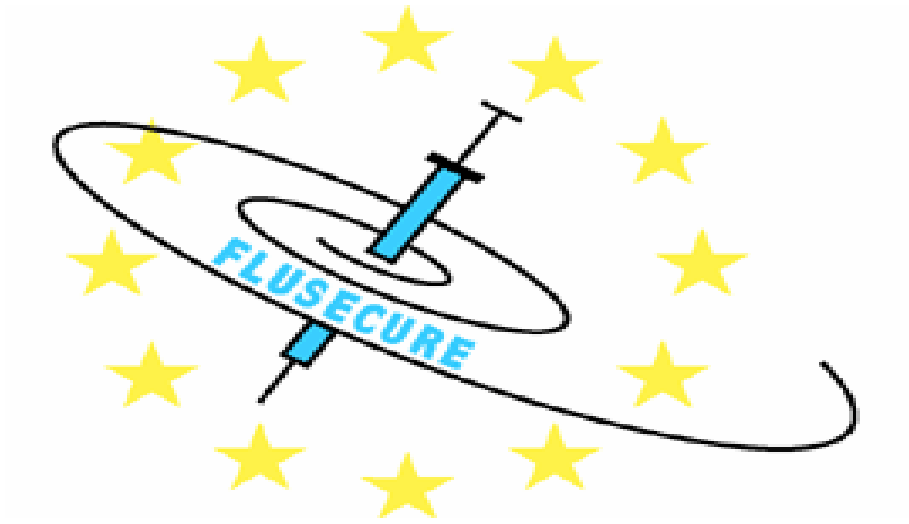


FLUSECURE

Towards sufficiency of Pandemic Influenza vaccines in the EU



TECHNICAL REPORT 2 (August 2006-February 2007)

FLUSECURE

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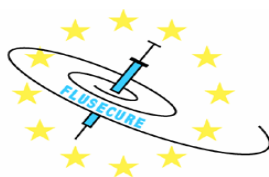


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FLUSECURE PARTNERS



Institute	Abbreviation	City	Country
Health Protection Agency	HPA	Porton Down	Great Britain
Statens Serum Institute	SSI	Copenhagen	Denmark
National Institute of Public Health	KTL	Helsinki	Finland
Norwegian Institute of Public Health	NIPH	Oslo	Norway
Cantacuzino Institute	CI	Bucharest	Romania
National Centre for Epidemiology	NCE	Budapest	Hungary
Slovenia National Institute of Public Health	IVZ	Ljubljana	Slovenia
Robert Koch Institute	RKI	Berlin	Germany
Netherlands Vaccine Institute	NVI	Bilthoven	the Netherlands
Institut Pasteur	IP	Paris	France

WORK PACKAGES AND CONTACT INFORMATION

FLUSECURE Project Work Packages and team leaders:

- WP1. Coordination of the Project
Prof. Dr. Ben van der Zeijst; Netherlands Vaccine Institute (NVI)
- WP2. Dissemination of the results
Dr. Ed Schmidt; Netherlands Vaccine Institute (NVI)
- WP3. Evaluation of the project
M.Sc. Claudine Coenen; Netherlands Vaccine Institute (NVI)
- WP4. Rapid development and production
Dr. Tim Brooks; Health Protection Agency (HPA)
- WP5. Effective vaccines
Dr. Else Marie Agger, Statens Serum Institute (SSI)
- WP6. Preclinical evaluation
Dr. Ernst Soethout; Netherlands Vaccine Institute (NVI)
- WP7. Clinical evaluation
Dr. Terhi Kilpi; National Public Health Institute Finland (KTL)
- WP8. Interface with Industry
Dr. Tim Brooks; Health Protection Agency (HPA)

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EXECUTIVE SUMMARY

TECHNICAL REPORT 2 (August 2006-February 2007)

FLUSECURE represents a network of European public health institutions, with the aim to support an efficient production chain of sufficient pandemic influenza vaccine doses in collaboration with the European vaccine manufacturers. The major objectives have been subdivided into eight different work packages, representing the rapid development and production of new vaccines, optimizing vaccine efficacy, quantitative pre-clinical testing, clinical network development, coordination, communication, evaluation and the establishment of a public private partnership with the vaccine industry.

Since the starting point most of the objectives are developing well and expand as planned. The only exception represents a reserved attitude of the European Vaccine Manufacturers (EVM) towards the FLUSECURE initiative. Solutions include the strategy of communication with individual vaccine manufacturers and the EVM organization simultaneously. Frequent communication and contacts provide the basis for efficient interactions between the different international institutes. Communication takes place in the form of meetings, telephone conferencing, emails and recently also in the form of webconferencing. In the near future we will extend the communication by the establishment of forum sites on the FLUSECURE website.

Progress has been made on a number of fields:

- The first pandemic strains have been selected for the FLUSECURE vaccine library and vaccine seeds are being produced together with the corresponding reagentia. Participating FLUSECURE institutes have direct access to copies of this library. A total of 4 vaccine strains has already been made available for the project, another 6 new strains will be produced within the FLUSECURE project.
- New technologies are being developed to improve the efficacy of vaccine seed stock production. This technology can be applied to increase the number of vaccine seed strains within the library.
- Vaccine formulations with different adjuvants have been used to characterize optimal profiles of immunogenic responses. Single vaccination experiments with cationic liposomes indicate that by using an appropriate adjuvant, the need of two vaccinations can be overcome.
- Parameters in the form of IFN- γ , IL-10 and granzyme B, have been selected as potential correlates of protection for influenza vaccines. Standardized detection protocols are being established for these cellular correlates.
- The establishment of a European network of clinical study sites for pandemic influenza vaccines is underway. So far six centres have been identified. Five have been considered to have adequate resources and experience of clinical trials and are selected for further evaluation.

INTRODUCTION

New emerging virus strains are a continuing health threat. Especially avian influenza is world-wide been considered as a most serious pathogen. The risk from avian influenza is generally low to most people, because the viruses do not usually infect humans. The extreme flexibility in the genetic make-up of influenza virus, caused by unpredicted genetic drift and genetic shift this virus displays, is a fact that can make it potentially the most lethal viruses in the world today. A large variety of animals acts as a reservoir of this virus from which new strains are evolving towards human infection adaptation.

Each year, with the appearance of new varieties, flu spreads around the world in seasonal epidemics. In non-pandemic years alone already hundreds of thousands of people are killed by the flu, whereas in pandemic years millions of people worldwide died. In the pandemic period of 1918, up to 50 million people have been reported to die as a result of such a pandemic, whereas in the mild pandemic periods of 1957 and 1968 approximately one million people were killed.

Influenza pandemics do not necessarily originate from an avian origin. The 1957 pandemic was due to a genetic reassortment between 3 H2N2 genes (including HA and NA) and 5 H1N1 genes from a human strain. The 1968 pandemic virus was the result of 2 H3N2 genes (including HA) and 6 H2N2 genes (including NA) from a human strain. The 1918 pandemic was an avian H1N1 strain adapted to humans.

