Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on 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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Influenza vaccine evaluation in macaques

2 Description of animal procedures

A. Experimental approach and primary outcome parameters
Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We have developed a general study protocol for the evaluation of influenza vaccines in macaques. Temperature and potentially movement and heart rate will be measured by telemetry. A device that records and transmits these parameters is surgically placed in the abdominal cavity. When all mentioned parameters need to be monitored during the immunization as well as the infection phase then a relatively large device is needed that is capable of performing all these measurements and can be used during a long period. This larger device has the advantage that it can be temporarily put on stand-by to safe battery life and then be reactivated when needed, so that it will last through the entire study period. Such devices will be placed at least 4 weeks before the first immunization. In case only the temperature needs to be monitored during the influenza virus infection phase then a small device suffices. Such a device has however a shorter life-span (28 weeks) and has to be placed in the abdominal cavity four weeks before infection. Subsequently the animals are immunized either once or they receive a number of immunizations over a certain period of time. Although the vaccines that are used in these studies have already been extensively evaluated in other animals and are expected to give no or only very limited adverse effects, macaques will be monitored for possible changes in general behaviour and health. The site of immunization will be checked for local reactions and blood will be drawn to measure clinical chemistry and haematology parameters. Before, between and after immunizations, blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. Both T-cell and antibody responses will be measured against several influenza viruses in order to establish the strength and breadth of the immune response. When adequate immune responses are induced that indicate that protection against infection might be achieved, the efficacy against infection will be tested by experimental infection with influenza virus. A group of non-vaccinated animals will be included as infection controls. Usually only one virus will be used for experimental infection. The choice of the virus strain to be used will depend on the outcome of the evaluation of the immune responses and the stage of development of the vaccine. An entirely new vaccine concept may require that in first instance protection against infection by a homologous virus strain is tested. However, for most vaccine candidates protection against infection with a heterologous virus will be tested. All animals in a specific vaccine group will be challenged with the same virus. If protection against two different viruses needs to be established than two groups of animals are immunized with the same vaccine and subsequently group 1 will be challenged with virus 1 and group 2 with virus 2. If animals are protected against infection, then a second challenge with a different virus may be performed. In this case an additional challenge control group is needed. In this manner the breadth of the immune response is both measured *in vitro;* via the evaluation of the induced immune response and *in vivo* via experimental infection with different virus strains. The infection will be performed as described in appendix 2.

The primary outcome parameters are:

- Absence of unexpected reactogenicity of the vaccine: effects of the vaccine on general behaviour, health, local reactions and blood parameters.
- Immunogenicity: Induction of cellular and humoral immune responses. The type and strength of the induced responses will determine if the objectives of the vaccine strategy are achieved and whether immune responses are sufficiently strong to proceed with viral challenge.
- Efficacy: Capacity to protect against viral challenge will be established in terms of: reduction in clinical symptoms, fever, virus multiplication and changes in blood parameters.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

A telemetry device is surgically placed in the abdominal cavity at least 4 weeks either before the first immunization or before the infection. This time frame is necessary for full recovery of the animals and to allow adequate recording of body temperature and/or heart rate and activity during a two to three-week period to establish normal values before immunizations start. Animals will receive one or more immunizations. If multiple immunizations are given then typically a 4 to 8-week time interval is used, although occasionally a longer time frame is needed between immunizations when different vaccine modalities are used for priming and boosting of the immune response. In rare occasions these limits may have to be exceeded, with a maximum of 6 immunizations. Specific rationale will then be presented to the animal welfare body (AWB). Immunizations will be done either by intradermal injection, intramuscularly, subcutaneously, intravenously, intranasally, intra-tracheally or via aerosol using a nebulizer. All vaccines have to be sterile and have to be given under aseptic conditions. At regular time intervals, usually two and four weeks after every immunization, blood is drawn to measure systemic adverse effects, which includes measurement of clinical chemistry and haematology, and to measure induction of cellular and humoral immune responses. The two-week interval after immunization is chosen, because this is optimal for measuring cellular immune responses, while four weeks is optimal for measuring humoral immune responses. In some studies, nasal washes and lung lavages (BAL) are taken after immunization in order to measure induction of local immune responses. BAL will be maximally collected three times after each immunization, resulting in total in 18 BAL collections if animals are immunized six times. Immune responses recorded after the final immunization will be used to decide whether protection against viral challenge can be reasonably expected. If these responses are inadequate then the study will be stopped and animals may be re-used in other non-influenza virus related experiments. Otherwise, experimental challenge will usually take place between 4 and 8 weeks after the last immunization. This time period is needed to allow immunological memory to form after the last immunization. Influenza infection may be done by intra-bronchial or intra-tracheal inoculation alone or in combination with oral, intranasal and intraocular inoculation or via aerosol using a nebulizer (as described in appendix 2). Clinical symptoms will be monitored twice daily during the infection phase. Nasal and tracheal swabs will be taken before infection, frequently during the first days after infection (daily and then every other day) and subsequently less frequently until day 21, to measure virus multiplication in the upper airways. Lung lavages may be taken at selected time points (max 5 times) to measure virus multiplication in the lungs. Blood is taken simultaneously with the swabs, to monitor changes in clinical chemistry and haematology parameters, leucocyte subsets and cytokine production. At the same time points body weight and physiological parameters (for instance pulse rate, blood pressure) are recorded and imaging (for instance CT or PET-CT) is performed to measure lung infiltration. After the virus is cleared (usually at day 21 after infection) animals may be exposed to a second challenge virus. All handling will then be repeated. However, this will be rarely the case and specific argumentation will be presented to the AWB. In general animals are either returned to the experimental stock or they are euthanized and a full necropsy is performed in order to establish lung pathology. Euthanasia is only performed when assessment of lung pathology is required to establish vaccine safety and in case pathogenic influenza viruses are used that are known to cause persistent lung pathology. Some telemetric devices, that have been designed to measure multiple parameters, can be quite large and require a substantial operation. These will not be removed and the animal will be euthanized instead. A small telemetric device can be surgically removed and animals may be re-used. A specific rational has to be provided to the AWB for using these devices. In case an animal should reach the humane endpoint during the study it will be immediately euthanized and a full necropsy will be performed to establish lung pathology and virus multiplication in the respiratory tract.

