Summaries proposals SOR starting in 2008

Title:	**Chlamydia positivity and prevalence**
Project number:	**S/210056**
Project leader:	Mirjam Kretzschmar (CIb – EPI)
Start:	1-7-2008
End:	30-6-2010
Total costs:	€195.000 (2008-2010)

Motivation

There is an urgent need to develop robust and efficient methods to monitor the success of population screening programs for genital *Chlamydia trachomatis* (CT) infection in reducing population prevalence. The start of a large scale pilot screening program for chlamydia in three regions of the Netherlands (Chlamydia Screening Implementation (CSI)) provides a unique opportunity to do this. First evidence from the English National Chlamydia Screening Programme (NCSP) that started in 2003 shows that positivity measured in population screening may actually be considerably higher than prevalence measured in an independent prevalence study. However, these studies were cross-sectional and did not consider factors associated with the chance of being screened and the risk of infection. This project aims at elucidating the relationship between the fraction of positive tests observed during a population screening program conducted over a longer time period and the prevalence in the target population.

Aim of the project

In the study proposed here we want to unravel the various factors that determine the relationship between positivity and prevalence by drawing together empirical data from various sources and combining it with a modeling study.

Strategic and innovative aspects[1]

The proposed study combines a number of innovative approaches. It uses data from a large scale CT screening project that is set up specifically to supply information about changes in population prevalence based on a stepped-wedge study. This unique data set will be combined with a state of the art mathematical modeling approach to describe the transmission dynamics of Chlamydia.

There is an ongoing debate in the scientific world regarding the explanation of increased notifications of Chlamydia. Therefore the results of this research are of importance not only for the CT screening policy in the Netherlands, but will contribute substantially to the understanding of the effects of screening on Chlamydia prevalence in other parts of the world.

Planned activities

The project can be divided in the following steps:

- Inventory of data sources to look at relationship between positivity and prevalence:
- Collection of additional sexual behaviour data about people being tested for chlamydia, and chlamydia testing practices in health care settings.
- Use results from CT screening pilot as a reference point for the prescreening situation. It will serve as input for model validation.
- Use simulation model to look at various screening scenarios and their effect on positivity and prevalence.

Related projects: Chlamydia-screening (PILOT CT) project; Chlamydia Screening Implementation (CSI) project; various international collaborations[1]

- Implement acquired immunity in the model and explore effects. Aim of this part of the study is to gain understanding of how immunity could impact on incidence if large scale screening is introduced.
- Using the model we will compare prevalence and positivity rates for various screening scenarios: The result will be an assessment of how to interpret CT positivity in terms of population prevalence of C. trachomatis.
- Writing of publications for international peer-reviewed journals.

Planned products

Foremost the project will provide an understanding of how various factors influence and determine the quantitative relationship between CT positivity and prevalence, and how this impacts the validity of using positivity measures to estimate Chlamydia prevalence in the course of long term population screening for Chlamydia infections. From these insights we will obtain:

- Information on what additional information is needed to obtain reliable prevalence estimates from positivity measures
- Conclusions for a more effective design of a screening program (e.g. with respect to screening intervals, re-screening policy for those tested positive, efforts nececessary for partner notification and treatment)
- Reliable prevalence estimates can then be used for estimating the burden of disease for Chlamydia and for cost-effectiveness analysis of intervention measures.

Finally, we think that the methodology and conclusions developed in this project can also benefit the evaluation of other large scale screening programs, because many questions will be encountered in similar ways when screening and testing for other infectious diseases.
In particular, we envisage the following articles as possible output:

- Epidemiological analysis of the data available in the Netherlands relating positivity and population prevalence
- Modelling study analyzing the temporal effects of population screening on the relationship between positvity and prevalence
- Modelling study analyzing the effects of acquired immunity on re-infection rates, positivity and prevalence

Manuscripts of these articles will be prepared by mid 2010. All publications will be prepared in close collaboration with the CSI project team and will include CSI project members as co-authors where appropriate.