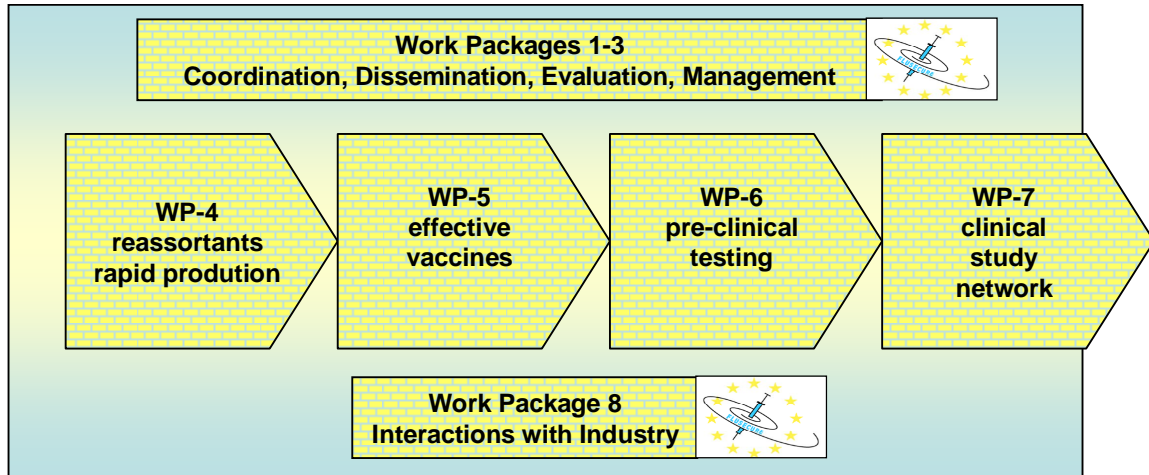
During recent outbreaks of avian influenza, there have been transmissions of H7N7, H9N2 and H5N1 to humans. H5N1 is the most deadly of those that have crossed the avian-human barrier. The H5N1 avian influenza A bird infection rate in Asia and parts of Europe, the Near East, and Africa is not expected to diminish significantly in the short term. It is likely that H5N1 infection among birds has become endemic in certain areas and that human infections resulting from direct contact with infected poultry and/or wild birds will continue to occur. There is little pre-existing natural immunity to H5N1 infection in the human population. If these H5N1 viruses gain the ability for efficient and sustained transmission among humans, an influenza pandemic could result, with potentially high rates of illness and death. Over 277 people worldwide have been infected by H5N1 so far, of which 167 have died as a direct result.

In order to establish an optimal response towards an influenza pandemic threat in Europe, a close collaboration between the public and the private sector is essential. A first step towards this is the formation of a European network of public health institutes in the form of FLUSECURE. This European consortium of influenza expertise will provide a communication platform between public bodies like the WHO, the Commission and national health authorities.

A subsequent major goal of FLUSECURE will be the establishment of a European platform for interactions between the public and the private sector. The vaccine manufacturers within the private sector are essential in the production phase of pandemic vaccine development, a role they already fulfill in the seasonal influenza vaccine production.

The timely production of sufficient vaccine is crucial, and can be shortened by fulfilling several contributions by the public sector as defined in the work packages of this project. With respect towards pandemic preparedness a mutual understanding of the differences in private and public needs is essential. The willingness and the

benefits obtained by collaboration in the preparation for pandemic vaccine development will certainly make a major difference in the case of a major outbreak.



OBJECTIVES

FLUSECURE has been established in order to enable the production and manufacturing of the most effective pandemic vaccine in the shortest possible time in sufficient quantity for the European population. FLUSECURE represents a framework that links the already existing efforts in the field of influenza preparedness planning and stimulates new activities in this field. The FLUSECURE consortium provides an interactive network of European specialists. The participating institutes have an extensive expertise on national vaccination programs or act as national influenza centers and WHO national reference laboratories.

The establishment of a European network of public health institutes in the form of FLUSECURE provides an interface for interactions with vaccine companies. Since these companies provide the essential infrastructure for the final production of pandemic vaccines they are an essential part of the complete chain of pandemic vaccine development. These industries themselves are dependent on the availability of Good Manufacturing Practice (GMP) vaccine seeds, and have to meet the standards set by the EMEA for vaccine efficacy and clinical trial procedures. The establishment of a public private partnership could be realized either with the European Vaccine Manufacturers (EVM) organization, or in direct contact with relevant industrial partners involved in vaccine production. Establishment of a continuous dialogue with these manufacturers will provide a partnership in which the resources for pandemic influenza vaccine development are optimal utilized. FLUSECURE will also provide a discussion platform for currently relevant issues like the industrial focus on pre-pandemic vaccines, cross-protection and new standards set for cross protection and vaccine efficacy.

Shortening the lead-time of vaccine development is possible by the production of a library of seed lots made by reverse genetics or by conventional reassortant methods. The collaborating effort between HPA and NIBSC will provide the basis for the production of a total of 10 (GMP) vaccine seed strains. Each year a set of relevant and potentially pandemic circulating virus varieties will be selected as the target for new GMP seed lot production. The library will primarily concentrate on European pandemic threats. The FLUSECURE library at this stage already contains two H5N, one H7N1 and one H9N3 viral seed strains. A combined strain, with elements of both H7N7 and H7N3, is currently being produced and another 5 reverse genetics strains in the PR8 backbone will be made during the coming years at the NIBSC. All these vaccine seed strains will be available for the participating institutes within the FLUSECURE project. This will provide an important and positive political signal for national governments.

As an alternative basis for seed lot production, a new strategy for reassortant production is being developed during the project by improved reverse genetics technologies.

Vaccine efficacy improvements are an essential part of the project by establishing a dose-sparing optimization protocol. Optimizing the formulation, adjuvants and vaccination strategies are major aspects in these studies. Dose sparing, immunogenic response and long term memory responses are under investigation on the most appropriate adjuvants available for influenza vaccines.

A clinical evaluation of available pandemic vaccines will be accelerated by the establishment of a multi centre clinical trial network throughout Europe. Industry will benefit from this infrastructure in their generation of data for efficacy and post-

marketing surveillance. The establishment of this network is in progress and a first set of study sites have been selected and are being evaluated.

MAIN EVENTS DURING REPORTING PERIOD

Advisory board meeting

November 14-15, Brussels, Belgium [advisory board and NVI, HPA, SSI and KTL]
An informative advisory board meeting took place with the work package leaders and the members of the advisory board:

David Salisbury, United Kingdom. EU Health Security Committee

Vytautas Bakasenas, Lithuania. EU Health Security Committee

Antoon Gijsens, DG SANCO European Commission

John Purves, European Agency for the Evaluation of Medicinal Products

Roland Dobbelaer, Chairman CPMP's Vaccine Working Party

Angus Nicoll, Influenza co-ordinator, European Centre for Disease Prevention and Control

Marie-Paule Kieny, WHO

David Fedson, Academic representative

Bruce Gellin, Department of Health and Humans Health Services (DHHS), USA – Liaison

The report of the meeting is added as annex 1.

Amendments

- The contract between HPA and NIBSC has been signed and work has commenced for production of library strains.
- Transfer of some work package 7 contributions from Institute Pasteur (France) towards the National Centre for Epidemiology (Hungary)
- Extension of the project justification time for 6 months
- Budget reallocations within institutes.

A number of proposed amendments have been incorporated into the grant agreement. The amended contract will be evaluated and adopted in the coming period.

The amended contract is added as annex 2.

Meeting on vaccines, antigens and reagentia production

November 28-29, Bucharest, Romania [RKI, CI, HPA]

A meeting was held at the Cantacuzino Institute to discuss the distributions of tasks within work package 4 in more detail. A report of the meeting is added as annex 3.

Meeting on animal model selection and cross protection studies

January 10, 2007 Bilthoven, the Netherlands [NVI, IP, SSI]

A constructive dialogue with Dr. Sylvie van der Werf of Institut Pasteur, France was started. Participation of IP in work package 5 and 6 of the FLUSECURE project was accomplished. During the talks specific and well-described participation of IP in the animal experiments and contribution in serological analyses was achieved.

A report is added as annex 4.

Meeting on strain selection criteria

January 23, 2007, London UK [HPA-NIBSC-NVI]

As a result of the HPA-NIBSC subcontracting a distribution of tasks between the partners has been organized: including strain selection, vaccine seed and reagentia production. A report of this meeting is added as annex 5.

PROGRESS

Work Package 1-3

MANAGEMENT, COORDINATION, DISSEMINATION, EVALUATION

Work Package 1, coordination of the project

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Work Package 2, dissemination of the results

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Progress and Challenges

Milestones

The first milestone represents the kick-off meeting. A detailed description of this meeting, which took place at March 16-17, 2006 at the NVI, has been provided in the first technical report. The second milestone, representing a dialogue between vaccine manufacturers and FLUSECURE, has been somewhat delayed due to the first reserved attitude of the EVM. Communication with individual manufacturers and with the EVM is ongoing (for details see work package 8).