The details of each study, regarding the interval between the immunizations, the number and time points of sampling, the specific criteria to proceed with a viral challenge, the time interval between the last immunization and viral challenge will depend on the actual type of vaccine that is being tested and this will be submitted to the AWB.

Table. Maximum number of repeats per procedure. Indicated is which procedure is performed under sedation and which procedure under deep anesthesia.

Procedure	Maximum	Duration	sedation	anesthesia
Recorder in/out	2	60 min		X
Vaccination	6	10 min	X	
Blood sample	44	10 min	X	
Bronchoalveola lavage (BAL)	28	30 min		X
Infection	2	10 min		X
Swabs	20	10 min	X	
CT-scan	10	15 min		X

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The number of animals will be based on statistical power analysis. Calculations take into account the number of animals needed to measure statistically significant induction of immune responses in relation to unvaccinated controls or, in case different vaccines are compared, whether significant differences between the vaccine groups can be obtained. In addition, calculations are performed to establish the number of animals needed to obtain a significant reduction in virus load in the trachea between the vaccine groups and the challenge control group. Experience in previous influenza vaccine evaluation studies performed at our institute indicates that statistically significant reductions in virus multiplication can be obtained with six animals per group. However, in order to be able to measure differences between the vaccine groups themselves, more animals per group may be needed. Only the minimum number of animals required will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the non-vaccinated challenge control group than in the vaccine groups.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Rhesus or cynomolgus macaque	Purpose bred	adult	90	M / F	no	Not applicable

Provide justifications for these choices

Species	Macaque species have been used in several influenza vaccine studies (1-6). The most frequently used species are the rhesus- (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*). Both species are semi-permissive to influenza infection. Therefore, both rhesus and cynomolgus macaques can be used for influenza vaccine evaluation studies.
Origin	All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier.
Life stages	Adult animals will be used because these allow larger volumes of blood to be collected.
Number	90 macaques in total (either cynomolgus or rhesus). The number of animals requested is based on the assumption that each study will contain two vaccine groups and 1 control group, with 10 animals per group. The group size will be determined per experiment, based on power calculation specific for the experiment. As stated above, probably fewer animals will be needed in the non-vaccinated challenge control groups and also in the vaccine groups in some of the studies. In all, we anticipate performing 3 such studies over a 5-year period.
Gender	Adult male and female animals can be used. Since there are immunological differences between males and females (7, 8), we prefer that for each individual experiment either all animals are male or all are female, in order to minimize the variation within the experimental test group, thereby increasing the probability of finding statistically significant differences between the experimental groups. This choice is also important with regard to the amount of blood needed to perform all assays. Male animals are usually larger than female animals and therefore more blood per time point can be drawn from males. In case assays need to be performed which consume large amounts of blood, male monkeys are preferred over females.

Genetic alterations	Not applicable
Strain	Not applicable

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

After placement of the recording device in the abdomen and after removal, which is needed in case the animal will not be euthanized after completion of the experiment, animals will receive analgesics for as long as necessary, typically 3 days. In previous studies we have observed that animals can experience some body temperature elevation during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation. Pain relieve will also be applied when substantial induration is seen at the site of vaccine injection. In case of the latter, analgesics known not to interfere with the induction of the vaccine response will be used.

Describe which other adverse effects on the animals' welfare may be expected?

1. Discomfort because of insertion or removal of the temperature recording device
2. Discomfort due to injection
3. Discomfort due to lung lavages
4. Discomfort due to virus installation
5. Discomfort due to PET-CTs
6. Stress because of sedation and recovery
7. Reduced food intake during the first days after infection
8. Disease symptoms due to the infection

Explain why these effects may emerge.

1. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
2. When vaccines are given by injection, this can cause local pain and irritation.
3. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
4. When virus is given intra-bronchially a bronchoscope is used and this combined with the inoculum volume will cause irritation. Aerosol application implies that animals have to be sedated and forced to breathe via a nebulizer device and this can give irritation.
5. For the PET-CT animals are sedated, intubated and mechanical ventilation with a forced breathing pattern is applied. No adverse effects are expected from the scan itself
6. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs, CTs and lung lavages. Nausea can sometimes be observed during recovery from the sedation.
7. Especially during daily sedation during the first 2 days after infection food intake might be reduced.
8. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, increased breathing rate, loss of appetite, inactivity.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
2. Animals will be sedated for vaccine delivery. Only rarely are strong adverse effects seen. Should granuloma formation be observed, then the animal will be sedated, the wound will be cleaned and analgesics are applied if necessary following veterinary consultation.
3. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
4. The same procedure as described under 3 will be followed
5. The animals are first deeply sedated and then receive a local mucosal relaxant before intubation. The mechanical ventilation & forced breathing patterns will be monitored and adapted on each animals characteristics.
6. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
7. Animals will receive supportive feeding with dense "brokkenballen".
8. Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms. When a certain pre-determined clinical score is reached then the animal will be humanely euthanized and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory tract.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms (9). When a clinical score of 35 is reached this indicates that the maximum duration of severity is reached then the animal will be euthanized. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort. Symptoms associated with score 35 that lead to an immediate endpoint are: open mouth breathing or cyanosis, lethargy as defined by minimal response to human approach.

Indicate the likely incidence.