Foreseen follow-up

On the national level, public health policy makers who have to take decisions on how to design future prevention strategies for Chlamydia infection will benefit from the results of this study.
On an international level, the results of this project will contribute to a better interpretation and evaluation of large scale chlamydia screening programs and will therefore benefit all countries that have already implemented or are planning to implement national chlamydia screening programs.
More generally, the analysis to be conducted in this project will contribute to evaluating large scale prevention programmes by generating insight into how well positivity of testing of a target population can represent population prevalence and what additional information is necessary to obtain reliable prevalence estimates.

Title:	**Ticks: Trojan horses with new surprises**
Project number:	**S/330116**
Project leader:	Hein Sprong (CIb – LZO)
Start:	1-5-2008
End:	30-4-2012
Total costs:	€400.624 (2008-2012)

Motivation

There are indications for increased prevalence of the bacterium *Rickettsia helvetica* in the Netherlands. These pathogens are maintained in natural cycles involving mammals and ticks. Ticks live on animal and human blood and are important vectors of diseases such as Lyme disease. *Rickettsia helvetica* was recently identified in European sheep ticks all over Europe. In our annual surveillance of tick-pathogens we have consistently identified *R. helvetica* in a high percentage of ticks over the last 5 years. The prevalence of *R. helvetica* was comparable to that of *Borrelia burgdorferi* (Lyme disease) and peaked up to 65% in certain areas. Human infections with *R. helvetica* have not been reported in The Netherlands, most likely because of unawareness and unspecific flu-like symptoms. These findings raise the question whether *R. helvetica* exposure through tick-bites constitutes a risk to human health. In this project research will be conducted to investigate if diseases caused by *R. helvetica*, are an emerging risk to public health.

Aim of the project

The general aim of the project is to setup and implement molecular and serological assays for the detection of Rickettsia species in ticks and human material.
The specific objectives are:

1. Determine the pathogenicity of *R. helvetica* infection after a tick-bite in a cohort of persons with recent tick exposure.
2. Determine the presence of different Rickettsia species in the Dutch tick population.
3. Determine whether parts of Ricketssial genomes have recombined with the tick genome.
4. Determine the prevalence of *R. helvetica* infections in the Dutch population.

Strategic and innovative aspects[2]

RIVM-CB has the task to monitor current and emerging infectious diseases. An increasing number of spotted fever Rickettsiae species have been associated with human diseases and may pose a threat to public health. At present *no R. helvetica* specific assays are commercially available. Tick-borne diseases like *Borrelia burgdorferii*, the Lyme spirochete are emerging in the Netherlands so it is essential to be actively involved in research regarding rickettsial infections. The extraordinary finding of *R. typhi* and other rickettsial DNA in Dutch ticks will undoubtedly have serious consequences: Either as (re) emerging zoonoses or as a potential source for virulent genes for currently non-pathogenic micro-organisms in ticks.

Planned activities

The project can be divided in 4 main activities:

1) Isolation of Rickettsiae species from the Dutch tick population

For the detection of Rickettsiae in ticks 16S RNA sequences are amplified by PCR, and subjected to Reverse Line Blotting or sequencing. Attempts will be undertaken to isolate *R.*

[2] This project is related toV/210690/01/SO Infectieziektebestrijding: ondersteuning en signalering ,S/232101/01/AA Bioveiligheid, V/600000/01/AE: Virale diagnostiek, S/230126/01/PP: SOR-project Proteomics for population screenings, V/330021/01/TZ: Landelijk tekenbeet onderzoek, V/330160/07/TE: tekenoverdraagbare aandoeningen (VWA)

typhi from ticks by cultivation in mammalian cells. In parallel, *R. helvetica* will be isolated from Dutch ticks and cultivated in mammalian cells for the generation of *R. helvetica*-specific antigens.

