Form
Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	50400
1.2	Provide the name of the licenced establishment.	[redacted]
1.3	Provide the title of the project.	Determining the immune modulating effects of therapeutics

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

- ☐ Basic research
- ☒ Translational or applied research
- ☐ Regulatory use or routine production
- ☐ Research into environmental protection in the interest of human or
- ☐ Research aimed at preserving the species subjected to procedures
- ☐ Higher education or training
- ☐ Forensic enquiries
- ☐ Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Background:
Humans are susceptible to a wide range of diseases, including infectious diseases, neurological diseases, autoimmune diseases, allergies and cancer. Both the diseases itself and the treatment and prevention thereof has been a major focus of scientists and clinicians. Since the first vaccine was prepared by Edward Jenner there has been a major progress in the development of methods to treat and prevent a wide range of diseases. This has resulted in many types of medical products such as medicines, vaccines and biological products capable of curing, managing or preventing disease. The basis of these beforementioned medical products is that they are capable of managing or clearing the disease in question. Some of the medical products directly activate the immune response by inducing a humoral (antibody-based) or cellular (T cell-based) response, for example. Other products are developed to modify an existing immune response, such as immunosuppressants, which directly target a component of the immune response (for example T cells) in order to inactivate or limit efficacy of that specific component of the immune response. Hereby, these products aid in combatting disease, either by preventing infection (e.g. vaccines), clearing the disease causing agent from the body (e.g. antiviral medicine), or by limiting inflammation caused by an individual's own immune response (immunosuppressants).

Generally, the first step in the development of a medical product is to identify a candidate capable of preventing, managing or curing the disease. In this candidate selection process, biomarkers for disease and the corresponding targets for the medical product are identified and a high throughput screening is performed to make a first selection of the most promising products. This phase often occurs in in silico models (e.g. use a computer to select candidates with similarities to products that are effective in similar diseases) or in in vitro models (e.g. by using cell lines to test the efficacy of the medical product in human cells).
The second step is to narrow the selection of possible candidates by performing potency and cytotoxicity test, often still in vitro, and to perform preliminary animal efficacy studies to confirm the potency of the medical product.
During the third step, more potency studies will be performed both in vitro and in vivo, followed by pharmacokinetics (PK), pharmacodynamics (PD), absorption, distribution, metabolism, and excretion (ADME), and toxicity (tox) evaluation of the most promising candidates.
The fourth step is to perform pre-clinical animal studies in which, in the case of pharmaceutical drugs, a pharmacological profile is formed (including determining the optimal dose), the optimal route of administration is determined and drug interactions are evaluated.
Following these research and development steps, the lead candidates are selected for further safety and efficacy testing in humans by performing clinical trials *(1, 2, 3)*.
As a contract research organization (CRO), we have several in vitro models available for the primary selection of candidates. In addition, we are involved in both the preliminary animal efficacy and toxicity studies to confirm the potency of the medical product and in the pre-clinical animal studies used to select the lead candidates and its optimal dose and route etc.

Motivation:
This project proposal is focused on a specific aspect of (pharmaceutical) product development, namely determining the immunogenicity or the immunomodulating properties of these products. Therefore, the studies performed under this proposal will contribute to steps two to four of the developmental pipeline of the product as described above.

Worldwide many pharmaceutical companies, biotech companies and even universities or spin-off companies thereof have a wide range of medical products in their developmental pipeline for the treatment or prevention of infectious diseases, neurological diseases, autoimmune diseases, allergies and cancer. The majority of this developmental work is performed by the companies/universities itself. However, in some cases (a part of) this work will be outsourced. For example because of lack capacity at the company/university or because of lack of expertise for a certain model.
In this respect, over the past years we have performed both in vitro and in vivo experiments that have aided in the development of vaccines and biological products for infectious diseases, neurological diseases, and cancer.