For the coming period, month 18, a number of milestones have been defined:

- Milestone 3 Midterm report
- 4 Development of evaluation questionnaires
 - 5 Effects of augmenting influenza vaccines identified
 - 6 Study centre network established
 - 7 Obtain manufacturing protocols from industry partners.

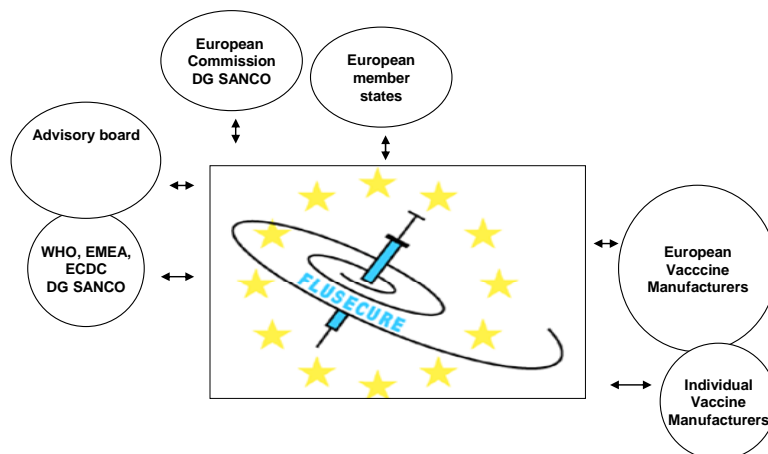
No problems or changes are foreseen in the forthcoming period with respect to these deliverables.

At the start of the consortium a number of problems were identified. Actions have been undertaken during the last 6 months period.

- The subcontracting of the NIBSC by the HPA
- Redistribution of wp 7 contribution from the IP towards the NCE
- Extension of the financial justification time for the project

- Redistribution of budget within individual partners.
All of these issues have been amended into the FLUSECURE grant agreement. This amended contract has been sent to DG-SANCO for approval.
- Communication problems with Institute Pasteur.
Contacts have been re-established with the Insitute Pasteur. Active participation of this partner in Work Packages 5-6 has been secured for the coming period (annex 4).

Communication



Communication within the FLUSECURE consortium, and interactions with public and private partners represents a major objective. In order to optimize communication channels the following actions have been initiated:

- **Meetings**

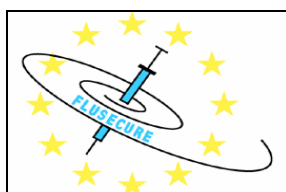
A number of meetings have been organized between FLUSECURE partners

- Advisory board meeting. November 14-15, Brussels, Belgium
- Meeting on animal model selection and cross protection studies.
January 10, 2007 Bilthoven, the Netherlands
- Meeting on strain selection criteria. January 23, 2007, London UK

(for details see the main events chapter and annexes).

- **Website**

In agreement with the project team, and in order to emphasize the European character of the project, the domain name WWW.FLUSECURE.EU has been registered. For direct international recognition of the project, a logo had been developed.



The logo symbolizes the execution of the project. The stars correspond to the EU co-financing. The needle symbolizes the flu vaccination.

The FLUSECURE website, an important tool in the communication network, is currently under development. The public site of the website is operational and new updates will concentrate on FLUSECURE information for the public and relevant links to other information sources.

- **Secured Website**

The secure area of the website will be developed in the coming period, and will provide a discussion forum for the FLUSECURE partners and a “Radar Screen” discussion forum in which the advisory board, DG-SANCO, and experts from the partner institutes will participate. The secure area will also contain a Work Package 8 database with information of the interactions with industry, and the available technologies, products and services for these collaborations.

- **Telephone / computer conferences**

Regular telephone meetings (at least on a monthly basis) take place between the work package leaders, the project coordinator and the project manager. Similar meetings take place between the partners of work package 6. Minutes are made of all meetings and distributed between the participants. Regular communication meetings will be continued and extended to work packages 4 and 5 in the coming period. These will be performed using either multi-telephone equipment or webconferencing technology. Web conferencing using Adobe Breeze will become operational within the forthcoming period.

Control Measures

During the first year, tasks for the main beneficiary mainly involved the coordination and the management involved in establishing an interactive and cooperating FLUSECURE consortium. Therefore, the majority of costs and capacity available to work package 1 have already been exhausted as expected. From the total budget of € 119.813,25, already € 108.231,78 has been realized. From the total available capacity of 265 mandays, already 169,25 have been realized.

The major tasks for the main beneficiary with respect to the remaining part of the project will concentrate on work package 2, dissemination. From the total available budget of € 138.672, so far only € 1.955,11 was realized during the first year. From the total available capacity of 250 mandays, 28,25 was realized so far.

Work package 3 so far has not been operational yet. During the coming period this work package will be involved in the development of questionnaires for evaluation.

Workpackage 1		
	Total three years	Realised first year
Costs	119,813.25	108,231.78
Mandays	265.00	169.25

Workpackage 2		
	Total three years	Realised first year
Costs	138,672.00	1,955.11
Mandays	250.00	28.25

Workpackage 3		
	Total three years	Realised first year
Costs	45,475.00	0.00
Mandays	100.00	0.00

TECHNICAL PROGRESS

Work Package 4 – RAPID DEVELOPMENT AND PRODUCTION

Partners involved

Health Protection Agency (HPA, United Kingdom), Statens Serum Institut (SSI, Denmark), Cantacuzino Institute (CI, Romania), Robert Koch Institut (RKI, Germany), NIBSC (subcontractor)

Workplan

A library of reassortants is being produced and safety tested for release to manufacturers. The resulting library will comprise representatives of high risk H and N types which could form the basis of an initial vaccine production until definitive seed viruses become available. The majority of the library will be derived using the 6:2 reverse genetics process. Classical reassortants in eggs and new technologies based on reverse genetics will also provide additional virus seeds. The strains will be made under a quality system that makes them suitable for use by vaccine manufacturers.

In addition, a small supply of reagents sufficient to allow manufacturers to accurately assess the yield, quality and formulation of any developmental production based on a library strain will be produced. The reagents comprise a sheep antiserum of standardized titer, made by injecting haemagglutinin into sheep (NIBSC) and a quantity of inactivated and sterile whole virus of the reference strain. For the FLUSECURE library strains, CI will manufacture the antigen and calibrate in collaboration with NIBSC.

Progress and Challenges

Library of strains and reagents

A subcontract has been signed with NIBSC to develop a library of strains for FLUSECURE. NIBSC currently has 4 strains in the FLUSECURE library:

H5N1 NIBRG 14: 6:2 reassortant, with HA and NA from A/Vietnam/1194/2004
H5N1 NIBRG 23: 6:2 reassortant, with HA and NA from A/turkey/Turkey/1/2005
H7N1 NIBRG 12: 6:2 reassortant, with HA and NA from A/Hong Kong/213/2003
H9N3 wilype A/Hong Kong/1073/99 (G1 lineage of H9).

A further 6 strains will be produced for this library of PR8 reassortants and safety tested for release to manufacturers. The library will comprise representatives of high risk H and N types. A meeting was held between HPA, NIBSC and NVI in January to decide what new for Europe relevant strains should be included in the library, given the lack of advice from the Advisory board on this issue. It was agreed that other H7 strains (e.g. H7N7, H7N3), H9 and H2N2 strains should be included.

Collaborations have been established between the NIBSC and Dr Derek Smith, Dept. of Zoology, University of Cambridge. Dr Smith previously developed a method called antigenic cartography which aids the analysis of antigenic relatedness of different influenza virus strains. This method should expedite and rationalize the choice of strains representative of various subtypes of influenza A viruses for inclusion in the library. Dr Smith has provided NIBSC with a preliminary antigenic map for H7 viruses. It was concluded from this analysis that strain A/mallard/Netherlands/12/2000 (H7N3) was a prime candidate for inclusion into the library. The virus A/mallard/Netherlands/12/2000, originally isolated in the lab of Dr Ron Fouchier, Erasmus Medical Centre, Rotterdam, Netherlands, was obtained from NIMR, London. A seed stock of the virus was established and is kept frozen at -70°C. In addition, RNA was extracted from the virus. We also received two plasmids from Dr Ron Fouchier: pRF562 (containing the HA gene of A/mallard/Netherlands/12/2000) and pRF581 (containing the NA gene of the highly pathogenic isolate A/Netherlands/33/03[H7N7]).