2) Detection of antibodies to Rickettsiae in the Dutch patients
The second step is to develop and implement *R. helvetica* specific serological assays. Validation of the final assays will be done with sera from our routinely used serological assays for the detection of antibodies to different rickettsia species and in collaboration with partner laboratories in Europe. When specific techniques are established a unique biobank of serum samples from a prospective tick study where people where included with a tick bite who visited the general practitioner will be tested.

3. Molecular identification of different Rickettsiae species
Several molecular targets for the sensitive and specific PCR amplification of different rickettsia species need to be established. Two essential and independent genes will be selected, and a PCR based technique will be set up. The different Rickettsiae genospecies in the Dutch tick population will be determined by Multi Locus Sequence Typing using Bionumerics. Finally, the prevalence of the different Rickettsia species in the Dutch tick population will be determined.

4. Lateral gene transfer between Rickettsiae and ticks
Similar approaches as described in Hotopp et al., 2007 will be used to look for lateral gene transfer: Fluorescence in situ hybridization with fluorescein-labeled probes of Rickettsia 16S RNA and other sequences should reveal their integration in the banded polytene chromosomes of ticks by fluorescence microscopy. A complementary approach for the (near) future is to obtain full access to the genome database of *I. scapularis* . Using BLAST searches with Rickettsial sequences on full-length sequences of the (partially) assembled *I. scapularis*, will allow us to see whether genetic integration occurred between Rickettsiae and ticks.

Planned products
1. Development and implementation of serological test for *Rickettsia helvetica* and molecular techniques for the detection of Rickettsia species in ticks and tissue samples at the RIVM, resulting in several standard techniques and assays for the detection of *Rickettsiae* in the future.
2. “Vital knowledge” concerning the prevalence and mechanisms of (re-)emerging zoonoses.
3. PhD thesis, with several (peer-reviewed) publications.
4. Network with researches involved in the development of techniques for human screenings purposes.

Foreseen follow-up
This project will have benefits for the RIVM and the public health services in the knowledge of a field that have been reported as emerging in Europe. Aim of this project is to develop and implement assays to be better equipped to study these emerging bacteria.
We try to implement the serological assays in ongoing SOR research regarding the development of proteomics based techniques because this system has enormous potential to be used as a tool to screen for many types of clinical syndromes or outbreaks.

Title:	**Transmission intervention stratagies**
Project number:	**S/230156**
Project leader:	Dr. Ir. E. Duizer (CIb – LIS)
Start:	1-1-2008
End:	31-12-2011
Total costs:	€331.100 (2008-2011)

Motivation
Food borne illness has been documented for several groups of pathogens that infect persons after oral ingestion. Viruses, and foremost noroviruses (NoV), are currently recognised as major food borne pathogens in industrialized countries. Contamination control often relies on methods detecting the presence of indicator organisms such as bacteriophages or E. coli. However, data obtained with these methods do not correlate with the presence of viruses. Moreover, molecular based tools to detect the presence of viral RNA (or DNA), does not necessary indicate infectious viruses. State of the art approaches are needed to obtain inactivation profiles for noroviruses (and other food borne viruses) to be able to determine viral infectivity reducing methods in the food production process and areas. Ultimately, this will allow us to draft protocols that will reduce the number of infections due to either food borne or environmental transmission.

Aim of the project
The aim of this project is to reduce the burden of (food borne) fecal-oral viral infections by presenting science based protocols for transmission intervention and to provide a tool for the assessment of the likelihood of food borne transmission of (emerging) viruses.

Strategic and innovative aspects[3]
The innovative aspect of this project is that human NoV strains will be used to determine the infectivity in stead of model viruses. The detection method is based on a novel low-shear-stress rotating cell culture system for three dimensional tissue-like aggregates.
An up to date tool-database system for the assessment of likelihood of food borne transmission of emerging viruses is relevant to enable implementation of effective intervention strategies. This approach is new and timely and the result will allow us to maintain our leading role in the world in the field of viruses and food safety.

