Please provide an executive summary. The length of this part cannot exceed 1 page.

Influenza is an extremely contagious infection of the upper and/or lower respiratory tract and is caused by distinct types and subtypes of influenza viruses. Depending on the pathogenicity of the virus and the susceptibility of the host, influenza can vary from a self resolving moderate disease to a life threatening illness. In Europe every year influenza viruses infect 10-15% of the population and account for about 100,000 cases of hospital admissions of which 20% die as either direct or indirect consequence of influenza infection, particularly the elderly and people suffering from chronic heart and respiratory diseases.

Humans are infected by influenza types A, B, and C viruses. Influenza A viruses can be further distinguished in different subtypes because of amino acid differences in the surface proteins hemagglutinin (HA) and neuraminidase (NA). To date 16 HA and 9 NA subtypes have been identified occurring in many possible combinations each representing a distinct virus subtype. The viruses currently circulating among people worldwide causing season influenza include H1N1 and H3N2 subtypes and B virus. Influenza viruses also infect a wide range of domestic and wild animals, particularly birds, where all known subtypes of influenza A viruses can be found. Human infection from bird viruses (avian viruses) occurs only upon exposure to infected poultry, wild birds or contact with surfaces contaminated with secretion/excretions of infected animals. The transmission of avian influenza viruses from man-to-man has been reported very rarely. However, the rapid spread in wild bird species of a highly pathogenic virus subtype, H5N1, that can cause a deadly infection in man, has generated the concern that a mutated form of this virus could acquire the capability to spread in humans and cause a pandemic of catastrophic consequences.

Rapid diagnosis of influenza infection is a key component of disease surveillance activity carried out by health authorities to monitor the presence of these viruses in the community. During the last years a number of influenza *in vitro* diagnostic tests have been developed because of the need to make a timely diagnosis of influenza for the optimal use of available antiviral treatments. Most of them utilize a lateral flow dipstick format. This technical solution allows the development of assays that are robust, affordable, easy to perform and therefore suitable for point-of-care. In spite of such clear advantages, rapid tests have two major limitations that restrict their use only during influenza season. First, most of the commercially available rapid tests have a sensitivity of approximately 70% and a specificity of 90%. Second, available rapid tests are capable to distinguish, in the best case, only between A and B viruses and do not have the capability to distinguish influenza A subtypes. The limited multiplex capability is again intrinsically linked to the format. Only a few assays and controls can be accommodated on a test strip and discriminated with confidence without the help of an instrument. Available rapid influenza test are then already at the limit of their performance and multiplex capability. The development of a point-of-care assay system that combines high technical performance (in terms of sensitivity and specificity) with the ability to distinguish a repertoire of different subtype can only be achieved using different and innovative technology. In this regard, the establishment of automated robust micro-deposition technologies has recently allowed the development of high density ordered arrays of proteins and nucleic acids. Protein chip immunoassays represent novel and powerful immunological tools that dramatically differ from single immunoassays in the number of antigen-antibody reactions that can be detected at one time. They can perform thousands of distinct antigen antibody reactions simultaneously, thus having clear advantages in terms of cost within overall national healthcare programs. In the field of in vitro clinical diagnosis the utilization of Microarray technology offers an opportunity to develop a new generation of in vitro diagnostic assays, capable of assessing multiple parameters simultaneously using individual samples. In this regard, a point-of-care assay that distinguishes virus subtypes will provide small laboratories, health offices, veterinary clinics and outposts (airports) with the diagnostic capability of major research institutions and reference centres. Most importantly, it will significantly facilitate the implementation of surveillance activities and will prove invaluable in guiding response measures (mass vaccination, preventive drug treatment, and containment) that are being designed to face a possible influenza pandemic caused by a highly virulent virus. This capability will have a tremendous impact in providing patients with better care and appropriate therapy.

This project exploits the knowledge and the expertise of the partners to convert microarray assays that have a powerful multiplex capability but are laborious, complex and expensive to perform, into a simple, robust and affordable automated point-of-care system for the diagnosis of influenza.

Please provide a summary description of the project context and the main objectives. The length of this part cannot exceed 4 pages.

Early diagnosis of influenza is increasingly recognized as a crucial instrument for disease treatment and control of transmission. A correct diagnosis can reduce the inappropriate use of antibiotics and provide the indication for using antiviral therapy that, if given within the first days of infection, can significantly reduce both morbidity and mortality particularly in susceptible individuals. Rapid diagnosis is also a key component of disease surveillance activity carried out by health authorities to monitor the presence of influenza viruses in the community. Diagnosis based on clinical examination is neither obvious nor rapid because the initial symptoms of influenza, such as high fever, headache, generalized malaise and respiratory symptoms, are similar to those caused by other infectious agents. Furthermore clinical diagnosis is also inadequate to implement surveillance measures as these require the identification of the predominant circulating virus types, subtypes, and possibly strains. There is an urgent need to develop technically innovative solutions for portable, robust, discriminatory devices which allow type and subtype influenza virus detection in low skill settings with little or no laboratory infrastructure.

A number of *in vitro* assays are currently utilized to help in the diagnosis of influenza. Among these, immunoassays detecting viral antigens, particularly those that utilise a lateral flow dipstick format (rapid tests), have become increasingly popular because they give rapid results and are easy to perform. These assays vary in their ability to distinguish influenza virus types. Different tests can detect 1) only influenza A viruses; 2) both influenza A and B viruses, but not distinguish between the two types; or 3) both influenza A and B and distinguish between the two. These tests do not have the capability to distinguish influenza A subtypes. None of the available influenza diagnostic assays combines the two key features that are deemed essential to effectively combat the disease: 1) a format suitable for point-of-care use and 2) a multiplex capability to identify a large repertoire of human and animal viruses.

During the last years a number of influenza *in vitro* diagnostic tests known as “Rapid influenza tests” have been developed. Most of them utilize a lateral flow dipstick format and produce results in about 30 minutes. Most of the commercially available rapid tests have a sensitivity of approximately 70% and a specificity of 90%. Furthermore, their use is linked to their capability to distinguish, in the best case, only between A and B viruses. New formats and technologies are emerging and microarray technology, in particular, offers an opportunity to develop a new generation of *in vitro* diagnostic assays, capable of assessing multiple parameters simultaneously.

The project aim is to develop an automated portable microarray assay system to distinguish influenza virus subtypes at point-of-care. The SMEs participating in this project have developed the knowledge and expertise to convert microarray assays, that have a powerful multiplex capability but are laborious, complex and expensive to perform, into a simple, robust and affordable automated point-of-care diagnostic system capable of executing, over a small surface area, hundreds of distinct parallel immunoassays, with minimal sample and no loss of performance. This technology has been validated in a number of different diagnostic applications including allergy, infections and autoimmune diseases where a number of different parameters must be evaluated to reach a correct diagnosis. We propose here to exploit such proprietary SME technology to develop a high throughput, point-of-care diagnostic system that is capable of unifying in a single format a number of assays for the detection of influenza viruses infecting man and animals. Micro-deposition, immunoassay chemistry, design moulding, optoelectronics, robotics and computational technologies will be employed to develop and assess a functional fully automated point-of-care system for the diagnosis of influenza consisting of three components: 1) a microarray immunoassay for the detection of viral antigens; 2) an innovative self-contained disposable lateral-flow device enabling automation of reagents incubation and washing steps; 3) a robust, affordable and simple automated equipment incorporating a novel optical reading module that overcomes the complexity of existing microarray reading instrumentation. To match the throughput capability provided by the this system, using current influenza diagnostic test, any laboratory would otherwise need the resources to manage a panoply of costly instruments, kits and reagents with different expiry dates, each requiring specific storage conditions. The proposed rapid test will offer significant benefits beyond the primary impact of improved diagnostic capability. Its portable nature and capacity to rapidly discriminate between influenza types and subtypes will also offer wider benefits for patient management, outbreak response management and broader virological surveillance.