Starting from viral RNA isolated from A/mallard/Netherlands/12/2000, the HA gene (subtype H7) was amplified by RT-PCR and cloned into reverse genetics plasmid pPolISapITerm (pPST) which contains the human RNA polymerase I promoter and is routinely used in reverse genetics experiments at NIBSC. Sequence analysis confirmed that the resulting plasmid contained a full-length copy of the HA gene and that its sequence was correct as compared to sequences deposited in sequence databases. The NA gene (subtype N7) of A/Netherlands/33/03 was amplified by PCR from the original plasmid pRF581 and inserted into pPST. Sequence analysis confirmed that the resulting plasmid contained a full-length copy of the NA gene and that its sequence was correct as compared to sequences deposited in sequence databases. These two plasmids were transfected into Vero cells together with 6 pPST plasmids containing the 6 “internal” genes of A/Puerto Rico/8/34 and with expression plasmids for the 3 polymerase subunits and NP from A/WSN/33 in order to generate a recombinant vaccine candidate virus. However, no recombinant virus resulted from this initial experiment.

Summary of the results so far:

7:1 reassortant containing the H7 HA from A/mallard/Netherlands/12/2000 and all other genes from PR8 -> successful generation of a virus.

7:1 reassortant containing the N7 NA from A/Netherlands/33/03 and all other genes from PR8 -> successful generation of a virus.

6:2 reassortant containing the H7 HA from A/mallard/Netherlands/12/2000 and the N7 NA from A/Netherlands/33/03 and all internal genes from A/Puerto Rico/8/34 (PR8) -> no virus obtained.

These results indicate that the H7 HA and N7 NA plasmids are viable but that there is an incompatibility between these two glycoproteins when trying to rescue them into a single virus. The viruses obtained above will be further analysed (genotyped) to ascertain the origin of their glycoprotein genes. The transfection/rescue experiments performed to date were done under ‘research’ conditions (at BSL 2+) without any additional quality measures to make them suitable for vaccine manufacture. This approach was chosen as a first and rapid procedure in order to assess the feasibility of generating these viruses by reverse genetics technology. For use as vaccine reference viruses the experiments will be reproduced with the optimized viral combinations

under the NIBSC quality system. This approach should make any viruses thus generated acceptable for all vaccine manufacture.

As alternative production platform on the delivery of vaccine seed lots is being established at the SSI. The goals from SSI are to develop protocols for a rapid reassortment between pandemic influenza stains and vaccine stains of the virus.

At this moment the available reassortant processes may be difficult and involves quite some valuable time, especially in case of a pandemic threat. Furthermore the present technique is directed against the production of vaccines in egg based systems. As a new pandemic virus may be derived directly from birds egg-based vaccines may difficult to produce in sufficient amounts due to low yield and difficulties in producing enough fertilized eggs. SSI is in the process of producing seed strains using 7:1 reverse genetics on viral backbones.

- For initial optimizations a new vector plasmid has been synthesized, in which HA gene have already been inserted.
- GMP certified production Vero cells have been infected with different influenza viruses and high yield viruses have been selected.
- In order to monitor the reassortment several real-time RT-PCR assays were developed. These assays counts specific target for H1, H3, H5, N1 (human), N1 (birds) and N2. Using these assays the SSI is able to detect, characterize and measure the amount of specific genome copies within a certain virus preparation.
- siRNA technology is being applied to optimize reassortment with selected 22mers of silencing-interfering RNA's. These molecules are made synthetically and added to the cells before the infecting virus. At this moment the reassortment efficacy improvements by the application of these interfering RNAs is being tested.

Improving yield

A work plan on improving yield of the current H5N1 - PR8 reassortant virus in collaboration with partners CI and HPA has been detailed and was discussed and approved on a WP4 meeting in Bucharest on November 28/29. Suggested molecular changes in NIBRG-14 include the replacement of the viral M segment of the PR8 parent with homologous segments from other strains as well as exchanges of the cytoplasmic tail and transmembrane regions of the viral surface glycoproteins. RKI has obtaining the required reverse genetic plasmid set for the production of PR8 reassortant viruses. An MTA was signed with the Mt. Sinai School of Medicine to obtain the recombinant PR8 plasmid set including constructs encoding HA and NA genes of a candidate H5N1 strain for research purposes.

Several plasmid constructs that enable the replacement of the PR8 M gene segment have been produced and confirmed.

One of the major tasks of the CI was to develop and to validate methods suitable for studying of the ability of viruses propagation in term on yield (HA and NA): in order to analyze multiple replication cycles on cells of a virus suspension of a suitable multiplicity of infection (MOI) of plaque forming units – by HA titer with different types of erythrocytes and QPCR, a standardization of plaque assays for vaccine strains on MDCK cell cultures/chicken's fibroblasts was performed.

Preliminary conclusion shows that:

- The viruses cause a cytopathogenic effect, thus making a plaque assay possible.
- Size and aspect of the plaques vary greatly depending on the virus strain. Small plaques have been obtained for H1N1 and H3N2 influenza virus strains, whereas large plaques have been obtained for type B influenza virus strains.

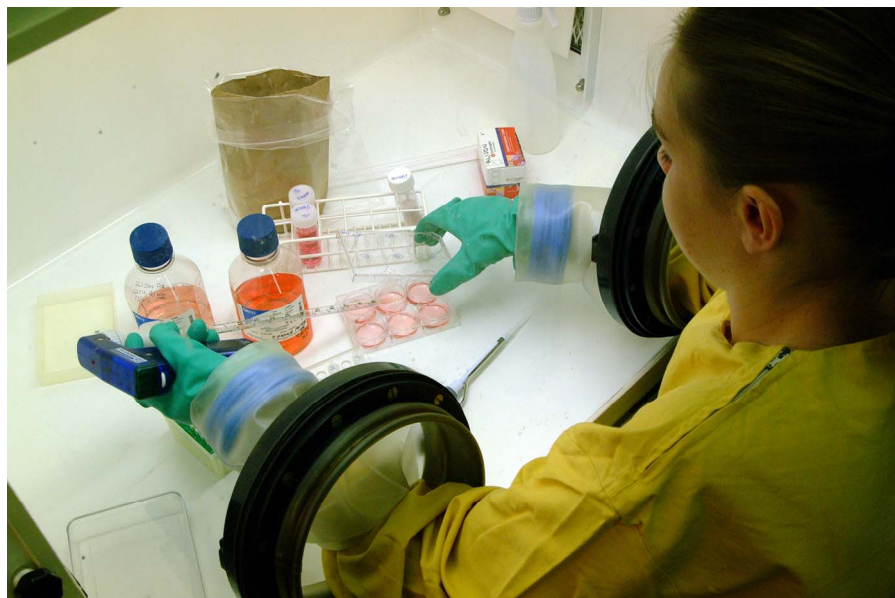
These results will be further extended in order to provide a standardization procedure for testing the performance of vaccine strains *in vitro*.

Seed stock, vaccine and reagent production

The CI activity was focused on optimization of preparation of the primary seed, purification methods suitable for small to medium scale in order to obtain good quality purified virus for further experiments. The activity is described below for each task:

I. Preparation of Primary seed (Master Seed lot) for human (type A, subtype H1N1 and H3N2 - highly growth reassortants, and type B) and avian (H5N1 - NIBRG – 14) influenza virus.

Influenza virus strains were obtained from NIBSC. The viruses used for preparation of primary seed stocks and also the working seed were grown in SPF eggs and incubated at 33-35°C for 48-72 hours. Allantoic fluids were aseptically harvested from embryos who survived inoculation. The mixture of allantoic fluids was aseptically split in 0.6ml portions in vials. The primary seed stocks are preserved either in form of allantoic liquid in liquid nitrogen, or at – 70°C. These conditions preserve influenza virus infectivity. Viruses collected were tested for identity of HA and NA, EID50.