The likely incidence for reaching a clinical endpoint depends on the virus strain that is used for infection. Human influenza viruses only rarely cause severe disease (<1% of the animals). Highly pathogenic avian influenza viruses can cause lethal disease in up to 75% of the non-vaccinated control animals.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

The total amount of discomfort is estimated as moderate. This is mainly caused by the surgical implantation and removal of the recording device and development of disease due to infection and the high maximum number of sedations.

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	The immune system is very complex and the *in vivo* interactions between virus and/or vaccine and host are not completely understood. At present there is no *in vitro* model available that can mimic the human immune system sufficiently to study the potential of a vaccine to induce protective immune responses. Due to the complex interaction of influenza virus with different tissues and the role of local immunity in eradication of the virus, the efficacy of an influenza vaccine to protect against infection can only be adequately established in an animal model. Several animal species have been used as a model for human influenza virus infection (10). However, mice are not naturally susceptible to influenza and usually lethal infection models are used, that are based on extremely adapted human viruses. Ferrets are naturally susceptible to most human influenza viruses and recapitulate the natural course of infection. However, infection is often restricted to the upper airways and this model has the disadvantage that limited immunological tools are available, especially to study innate and cellular adaptive immune responses, which are especially important in the evaluation of "universal" vaccine strategies. NHP have an immune system that most closely resembles that of humans. Also the availability of many cross reactive reagents makes it possible to study in detail the contribution of the innate immune system and to analyse vaccine induced immune responses and evaluate their role in control of infection. In addition, vaccine strategies that aim to trigger immune responses through targeting of specific cell surface molecules or innate immune receptors have either limited cross reactivity to other animal species or trigger different cell types or different responses because of differences in receptor expression pattern or cell signalling pathways. These aspects are essential for the evaluation of so called "universal" influenza vaccines. For this type of vaccines new vaccination strategies as well as vaccine modalities are used that aim for the induction of cross protective cellular immune responses or induction of broadly cross neutralizing antibody responses or non-neutralizing antibody responses that become effective through interaction with innate immune cells. Here, the close homology between the immune system in NHP and humans is essential for proper induction and adequate analysis of these responses, allowing a better extrapolation to the human situation. Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this last validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans.
Reduction	The number of animals needed per experiment will be based on statistical power calculation for achieving statistically significant induction of immune responses and a significant reduction in virus load in the respiratory tract between the vaccine groups and the challenge control group. Only the minimum number of animals needed will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the challenge control group than in the vaccine groups. Furthermore, we aim to evaluate multiple vaccine candidates in a single experiment, so that a single challenge control group can be used. This has to be weighed against the fact that in order to obtain significant differences in immune response between the vaccine groups, more animals per vaccine group may occasionally be required.

Refinement	The use of recording devices enables the monitoring of body temperature, heart- and breathing-rate every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by influenza infection (2). With this method we have observed a significant reduction in fever by influenza vaccine candidates (5). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgical procedure, which will be done under anaesthesia. Subsequently, animals will receive analgesics as long as required. Animals are trained to cooperate as much as possible for the invasive handlings, such as receiving the sedation. Animals will be socially housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food. During the study animals will be observed daily by qualified and competent animal caretakers. Should changes occur in behaviour, appetite or stool then a veterinarian will be informed and measures will be discussed with the investigator and implemented. Possible local reactions on the injection site of the vaccine will be recorded at multiple time points using a scoring system that includes redness, swelling and induration. In case substantial induration is seen, then the wound will be treated and analgesics will be applied. During the infection phase, animals will be observed twice daily and clinical symptoms will be scored using a well-established clinical scoring list adapted from Brining et al.(9) . On the basis of the scoring system a humane endpoint is defined. In addition, a sudden strong decrease in body temperature is taken as a humane endpoint. When this endpoint is reached the animal will be immediately euthanized and a full necropsy will be performed to determine the cause of clinical disease. All handlings will be performed under sedation. On every time point where a handling is performed the animal will be weighed and closely examined. During the first 2 days of the infection the animal will receive supportive feeding with dense "brokkenballen". This is necessary, because animals have to fast the night before the sedation and since animals will be sedated daily during the first days after infection the food intake during this period would otherwise be very limited.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☐ No > Continue with question I.

☒ Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that will be used in these experiments, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infection studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life-span of the animals of this species re-use of animals will take place within the limitations described in art 1e of the Wet op de Dierproeven.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

In most studies the animals will be re-used after the virus is cleared (usually at day 21 after infection). However, in cases where possible adverse effects of the vaccine have to be studied animals are euthanized and a full necropsy is performed.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Euthanasia is done by injection of an anaesthetic dose of ketamine followed by an overdose of barbiturate intravenously

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

References

1. 10.2.e [redacted]

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5. Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science. 2015;349:1301-6.
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7. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16:626-38.
8. Klein SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg. 2015;109(1):9-15.
9. Brining DL, Mattoon JS, Kercher L, Lacasse RA, Safronetz D, Feldmann H, et al. Thoracic radiography as a refinement methodology for the study of H1N1 influenza in cynomologus macaques (Macaca fascicularis). Comp Med. 2010;60:389-95.
10. Bodewes R, Rimmelzwaan GF, Osterhaus AD. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines. 2010;9(1):59-72.