The foreseen objectives will be achieved through the following research activities:
1. Microarray immunoassay– A sandwich competition microarray immunoassay is being developed with the capability to distinguish different hemagglutinin (HA) and neuraminidase (NA) variants from major human and animal viruses in biological specimens (i.e., throat, nasopharyngeal, or nasal aspirates, swabs, or washes).
2. Immunoassay validation - The analytical parameters of the micro-array immunoassay (specificity, sensitivity, reproducibility and accuracy) are being evaluated and compared to available diagnostic assays including the rapid diagnostic tests. Specimens collected from humans and animals with a documented history of infection with distinct virus subtypes have been used.
3. Device design and validation - A lateral flow device has been developed to control the sequential addition of specimen and reagents to the microarray in a temporally controlled manner. This, unlike classical lateral flow devices, accommodates the need to vary incubation times and wash volumes to maximize assay sensitivity and its overall design is being assessed for its capability to process the influenza antigen competitive assay. The device performance is currently being optimised, in particular the shape of the chambers, the thickness of the capillary gap and the use of different materials disclosed a significant influence on the immunochemical reactions, the flow of both specimens and reagents, the efficiency of washing and the detection of fluorescent signals and have been modified accordingly.
4. Instrument engineering and production – A low cost and simple instrument is being built to automatically load reagents on the assay device and read the micro-array immunoassay. MtM and SEAC have carried out pioneering work that lead to a prototype incorporating confocal optics and light emitting diodes (LED) that could read microarray immunoassays with a sensitivity and dynamic range similar to that of the most sophisticated microarray scanners. Different combinations of LEDs, filters and photomultiplier tubes have being assessed for their performance in terms of beam profile, alignment and signal-to-noise ratios. Efforts are also being made to reduce the size of the optical module to facilitate its assembly into a small robotic system able to automatically deliver specimens and reagents into the lateral-flow device. SEAC's work is focusing on the production of prototype instruments that will be assessed for performance.

The expected results are:

i. Define immunoassay, device and instrument specification
The objective is to set the specifications of a rapid point-of-care influenza multi-parametric diagnostic system. We aim to align our activities with ongoing international efforts to improve influenza diagnostic assays and to build a response to the threat of a pandemic. International experts, veterinarians and clinicians as well as representatives of international agencies will be consulted and asked to provide feedback on the different components of the diagnostic system, the assay, the device and the instrument.

ii. Assay concept
The assay will be designed to detect in clinical specimens conserved antigens, such as nucleoproteins (NPs), which can discriminate between the Influenza A and B types, as well as the type A subtypes. The assay aims at identifying virus subtypes and not virus strains and as such it is anticipated that the targeting of conserved antigens will offer suitable stability to justify this approach and the diagnostic value of the end product. The assay key analytical parameters such as sensitivity, specificity, reproducibility and accuracy will be assessed.

iii. Tooling and optimization of a lateral flow device
The objective is to optimize the proposed lateral flow device and assess its performance in detecting influenza antigens as well its compatibility with clinical specimens (i.e., throat, nasopharyngeal, or nasal aspirates, swabs, or washes).

iv. Instrument development and optimization
The aim is to develop, from the stage of proof-of-principle/prototype, a small, simple, robust and easy to operate microarray reading/processing instrument that can be utilized in small laboratories (point-of-care)

and if necessary in non-medical environments such as airports. It has been estimated that the validation of the assay system will require the initial production of 10 instruments.

iv. Point-of-care system performance

We have planned to carry out a clinical validation of the integrated point-of-care system (assay, device and instrument) utilising both reference and clinical samples in comparison with available diagnostic procedures (virus culture, PCR and rapid diagnostic test). The clinical validation has not been carried out due to delays in the production and distribution of the prototypes.

The commercial opportunities arising from the exploitation of the technology are potentially very large with the current acute respiratory diagnostics market standing at $400 million per annum and exhibiting 10-20% annual growth. How the market will respond to the proposed point-of-care system can only be gauged when such products are launched, however several critical issues can be identified such as the impact of multiplex testing on other diagnostic markets as well as political, economic and regulatory pressures to control spending in the current healthcare industry.

Please provide a description of the main S & T results/foregrounds. The length of this part cannot exceed 25 pages.

This project proposes to develop an automated portable microarray assay system to distinguish influenza virus subtypes at point-of-care. This assay will consist of three components: 1) a competition microarray immunoassay for the detection of viral antigens; 2) an innovative self-contained disposable lateral-flow; 3) a robust, affordable and simple automated equipment incorporating a novel optical reading module that overcomes the complexity of existing microarray reading instrumentation.

A **sandwich format immunoassay** has been developed with the capability to distinguish different hemagglutinin (HA) and neuraminidase (NA) variants from major human and animal viruses in biological specimens (i.e., throat, nasopharyngeal, or nasal aspirates, swabs, or washes).
The analytical parameters of the micro-array immunoassay (specificity, sensitivity, reproducibility and accuracy) have been evaluated and compared to available diagnostic assays including the rapid diagnostic tests. For this purpose we have utilized specimens collected from humans and animals with a documented history of infection with distinct virus subtypes.

A **proprietary lateral flow device** has been developed to control the sequential addition of specimen and reagents to the microarray in temporally-controlled manner. The innovative technical solution adopted, unlike classical lateral flow devices, accommodates the need to vary incubation times and wash volumes to maximize assay sensitivity. The device combines the ease-of-use and design simplicity typical of a dipstick test with the capability to carry out high sensitivity complex multiplex assays. Individual device components and its overall design have been assessed for its capability to process the influenza antigen competitive assay. Experiments have been carried out to optimize the performance of the device and its components for the influenza antigen assay. In particular the shape of the chambers, the thickness of the capillary gap and the use of different materials have been investigated to assess how they influence the immunochemical reactions, the flow of both specimens and reagents, the efficiency of washing and the detection of fluorescent signals.

An **affordable and simple instrument** have been built to automatically load reagents on the assay device and read the micro-array immunoassay. The SMESs participating to the project have carried out pioneering work showing that a prototype incorporating confocal optics and light emitting diodes (LED) could read microarray immunoassays with a sensitivity and dynamic range similar to that of the most sophisticated microarray scanners. This technical solution combined with the use of the lateral flow devices dramatically simplifies the design and reduces the cost of an instrument thus making it suitable for a point-of-care setting.

Production and characterization of MAbs

The assay requires a large collection of monoclonal antibodies (mAbs) capable to recognize epitopes not cross-reacting among the virus subtypes. To this aim, mice have been immunized with five different subtypes of whole influenza viruses (H1N1, H3N2, H5N3, H7N3, H5N1 and type B viruses) as source for the fusion procedure used to generate hybridoma cells secreting mAb directed against specific influenza epitopes.
The first group was injected with a mix containing the inactivated H1N1, H5N3 and type B viruses. The second group was immunised with the H3N2 and H7N3 viruses, whereas the H5N1 subtype virus was single injected to the third group as it was provided later. After the immunization schedule was completed (a first priming injection followed by 4 boosts), the sera from each immunized mouse were analyzed to select the mouse that had raised the strongest reactivity against the injected viruses. The specificity of monoclonal antibodies produced was analyzed using microarray technology instead of the classic ELISA test, as it allows detecting the reactivity against a large number of antigens in a single test.