Planned activities
The project can be divided in two research lines (1 and 2) of which each has several objectives:

1. Development of tools for the assessment of food borne transmission of viruses.
    - Implement the NoV infectivity assay.
    - Perform laboratory experiments to assess temperature, chlorine and pH dependent inactivation, the effectivity of commercial available disinfectants and the sensitivity for freezing/thawing cycles, drying and UV radiation of several enteric viruses.
    - Perform laboratory experiments to fill the gaps in data needed for the database on likelihood of food borne transmission.
    - Draft criteria for the extrapolation of inactivation data obtained by molecular detection methods to rates of reduction in infection.

[3] Related projects:V/230461/01/EV Transmission and control of enteric viruses, V/300100/07/AA Voedsel gerelateerde virusinfecties, ENVIRONET, V/330140/07/VO Virussen in Voedsel

- Develop criteria and features that determine the success of viruses for food borne transmission.
- Produce a tool to assess the likelihood of food borne transmission.

2. Production of protocols for intervention of the transmission of food borne viruses.
- Analyse food production chains
- Implement detection methods for viruses on food
- Assess viral load reduction in post-contamination treatments of foods.
- Test measures to prevent food contamination.
- Draft protocols to increase bacterial and viral food safety.

Planned products
- Techniques and methods, standard operating procedures for the detection and inactivation of noroviruses and protocols for the prevention of contamination
- A database containing structural virological information and inactivation profiles
- Vital knowledge on effective disinfection methods for the enteric viruses will be generated as well as a tool (database) to support in assessment of likelihood of food borne transmission of emerging viruses.
- Development of networks. The interchange of expertise's and strengthening the collaboration between RIVM and other research groups is a major asset of this project.
- Fact-sheets with the data obtained on the inactivation profiles.
- Progress reports for the graduate school VLAG and the SOR programme.
- Five peer reviewed publications
- PhD thesis

Foreseen follow-up
This project will contribute directly and indirectly to the production of science based protocols for the intervention of transmission of enteric viruses. As such the CIb (foremost LCI), the public health services (GGD) and food handlers and food industry will benefit from the results. Since part of the project will aim at developing, evaluating and implementing assays to study the effectivety of intervention/inactivation methods for viruses, we will be better equipped in the future to produce data needed for risk assessment of emerging viruses. Additionally, the database that will be constructed for the assessment of likelihood of food borne transmission of viruses will be maintained and put to use if viruses do emerge.

Title:	**Zoonotic helminth infections and allergy**
Project number:	**S/230166**
Project leader:	Dr E. Pinelli (CIb – LIS)
Start:	01-01-2008
End:	31-12-2011
Total costs:	€220.000 (2008-2011)

Motivation

In the Netherlands, different animal species are infected with helminths such as *Trichinella*, *Toxocara, Echinococcus*, and *Ascaris* that can also infect humans. A study carried out at the RIVM showed that the *Toxocara* seroprevalence is 19 % on average, with 4 % to 15% in people younger than 30 years up to 30 % in people older than 45. A recent serological survey revealed that pigs are often infected with *A. suum*. Dutch citizens could be exposed since eggs of *A. suum* have been found in sewage sludge that is widely used as fertilizer.
Evidence from various studies suggests that helminths modulate the host immune response and affect other immunopathologies such as allergies. To get a better understanding of the mechanisms involved in the negative or positive correlation between allergic asthma and helminth infection, early immune responses have to be investigated in detail.

Aim of the project

The aim of the present project is to identify helminth antigens and their role in immunoregulation (studies carried out within Cib-6) and to use the identified antigens in order to:

- Improve and/or develop new assays for the serodiagnosis of endemic helminth infections.
- To evaluate the effect of different helminth antigens on experimental allergic asthma. This study will allow us to test the working hypothesis: The effect of helminth infection on allergy depends on the helminth species involved. Therefore, antigens derived from different helminth species will affect allergic manifestations differently.