Category 4
Reverse Genetics

II. Generation of associated reagents

For the preparation of working reagents the main focus was on obtaining good quality haemagglutinin antigen. The haemagglutinin protein was purified on sucrose gradients and the purified HA was used to immunize rabbits for the preparation of an anti-serum reagent for single-radial-diffusion assay of NIBRG-14. For the single radial immunodiffusion assay using the antigens containing 60 micrograms of HA activity/ml (NIBSC: A/Vietnam/1194/2004, influenza virus haemagglutinin code

05/204) serum could be used in a dilution 1:10, approximately 15-20µl for 1 ml agarose.

Relationship with Other Work Packages

- Library strains and reagents produced from this work package will be supplied to partners of other work packages as required. FLUSECURE partners in work packages 5 and 6.
- Partners from the other work packages will apply both the vaccine and wildtype viruses in their *in vitro* and animal experiments on vaccine delivery cross-protection and challenge studies.
- Virus neutralization assays will be made available to the partners of work package 5.

Proposed Actions

- It appears that NIBSC is receiving funding from an independent source to look at the problem of improving yields from the reverse genetics strains. This work was also planned by RKI as part of Work Package4. Rather than duplicate the effort on this, a new work plan for this part of the work package that provides added value for both projects and utilizes the skills and capabilities to enhance the projects will be produced. A meeting is being organized by the HPA in March to optimize the collaborative efforts of the different partners with respect to the yield optimization studies.
- The reverse genetics reassorting with siRNA is advancing and it is expected that during the next 6 month a functional system for reassorting new influenza strains has been established. The selection of strains to be reassorted will be coordinated between the members of the WP4 package.

Control Measures

Work package 4 has a total budget of € 1.739.248,74 for the three year period and a capacity of 3.682,5 days. During the first year, total costs of € 230.845.36 and capacity of 566,78 days have been realized. Due to the fact that the expected merging between NIBSC and the HPA has been cancelled, a subcontracting agreement proved to be necessary for the project plans to continue. This resulted in some delays in the actual finances and capacity allocated to these tasks. Extensive details for the justification are available under annex 6.

Workpackage 4		
	Total three years	Realised first year
Costs	1,739,248.74	230,845.36
Mandays	3,682.50	566.78

Work Package 5 –EFFECTIVE VACCINES

Partners involved

Statens Serum Institut (SSI, Denmark), National Centre for Epidemiology (NCE, Hungary), Cantacuzino Institute (CI, Romania), Institut Pasteur (IP, France).

Workplan

An adjuvant formulation system will be used to augment the B- and T-cell responses to influenza vaccines. This will allow the establishment of an immunization strategy in mice.

Influenza vaccine (either commercially available influenza vaccine or H1N1 from Cantacuzino Institute) will be formulated with available adjuvants to test for the ability to augment vaccine-induced response by these adjuvants. In this first set of experiments, BALB/c mice will be administered one or two injections with the influenza vaccine with or without adjuvant. The immunization routes intra muscular, intradermal, intranasally and subcutaneous will be compared for all of the adjuvants.

The specific immune responses will be followed over time after vaccination. Parameters included will be cell mediated immune response, CD8 T cell induction, Cytotoxic responses, serum antibody levels, IgG isotypes, HI assay, virus neutralisation and longevity of the response.

In the next round of experiments the most promising adjuvant and immunisation route will be selected and a more detailed analysis will be performed to investigate the immune response to the different components of the vaccine. This work will identify an optimised vaccine formulation as well as immunisation strategy to be tested in the ferret model in collaboration with work package 6.

Progress and challenges

Optimizing optimal adjuvant profile

As anticipated it was associated with several difficulties to obtain adjuvants for testing with the influenza vaccine. Ideally, the vaccine should be tested in combination with different adjuvants. Adjuvants themselves are only effective with some antigens and not with others. Although there are a huge number of experimental adjuvants, the majority of effective and well-characterised adjuvants are presently under intellectual property rights protection and hence not available for public research. Adding to this, it has become obvious that very few companies involved in adjuvant research/development will render their adjuvant available for comparative evaluations. Initial studies have therefore been limited to a panel of currently available adjuvants: Aluminium, Montanide and cationic liposomes based on DDA/TDB. These three adjuvant formulations induce highly diverse immune responses and therefore provide an indication of the characteristic profile for an ideal influenza adjuvant.

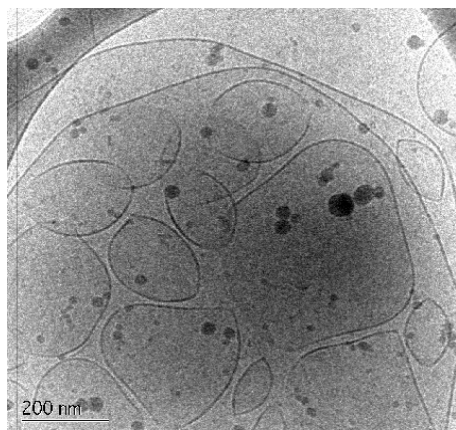
Prolonged effects of adjuvants

The long term response induced by the different formulations has been evaluated 5 months post vaccination. At this time point potent T cell responses were still detected in splenocytes from mice vaccinated with influenza vaccine mixed with adjuvant. Vaccination without adjuvant did not induce a long lasting T cell response. In fact, vaccination without adjuvants failed to induce a detectable IFN- γ response at any measured time-point. The long lasting serum IgG levels were enhanced by adjuvants. All three tested adjuvants enhanced both long term T cell responses as well as serum IgG levels.

Dose regime

The positive effect on the immune response of two vaccinations, as compared to one vaccination, was investigated. As expected, the immune response was boosted by a second vaccination. Serum IgG levels were increased by the booster vaccination both with and without adjuvants. Interestingly, a single vaccinating with the cationic liposome was still more potent than two dose vaccination without adjuvant. This indicates that by using an appropriate adjuvant, the need of two vaccinations can be overcome.

The cell mediated immunity response was also enhanced by a second vaccination when adjuvant was included in the vaccine. However, boosting without adjuvant still could not induce a detectable IFN- γ response. This indicates that it is essential to use adjuvants to induce a sufficient cell mediated immunity response to influenza vaccines.



CryoTEM picture of cationic liposomes

The previous studies have been performed using commercially available vaccines. As this vaccine is already formulated, whole inactivated or a split vaccine was provided by Cantazino Institute and evaluated in parallel. These vaccine preparations were formulated with liposomes. Mice were vaccinated subcutaneously, shown in above experiment to be a potent route of administration for the liposomes. Preliminary testing of these preparations indicated a reduced immunogenicity. However, a dose optimization may improve the performance.

The influenza vaccines from Cantazino Institute were prepared as follows. Preparation of influenza virus type A, subtype H1N1 IVR – 116 (high growth reassortant of A/New Caledonia/20/99, originated from NIBSC), whole virion, inactivated and split virus. Embryonated eggs were inoculated in allantoic cavity with influenza virus strains. After incubation at 35°C for 48 hours, the allantoic liquid was harvested,

clarified at 10.000 g and purified by zonal ultra centrifugation in sucrose gradient. The virus was inactivated with 0.025% formaldehyde, concentrated by ultrafiltration and fragmented with tween-ether. Final filtration was used in order to obtain a sterile viral suspension. The two virus suspensions (whole virion and split virus) were standardized by single radial immunodiffusion assay. The content of the haemagglutinin in the two virus suspensions (whole virion and split virus) have been tested by single radial immunodiffusion assay, using an influenza reference haemagglutinin antigen reagent (originated from NIBSC). List of excipients: sodium chloride, sodium dehydrogenate phosphate, potassium phosphate, 1:10.000 thiomersal; 1:12000 formalin.

Proposed actions:

Further analysis of mouse sera from above vaccination experiments will continue. The isotype of the IgG responses, as well as IgA responses, induced by the various adjuvants is currently under investigation. The Pasteur Institute will evaluate the sera in a HI assay. WP4 will provide a virus neutralisation assay.