All influenza virus subtypes were printed on microarray slides to generate chips containing all virus subtypes. Recombinant NP proteins from human A and B, avian viruses, purified HA and NA subunits from H1N1, H3N2 and B viruses, were also printed on the same chip. These chips were processed against each mouse serum and the antibody reactivity was detected by a second incubation with a secondary antibody conjugated to a fluorochrome (Alexa 555) directed against mouse IgG. The slides were analyzed using a scanner. In each immunization group the mouse showing the highest antibody titer was selected as source for the fusion procedure used to generate hybridoma cells secreting mAb directed against specific influenza epitopes. The first fusion was performed using the spleen of a mouse immunized with H1N2, H5N3 and type B viruses. This fusion produced 480 wells containing hybridoma clusters. The supernatant from each of these wells were tested using the microarray assay used to analyze the mice sera as described above. This analysis allowed the identification of 16 positive hybridoma clusters reacting against single or more virus subtypes. The second fusion was obtained using the spleen of a mouse immunized with the H3N2 and H7N3 viruses and resulted in 354 hybridoma clusters positive wells. Supernatants from 219 positive wells were tested by microarray assay for reactivity against all virus subtypes. Nine out of 219 supernatants showed specific reactivity against single or groups of virus subtypes. In the third fusion, the spleen originated from a mouse immunized against the H5N1 virus. This experiment produced 250 wells containing growing hybridoma cells and the microarray screening identified 11 positive wells, most of them showing a specific reactivity only against the H5N1 virus or the avian viruses. Single clones from each positive hybridoma cluster were obtained by limiting dilution and the reactivity of each clone was tested by microarray assay. In summary the above described fusions results in the isolation of 36 hybridoma clones secreting antibodies with specific reactivity against single or more influenza viruses' subtypes.

In the second phase of the project a second round of immunisations was carried out with the aim to identify additional antibodies directed against specific influenza epitope. Five groups of mice were injected with single whole inactivated subtypes of influenza virus and two groups were injected with the recombinant nucleoproteins of Human and Avian influenza. In this phase of the project 97 new monoclonal antibodies were identified thereby increasing the panel of the consortium anti influenza mAbs collection to a total of 133. Fusion procedures from H1N1 and H3N2 immunizations produced 12 and 18 new influenza reacting mAbs, respectively. Two mAbs, 61A5 and 62F10, from the H1N1 fusion showed reactivity exclusively with the H1N1 influenza subtype and two mAbs from the fusion H3N2 immunization showed reactivity restricted to the only H1N1 subtype: 45A1 and 45C3. The experiments of fusion performed with spleens of mice immunized with H7H3, H5N3 and H7N1 subtypes produced 11, 19 and 6 hybridomas secreting monoclonal antibodies able to detect different influenza avian subtypes. Only one mAb, 131E3, from the H7N1 fusion showed specific signal from H7N1 antigens.

The two last fusions were obtained using the spleens of mice immunized with recombinant nucleoproteins of human influenza A (NPA) and recombinant nucleoproteins of avian influenza A (NPA Avian). The microarray screening assay identified 11 and 20 positive hybridomas producing antibodies that recognize type A NPs. Although most of these antibodies show a cross-reactivity against both NPs of Human and Avian influenza, was possible obtained two mAbs specific for NPA (126A5 and 128C1) and five mAbs able to detect only NP A Avian (103D7, 105G2, 108A10, 109A8, 109D12).

A total of 133 hybridomas secreting antibodies were isolated from all fusions. The reactivity was analysed using microarray chips generated by printing whole virus, HA/NA mixtures and single NPs. This screening strategy allowed the rapid identification of the reactivity pattern of each antibody against influenza type(s) or subtype(s). However, it was not possible to identify which specific antigen was recognized. To better characterize the reactivity of the mAbs selected, western blot experiments were performed against influenza virus antigens. For this, 8 influenza structural proteins were size-separated by SDS-PAGE procedure and then they were transferred on a nitrocellulose membrane and revealed. Some of the 36 mAbs isolated in the first phase of the project, showed unusual reactivity as they recognized a broad variety of influenza subtypes. Particularly, some hybridomas showed reactivity to either all set of influenza viruses analysed (hybridoma 1H11), or one subset such as, for example, human viruses (i.e. hybridomas 3F8, 13E9 and 15C4) or avian viruses (i.e. hybridomas 16G6, 19D10 and 3C9).

Investigations proved that a panel of mAbs react with glycosyl residues rather than with amino acid epitopes. In fact the influenza glycosylation pattern obtained when the virus was grown in chicken or in

mammalian cells was different, and thus the antibodies would react against the glycosil residues. These candidates were not suitable for the diagnostic platform to be developed and had to be discarded.

The subsequent set of antibodies obtained from the second round of immunization were so analysed by immune blot. Most of them showed reactivity towards more subtypes of viruses. Signals from untreated influenza antigens were compared with the reactivity from deglycosylated antigens. Results confirmed that their targets might be glycosyl residues. For some other antibodies, in particular those produced from mice immunised with avian viruses, reactivity was observed not only against avian antigens but also against alloantoide liquid and "non influenza viruses" (Adenovirus and Parainfluenza virus) used as negative controls. Following these results the attention was focused on the mAbs that react with amino acid epitopes.

The mAbs were classified in two main groups according to their capability to recognize nucleoproteins, one single subtype. The first group is composed by mAbs that detect NPB and NPA either of Human or of Avian influenza. Among these mAbs, 33G2 showed the rare feature to detect both NPA and NPB, while 3F6 seemed to recognise only nucleoprotein B. Investigations carried out by ISS and MtM reported poor performance probably due to low affinity and therefore these candidates were considered non-optimal for the designed system and were discarded. Characterization studies on mAbs 11B11 and 42F6 proved specificity against NPA and NPA Avian respectively and isotyping determination performed by MtM identified them as IgM. Because of the typical pentameric nature of immunoglobulin M the mAbs 11B11 and 42F6 were judged unsuitable in the role of NPA capture substrate.