Centrale Commissie Dierproeven

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- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on our website (www.centralecommissiedierproeven.nl).
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1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Establishment of a new influenza infection model in macaques

2 Description of animal procedures

A. Experimental approach and primary outcome parameters
Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

In order to establish the capacity of a vaccine to protect against influenza virus infection it is necessary to have a well-defined influenza virus infection model. Previously we have established a model for infection of macaques with pandemic H1N1 and highly pathogenic avian H5N1 viruses (1-3). For new influenza viruses that have not yet been tested at our institute it is necessary to establish infectivity and pathogenicity in macaques before they can be applied in influenza vaccine efficacy evaluation studies. The main objective is to obtain an infection model that is sufficiently robust to allow adequate evaluation of vaccine efficacy in terms of reduction in clinical symptoms, fever and virus multiplication. In cases viruses are used that are known to cause persistent lung pathology, this will also be a primary outcome parameter. In general, the study set-up is as follows: a small number of animals will be infected and monitored for clinical symptoms, fever, body weight and changes in blood parameters. Nasal and tracheal swabs will be taken to determine if the animals have become infected and determine the magnitude of virus multiplication. To evaluate a new virus, the virus is either inoculated by a combination of routes; for instance intra-bronchial, oral, intranasal and intraocular or by aerosol exposure using a standard dose. Proper application for vaccine evaluation requires that more than 80% of the animals become infected and that the amount of virus produced in the trachea over the infection period is clearly measurable and that the variation between the animals is sufficiently low to allow measurement of reduction in virus load in vaccinated animals with less than 10 animals per group. In case these parameters are not achieved then the experiment will be repeated with a 10-100 times higher dose. In case any of the animals reaches the clinical endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The mode of exposure that will be tested will be identical to the method to be used in the vaccine evaluation study. Evaluation of new influenza viruses as described in this appendix is only performed when a vaccine evaluation study is already planned with the same virus.
Primary outcome parameters are:
Clinical symptoms, fever, virus multiplication.
Pathology in case viruses are used that are known to cause persistent lung pathology.
Secondary outcome parameters are:
Bodyweight, changes in leucocyte subset composition in peripheral blood.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

Temperature and potentially activity and heart rate will be measured by telemetry. A device that records and transmits these parameters is surgically placed in the abdominal cavity at least 4 weeks before the infection. This time frame is necessary for full recovery of the animals and to allow adequate recording of body temperature and/or heart rate and activity during a two to three week period to establish normal values before infection. Influenza infection may be done by intra-bronchial or intra-tracheal inoculation alone or in combination with oral, intranasal and intraocular inoculation or via aerosol delivery using a nebulizer. Clinical symptoms will be monitored twice daily. Nasal and tracheal swabs will be taken before infection, frequently during the first days after infection (daily and then every other day) and subsequently less frequently until day 21, to measure virus multiplication in the upper airways. Lung lavages may be taken at selected time points (max 5 times) to measure virus multiplication in the lungs. Blood is taken simultaneously with the swabs, to monitor changes in clinical chemistry and haematology parameters and leucocyte subsets. At the same time points body weight and physiological parameters (for instance pulse rate, blood pressure) are recorded and imaging (for instance (PET-)CT scan) is performed to measure lung infiltration. After the virus is cleared (usually at day 21 after infection) animals are either returned to the experimental stock or they are humanely euthanized and a full necropsy is performed in order to establish lung pathology and virus multiplication in the different parts of the respiratory tract. Euthanasia is only performed when assessment of lung pathology is required in case pathogenic influenza viruses are used that are known to cause persistent lung pathology. However, when animals are not yet virus negative at day 21 an extra tracheal swab will be taken at day 28. When that is also virus positive, which is very unlikely, the animals will be euthanized in order to preclude further discomfort. In case an animal should reach the humane endpoint during the study it will be immediately humanely euthanized and a full necropsy will be performed to establish lung pathology and virus multiplication in the respiratory tract. In case animals are returned to the experimental stock the recording devices are surgically removed and body temperature and/or heart rate and activity data are analysed.
The details of each study, regarding the route of infection, dose used, species and whether animals are to be euthanized at the end of the study will be submitted for approval to the AWB.
Table. Maximum number of repeats per procedure. Indicated is which procedure is performed under sedation and which procedure under deep anesthesia.

Procedure	Maximum	Duration	sedation	anesthesia
Recorder in/out	2	60 min		X
Blood sample	10	10 min	X	
Bronchoalveola lavage (BAL)	5	30 min		X
Infection	1	10 min		X
Swabs	10	10 min	X	
CT-scan	5	15 min		X

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The initial experiment will be performed in four animals. Experience in the pandemic H1N1 and avian H5N1 influenza infection models have shown that with this number of animals an adequate assessment can be made on the reproducibility of infection (all 4 animals need to show virus multiplication in the trachea), the variability of virus production in the trachea and the amount of fever induction. On the basis of these data a power calculation can be made about the number of animals needed in a vaccine evaluation study. Should the result of these calculations be that more than 10 animals are needed per group or should not all four animals have become infected than a new experiment with 4 animals is needed with a higher virus dose. If also at a high virus dose the variation between the animals is still too high then it may be necessary to repeat the experiment in another macaque species. Experiments will preferentially be performed in cynomolgus macaques. However, if after virus inoculation the levels of virus production are too low (below the detection limit) or too variable (>10 animals needed/group) then the virus will subsequently be tested in rhesus macaques.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain

1	Rhesus or cynomolgus macaque	Purpose bred	adult	24	M / F	no	Not applicable

Provide justifications for these choices	
Species	Macaque species have been used in several influenza vaccine studies (1, 2, 4-7). The most frequently used species are the rhesus- (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*). Both species are semi-permissive to influenza infection. Therefore, both rhesus and cynomolgus macaques can be used for influenza vaccine evaluation studies. Previous studies have shown that cynomolgus macaques are more susceptible than rhesus macaques for infection with pH1N1 influenza virus. Cynomolgus macaques show higher levels of virus replication and more fever than rhesus macaques (2). However, for other influenza viruses it is not known which of the two species is the most susceptible. Therefore, experiments will preferentially be performed in cynomolgus macaques. However, if after virus inoculation the levels of virus production are too low (below the detection limit) or too variable (>10 animals needed/group) then the virus will subsequently be tested in rhesus macaques.
Origin	All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier.
Life stages	Adult animals will be used because these allow larger volumes of blood to be collected.
Number	24 macaques in total (either cynomolgus or rhesus). The number of animals requested is based on the assumption that each study will comprise two challenge doses, with 4 animals per group. We anticipate to perform 3 such studies over a 5-year period.
Gender	Adult male and female animals can be used. Since there are immunological differences between males and females (8, 9), we prefer that for each individual experiment either all animals are male or all are female, in order to minimise the variation within the experimental test group, thereby increasing the probability of finding statistically significant differences between the experimental groups. This choice is also important with regard to the amount of blood needed to perform all assays. Male animals are usually larger than female animals and therefore more blood per time point can be drawn from males. In case assays need to be performed which consume large amounts of blood, male monkeys are preferred over females.
Genetic alterations	Not applicable
Strain	Not applicable