Strategic and innovative aspects[4]

In The Netherlands serodiagnosis of infections with *Toxocara spp., Ascaris spp.* and *Trichinella spp.* is only carried out at the RIVM. The use of purified antigens derived from different helminths will be crucial in the attempt to improve the currently available assays. Serodiagnostic assays with higher specificity and sensitivity may have worldwide application. Emerging evidence indicate that infection with helminth affect the outcome of allergic manifestations. Identifying parasite antigens capable of suppressing allergic manifestations will open possibilities to develop novel therapeutics to prevent allergic diseases

Planned activities

Effect of helminth antigens on APC maturation

This research project will start by characterizing the effect of helminth antigens on APC maturation. Maturation of APC will be evaluated by measuring expression of cell surface molecules and induction of cytokines by flow cytometry and ELISAs/RT-PCR respectively.

Evaluation of purified helminth antigens and synthesized neo-glycans on APC maturation and T cells stimulation.

Different glycan molecules present in *T. canis* E/S antigen have already been identified in a previous study and have been synthesized. The effect of neo-glycans TSL-1 and gp45 on DC

[4]Related project: SOR project Cib6; Diagnosis of human parasitic infections (Cib/LIS/PAM); Surveillance of parasitic animal infections (Cib/LZO/Parasitology).

and macrophage maturation will be followed by measuring expression of cell surface molecules and cytokine induction as mentioned above.

Evaluation of the effect of purified helminth antigen on experimental allergic asthma
Mature DCs that have been exposed to the helminth antigens of interest (based on results from *in vitro* studies) will be transferred by intraperitoneal injections to naïve BALB/c mice. Mice will then be exposed to the OVA-sensitization/challenge treatment and experimental allergic asthma will be evaluated by histological examination of lung tissue, cytokines and antibody production.

Improvement of serodiagnosis of helminth infections
Several antigens will become available during the first years of this project which will be used to improve the serodiagnosis of helminth infections.

Planned products
Our study will lead to the following publications in peer reviewed journals in the field of immunology/parasitology/glycobiology:

- Effect of different Helminth antigens on APC maturation. To be submitted December 2008.
- Effect of helminth antigens and neo-glycans on APC maturation and T cell priming. To be submitted November 2009.
- Effect of helminth antigens on experimental allergic asthma. To be submitted December 2010 .
- Improvement of serodiagnosis of helminth infections. To be submitted December 2011.

Other foreseen products are:

- Availability of purified helminth antigens that could be used in the development of diagnostics tools for helminth infections.
- The methodology for DC transfer could be used with other models and molecules of bacterial and viral origin with immunomodulatory potential.
- "Vital knowledge" at the RIVM and increased collaboration with universities in the Netherlands and abroad (UK, Italy, France).
- Building and extending a network in the field of parasitology and Immunology will facilitate knowledge exchange and the possibilities for future grants.
- PhD thesis.

Foreseen follow-up
Results from this project will contribute to the fields of:

- Immunology, by getting a better insight into the role of helminth antigens in immunomodulation. Studies on the identification and characterization of purified helminth antigens are limited. However, the availability of molecules that could suppress immunopathologies such as allergies could have therapeutic application.
- Parasitology. Results from these studies will contribute to our knowledge on the characterization of purified helminth antigens and on information regarding parasite-host interactions.
- Glycobiology. Studies carry out in this study will provide information on the function of the synthesized neo-glycans and their use in immunomodulation serodiagnosis.

The Cib/RIVM will also benefit from this project since the immunological studies carried out in this study fits within the efforts in stimulating and improving immunological research within the Cib/RIVM. This will materialize in the future in the form of publications in peer reviewed journals, additional grant applications, PhD thesis and in the field of diagnostics in SOPs describing new tools for the diagnosis of toxocariasis and trichinellosis both important for humans and veterinary parasitology.