Two very promising candidates, are the clones 2B4 and 4A11. These proved very specific reactivity against the nucleoproteins of influenza type B. Immunoblot experiment excluded cross-reactivity of 2B4 against HA and NA, as no reactivity was observed towards HA and NA purified subunits. Results conducted by MtM evidenced this candidate produced significant signal/noise when probed against the recombinant NPB. Similarly, for the mAb 4A11 both microarray and immunoblot analysis showed reactivity directed against nucleoprotein of influenza type B and no signal was observed when probed against from HA, NA.
Five mAbs 103D7, 105G2, 108A10, 109A8, 109D12 were considered good candidates for the detection of the avian influenza. In fact, western blot analysis confirmed that the five antibodies were directed exclusively against recombinant nucleoprotein of avian influenza, both cleaved and un-cleaved. Also, these five candidates were shown on microarray experiments to reveal the spotted NP avian. However, while all these candidates produced significant S/N on the NP avian spots, two, 105G2 and 109D12, also produced fluorescence on the NPA of Human influenza. This could be explained by the high percentage of identity between the amino acid sequences of the avian and human NPA. According to ISS cross-clade analysis the clone 108A10 was indicated as the best candidate to discriminate nucleoprotein of a potential influenza avian infections.
The very last experiment of fusion produced two promising hybridomas secreting antibodies reacting exclusively with nucleoprotein type A from the Human influenza virus. Both mAbs, 126A5 and 128C1, produced fluorescence on microarray spots with NPA of Human influenza. In western blot experiments the bands corresponding to NPA of Human influenza were clearly detected while no bands corresponding either to NPA Avian or NPB were revealed.
The second cluster of mAbs consists of a total of 5 mAbs specific for one subtype of influenza virus. The microarray and immunoblot data showed that the mAbs 45A1 and 45C3 reacted only with the H3N2 subtype while 61A5 and 62F10 reacted only against H1N1 subtype. Both monoclonal antibodies 45A1 and 45C3 should be directed against the HA1 peptide of the Hemagglutinin 3 since HA and NA purified subunits are strongly detected by both mAbs and the molecular weight of the band observed in western blot correspond to that of the fragment HA1 of the hemagglutinin. The 61A5 and 62F10 mAbs displayed a strong and specific reaction against purified HANA subunit of H1N1 subtype, also in this case both antibodies are expected to bind some epitope in HA1 peptide of Hemagglutinin 1.
The fifth antibody identified as specific for one single subtype is the 131E3. Protein microarray test revealed significant signals against H7N1 and H5N1 whole viruses. Images of membranes probed with 131E3 proved cross-reactivity with both avian subtypes and moreover visualised a third band corresponding to the lane

where H1N1 viurus were loaded. According to the molecular weight of the band detected, 131E3 seems to react with the fragment NA1 of the avian hemagglutinin. Since purified subunits of influenza avian subtypes were not available, it was not possible to perform deeper investigation.
The monoclonal antibodies (mAbs) originally screened on microarrays have been subjected to a second expanded analysis, requiring the use of purified viral antigens, and analysis with a number of different influenza subtypes.
HPA received a total of 23 mAbs (with more than one batch of some of the 23 mAbs received and tested) from UNIPG, generated following immunization with inactivated seasonal H1N1, H5N3 and influenza B viruses and primary screening by microarray analysis at UNIPG. These were subjected to detailed analysis at HPA by western blotting and ELISA against influenza antigens, and those mAbs showing good reactivity were further analyzed by immunoprecipitation to confirm specificity and sensitivity.
This work has led to the identification of five mAbs (Table 1) that are specific to the nucleoprotein (NP) of either influenza A or B viruses.

Table 1.Influenza virus specific monoclonal antibodies identified

mAb ID	Specificity	Isotype (UNIPG/HPA determined)
2B4	NP B	IgG1
4A11	NP B	IgG1a
3F6	NP B	IgA
11B11A10	NP A	IgM
42F6F7	NP A	IgM

The remaining 18 mAbs showed aspecific reactivity with egg grown influenza viruses or with other respiratory viruses in western blotting analysis.

ISS also received MAbs from UNIPG and set up two different ELISA methodologies to test the specificity of the MAbs produced and to analyze and compare the results of these tests, with those previously obtained by UNIPG using microarray procedures.
The first one is a conventional ELISA assay, which used different antigens (H1N1, H3N2, B, H5N3, H7N3, H5N1 egg-grown strains and H1N1, H3N2, B whole and sub-unit monovalent vaccine bulks) fixed to the well surface of a microtiter plate.
The second one, a variant of the previous ELISA method, had been performed using MDCK cells infected with H1N1, H3N2, B, H5N3, H7N3 egg-grown strains to coat the plate. Hemagglutination inhibition (HI) and Neuraminidase Inhibition (NI) tests had been also carried out to analyze the reactivity of these mAbs.
ISS received a second set of 8 mAbs from UNIPG (10B10, 27B3, 8E2, 22C3, 22F4, 28C8, 27A10, 3C9), obtained from the third group of mice immunized with the A/H5N1 antigen. As agreed with HPA, Western Blot analyses had been also performed in order to better characterize the fine antigenic specificity of the mAbs received, but the 8 mAbs showed a non-specific and not clear reactivity when tested against different influenza viruses and they were considered to be not useful for the diagnostic Fluarray chip.
ISS carried out the cross-clade analysis of the following 13 mAbs sent by UNIPG, using the conventional ELISA assay:

anti H1N1: 62F10, 61BisA5,
anti H3N2: 45 A1, 45C3
anti NP B : 3F6 , 2B4
anti NP A : 34C11
anti-avian (H5N1, H5N3, H7N3): 22C12, 26H9, 22F4, 24C5, 27B3, 2 A12

ISS started working on the MAbs directed against the surface glycoproteins HA and NA (62F10, 61BisA5, 45A1, 45C3) to see whether they would show any inhibition of HA and/or NA activity by HI and NI assays

and they were all negative, with the exception of the mAb 62F10, which showed a positive result in HI for the H1N1 virus A/Solomon/3/06 (used for the mice immunization).
Cross-clade analysis on the first 7 mAbs, has been performed in ELISA, coating the plate with the influenza monovalent vaccine bulks (purified whole virus and/or subunit HA/NA) representing the predominant circulating viruses of the past ten years.
The mAbs 62F10 and 61BisA5, 45A1, 45C3 were found to be specific for the H1N1 and H3N2 subunits respectively and they recognized in ELISA most of the antigens used. ISS also found out that the mAb 61BisA5 appeared to be able to recognize the current H1N1 swine-origin pandemic strain, which is particularly important given the latest number of lethal cases.
The mAbs 3F6 and 2B4 bound the NP of B viruses, even if they did not show a broad cross clade reactivity.
Western Blotting analysis, in the case of mAb 2B4, confirmed the results obtained. The same protein was recognized in Western blotting experiments as in the previous ELISA tests. In particular, an influenza B virus protein having molecular weight of 70 KDa (hemagglutinin-HA) was found to react strongly with that mAb.
Regarding the anti NPA, the mAb 34C11 was negative against all the antigens used in the ELISA test.

With regard to the 6 anti-avian mAbs, the not availability of vaccine bulks made it necessary to select, grow in eggs and purify a panel of viruses including different avian influenza subtypes.
Preliminary results showed that some of these mAbs bound many different antigens (H5N2, H5N3, H7N3, H7N1, H7N7), including the seasonal egg grown virus A/Solomon/3/06 (H1N1) and they were negative for the purified whole virus H5N1 (3 different clades used). Their broad cross-reactivity could be due to the proteins present in the allantoic fluid. Trying to sort this problem out, ISS tested these mAbs against non-infected allantoic fluid and they were found positive.
Additional comparative work was done by ISS to test the reactivity of the anti-avian mAbs against not purified as well as purified viral antigens. This confirmed the not specificity of these mAbs which recognized, in ELISA, egg-derived components and did not show any reactivity against purified viruses.
There was concordance in terms of specificity and reactivity between the tests performed at UNIPG and ISS as reported during the meeting at the Imperial College of London, in February 2011.
In April 2011, ISS received a fourth set of 6 mAbs from UNIPG 103D7,109A8,109D12,108A10,105G2,105H4), all obtained from mice immunized with the NP-avian recombinant protein.
The 6 mAbs were tested, in ELISA, against different egg-grown purified avian viruses, belonging to the subtypes H7N3, H7N1, H5N3, H1N1. With the exception of the mAb 105H4, which did not show any positivity, the other 5 mAbs showed similar relativities and they were positive against all antigens used in the test.
To better understand the reactivity profile of these anti-avian mAbs, two human strains (H1N1 and H3N2) were also included in the test. According to the high similarity (80-90%) between the amino acid sequences of avian and human nucleoproteins, most of the mAbs also recognized the two human strains in ELISA.
The only mAb that appeared to be specific for the avian NP was the 108A10.