The expansion of cytotoxic CD8 T cells by the use of adjuvants will be investigated by FACS analysis with NP-pentamers.

The mouse immunogenicity studies will be followed with challenge studies. Hence, Hungary will provide influenza virus adapted for use in the mouse model (H1, H2, H3) and challenge studies will be performed at the Pasteur Institute. This will include studies on cross protection.

To provide input for WP6 engaged in the pre-clinical evaluation of vaccines, we will initiate testing of influenza vaccine/adjuvant in the ferret model. For this study, only the most promising formulation will be tested.

Parallel experiments using aluminium phosphate instead of aluminium hydroxide will be carried out. These studies are based on an influenza vaccine H5N1 (NIBRG – 14 reassortant of A/Vietnam/1194/2004) prepared to contain whole or split virus with different contents of the haemagglutinin (1, 3 or 15 µg HA/dose), adjuvanted or not with aluminum phosphate in order to test the humoral systemic response after parenteral inoculation in mice (5 animals/lot).

The advisory board recommended the studies to be limited to approved adjuvants only as there is limited time in FLUSECURE that does not allow awaiting approval of new adjuvants. Thus, the future investigations within Work Package 5 will focus mainly on Alum, and cationic liposomes (DDA/TDB). The studies with Montanide will not continue. Further the advisory board recommended to include MF59 if available. Contacts with Chiron/Novartis will be re-established.

Relationship with other work packages

- Work package 5 will provide a virus neutralisation assay for monitoring the efficacy of the vaccine formulations in work package 5.
- Cross protection studies will be performed in collaborations with work package 4 and work package 6.
- The optimized formulations will be available for manufacturers for GMP production, allowing testing in trial experiments in work package 7.

Control Measures

Work package 5 has a total budget of € 975.612,36 for the three year period and a capacity of 2.940 days. During the first year, total costs of € 252.695,83 and capacity of 1.222,61 days have been realized. Comparative studies early in the project have contributed to significant capacity input in this period. The costs at this early inventory stage of the project are limited, but will expand during the later stages. Extensive details for the justification are available under annex 6.

Workpackage 5		
	Total three years	Realised first year
Costs	975,612.36	252,695.83
Mandays	2,940.00	1,222.61

WP 6 PRECLINICAL EVALUATION

Partners involved

Netherlands vaccine Institute (NVI, the Netherlands), National Public Health Institute Finland (KTL), National Public Health Institute Norway (FHI), National Centre Epidemiology Hungary (NCE), Institute Pasteur France (IP)

Workplan

The identification of new Correlates of Protection (COP) in work package 6 is based on 2 complementing strategies, which are based human and animal experimental work.

- The human research focuses on the development, pre-validation and validation of the assays in men. Subsequently, the validated assays will be evaluated for their predictive potential in a clinical trial. It is expected that afterwards, the newly developed tests will be examined in a clinical trial with a suitable pandemic influenza strain. In this trial, the predictive value of the new COP will be compared to the classical HI-tests.
- The animal research will use the same COP as those which are being developed in humans, and will correlate these to protection after experimental infection with H5N1 influenza. Initially, a mouse-model will be used, since in this model the immunological tools are well developed and defined.

To safeguard applicability of the tests that are being developed and most importantly, acceptability of the tests for European authorities, a constructive dialogue with several international organizations will be pursued. A dialogue will be initiated with European legislative organizations to guarantee the acceptability of the essays in the development of vaccines. To this end, contacts with the EDQM and EMEA through the Vaccine Working Party (VWP) are important. In addition, regular contacts with the WHO should enable broad international support and acceptability of the work that is being done in WP6. An established collaboration with the University of British Columbia (Vancouver, Canada; Dr. J. McElhaney) will be important as an external scientific reference, since this group is internationally leading in COP research.

It is to be expected that the newly developed technologies resulting from the research efforts in this work package, will provide a highly improved determination of vaccine efficiency compared with the classical HI-tests. It will not only provide an improved instrument in the pre-clinical evaluation of pandemic vaccines early during development but will also be applicable for seasonal influenza vaccines.

Progress and challenges

Human assays

Previous research performed by the NIPH, the Norwegian partner in the FLUSECURE network, and published findings by UBC (Ca) allowed the identification of IFN- γ , IL-10 and granzyme B production as important predictive

parameters in the cellular immune response. The identification of human COP based on these described findings was divided in four phases:

- a. Practical development of the protocols
- b. Pre-validation
- c. Validation
- d. Testing in clinical trials

An inventory was made of the technical equipment from all partners in the project, in order to define standardized conditions and availability of specific equipment for the assays.

In three of the four phases that were defined in the identification of human COP, progress was made during the last 6 months:

a. Practical development of the protocols.

The basis for the development of these protocols was the literature publications, the technical expertise at NIPH Norway, NVI The Netherlands and at the other partners within the consortium. Several protocols were determined that are being applied in the study design:

- Isolation and counting of peripheral blood mononuclear cells (PBMC)
- Cryo-preservation of PBMC
- Thawing of cryo-preserved PBMC
- Stimulation of PBMC with live influenza virus for Granzyme B and cytokine assays.

b. Pre-validation.

A concise clinical trial was started at the NCE, Hungary. Eight healthy, adult individuals were vaccinated with an aluminium adjuvanted whole virus vaccine. Before vaccination, PBMC were isolated and stored. After vaccination, PBMC will also be isolated. These samples will be used as positive controls in the pre-validation.

c. Validation.

-

d. Testing in clinical trials

At the NVI The Netherlands a clinical trial was started in December 2006, after approval of the trial design by the medical ethical commission (Protocol NVI-240, 'Activation of the cellular response as a correlate of protection for the annual trivalent subunit influenza vaccination in healthy adults between 18 and 60 years of age', see figure). In total 188 healthy individuals enrolled 18-60 years of age, who were vaccinated with the standard trivalent inactivated split vaccine. PBMC were isolated and stored before vaccination and four weeks after vaccination in the second week of January, which was immediately before the start of the influenza season.

The trial is currently ongoing. At the end of the influenza season, the third and last blood sample will be isolated. During the influenza season, all individuals will be monitored for infection with influenza. People will report when symptoms of influenza-like illness occur, after which a nasal swab will be taken for virus identification. Another applied method to detect influenza infection will be based on determining a significant rise in influenza-specific antibody titers during the

influenza season. At the end of the influenza season, a group of influenza-infected individuals and a group of non-infected individuals will be distinguished. The validated assays will be run in both groups, and the results will be compared to determine a correlation between infection and the outcome of the assays.

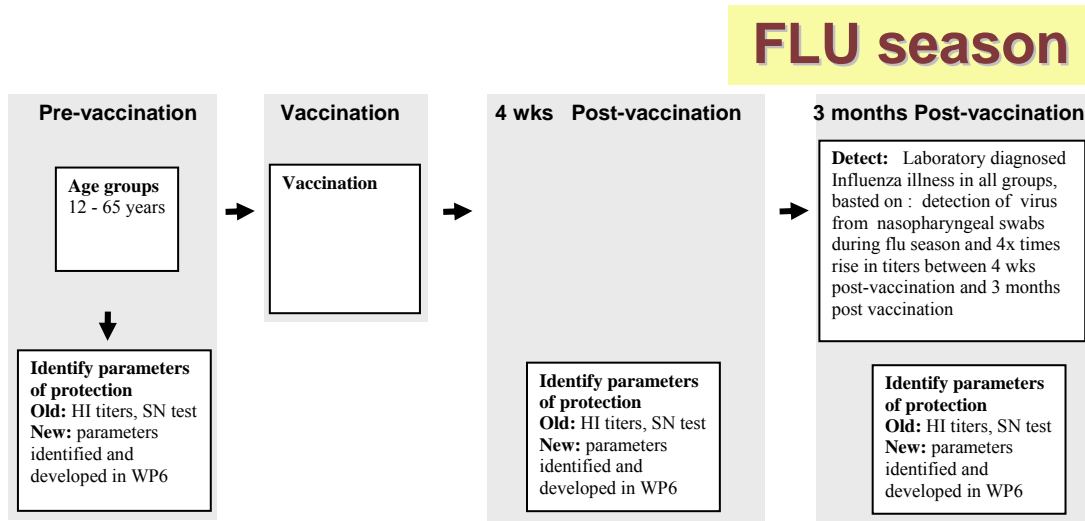


Figure: design of clinical trial NVI-240 for evaluating of correlates of protection

The challenges for the coming period will be to make significant progression in the practical development and pre-validation to enable at a later stage a swift validation and application of the validated assays in the clinical trial.