Title: **VITAL: Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains**
Project number: V/330274/01/VI
Project leader: dr. Ana Maria de Roda Husman (LZO)
Start: April 1st 2008
End: March 31st 2012
Total costs: €200.000,-

Motivation
In recent years, Europe has seen several outbreaks of viral infection where contaminated food was implicated as vehicle of transmission. Although the associated diseases are so far mild, such outbreaks have economic implications to communities. The types of foodstuff most often implicated in viral disease outbreaks are those which are eaten raw or only lightly cooked, such as soft fruit, salad vegetables, and shellfish. Contamination of foodstuff can occur at different stages of food production. Current HACCP measures in food production are directed towards reduction of bacterial – not viral – contamination. The existence of enterically transmitted viruses from both human and animal sources, and susceptible routes whereby they may contaminate the foodstuffs, thus constitute a present threat to European food safety. Without active measures to monitor and control virus contamination of food, the impact of foodborne viral disease will remain, and possibly increase if current or new viruses emerge.

Aim of the project
The concept of VITAL is the integrated risk assessment and management of contamination of the European farm to market food supply chain by pathogenic viruses to:

1) acquire data on virus contamination of food and environmental sources suitable for quantitative viral risk assessment
2) assess foodborne viral risks for determining high risk situations and efficacy of interventions
3) develop new measures to prevent virus contamination of foods and the environment
4) develop and assess measures for virus reduction and control in case of virus contamination.

Strategic and innovative aspects[5]
The output of the project relies on quantitative virological risk assessment, which in the end will direct the identification of good management practices that reduce viral contamination of foodstuffs. CIb plays a vital role in the output of the project by leading the data analysis work package, which includes the risk assessment. Because the quality of the risk assessment depends on the quality of included data, CIb will automatically play a vital role in the data gathering work packages by developing effective sampling schemes for the respective food chains. This provides CIb with a central role in the development of quantitative virological risk assessment, a currently unexplored discipline worldwide. Furthermore, within VITAL three PhD students are contracted, one of whom will be supervised by the RIVM. The assigned SOR budget will be used to fund this PhD-student and his/her research.

Planned activities

[5] This project is a cooperation between the RIVM and the Central Science Laboratory (UK), Veterinary Laboratory Agency (UK), Catholic University of Leuven (Belgium), University of Barcelona (Spain), Instituto Tecnológico Agrario de Castilla y León (Spain), University of Ljubljana (Slovenia), Veterinary Research Institute (Czech Republic), University of Helsinki (Finland), University of Patras (Greece), Instituto Superiori Sanita (Italy), Wageningen University and Research (Netherlands), National Veterinary Research Institute (Poland) and the Scientific Veterinary Institute "Novi Sad" (Serbia).

The project focuses on norovirus (NoV), hepatitis A virus (HAV) and hepatitis E virus (HEV) as specific foodborne viruses. As these viruses may be present at undetectable levels, source-specific adenoviruses (human, bovine and porcine) are monitored as well as indicators for faecal contamination from respective sources. The presence of these viruses will be examined during production, processing and retail for the following food chains in three or four different European countries: soft fruits, vegetables and pork. Furthermore, presence of viruses in shellfish will be monitored at retail in three different European countries. In addition to the monitoring of viruses along the food chain, effects of specific measures to reduce or eliminate viruses will be studied.
VITAL focuses on all phases of food production: primary production, processing and retail. Four distinct tasks will be completed within VITAL. First, a preparatory phase will be initiated to harmonize virus-detection procedures including proper internal and external quality controls, and to identify critical control points according to HACCP guidelines for the production and processing phase. Second, samples will be collected at these critical control points for each food production chain and analyzed for viral contamination. By assessing viral load before and after a critical control point, the increase or decrease of viral contamination can be estimated. In addition, laboratory and pilot-plant experiments will be conducted to obtain more refined virus-specific inactivation and reduction data for the control points. These studies are the three PhD projects initiated within VITAL. The SOR-funded PhD-student will specifically study virus inactivation. The data needs in the risk assessment will direct the choice of specific measures or treatments to study. Fourthly, data will be sent to CIb for statistical analysis and use in quantitative virological risk assessment. The final risk assessment model will subsequently be used to estimate effects of possible intervention measures. The effects will be evaluated ultimately in the field.