Validation of the assay

One of the objective of the FLUARRAY project was the development of a competition microarray immunoassay with the capability to distinguish different hemagglutinin (HA) and neuraminidase (NA) variants from major human and animal viruses in biological specimens (i.e., throat, nasopharyngeal, or nasal aspirates, swabs, or washes). The assay utilizes a capture substratum (a microarray of monoclonal antibodies directed against distinct HA and NA variants), HA and NA recombinant tracers (engineered to contain a common amino acid epitope tag) and a secondary fluorophore-labeled antibody directed against the epitope tag, that is therefore able to recognize all HA and NA tracers irrespective of their subtype. Viral derived antigens, if present in the sample, would displace the cognate recombinant tracer from binding to the corresponding antibody thus causing a reduction in the signal.

Expression and cloning of influenza proteins.

During the first period the research activities had been focused on the development and optimization of the protocol of production, purification and tag removal of the different nucleoproteins. The selected

influenza proteins had been successful cloned and expressed and a mg scale of protein (12 batches in total) has been produced. For InfB nucleoproteins, removal of the His-tag had also been performed in order to deliver untagged proteins. This course of action allowed the recovering of the full length protein devoid of His tag but also a form devoid of the last 68aa due to the presence of an internal Xa site.
It had been evaluated the expression level of His tagged HA and NA proteins from influenza viruses H3N2, H1N1, H5N3, H5N1 and H7N3 and B virus by separately transfecting in COS-7 the corresponding genes cloned into pCMV. However, it failed to obtain any expression of all the proteins. Following these negative results obtained, the Consortium only focused its attention on H1N1 and assesses the expression using different cell lines: adherents HEK293 or using cell line adapted to suspension, HEK293-FS and CHO-S. Despite correct transfection efficiency, Western-Blot analysis using anti-His of supernatants or cell extracts of transfected or co-transfected cells does not show expression or secretion of NA and HA.
H1 and N1 encoding genes were subcloned to remove the His tag and. In addition to HEK293 and COS7, VERO and MDCK cells have been transfected with different combination of gene expressing NA and HA proteins: H1, N1, H1-his, N1-His, H1+N1+m2 and H1+N1+m1+m2. However, no secretion was again detected except in the case of the co-transfection of HEK293 with H1+N1+m1+m2 where a weak immunofluorescence signal has been detected. This might result of a release of the virus in the supernatant but at a too much diluted concentration. The reasons for failing were due to the intrinsic difficulties represented by the production of recombinant HA and NA proteins: the Consortium tried several approaches (i.e. different cell lines, transfection vs co transfection, protein expression strategy vs virus like particle expression) but expected in HEK293, and did not succeeded in achieving encouraging/positive results.

Due to the discouraging results reported by PX, which did not succeed to express and purify the recombinant tracer proteins using virus infection, the development of a competitive microarray immunoassay has been evaluated and the Consortium decided that sandwich competitive and non competitive immunoassays would produce more positive results.

A competitive-format immunoassay was initially evaluated for NPs. In this format, 6xHis-tagged antigens (tracers) are captured by specific monoclonal antibodies (mAbs) spotted onto the microarray, and then detected with an anti-6xHis mAb (clone 4D11) and reagents that generate localized fluorescence. The tracers are then displaced by the viral antigen, thereby causing a recordable drop of the recorded fluorescence. The detection range permitted by the HRP-conjugated 4D11 was in the range of tens of µg/ml of tracer, far higher than the concentration range of antigens in biological samples (pg/ml to ng/ml). A possible explanation for the poor results, as indicated by western-blot analysis, could be a non-optimal HRP conjugation, and the consequent presence of unconjugated 4D11 in solution. An alternative signal generation strategy was therefore evaluated in order to improve the detection limit. 4D11 antibody was biotin-conjugated and employed along with a commercial streptavidin-HRP solution. Experiments were performed to optimize the protocol and to achieve the best detection limit within a total processing time of 25 minutes. Parameters evaluated for the protocol optimization included the concentration of the biotinilated 4D11, the streptavidin-HRP, and the HRP-substrates solution. The improved protocol permitted to differentially reveal approximately 10 ng/ml of 6xHis-NPA and 6xHis-NPB4 (NPB variant which resembles that of the Victoria lineage). Lower concentrations were tested, but did not produce significant signal compared to the negative controls. Microarrays are generated with commercial anti-NPA and anti-NPB reagents printed in selected buffers. When the microarrays were incubated with 100 ng/ml, 10 ng/ml, and 1 ng/ml of 6xHis-NPA, the first and second dilutions produced significant signal compared to the negative control. Similarly, the array could reveal 10 ng/ml of 6xHis-NPB4. The concentration range revealed by the new detection method represents a 1000-fold improvement of the 4D11 detection capability. Using the new detection method then, competition experiments were set up in order to evaluate the kinetics of the tracer-vs-antigen competition.
Initial tests proved competition was taking place on the array, however, only at concentrations levels (ug/ml) several orders of magnitude higher than those expected in clinical samples (pg/ml to ng/ml). Increasing concentrations of the antigen in the low ng/ml range were tested, but they failed to significantly modify the S/N (data not shown).

The difficulties encountered in setting up the competitive assay, as well as the theoretical limitations associated with this format, led to the decision to exploit the incoming reagents produced by UNIPG to focus on the immunometric ('sandwich') format.

Evaluation of the sandwich format immunoassay for the detection of the NPB and NPA

Detection of the NPBs previously achieved using anti-6xtag 4D11 was re-evaluated using anti-NPB consortium-produced mAbs. 4A11 and 2B4 were employed as capture agents, and the biotin-conjugated form of either antibody was used as detector. Biotin-conjugation of 4A11 and 2B4 followed the same procedure previously applied to 4D11, and the NPB reactivity of the biotin-conjugates was confirmed.
To develop the designed 'sandwich' assay, arrays were generated with 4A11 and 2B4. The same printing conditions selected for 4A11 were used to print 2B4. The NPB4 was incubated on the array at different concentrations in the low ng/ml range, and then revealed with the biotin-conjugates, a commercial streptavidin-HRP reagent, and fluorescent HRP-substrates. Of the four possible sandwich conformations made possible with the two antibodies (capture/2B4 and detector/biotin-4A11, capture/2B4 and detector/biotin-2B4, capture/4A11 and detector/biotin-4A11, capture/4A11 and detector/biotin-2B4) one was chosen for future experiments: capture/2B4 and detector/biotin-4A11. Not only this format produced good S/N values (Fig. 8 bottom), it is also expected to be a more reliable approach compared to that of revealing the antigen with one same antibody (for example capture/4A11 and detector/biotin-4A11).
This immunoassay developed with this combination performs with a sensitivity of 1-10 ng/ml NPB4 with a total assay time of approximately 45 minutes.

Experiments for detection of the human and avian NPAs relied at the beginning on commercial reagents. By the end of the project we manage to performed the assay using only the antibodies produces by the Consortium (109 D12, 105 G2).