Animal assays

An intimate collaboration with the partners from SSI was started, to embark on joint animal assays which will serve the goals of both WP5 and WP6.

The animal assays will be started off applying the mouse infection model, since in this species all relevant assays can be performed and because ample experience at the different institutes with the species is available. The experiments and the assays will be based on the COP assays that are being developed for humans. These assays will be adapted to the murine system by the SSI, Denmark and will be described in murine protocols for identification of COP. These protocols will be transferred to IP and will be used for infection studies.

The mouse infection studies will be designed similar to the studies in humans. Cells will be isolated before and after vaccination and the assays will be run on the samples following the murine COP protocols. Subsequently, the mice will be challenged and afterwards they will be followed for protection and partial protection. The outcome of the assays will be correlated to protection from infection.

When the mouse model is successful, several variants will be applied:

- Mice will be infected with avian influenza and COP for avian influenza will be determined.
- An outbred mouse strain will be used in the model to mimic the diverse genetic background in humans

The challenge in the animal assays will be to adapt the human assays to the murine background. Another challenge will be to determine whether the infection model in mice is suitable for these purposes.

Relationship with other work packages

- Strategies to characterize similar COP in a ferret model will later be developed within the FLUSECURE network with partners from work packages 4, 5 and 6. The ferret model is regarded as the standard accepted animal model for mimicking influenza infections in humans.
- Animal experiments will be done in collaboration with WP 5. This will allow a direct coupling of the vaccine/adjuvant optimization studies with the identification and analyses of COP.
- Cross protection studies in animals will be performed in collaboration with work package 4 and 5.
- To connect the results of WP6 to the initiation of a clinical trial network, the results and the tests that will be developed will be made available to the partners in WP7. These tests can then be applied in the vaccine trials provided by WP7.

Proposed Actions

We will proceed in the actions undertaken as described in the roadmap. This means that for the human and animal experiments, the steps will be taken as described. An important factor that will be monitored closely is acceptability of the COPs that are

being developed by important international organizations. Therefore, a dialogue with external organizations such as EMEA and also EDQM and WHO will be initiated and where feasible will be continued for a longer period of time to safeguard acceptance by the international community of the COP that are being developed. In addition, to safeguard acceptance of the COP by the Industry, important vaccine manufacturers as well as the EVM will be contacted regularly to discuss these issues and to inform whether the Industry would be interested in participation in the identification of new COP.

Control Measures

Work package 6 has a total budget of € 1.184.886,01 for the three year period and a capacity of 2.837 days. During the first year, total costs of € 131.468,18 and capacity of only 248.56 days have been realized. This was due to delays in the employment of personnel on the project at the participating institutes. For the coming period the project will be fully staffed and operating at full complement. Extensive details for the justification are available under annex 6.

Workpackage 6		
	Total three years	Realised first year
Costs	1,184,886.01	131,468.18
Mandays	2,873.00	248.56

WP 7 - CLINICAL EVALUATION

Partners involved:

National Public Health Institute Finland (KTL, Finland), Statens Serum Institut (SSI, Denmark), Slovenian Public Health Institute (IVZ, Slovenia), Norwegian Institute of Public Health (NIPH, Norway), Netherlands Vaccine Institute (NVI, the Netherlands), National Centre for Epidemiology (NCE, Hungary).

Workplan

The specific aim of the WP7 is the evaluation of pandemic vaccines in use, by setting up a multi centre clinical trial network and a post marketing surveillance system. After the Kick-off meeting in March 2006, the following action plan was produced:

1. Identification of study centres:
 - When the contract has been signed, the WP leader will contact the other partners by e-mail (month 1).
 - WP leader will request the partners to provide information on potential study centres using a structured questionnaire (month 2).
 - Initial screening of study centres. WP leader will visit the study centres (month 6).
 - Selection of study centres (month 8).
2. GCP audit of study centres:
 - GCP audits will be performed by KTL staff not otherwise involved in FLUSECURE (month 12).
 - The auditors will make the study centres suggestions on improvements needed to meet the GCP standards (month 14).
3. Description of study centres:
 - WP leader will provide a detailed description of the study centres after receiving the audit reports (month 14).

Negotiations with interested vaccine manufactures

- A meeting between the FLUSECURE coordinator, WP leaders and the EVM will be arranged as soon as the contract has been signed (month 1-2).
- Negotiations with individual vaccine manufacturers will also be started as soon as possible after the contract has been signed (month 1-2).

Work plan for clinical trials

- The first version will be produced by month 20 and thereafter the work plan will be continuously updated during the course of the project.

Study protocols

- Each study protocol will be prepared in collaboration between the investigator and the vaccine manufacturer involved in the planned study.

Plan for postmarketing surveillance and clinical studies of a pandemic vaccine

- The plan will be prepared during the third year of the project.

Report on the status of the clinical trials

- The report will be completed by month 36.

Roles and responsibilities of different partners

The WP leader will coordinate establishment of the study centres, provide guidance on adherence to GCP standards, and assist in negotiations between the vaccine manufacturers and individual partners, if needed. The WP leader will also establish one study centre itself. The individual partners are expected to identify study centres and prepare a plan on the steps needed to run a clinical trial in the selected centres. The partners are expected to negotiate with vaccine manufactures on conduct of clinical trials, and run or supervise the clinical trials potentially carried out in the centres.

Progress and challenges

In September 2006, the WP leader, National Public Health Institute (KTL), sent the first structured questionnaires to the WP7 partner institutes for collecting initial information on the study centres potentially included in the study centre network. The identification of suitable study centres has lasted for somewhat longer than expected. Thus far, altogether seven centres in Norway, Slovenia, Netherlands and Finland have been identified. Of these, one centre in Norway has later withdrawn for reasons not known to the WP7 coordinator and another centre in Norway has been identified instead. Of the six centres, five have been considered to have adequate resources and experience of clinical trials and selected for further evaluation.

Detailed questionnaires have been formulated for further evaluation and screening of the potential study centres during the screening visits made by a KTL group. The questionnaires are also used for collecting information for the descriptions of the study centres to be provided to the industry as background information for negotiations on the conduct of mock-up vaccine clinical trials (Deliverable D16). The questionnaires have been/will be sent to the potential study centres before the screening visits. They cover:

- The clinical trial experience of the investigator/organisation conducting the potential trial and competence to fulfil the GCP responsibilities of the investigator.
- GCP and other relevant skills of the staff representing different professions potentially needed in clinical vaccine trials
- The study facilities, equipment, regular practices and services needed for different functions of a vaccine trial, with special emphasis on vaccine and sample logistics and information security
- The size and demographic characteristics of the eligible population, previous experience of recruitment and potential methods of recruitment
- Ethical review, requirements of international, national and local regulations and competence of the investigator/organisation conducting the potential trial to fulfil these requirements

All potential study centres are institutes that currently are busy in providing health care or medical treatment for patients and/or running clinical studies, and it has turned out to be difficult to make appointments for a visit for further evaluation of the centres.

Thus far the KTL group has visited one study centre, the Department of Infectious Diseases, University Medical Center Ljubljana, proposed by the Institute of Public Health of the Republic of Slovenia (IVZ). Collaboration with this centre will be continued. Appointment for a visit has been made with Bekkestualegene in Norway, proposed by Norwegian Institute of Public Health (NIPH, FHI), and negotiations on suitable time for a visit are ongoing with University Medical Center Utrecht in the Netherlands, proposed by Netherlands Vaccine Institute (NVI). Negotiations on the participation of the other Norwegian centre are ongoing. National Public Health Institute (KTL) has identified one study centre in Finland, affiliated by KTL itself. Statens Serum Institut (SSI) is paying efforts to identify a study centre in Denmark.