Planned products

1. Guidelines for collection of data that feed directly into quantitative viral risk assessments
2. A statistical tool to quantify virus counts and to estimate elimination and reduction of viruses due to treatment
3. Insight in virus inactivation and elimination for HAV, HEV, NoV and adenoviruses
4. A modular process risk model for quantitative virological risk assessment
5. Handbooks for the food industry prescribing HACCP measures that reduce virus concentrations

Foreseen follow-up

After handing the handbooks and protocols to the industry, actual effects of intervention measures on viral contamination of foodstuff should ideally be examined in the long run. Furthermore, prolonged support to the food industry is wanted to ensure correct and consistent implementation of proposed measures. A subsequent project may be initiated with interested partners.

Title:	**Preparatory study for a National Survey of primary care**
Project number:	**S/270176**
Project leader:	Dr. N. Hoeymans (VenZ – VTV)
Start:	01-01-2007
End:	31-12-2008
Total costs:	€ 176,800

Motivation

The National Survey of primary care is a continuation of the first and second National Survey of General Practice. These surveys are performed by the National Institute for Health Services research (NIVEL) in 1987 and 2001. Because the results are relevant for the RIVM, this institute cooperated in the Second National Survey. A special feature of the national surveys is that the demand for care and the supply of care are studied from the perspective of both patients and caregivers. The research therefore consists of several modules, as registrations of contacts between patient and doctor, interviews and videotaping of consultations.
The current project is a preparatory study for a third National Survey, by now called the 'National Survey of primary care'. An important difference with the former two National Surveys is that the scope has been widened from the general practice to primary care in general. This means that the study also includes care by physiotherapists, primary care psychologists and obstetricians. The RIVM will also cooperate in this 'third' National Survey.

Aim of the project

The goal of this preparatory study is to write a project plan for the National Survey of primary care. This takes the following steps:

- Describe the societal developments and changes in the health care system
- Consult important partners in this survey: representatives of care givers, patients and policy makers
- Incorporate research questions of scientific partners
- Formulating the research questions
- Work out the design and estimated budget of the survey

Strategic and innovative aspects

Investing in a new National Survey is of strategic importance for the RIVM, because this study delivers information for both the Public Health Status and Forecast and the Dutch Health Care Performance Report. These products come out at a continuous basis. Therefore it is vital that the information can be updated at a regular basis. One of the innovative aspects of this Survey is that it results in a continuous registration in primary care, just like the Second National Survey resulted in a continuous morbidity registration in General Practice. Other innovative aspects of this survey are the study of prevention in primary care and innovative forms of collaboration within primary care.

Planned activities

1. Describe the importance of the study in the context of societal changes, and make a first draft of the research questions.
2. Consult important partners in this field of study with the question what this study can mean to their practice. With this inventory we can refine the research questions.
3. Think through the design of the study and the budget.
4. Find financing for this project

Planned products

The product of this project is first of all a project plan describing the importance, the research questions and the design of the survey. The real product is or course a scheme for the performance of the National Survey in primary care, in such a way that the study can start in the beginning of 2009.

Foreseen follow-up

If this preparatory study succeeds, a new National Survey will be performed. This survey will bring relevant information for different products of the division of public health and health care of the RIVM. In addition, because the study is designed on the basis of questions from policy makers, care givers, patients and scientists, the results will be relevant for enhancing health and health care policy, practice and scientific research. Just like its predecessors, the National Survey will be the source of many policy documents, reports, scientific publications and doctoral theses.