The two commercial reagents chosen were C01321M and C01323M (Meridian Lifescience), indicated for use as capture/detection pair. These two reagents were first reactivity confirmed on arrayed NPs. Both candidates produced fluorescence against human and avian NPAs, and no reactivity was detected on the NPBs (results not shown). C01323M was then biotin-conjugated and evaluated as detector with a procedure very similar to that used for the previously biotin-conjugated antibodies. Finally, C01321M was arrayed and used to capture the NPA. This was revealed with biotin-C01323M following the same procedure used in the anti-NPB assay. Incubation of the NPA produced localised fluorescence on the anti-NPA spots. The same experiments have been performed with the antibodies produced by the consortium and we obtain exactly the same results.

The constructed anti-NPA and anti-NPB immunoassays were evaluated for detection of spiked influenza samples provided by HPA. Three samples were sent to MtM, two influenza A (A/NewCaledonia/20/1999, and A/Wisconsin/67/2005) and one influenza B (B/Malaysia/5606/2004). Each sample was received at the concentration of 10 e^0, 10xe^1, 10xe^2, 10xe^3, 10xe^4, 10xe^5, 10xe^6, and 10xe^7 PFU/ml prepared in Viral Transport Medium by HPA. The samples at 10xe^7 PFU/ml were tested on the assay where they replaced the recombinant NPs. Samples were tested neat and at serial dilutions achieved with two alternative buffers mix. The first is the same buffer that was utilised to dilute the recombinant NPs during the assay optimisation. The second is an extraction mix included in one rapid detection kit (Directigen EZ Flu A/B, BD). The aim of the experiments was to, 1) demonstrate the immunoassay with 'real' samples, and to 2) select a panel of buffers and extraction mixes to solubilise the NPs from the outer layers of the virus. For every condition, background noise associated with each buffer was controlled with one negative control test. In this case, fluorescence recorded on the spots with relevant anti-NP antibody was recorded from the negative control slide, averaged across identical spots, and accounted for as 'noise'.
The neat sample produces very good S/N (50,000 to 60,000). The 1:2 dilutions with both buffers produced recordable S/N, with the sample treated with the Directigen buffer being more reactive than that treated with standard salt buffer. Standard deviation was calculated based on the values measured on the spots containing only printing buffer. Low non-specific fluorescence is revealed on the negative controls. Of the

two influenza A samples, one, A/NewCaledonia/20/1999 was successfully detected (data not shown), although specific fluorescence was very low. Moreover, four samples were send to MtM by University of Bologna and used to validate the assay for the detection of NPA.

The successful detection of the A and B viral strains demonstrates that the developed assay is feasible to capture and reveal the influenza virus in samples that resembles those to be assayed in a real-case scenario.

Tooling and production of device.

Experiments were also performed to optimize the flow of samples and reagents in the capillary chamber and a re-design of the device was being conducted to sort out several issues concerning the processing of the immunoassay. The draft specifications for the immunoassay and the corresponding instrument specification of the diagnostic system have been released. A prototype automated microarray reading instrument that has the capability of adding samples and reagents to the device has been constructed. The instrument contains an optical module incorporating light emitting diodes (LED) and reads microarray immunoassays with a sensitivity and dynamic range similar to that of the most sophisticated microarray scanners. The optical design was based on the principles of a fluorescence microscope. Different combinations of LEDs, filters and photomultiplier tubes have been characterized and assessed in terms of beam profile, alignment and signal-to-noise ratios. A high intensity LED is used as the light source and fluorescence emission from the whole assay spot is collected by the focusing optics and after transmission through the dichroic beamsplitter and an emission filter is directed onto a photomultiplier detector. The fluorophore currently used is Alexa555 or equivalent. Minor issues relating bad optical sensitivity and too long acquisition time were successfully managed. The result is an optical reader with enounced sensitivity and rapidity that can perform a 7x7 spots slide read in 1 minute and 30 seconds.
An automated microarray reading instrument that has the capability of adding samples and reagents to the device is being constructed bringing together some important features such as the ease-of-use, the automation, robustness, affordability, low cost and small size. The contribution to the instrument small robotic system has focused on the prototype module for automatically deliver specimens and reagents into the lateral-flow device, and on the optics and scanning with the LED-based optics to assess the performance of the module and its suitability for clinical diagnosis.
The reagent/sample module provides an affordable and simple system to automatically load reagents on the assay device. Standard robotic solutions are utilized to control the addition of reagents to the device as part of an automatic routine. The optical module incorporating light emitting diodes (LED) reads microarray immunoassays with a sensitivity and dynamic range similar to that of the most sophisticated microarray scanners. Different combinations of LEDs, filters and photomultiplier tubes have been characterized and assessed in terms of beam profile, alignment and signal-to-noise ratios. These technical solutions, combined with the use of the lateral flow devices, simplify the instrument design and reduce its cost, making it suitable for a point-of-care setting as proposed in this project.
The major work on tooling and assembly of prototype instrument has been dedicated to the optics, in order to obtain the performance (reproducibility in manufacturing) and cost required agreed in the product specification, and to the reagent module, in order to obtain a simple and reliable loading of the reagent from the reagent reservoir to the device.
The 10 ADAM prototypes have been produced. The major result obtained in the optimization of the 10 prototypes has been the enlarging the scanning area from 7x7 to 17x17 spots achieving and maintaining the requested scanning time <10 minutes without influencing the reproducibility performance of the system. The extension of the scanning area from 7x7 to 17 x17 and the use life test during the reading process positioning on each individual spot showed a deterioration of the positioning with the increasing the number of cycles (400 slide).
The operating Manual has been completed and it has been released in the Italian version. The English version has not been completed and the translation will be completed either internally or with external support, if needed.

Validation of integrated diagnostic system.

A specificity panel of simulated respiratory samples has been constructed at HPA as part of preparing for technical validation (specificity testing) of the diagnostic device. Firstly, respiratory virus negative clinical respiratory material has been identified at HPA by testing combined nose and throat swab material by PCR analysis for respiratory viruses. Nose and throat swab specimens known not to contain influenza viruses were made into 30 pooled samples and tested by multiple RT-PCR and PCR assays specific for the presence of other respiratory viruses. Pools which were negative for these viruses were identified and combined to provide diluents for making spiked samples for the panel. The panel comprised 11 influenza viruses of different types and subtypes, and 21 other respiratory viruses (different strains and serotypes of RSV A, RSV B, coronavirus, parainfluenza virus, rhinovirus and adenoviruses). Each virus was grown in a permissive cell line or in eggs for culture of influenza viruses, and influenza viruses were titrated by plaque assays. All viruses were prepared as a 10-fold dilution series and pre-tested in duplicate by RT-PCR and PCR assays to determine suitable standardized dilutions of each virus to use in the panel aliquots. The 32 viruses were then spiked at the chosen dilutions into the pooled negative clinical material and stored frozen. The panel has not yet been used to evaluate the diagnostic system, as this was not available.

The constructed anti NPs immunoassays were evaluated for detection of spiked avian influenza samples provided by Bologna University. The samples were tested on arrays generated with 105 G2, 109 D12 and a commercial anti-NPA (C01321M). These antibodies were arrayed along with anti-NPB reagents (2B4, 4A11, and a commercial anti-NPB). Each viral sample was incubated neat, and then captured virus particles were revealed with a biotin-conjugated 109 antibody, streptavidin-HRP, and fluorescent HRP-substrate. All strains tested were detected in the ADAM system. The results demonstrated that we developed a reliable protocols to detect avian influenza proteins based completely on the reagents produced by the consortium and we validated the integrated diagnostic system using the clinical samples.