References:
(1) https://www.fda.gov/ForPatients/Approvals/Drugs
(2) WHO guidelines on nonclinical evaluation of vaccines, WHO Technical Report Series No. 927, Annex 1.
(3) ICH guideline: Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals.

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

Over the past years we have contributed to the development of medical products such as vaccines and biological products for infectious diseases, autoimmune diseases, and cancer by performing animal experiments crucial for the selection of lead candidates. Continuously, progress is being made in the development of new or improved products for a wide range of diseases. In the coming years, we expect to keep contributing to the development of lead candidate vaccines and other medical products by evaluating the immunogenicity or immunomodulating properties of these products. In addition, we expect to contribute to the development of medical products for oncology, for example, as this is a growing market in the developmental pipeline of many pharmaceutical companies. Therefore, the objective of this project is to evaluate the immunogenicity or immunomodulating properties of (novel) medical products, which is needed to bring these products to the market.

The common derivative of the studies that will be performed under this project is that we will test products that are designed to activate, suppress, or modify the human immune system. The main read-out in these studies is the evaluation of the immunogenicity, i.e. the ability of a particular substance to provoke an immune response or the ability of a substance to modulate an immune response in the body. This read-out can be performed in house or, if this is not an option, we can provide the logistics to transport the materials to the site where the evaluation of the product on the immune response is performed. The procedures underlying these activities are described more elaborately in the appendices.

We will achieve this by offering experienced staff, including scientifically trained staff who can aid in selecting the appropriate model. We have trained immunologists available who have a thorough understanding of the immune response in both animal models and in humans and can therefore contribute to obtaining an optimal study design. In addition, we have experienced biotechnicians trained to perform both the necessary procedures and to monitor the animals carefully to observe any possible adverse effects. The latter is especially an important criterium in the preliminary animal studies, as this may be a reason to halt the development of that candidate or to make changes necessary to prevent adverse effects.

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

The animal studies described under this project will contribute to the development of medical products designed to limit occurrence of disease and/or death due to preventable diseases. These studies will do so since the immunogenicity/immunomodulatory properties of the products needs to be evaluated in order for the medical products to proceed in the developmental pipeline.

The project will provide knowledge on the effect of medical products to activate or modify the immune response. Hereby contributing to new insights in and the development of products to treat or prevent e.g. infectious diseases, autoimmune diseases, and cancer.
Furthermore, the results of these animal studies will determine which candidates have the potential to be selected for further development and will eventually be evaluated in clinical trials.

3.4 Research strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

As described under section 3.1, during the selection of candidate medical products, there are several crucial steps:

- Identify a candidate capable of preventing, managing or curing the disease.
- Narrow the selection of possible candidates by performing potency and cytotoxicity tests.
- Perform potency, PK/PD, ADME and toxicity studies to evaluate the most promising candidates.
- Perform pre-clinical animal studies.

This proposal will contribute to selection of lead candidates as the potency of the product will be tested which will narrow the selection to obtain a lead candidate. In addition, pre-clinical animal studies will be performed to optimize the dose regime before entering into efficacy studies and/or clinical trials.

Before a study is initiated, the information needed to evaluate the necessity for this study is collected via literature research and discussions with the customer. Hereby, the focus will be specifically on the preparative work performed on the product to be tested, both in vitro and in vivo, to prevent unnecessary animal studies.
The subsequent step is to select the appropriate study design, which mostly depends on the type of product to be tested.

In general, the following types of medical products can be distinguished of which the immunogenicity or immunomodulating properties will be tested under this proposal:
- Small molecules, chemical substances, e.g. protein kinase inhibitors that inhibit inflammatory responses.
- Biopharmaceuticals, e.g. proteins, vaccines, blood products, monoclonal antibodies, cells etc.
- Live organisms (attenuated or not), e.g. orally ingested live commensal bacterial strains that amplify cancer therapy by modulating the immune response.