After withdrawal of Institut Pasteur (IP) from participating in WP7, European Commission has permitted the FLUSECURE coordinator to reallocate some of the funding to the National Centre for Epidemiology (NCE, OEK) in Hungary. In December 2006, NCE signed a letter of mandate for contribution in the WP7. A study centre in Hungary is being searched for.

Relationship with other Work Packages

The first contact and expression of interest from an individual vaccine manufacturer has arrived through Work Package 8. The negotiations of KTL on conduct of a vaccine trial in Finland have been delayed because the manufacturer has problems with the vaccine production, but the negotiations are ongoing. Since there are no concrete plans for clinical trials currently in progress, the collaboration of WP7 with other Work Packages has been practiced on the level of preliminary discussions.

Proposed actions

New contacts with the vaccine manufacturers to start negotiations on clinical trials will be searched for in collaboration with Work Package 8, when the clinical trial centres included in the network have been identified and the information of the study centres for the descriptions is available.

Control Measures

Work package 7 has a total budget of € 1.488.006,54 for the three year period and a capacity of 5.485 days. During the first year, total costs of € 77.693,35 and capacity of 199.36 days have been realized. During the first period, international study centra have been identified. This process started from September 2006 onwards and at this stage, not a major investment in time and budget was involved. The re-allocation of tasks from one partner within this work package (from IP towards NCE) resulted in a delayed start. In the coming period, GCP audit of study centres and descriptions of the centra will be performed, involving a major investment in time and budget (visits, GCP audits). Detailed descriptions for the justification are available under annex 6.

Workpackage 7		
	Total three years	Realised first year
Costs	1488006.54	77,693.35
Mandays	5,485.00	199.36

WP 8 - INTERFACE WITH INDUSTRY

Partners involved

Health Protection Agency (HPA, United Kingdom), National Public Health Institute Finland (KTL), Robert Koch Institut (RKI, Germany)

Workplan

This Work Package intends to set up the links between industry, other key institutions such as EMEA and WHO and the project partners to:

- Tailor the outputs from the FLUSECURE project to the requirements of industry
- Assist industry in contributing to the completion of core dossiers
- To obtain industry approval of FLUSECURE products
- Provide a link between the project, industry and the Advisory Board

Progress and Challenges

Milestones

The first milestone within this work package represents the establishment of a dialogue between manufacturers and consortium. Given EVM's previous reluctance to show any support for FLUSECURE, it is intended to approach individual manufacturers individually in order to secure support for the project and ask the manufacturers how they would benefit from FLUSECURE's outputs.

Collaboration individual manufacturers

Tim Brooks and Jackie Duggan (HPA) met with Solvay in January to discuss FLUSECURE. Solvay expressed interest in Work Package 7 Clinical Trials and this information was sent to KTL. Further negotiations on collaborations between Solvay and FLUSECURE are ongoing. New collaborations will be initiated in the coming period.

Collaboration EVM

Luc Hessel (chairman of the the influenza pandemic working group of the EVM) has expressed interest to Marie-Paule Kieny (WHO) of possible interest in Work Package 6 Correlates of Protection. Similarly, Terhi Kilpi from Work Package 7 has been approached. Luc Hessel has been approached by representatives from FLUSECURE to explore this further and to try to re-establish connection and interest with EVM. A meeting has been organized for the coming period (March 30, 2007) between Luc Hessel and representatives from HPA, KTL and the NVI.

Relationships with other Work Packages

This Work Package acts as a link between industry, related bodies and all the Work Package partners from WP 4-7, allowing key results to be disseminated and negotiating with industry for buy-in into the project, so that the outputs can be tailored to their requirements.

Proposed Actions

- Luc Hessel, chairman of the the influenza pandemic working group of the EVM, has been contacted to discuss the interest of the EVM in FLUSECURE. A meeting has already been planned in the new project period, March 30, 2007, between representatives of the EVM (Luc Hessel) and FLUSECURE (HPA, KTL, NVI).
- HPA will organize to meet individual manufacturers to discuss FLUSECURE project.
- A database will be established on the secured area of the FLUSECURE website in which information will be made available of all possible services, products and technologies developed within the consortium. Communications between partners and manufacturers will be included within this database. Information on established collaborations will be linked to the same database.

Control Measures

Work package 8 has a total budget of € 558.283,63 for the three year period and a capacity of 1.626 days. During the first year, total costs of € 9.092,25 and capacity of 15 days have been realized. In the coming period, the input will be directed mainly on approaching individual manufacturers, which will be a time-consuming effort. Detailed descriptions for the justification are available under annex 6.

Workpackage 8		
	Total three years	Realised first year
Costs	558,283.63	9,092.25
Mandays	1,626.00	15.00

EXPERIENCED OR EXPECTED PROBLEMS

Milestone work package 8

Establishment of a dialogue between manufacturers and FLUSECURE

As stated already in work package 8, the EVM displayed an initial reservation towards the FLUSECURE initiative. Its recent positive attitude, personified in the person of Luc Hessel, chairman of the influenza pandemic work group of the EVM, will be further elaborated on within the coming period.

Individual vaccine manufacturers do show a keen interest in the objectives of the FLUSECURE initiative. Therefore, open discussions have already started with individual manufacturers, not only with the members of the EVM, but also with the significant number of non-EVM companies contributing to pandemic vaccine production.

FINANCIAL JUSTIFICATION

Amount(euro)	Partner	Date
56,672.55	IP FIRST PAYMENT FLUSECURE	17-11-2006
259,126.48	HPA FIRST PAYMENT FLUSECURE	12-09-2006
59,946.78	RKI FIRST PAYMENT FLUSECURE	07-07-2006
194,998.94	SSI FIRST PAYMENT FLUSECURE	07-07-2006
195,001.57	KTL FIRST PAYMENT FLUSECURE	07-07-2006
53,966.60	FHI FIRST PAYMENT FLUSECURE	21-07-2006
47,700.14	CI FIRST PAYMENT FLUSECURE	21-07-2006
35,669.52	OEK FIRST PAYMENT FLUSECURE	21-07-2006
27,761.54	IVZ FIRST PAYMENT FLUSECURE	07-07-2006

Transfers of pre-financing payments

Transfers of pre-financing payments have been made from the main beneficiary to all the participating institutes: Due to the delays in the communication and activities of Institute Pasteur, the first contributions have been transferred to this institute in November, 2006. For the coming period, a further pre-financing payment of € 749.937,18, representing 20% of the amount specified in Article 1.3.3. of the FLUSECURE grant agreement, will be applied for by the main beneficiary. This application is accompanied by the first interim report, covering the technical progress report of and the financial justification for the first year. An overview of the budget and capacity justification has been presented in the description of the work packages. The relatively late start of the employment of FLUSECURE staff has been mainly due to the time-point at which the pre-financing budgets have become available to the partners. This has already been described in the first technical report. In order to solve this problem, a proposal for an extended period for financial justification has been amended into the grant agreement (annex 2).

A summary of the global first year budget is provided in the figure below. A detailed description of the financial justification for the partners and the work packages is available under annex 6.

E1. Staff	570,695.18
a. Costs not pertaining to national officials	271,199.73
b. Costs pertaining to national officials	299,495.44
E2. Travel and subsistence	17,626.70
E3. Equipment	9,046.47
E4. Consumables and supplies	64,000.05
E5. Subcontracting costs	1,955.11
E6. Other costs	81,280.22
Total direct eligible costs	744,603.73
E7. Overheads	23,574.38
Total Expenditure	768,178.11

Global budget first year FLUSECURE

ANNEXES

- Annex 1: Advisory board meeting
- Annex 2: Amended grant agreement
- Annex 3: Meeting on vaccines, antigens and reagentia production
- Annex 4: Meeting on animal model selection and cross protection studies
- Annex 5: Meeting on strain selection criteria
- Annex 6 Financial justification

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