The results achieved during the lifetime of the Fluarray project reflect quite close the schedule presented in the proposal, most of the objectives have been accomplished even though minor delays have been recorded. A wide collection of mAbs has been generated, the key reagents needed to develop the immunoassay. Some of this mAbs represent also a potential source for other application such as human therapy and research in the influenza field. Ten prototypes of the automated microarray reading instrument has been produced and assembled. The clinical validation of the immunoassay have been carried out at MtM and not at the selected point-of-care due to unexpected delays in the production of the diagnostic system. The technical issue caused the delay in the completion of the construction and distribution of the prototypes.

Please provide a description of the potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and the exploitation of results. The length of this part cannot exceed 10 pages.

According to World Health Organisation statistics, influenza is one the most common causes of global morbidity in the world. Influenza viruses are responsible for enormous economic and social burdens. Each year influenza viruses infect millions of individuals and are either directly or indirectly responsible for the death of a significant fraction of the elderly and debilitated population. The virus also has the ability to undergo a continuous genetic change that results in the emergence of new antigenic and virulent variants. WHO, together with a number of governments, are involved in an effort to build a response to the influenza threat that includes diagnosis, continuous surveillance, early identification of new virus variants and the development of vaccines. These measures would be significantly enhanced by the availability of a high sensitivity diagnostic assay suitable for point-of-care settings that has the capability to distinguish different virus variants of human and animal origin. Point-of-care assays are also recognised as an essential component to implement a quick and effective response to the emergence of new virulent virus strains.
Assay performance and profitably has been driving the development of both new tests for disease markers and improved configurations of tests already available. For decades this model has generally been considered sustainable, though now it is under the combined pressure of budgeting constraints of national health care systems and spiralling treatment costs. The main priority has become the effective allocation of resources by maximising diagnostic efficiency, increasing assay throughput and hence treatment at an affordable cost. This is usually best achieved at the point-of-care. This scenario overlaps with the growing clinical need in many pathological conditions and diseases to determine numerous parameters at the same time. In the case of influenza, the need is to distinguish amongst a number of possible variants of the virus subtype that is causing the infection in man or animals. There are no diagnostic assays and procedures routinely employed for the point-of-care diagnosis of influenza that have the throughput and the sensitivity performance characteristics to address this need. Current assay formats are either procedure-intensive or perform individual or small numbers of antigen detection tests at any one time. This project will deliver a high sensitivity point-of-care diagnostic system that combines key features of dipstick technology such ease-of-use, low cost and robustness with a quantitative, multiplexing capability. This will be achieved by exploiting a proprietary technology that dramatically simplifies the use of microarray-based assays without compromising the assay performance and throughput.
The availability of the proposed system will provide the health system with an invaluable tool for facilitating the diagnosis of influenza. Such a system is also anticipated to dramatically help in implementing the response in the case of a pandemic as it could be easily deployed in different settings and operated by untrained personnel. The proposed rapid test will offer significant benefits beyond the primary impact of improved diagnostic capability. Its portable nature and capacity to rapidly discriminate between influenza types and subtypes will also offer wider benefits for patient management, outbreak response management and broader virological surveillance. A prompt diagnosis at the onset of symptoms is critical for the appropriate patient management with antiviral therapy and can therefore help in reducing the inappropriate use of antibiotics as well as in preventing nosocomial outbreaks through isolation of confirmed cases. Discernible benefits can also be readily envisaged for the deployment of a rapid diagnostic system to potential institutional/semi-closed outbreaks of influenza infection – situations where early confirmation of influenza is critical in containing outbreak progression. Furthermore, as a first-of-its-kind rapid influenza test with type and subtype discriminatory capacity, additional value may also materialize for the use of the test in surveillance programs aimed at providing an “early warning” system for characterizing shifts in influenza activity. The laboratories of Professor Zambon (HPA) and Dr. Donatelli (ISS) serve as the registered National Influenza Centre to the UK and Italy, respectively, and as such form part of the WHO Global Influenza Surveillance Network. The consortium is, therefore, well positioned to continually monitor and assess the benefit of the system in supporting or improving the existing global surveillance mandate.

Benefit for SMEs
SMEs will benefit in technology sharing and development and integration of activities. None of the SMEs alone would have the dimension, the scientific background and the resources to develop such competitive

research activities. The proposed project will give to the participating SMEs a competitive advantage not only in terms of technological development and IP but also in terms of interactions and links with the scientific community that will extend beyond the duration of the project. The project will help the SMEs in strengthening their research capability, integrating their technology and translating it into products addressing a clear unmet need that represents an attractive commercial opportunity. The proposed experimental and integration activity will generate additional intellectual property (IP) and proprietary reagents in the field influenza diagnosis, assay development and instrument design.

European dimension

In Europe, research and development activities in the fields of infectious diseases and associated diagnostic assays is currently conducted essentially at the Member and Associate State level, under national or regional research programs, and through private Research Institutions and Universities. In the case of influenza this has resulted in a leading scientific position in several areas including influenza genetics, epidemiology and vaccine development. European leadership has not yet translated into competitive products, particularly in the development of diagnostic assays, because of fragmentation of research at the national and European level. It is emblematic that all existing rapid diagnostic influenza assays that are on the market have been developed and introduced by American companies. The market opportunities in terms of new diagnostic tests are considerable. However, European SMEs will have little chance to exploit this commercial opportunity because of the lack of adequate resources, research capability and dimension. Access to appropriate finance and research facilities is not the only challenge for SME development in the EU. SMEs are still under-capitalised, do not invest enough in research and development and often do not have sufficient links with University and research institutions.

This proposal brings together highly qualified research institutions with a group of European SMEs that have developed a valuable portfolio of technology. The proposed activities will be instrumental in implementing technological developments while stimulating flexibility, investment in knowledge, risk-taking, and the dissemination of information and know-how. This project will provide the SMEs with a complete new dimension in terms of research capability, innovation and interactions thus meeting the vision of multi-dimensional and horizontal innovation policy for the future EU where innovation has to become an integrated dimension of SMEs activities. Here SMEs will benefit with new opportunities for innovation in terms of technological aspects of products and services as well as with intangible value added improved market position and increased research capability.

Dissemination of project results

Internal and external communication is central to the management scheme of this project. The Executive Committee of Fluarray elaborated a communication plan to convey activities, results and accomplishments to the scientific community, health authorities and to both international agencies (WHO, Wellcome Trust, Gates Foundation, NIH, JICA) and national health agencies and funding bodies. Communication to the scientific community included scientific publications as well as the participation in scientific meetings. The project's communication activities also included a web site. Communication to the public has been developed to promote the understanding of new technologies using press releases, annual reports (internet), demonstration visits and lectures for the general public. A project website has also been created and maintained aimed at publicising the aims and outcome of the project. Most participant institutions have a communication office that has excellent interactions with the national and international press and television stations. Fluarray made ample use of these infrastructures to raise awareness in the public and policy makers.

During the whole duration of the project different communication and dissemination strategies were set out depending on the targeted audience: scientific community, the general public, European Commission representatives, public and private sector representatives, policy makers, etc.