The immunogenicity or immunomodulating properties of these products can be tested in several animal models. The model most often used for these types of studies are mice. As there is a lot of knowledge on the immune system of mice available, a large set of historical data (in literature), and a wide range of reagents available. We offer our services for demonstrating the effectiveness and mechanism of action of immune modulatory agents in healthy animals.

The optimal study design will mainly depend on literature research, the eventual disease model needed to prove the efficacy of the product, previous experience with similar models (i.e. historical data on similar products that were tested previously), and discussions with the customer. For example, small molecules are mostly provided orally, vaccines are mostly provided intramuscularly, and monoclonal antibodies are mostly provided intravenously.

Upon receipt of a study request, a study director (SD, project leader) is appointed, who will form a project team and the project proposal will be discussed scientifically and ethically with the customer. Before each study is initiated, together with the customer, the need for an animal study will be evaluated in a project team which consists of (senior) scientists, biostatisticians and/or pharmacologists. The project team will evaluate historical data and make recommendations on the number of animals per treatment group, doses used, and also on the need for an animal study, etc., to maintain the scientific quality of the project. The animal welfare body and the designated veterinarian will be consulted on issues of animal welfare. For most studies a 'pre-study briefing' is performed by the study director to ensure understanding of the study objectives and the study design by the staff involved. During the study, any (unexpected) adverse effects or other concerns concerning animal welfare will be monitored by the study director and discussed with relevant staff.

Summarizing, together with the customer, the optimal model will be selected based on the following questions:

a. Was the immunogenicity/immunomodulating properties of the product tested in other studies in vitro or in vivo? If yes, how are these results translated into the choice of the animal model and the study design. If no, see points b-f
b. What type of product will be tested? Here, background information on the product will be asked, such as the preparative work, if similar products have been tested previously, etc.
c. What is the most desirable route of administration?*
d. How is the dose of the product determined?*
e. How is the dose schedule determined?*

*This could also be a secondary objective of the study, in that case the results may be used for follow-up studies.

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

When testing a medical product there are two categories, i.e. medical products that activate the immune response (immunogenicity testing) and products that will modify (e.g. block or suppress) the immune response (testing of immunomodulating properties). Depending on the product to be tested, two models (components) may be used:

1. Immunogenicity testing: The product is administered for a number of times (mostly 1 or 2 times) in order to activate the immune system and (immunological) read-outs will be performed to evaluate the immunogenicity of the product.
2. Testing of immunomodulating properties: The effect of the product on the immune response will be determined: A general immune response will be initiated by administration of a general activator (for example an unrelated protein) or by passive transfer of immune cells, then the medical product will be administered to determine whether the immune response can be modified by that product (animal models: mice, including immunodeficient mice).

The study design of each individual study will be performed in accordance with the code of good practice (Diehl et al, J. Appl. Toxicol. 21, 15–23 (2001); A Good Practice Guide to the Administration of Substances and Removal of Blood, Including Routes and Volumes).

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points.

In both components, the medical product will be administered to the animal and read-outs will be performed to determine the effect of the medical product on the immune response. In the first component, mostly ex vivo read-outs on blood products or organs will be performed. In the second component, in vivo read-outs, such as measurement of ear thickness may be used. In the latter case the product can be administered before, during, or after initial activation of the immune response.

Based on the outcome of the studies performed under this project, the product will or will not proceed in the developmental pipeline:

- The product may not be immunogenic in vivo or may not show the expected immunomodulatory capacity, then the product development will be stopped or will go back to an earlier phase to adjust the product before further testing under this project.
- The product is immunogenic/can modulate the immune response as expected and additional tests need to be performed to enhance the effect of the product by testing e.g. different doses or dose regimes before proceeding to the next step.
- Both the product and the corresponding dose and dosing regime are optimized: Then the product will be tested in an efficacy model (if applicable) or proceed to the clinic (these tests are not part of this project).

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Immunogenicity testing
2	Testing of immunomodulating properties
3	
4	
5	
6	
7	
8	
9	
10	