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

After placement of the recording device in the abdomen and after removal, which is needed in case the animal will not be euthanised after completion of the experiment, animals will receive analgesics for as long as necessary, typically 3 days. In previous studies we have observed that animals can experience some body temperature elevation during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation. Pain relieve will also be applied when substantial induration is seen at the site of vaccine injection. In case of the latter, analgesics known not to interfere with the induction of the vaccine response will be used.

Describe which other adverse effects on the animals' welfare may be expected?

1. Discomfort because of insertion or removal of the temperature recording device
2. Discomfort due to lung lavages
3. Discomfort due to virus installation
4. Discomfort due to PET-CTs
5. Stress because of sedation and recovery
6. Reduced food intake during the first days after infection
7. Disease symptoms due to the infection

Explain why these effects may emerge.

1. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
2. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
3. When virus is given intra-bronchially a bronchoscope is used and this combined with the inoculum volume will cause irritation. Aerosol application implies that animals have to be sedated and forced to breathe via a nebulizer device and this can give irritation.
4. For the PET-CT animals are sedated, intubated and mechanical ventilation with a forced breathing pattern is applied. No adverse effects are expected from the scan itself
5. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs, CTs and lung lavages. Nausea can sometimes be observed during recovery from the sedation.
6. Especially during daily sedation during the first 2 days after infection food intake might be reduced.
7. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, increased breathing rate, loss of appetite, inactivity.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
2. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
3. The same procedure as described under 3 will be followed
4. The animals are first deeply sedated and then receive a local mucosal relaxant before intubation. The mechanical ventilation & forced breathing patterns will be monitored and adapted on each animals characteristics.
5. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
6. Animals will receive supportive feeding with dense "brokkenballen".
7. Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms. When a certain pre-determined clinical score is reached then the animal will be humanely euthanized and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory tract.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms (10). When a clinical score of 35 is reached this indicates that the maximum duration of severity is reached then the animal will be euthanized. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort. Symptoms associated with score 35 that lead to an immediate endpoint are: open mouth breathing or cyanosis, lethargy as defined by minimal response to human approach.

Indicate the likely incidence.

The likely incidence for reaching a clinical endpoint depends on the virus strain that is used for infection. Human influenza viruses only rarely cause severe disease (<1% of the animals). Highly pathogenic avian influenza viruses can cause lethal disease in up to 75% of the non-vaccinated control animals.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).
The total amount of discomfort is estimated as moderate. This is mainly caused by the surgical implantation and removal of the recording device and development of disease due to infection.

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.	
Replacement	Due to the complex interaction of influenza virus with different tissues and the role of local immunity in eradication of the virus the efficacy of an influenza vaccine to protect against infection can only be adequately established in an animal infection model. Several animal species have been used to study influenza virus infection (11, 12). However, of these different species NHP have the advantage that they physiologically and immunologically most closely resemble humans. This has important implications, both for vaccine evaluation (explained in appendix 1), as well as for the interaction with influenza virus, since this is affected both by physiology and by the reaction of the innate and adaptive immune system. As explained in appendix 1, these aspects are especially important for the evaluation for "universal" influenza vaccines. The proper evaluation of these vaccines requires adequate infection models in NHP, which is the purpose of the studies proposed here.
Reduction	Experience from previous experiments has shown that when the virus is inoculated by a standard combination of routes at a standard dose, four animals per test group is sufficient in order to determine whether a suitable infection model has been achieved and to perform a power calculation to determine the number of animals needed in a vaccine evaluation study. In case the criteria, as outlined under A are not met, a second experiment may be needed with another dose or in another NHP species. Only the minimum number of animals required, will be used.

Refinement	The use of recording devices enables the monitoring of body temperature, heart- and breathing-rate every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by influenza infection (2). With this method we have observed a significant reduction in fever by influenza vaccine candidates (6). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgical procedure, which will be done under anaesthesia. Subsequently, animals will receive analgesics as long as required. Animals are trained to cooperate as much as possible for the invasive handlings, such as receiving the sedation. Animals will be socially housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food. During the study animals will be observed daily by qualified animal caretakers. Should changes occur in behaviour, appetite or stool then a veterinarian will be informed and measures will be discussed with the investigator and implemented. During the infection animals will be observed twice daily and clinical symptoms will be scored using a well-established clinical scoring list adapted from Brining et al. (10). On the basis of the scoring system a humane endpoint is defined. In addition, a sudden strong decrease in body temperature is taken as a humane endpoint. When this endpoint is reached the animal will be immediately euthanized and a full necropsy will be performed to determine the cause of disease. All handlings will be performed under sedation. On every time point when a handling is performed the animal will be weighed and closely examined. During the first 2 days of the infection the animal will receive supportive feeding with dense "brokkenballen". This is necessary, because animals have to fast the night before the sedation and since animals will be sedated daily during the first days after infection the food intake during this period would otherwise be very limited.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☐ No > Continue with question I.

☒ Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that will be used in these experiments, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infection studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life-span of the animals of this species re-use of animals will take place within the limitations described in art 1e of the Wet op de Dierproeven.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

In most studies the animals will be re-used after the virus is cleared (usually at day 21 after infection). However, in cases where possible adverse effects of the vaccine have to be studied animals are euthanized and a full necropsy is performed.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes > Will a method of killing be used for which specific requirements apply?