They will continue to be addressed and efforts will be focused on the following objectives to exploit the Fluarray results after the conclusion of the project:

i. To disseminate the scientific outputs of FLUARRAY to the international scientific community through the project website, publications, organization of workshops and talks, press releases, technology demonstration visits, etc.

ii. To promote new and strengthen existing collaborations and networks in the field of influenza and infectious diseases during and beyond the project lifetime.
iii. To contribute to the international health scene with tangible scientific results and facts on infectious diseases issues as well as address current pressing issues in the health sector.
iv. To expand the project scientific results to other fields of application. The project expected benefits will interest both the public and the private sector as well beyond the project lifetime.
v. To expand the project scientific results to other fields of application. These will be easily transferrable to other fields of clinical applications where multiplex assays are needed. The benefits will interest both the public and the private sector.

The FLUARRAY website (www.fluarray.eu) was finalized in January 2009 (month 12) and it is currently only available in English. The Home page provides general information about the project (call identifier, funding scheme, funding received by the EC, start date, project coordinator) and a short summary.
The project brochure is available in PDF format on this page. Further information is provided through the following links: Fluarray Project, Partners, Links & Publications, Member Sections and Contacts and can be downloaded in PDF version or printed by users.
A total of five consortium meetings took place during the lifetime of the project in the countries of the project partners to discuss the status of the project and its progress. FLUARRAY representatives participated in peripheral meetings of other satellite projects.
Attendance to conferences, seminars and other projects events did not only serve as a communication and dissemination tool to both the public and the scientific community but also ensured that the FLUARRAY consortium was informed about other project activities.
The UNIPG and MtM teams presented a poster at the International Meeting on Emerging Diseases and Surveillance (IMED 2011) held in Vienna (Austria) from the 4th to the 7th February 2011. A poster titled *"Role of the protein microarray technology in the development of a rapid immunoassay for the influenza virus"* has been presented, describing the project background and results achieved so far. The poster reported that the project was funded by the European Seventh Framework Programme for research and technological development (FP7), and presented the consortium composed by 6 European partners that cooperated to develop a rapid diagnostic system for the detection and type-differentiation of the influenza virus. Meetings and events organized and hosted by the European Commission will be attended where appropriate after the end of the project. A training session for FP7 reporting offered to coordinators from HEALTH-2007-A calls and NCPs was attending by the Project Manager.

FLUARRAY disseminated project information and proceedings on different platforms, peer review journals, posters, including networking with third parties. Updates and further information will continue to be provided in a dedicated Links & Publications section of the project website, where participation to international congresses and other events will also be publicized.
The first project-related publication titled "*An antigen microarray immunoassay for multiplex screening of mouse monoclonal antibodies"*was published on Nature Protocol, Nov 2010 issue.
The Commission undertakes to promote European-funded health research as much as possible through various media, particularly the web and the press. Financial support is given to research that falls under three pillars: 1) Biotechnology, generic tools and medical technologies for human health; 2) Translating research for human health and 3) Optimising the delivery of health care to European citizens. Under the second pillar particular emphasis is given to Emerging and re-emerging infectious diseases and particularly to influenza. An executive summary of FLUARRAY, along with other influenza related projects, is provided in this section (http://ec.europa.eu/research/health/infectious-diseases/emergingepidemics/projects/177_en.html).

On the "Parliament Magazine" of the 7 February 2011 has been published a presentation of the FLUARRAY project and the main objectives achieved so far. The Parliament Magazine is the magazine for the European Parliament and European Commission. It is distributed once every two weeks to all Members of the European Parliament, senior members of the Commission, the Council and various EU institutions. The Parliament Magazine has a hard copy distribution of 3,000. Additionally, the digital magazine is distributed to 30,000 contacts including the public affairs contacts from EPAD (European Public Affairs

Directory), TheParliament.com bulletin subscribers, from EU officials/Commission staff to project coordinators, university and research specialists, public affairs consultants and journalists worldwide.
The 7th February edition had a focus on the *International meeting on emerging diseases and surveillance (IMED).*

Exploitation of project results

The generation of a number of MAbs each recognising a different HA and NA variant will dramatically facilitate the development of a multiplexed microarray assay capable of distinguish influenza subtypes in specimens. In addition, the development of a robust affordable instrumentation will transfer the use of microarray assays to the point-of-care thus addressing an increasing clinical need for multiple assays, increased speed and cost reduction. It is anticipated that this project will provide SMEs with background information, technical skills and innovative technology to generate the following individual products:

1. A portfolio of immunological reagents consisting of MAbs with defined specificity against HA and NA antigens. These MAbs represent a valuable set of tools for research and specific diagnostic applications.

2. SEAC and MtM will produce an instrument that automatically processes the assay device and performs the reading of the array. The instrument will incorporate a LED optical module to read the array and simple robotics to add specimens and reagents to the lateral flow device. This design dramatically simplifies the complexity of the instrument thus making it suitable for point-of-care settings. This instrument will make diagnosis of influenza subtypes affordable and will contribute in bringing microarray technology to clinical laboratories.

3. The device is a key component of the point-of-care system. It combines the simplicity and ease of use of dipstick lateral flow test with the capability to control the incubation time of specimens and reagents over the capture substratum as well as the wash volumes. The technical solution adopted by the device allows the implementation of assay protocol that maximize sensitivity and specificity of the tests that a classical lateral flow device would not permit.

4. An antibody microarray containing a repertoire of MAbs directed against the most common HA and NA influenza variant. This array forms the core of the influenza antigen detection assay. This product meets a clear clinical need for the differential diagnosis of influenza and could be easily transferred to SMEs such as SEAC and MtM that have a vast experience in diagnostic assay production and commercialisation. Information for antigen production, array printing and clinical validation data will be made available to support the development of this product.

5. A number of recombinant influenza antigens will be produced and purified to screen MAbs and generate tracers for the competitive assay. These proteins will find a number of applications outside the scope of the project alone or in different combinations to study the target of the protective B and T immune response. SMEs like P'X and MtM have both the expertise and the commercial interest to further assess these products in collaboration with the pharmaceutical industry.

The technology and the products arising from this project can be easily transferred to other fields of clinical applications where multiplex assays are needed. The SMEs will exploit their position to expand in these niches and to establish new alliances with strategic commercial partners.

The disclosure of foreground is regulated by the Consortium Agreement and the Grant Agreement and the following strategies are compatible with the protection of any IPR, confidentiality or interest of all project partners. Any project results and foreground must be disseminated to the consortium in a timely fashion. The disclosure of any Project proceedings, notices or publications undertaken by individual partners or by the consortium as a whole, must specify that the project has received research funding from the EU's Seventh Framework Programme.

Future dissemination activities

The project results will be disseminated as well after the end of the project. Most of the Europeans are interested in Medical and Health research as the results and the discoveries connected to those researches can affect their quality of life. The visibility on the use of EC contribution in the framework of research projects would have an impact on future research funding and on the increase of the appeal of scientific careers on the young generation. The dissemination activities would remain at European and International level and will be focused on specialized and general audience, in order to involve the scientific community from one side and to improve the awareness and knowledge on specific health topics of the citizens. The project results visibility will be increased thorough the use of information channels and instruments, as carried out during the lifetime of the project. Any notice or publications about the project, including at conference or seminar, must specify that the project has received research funding from the EU's Seventh Framework Programme.

The dissemination of the results after the conclusion of the project will improve the visibility of the topics studied under the project and would create future opportunities to implement the results and achieved additional and more ambitious objectivities in the next future.