Title:	**BIOTHREE**
Project number:	**S/370030**
Project leader:	Dr. D. M. Barends (VGC – KCF)
Start:	1-4-2008
End:	1-10-2010
Total costs:	€276.469

Motivation
In vivo bioequivalence (BE) testing is the classical methodology for assuring efficacy and safety of generics. But *in vivo* BE testing is relatively expensive and reliable *in vitro* BE tests could be a very important contribution to the availability of affordable, yet effective and safe medicines. Amidon and co-workers (1995) developed a comparative *in vitro* dissolution testing of the generic drug product versus the innovator as a surrogate BE test. What, however, is lacking is a validated surrogate test able to detect differences in GI permeability *in vivo* between two drug products. BIOTHREE will develop such an *in vitro* BE test.

Aim of the project
BIOTHREE will develop a new *in vitro* BE test and hence extending the possibilties of *in vitro* BE testing to BCS Class III APIs.

Strategic and innovative aspects[6]
Generic substitution is the single major tool available to limit the costs of pharmaceutical care. This holds for all countries, and also for The Netherlands as generic substitution is strongly promoted by the government and health assurance companies. So, biowaiving of BCS Class III immediate release drug products is important for all countries but most urgent for developing countries and a major objective of the WHO.
In addition to the scientific outcomes, the additional benefits for RIVM, will be:
• Ph.D. opportunity for an RIVM employee.
• Sponsored practical work for KCF laboratory.
• New field of competence for KCF laboratory: bioanalytics.
• More, and more important, assessments of claims of BE.

Planned activities
The methods used in BIOTHREE are the Caco-2 cell-culture membrane system will similar as the methods used at the Pharmaceutical Quality Research Institute (PQRI) in the USA.

- From the results of the two PQRI studies, three APIs from the five studied by the PQRI will be selected as model APIs for BIOTHREE.
- From the results of the PQRI studies 9 excipients studied in BIOTHREE will be selected.
- Reference drug products will manufactured from purchased innovator tablets that will be grinded and formulated in capsules.
- Worst-case test drug products will be manufactured; being the same grinded innovator tablets, but with added known amounts of excipients to be tested.
- From each model API, one reference product and two test products will be manufactured.
- Reference and test products will be manufactured under a license to produce clinical test material.

[6]BIOTHREE is a project under the umbrella of the International Pharmaceutical Federation FIP, in cooperation with the WHO; International partners are: Universitat Mainz and Frankfurt, University of Maryland, Mahidol University and TNO.

- Test and reference products will be subjected to comparative in vitro dissolution studies
- Test and reference products will be subjected to comparative in vitro Caco-2 transport studies.
- Test and reference products will be subjected to a comparative in vitro bioaccessibility profiling during GI passage in TNO's gastroIntestinal Model (TIM).
- The results of the TIM studies will be integrated with the results of the Caco-2 studies using in silico modeling
- Test and reference products will be entered in three in vivo BE studies
- The results of the in vivo BE studies are compared to the results of the comparative Caco-2 testing.
- The results of the in vitro TIM studies alone and in combination with the Caco-2 studies will be compared with the results of the in vivo BE studies.

Planned products
The following scientific publications are foreseen:

- Permeability as a critical parameter in bioequivalence: a literature review.
- Comparative permeability testing by CaCo-2 as a surrogate for bioequivalence studies in humans.
- Comparative integrated approach of TIM and Caco-2 studies as surrogate for bioequivalence studies in humans.
- Comparative in vitro permeability testing as a predictor for bioequivalence.
- Ph.D.: Comparative in vitro permeability testing as predictor for bioequivalence
- Recommendations for revisions of the regulatory guidance's.

Foreseen follow-up
See strategic and innovative aspects.
For this project, co-funding is needed, which is yet not assured. The project will start only if the sponsoring is allocated.