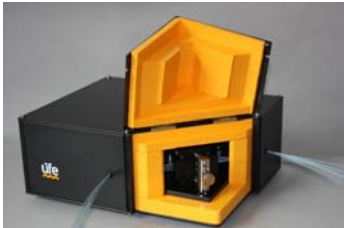


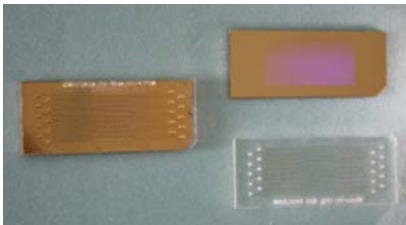
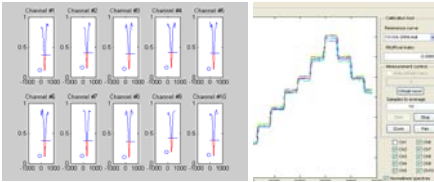
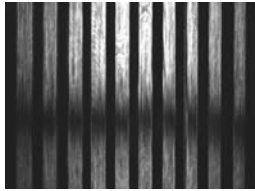
For each individual result, the current pre-competitive status was defined and the suggested route for competitive development was identified. With this information the IAGs have in their hands a basic route map to follow the future steps in the use of the generated knowledge. In that sense they have decided to jointly own all results which will exploit using with their own resources, establishing alliances with technical SMEs of the PATHOMILK consortium and with third parties they see fit to aid them in their exploitation efforts. The IAGs will also develop a business plan using the final results of PATHOMILK, in order to seek investors and/or to seek licensing opportunities. This way they expect to share the risks involved in all the R&D effort necessary to take the developed technology from a pre-competitive state to a competitive one, and finally undertake all actions to reach the market.

The technical SME EMBIO presented a report concerning the results of 708 patent applications (US, EP, WIPO) and 800 scientific publications related to the deliverables of the PATHOMILK Project. The study concluded that in view of the current status of patent applications in the field of milk quality testing, there is no apparent threat for the pending IP protection of the PATHOMILK products.

The high-throughput SPR sensor developed with the PATHOMILK Project seems to be at least as efficient as or even superior than existing SPR systems, in particular SPR immunoassays. The sensor has two main advantages, namely speed (15 min total assay time) and capacity (parallel testing of 10 samples). The sensor's scope of applications could benefit by the incorporation of novel, though system-compatible biorecognition elements, such as aptamers, which could allow for the detection of a vast palette of pathogens and proteins of both scientific and commercial significance.

## 2. DISSEMINATION AND USE

This section provides a publishable summary for exploitable results generated by the project, which are already properly protected. Each result presented represents one component of the total PATHOMILK system, meaning that individually do not possess a specific market value. Only the sum of the parts, forming the integrated PATHOMILK system has a potential market application:

Partial Result	Short description
  <p data-bbox="268 1003 588 1039"><b>PATHOMILK SPR Prototype</b></p>	<ul style="list-style-type: none"> <li>i. SPR sensor dimensions: 29x22x12 cm</li> <li>ii. Weight: 2.4 kg</li> <li>iii. Sensitivity: 95 deg/RIU</li> <li>iv. Dynamic range: 0.025 RIU</li> <li>v. Channel-channel reproducibility: 90%</li> <li>vi. Temporal drift: <math>&lt; 2 \times 10^{-6}</math> RIU/min</li> <li>vii. Resolution: <math>6 \times 10^{-7}</math> RIU</li> <li>viii. Detectable surface coverage: 0.6 pg/mm<sup>2</sup></li> <li>ix. Result reproducibility: <math>\geq 90\%</math></li> <li>x. Sensor regenerability: at least 10 cycles</li> <li>xi. Specificity: up to 3-mismatches in DNA molecule</li> <li>xii. Limit of detection: 100 pM DNA</li> </ul>
  <p data-bbox="252 1487 604 1523"><b>PATHOMILK Sensor Cartridge</b></p>	<ul style="list-style-type: none"> <li>i. Sensor cartridge consists of sensing chip (gold-coated diffraction grating) and microfluidic chip.</li> <li>ii. 10 independent channels (0.84mm wide, 0.4mm inter-channel spacing).</li> <li>iii. Functionalization before the cartridge assembling is possible.</li> <li>iv. Special cartridge-handling system enables convenient insertion/removal of the cartridge by an operator.</li> <li>v. Developed cartridge holder assures reproducible position of the cartridge with respect to the sensor optics.</li> <li>vi. The sensor microfluidics was tested and found reliable for flow rates: 5 – 150 <math>\mu</math>l/min.</li> </ul>
  <p data-bbox="300 1953 555 1989"><b>PATHOMILK Software</b></p>	<ul style="list-style-type: none"> <li>i. Real-time data acquisition</li> <li>ii. 10 channel operation</li> <li>iii. Automatic detection of sensing channels position</li> <li>iv. Easy export of measured data</li> </ul>

<b>Result</b>	Integrated PATHOMILK system
<b>Short description</b>	Fast microbiology analytical system based on SPR and nucleic acids detection
<b>Market applications</b>	Microbiology analysis in milk and other foods
<b>Stage of Development</b>	Laboratory prototype
<b>Collaboration/ Collaborator sought</b>	R&D partners (competitive prototype development) and Investors (commercial product development)
<b>Intellectual property rights</b>	Current IP belongs to IAGs (RABDF, LEICAR, LLET and AIC).
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