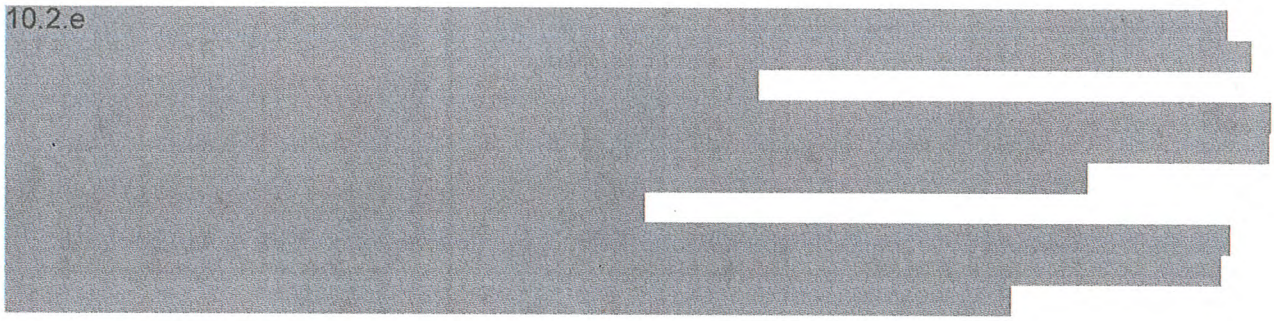
☒ No > Describe the method of killing.

Euthanasia is done by injection of an anaesthetic dose of ketamine followed by an overdose of barbiturate intravenously

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

References



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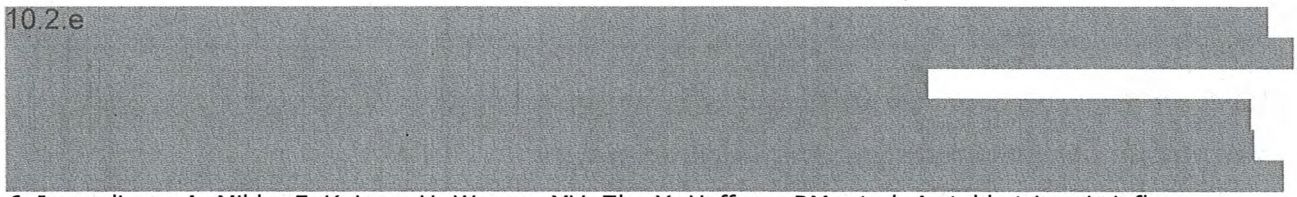

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Format DEC-advies

Maak bij de toepassing van dit format gebruik van de bijbehorende toelichting, waarin elke stap in het beoordelingsproces wordt toegelicht

A. Algemene gegevens over de procedure

Aanvraagnummer: AVD10.2.g202114680

1. Titel van het project: Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza infection in macaques

2. Titel van de NTS: Onderzoek naar de beschermende werking van nieuwe griepvaccins.
3. Type aanvraag:

   X nieuwe aanvraag projectvergunning

4. Contactgegevens DEC:
   - naam DEC: 10.2.g
   - telefoonnummer contactpersoon: 10.2.g
   - mailadres contactpersoon: 10.2.g

5. Adviestraject (data dd-mm-jjjj):
   - ✓ ontvangen door DEC: 03-03-2021 (11-03-2021: Verzoek om advies van CCD)
   - ✓ aanvraag compleet: 18-03-2021
   - ✓ in vergadering besproken: 11-03-2021
   - ✓ anderszins behandeld
   - ✓ termijnonderbreking(en) van/tot 15-03-2021 tot 18-03-2021
   - ✓ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen nvt
   - ✓ aanpassing aanvraag 18-03-2021
   - ✓ advies aan CCD: 25-03-2021

6. Geef aan of de aanvraag is afgestemd met de IvD en deze de instemming heeft van de IvD. De aanvrager heeft het projectvoorstel afgestemd met de IvD en het met instemming van de IvD ingediend.

Bij de punten 8 t/m 10 kan worden volstaan met 'n.v.t.' wanneer de betreffende acties niet aan de orde zijn geweest. Bij vragen die gericht zijn op het compleet maken van de aanvraag (aanvullingen achtergrond informatie etc) kan bij punten 8 en 9 worden volstaan met de vermelding van het type vragen en de vermelding dat de aanvraag op de desbetreffende onderdelen is aangepast of dat de antwoorden in de aanvraag zijn verwerkt. Bij vragen die gericht zijn op het verkrijgen van verklaringen voor keuzes die door de aanvrager gemaakt worden, kan niet worden volstaan met het weergeven van de strekking van de antwoorden tenzij de antwoorden volledig in de aanvraag zijn opgenomen. Als dat het geval is, moet dat in het DEC advies worden benoemd en in de aanvraag inzichtelijk worden gemaakt.

7. Eventueel horen van aanvrager n.v.t.

8. Correspondentie met de aanvrager
   - Datum: 15-03-2021
   - De gestelde vragen betroffen de volgende onderwerpen:
     - Entree criteria voor de vaccins die onderzocht zullen worden
     - Onderbouwing en uitleg mucosale toediening
     - Toedieningswijzen vaccins
     - Tijdstip van plaatsten telemetrie apparaten
     - Keuze sedatie of anesthesie voor experimentele handelingen
     - Beschrijving humaan eindpunt criteria
     - Keuze tussen soorten makaken (criteria)
     - Tekstuele aanpassingen aan NTS
   - Datum antwoord: 18-03-2021
   - Verstrekte antwoorden: De gestelde vragen zijn naar tevredenheid beantwoord.
   - De antwoorden hebben geleid tot aanpassing van de aanvraag.

9. Eventuele adviezen door experts (niet lid van de DEC). N.v.t.

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunning-plichtig (dierproeven in de zin der wet).

2. De aanvraag betreft een nieuwe aanvraag.

3. De DEC is competent om hierover te adviseren: De commissie heeft voldoende expertise, inbegrepen kennis over immunologie en kennis over vaccins tegen influenzavirussen, onderzoek met niet-humane primaten, en toepassing van alternatieven op deze gebieden. Ook is er voldoende expertise op het gebied van ontwerp van proeven, statistiek, de proefdiergeneeskundige praktijk, het houden en verzorgen van dieren, van proefdieren en hun bescherming en ethiek.

4. Geef aan of DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, zijn uitgesloten van de behandeling van de aanvraag en het opstellen van het advies. Indien van toepassing, licht toe waarom. Een van de leden is betrokken bij dit onderzoek. Dit lid heeft de vergadering verlaten tijdens de bespreking van het project en was niet betrokken bij de advisering aan de CCD.

C. Beoordeling (inhoud)

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft.
   Dit project betreft preklinisch onderzoek naar de mogelijke bijwerkingen, immunogeniciteit en effectiviteit van nieuwe (vormen van) vaccins die ontwikkeld worden ter voorkoming van ziekte veroorzaakt door influenzavirussen in makaken. Het betreft vaccins die brede bescherming kunnen bieden tegen verschillende varianten van influenzavirussen en/of vaccins die gebruik maken van nieuwe vaccinatie strategieën. In de aanvraag is (terecht) een aantal voorwaarden gesteld waaraan kandidaat vaccins moeten voldoen alvorens tot verdere evaluatie in makaken kan worden overgegaan.
   Indien gebruik gemaakt moet worden van een nieuwe virusvariant voor de besmetting, zullen eerst experimenten moeten worden uitgevoerd om te bepalen wat de juiste besmettingsdosis en route is voor die variant en eventueel welke makaak-soort hiervoor geschikt is. Immunisaties met de kandidaat vaccins zullen eerst worden uitgevoerd en geëvalueerd voordat besmetting plaatsvindt. Alleen wanneer de immuunrespons voldoende is, zal besmetting plaatsvinden om te onderzoeken of het vaccin voldoende kan beschermen tegen infectie en/of de ziekte.
   De aanvraag heeft een duidelijk omschreven en concrete doelstelling en kan getypeerd worden als een project. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is het ook duidelijk met welk ongerief individuele dieren zullen worden geconfronteerd. De DEC is ervan overtuigd dat er gedurende de looptijd van het project op zorgvuldige wijze besluiten zullen worden genomen over de voortgang van het onderzoek en dat er niet onnodig dieren zullen worden gebruikt. De DEC vindt dat het project toetsbaar is en voldoende samenhang heeft om aangemerkt te worden als een project.

2. Signaleer of er mogelijk tegenstrijdige wetgeving is die het uitvoeren van de proef in de

weg zou kunnen staan. Het gaat hier om wetgeving die gericht is op de gezondheid en welzijn van het dier of het voortbestaan van de soort (bijvoorbeeld Wet dieren en Wet Natuurbescherming).

Er is, zover de DEC kan overzien, geen tegenstrijdige wetgeving die het uitvoeren van de proef in de weg zou kunnen staan.

3. Beoordeel of de in de projectaanvraag aangekruiste doelcategorie(ën) aansluit(en) bij de hoofddoelstelling. Nevendoelstellingen van beperkt belang hoeven niet te worden aangekruist in het projectvoorstel.

   De aangegeven doelcategorieën, te weten 'translationeel of toegepast onderzoek' sluiten aan bij het projectvoorstel. In dit projectvoorstel zal worden onderzocht of nieuwe types kandidaat influenzavaccins bescherming bieden tegen (de gevolgen van) een influenzavirus infectie. Wanneer een vaccin voldoende bescherming biedt, kan dit vervolgens in een klinische studie getest worden. Het uiteindelijke doel is een universeel influenzavaccin te ontwikkelen dat slechts één keer in de circa vijf jaar gegeven hoeft te worden aan risicogroepen (ouderen, mensen met onderliggend lijden) en dat bescherming biedt tegen meerdere varianten van het virus.

Belangen en waarden

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een directe en reële relatie is tussen beide doelstellingen. Beoordeel of het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.

   Influenza veroorzaakt jaarlijks een griepgolf die vooral voor ouderen en kwetsbare mensen met onderliggend lijden ernstige ziekte kan veroorzaken en daarmee behalve deze mensen ook de zorg extra belast. Daarom wordt deze groep mensen jaarlijks gevaccineerd. Het influenzavirus muteert echter veelvuldig en sommige varianten omzeilen ongewenst de afweer, die door eerdere infecties of vaccinatie met een vaccin tegen andere varianten dan de op dat moment circulerende variant(en) is ontstaan. Een mogelijke oplossing hiervoor is de ontwikkeling van nieuwe soorten vaccins die kunnen beschermen tegen vele varianten, inclusief toekomstige varianten van het virus. Op dit moment zijn zulke universele vaccins nog niet beschikbaar. Wel worden dit soort vaccins ontwikkeld en deze moeten voordat ze in de mens getest kunnen worden in relevante proefdiermodellen op veiligheid en effectiviteit getoetst worden. Het directe doel van dit project is de evaluatie van nieuwe (vormen van) influenzavaccins op bijwerkingen, immunogeniciteit en beschermende werking in resus of Java apen. Het uiteindelijke doel is een werkzaam vaccin op de markt te brengen dat kan worden toegepast in de relevante doelgroepen. Er is binnen dit onderzoek een reële relatie tussen het directe doel en het uiteindelijke doel.

5. Benoem de belanghebbenden in het project en beschrijf voor elk van de belanghebbenden welke morele waarden in het geding zijn of bevorderd worden *(Zie Praktische handreiking ETK: Stap 2.B en tabel 1; zie bijlage I voor voorbeeld).* De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de zorginstellingen, maatschappij en met name kwetsbare mensen met onderliggende ziekte en ouderen, de aanvragende onderzoeksinstelling, de bedrijven die influenzavaccins ontwikkelen en de wetenschap.

   Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. Er is sprake van instrumenteel gebruik; de dieren zullen door de huisvesting in een onderzoeksfaciliteit beperkt worden in hun natuurlijke gedrag. De dieren zullen stress ondervinden door biotechnische handelingen en mogelijk enige mate van pijn na het plaatsen van een datalogger. Daarnaast kunnen dieren ziekteverschijnselen ontwikkelen ten gevolge van de virusinfectie, met name koorts, hoesten, niezen, ademhalingsproblemen, benauwdheid en verlies aan eetlust.

   Voor de potentiele patiënten, de zorginstellingen en de maatschappij is dit onderzoek van groot belang, omdat een vaccin dat niet elk jaar moet worden aangepast aan de circulerende virusstam en niet jaarlijks hoeft te worden gegeven er minder druk op de zorginstellingen zal ontstaan. Met een 'universeel griepvaccin' wordt de kans dat het vaccin niet beschermt tegen de nieuwe virusvariant die op dat moment circuleert sterk verkleind. Dit zal er voor zorgen dat er betere bescherming is tegen de gevolgen van een infectie met influenzavirus wat voor zowel individuele patiënten als voor de maatschappij van groot belang is. Voor de bedrijven die vaccins ontwikkelen geldt dat het op de markt brengen van een werkzaam vaccin van economisch belang is.

   Het onderzoeksveld krijgt nieuwe informatie over de werkzaamheid van nieuwe types van vaccins die door hun ontwerp alleen goed in een niet-humane primaten diersoort geëvalueerd kunnen worden. Het is evident dat dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis).

6. Is er aanleiding voor de DEC om de in de aanvraag beschreven effecten op het milieu in twijfel te trekken? Er zijn geen substantiële milieueffecten te verwachten binnen de kaders of ten gevolge van dit project. De risico's worden beheerst door goede biologische inperking.

Proefopzet en haalbaarheid

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw beoordeling toe.

   De kennis en kunde binnen de instelling en de directe betrokkenen bij de dierproeven met primaten zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, zoals blijkt uit de jarenlange ervaring van de instelling met het testen van vaccins in makaken.

De DEC concludeert dat de aanvragers voor de uitvoering van de voorgestelde experimenten beschikken over voldoende kennis en kunde om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van de voorgestelde dierproeven.

8. Beoordeel of het project goed is opgezet, de voorgestelde experimentele opzet en uitkomstparameters logisch en helder aansluiten bij de aangegeven doelstellingen en of de gekozen strategie en experimentele aanpak kan leiden tot het behalen van de doelstelling binnen het kader van het project. Licht uw beoordeling toe.

De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet sluit hier logisch op aan. Eerst zullen de kandidaat vaccins getest worden op bijwerkingen en immunogeniciteit. Alleen als een vaccin geen onverwachte negatieven bijwerkingen heeft en voldoende immunogeen blijkt te zijn, zullen gevaccineerde dieren besmet worden met influenzavirus. De commissie is ervan overtuigd dat met de voorgestelde aanpak de doelen gehaald kunnen worden.

Welzijn dieren

9. Geef aan of er sprake is van één of meerdere bijzondere categorieën van dieren, omstandigheden of behandeling van de dieren. Beoordeel of de keuze hiervoor voldoende wetenschappelijk is onderbouwd en of de aanvrager voldoet aan de in de Wet op de Dierproeven (Wod). voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw beoordeling toe *(Zie Praktische handreiking ETK: Stap 1.C1; zie bijlage I voor toelichting en voorbeelden).*

☐ Bedreigde diersoort(en) (10e, lid 4)
✓ Niet-menselijke primaten (10e)
☐ Dieren in/uit het wild (10f)
☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
☐ Zwerfdieren (10h)
✓ Hergebruik (1e, lid 2)
☐ Locatie: buiten instelling vergunninghouder (10g)
☐ Geen toepassing verdoving/pijnbestrijding (13)
☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c, lid 3)

De keuze voor deze diersoort is gebaseerd op de noodzaak om niet-humane primaten te gebruiken voor de evaluatie voor influenzavaccins. Het gaat hierbij om nieuwe vaccins waarvan niet eerder is aangetoond dat deze werkzaam kunnen zijn in primaten. Ook zal dit vaccins voor humane toepassing betreffen die alleen in apen getest kunnen worden omdat componenten van de vaccins zeer soort-

specifiek zijn en niet kunnen worden geëvalueerd in andere proefdiersoorten. De DEC is het eens met het gebruik van apen voor dit onderzoek om deze redenen.
Hergebruik zal plaatsvinden binnen de wettelijke kaders beschreven in de Wet op de Dierproeven. De DEC is het eens met hergebruik van de dieren om deze redenen.

10. Geef aan of de dieren gehuisvest en verzorgd worden op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU. Indien niet aan deze minimale eisen kan worden voldaan, omdat het, om redenen van dierenwelzijn of diergezondheid of om wetenschappelijke redenen, noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe.
De huisvesting en verzorging voldoen ten volle aan de vereisten in bijlage III van de richtlijn.

11. Beoordeel of het cumulatieve ongerief als gevolg van de dierproeven voor elk dier realistisch is ingeschat en geclassificeerd. Licht uw beoordeling toe.
Het ongerief voor de dieren is correct als matig ingeschat. Het ongerief zal vooral veroorzaakt worden door het frequent bloed afnemen bij de dieren, het plaatsen van een datalogger, het uitvoeren van CT scans en het besmetten met influenzavirus met de daarop volgende klinische symptomen. De DEC is het eens met deze inschatting.

12. Het uitvoeren van dierproeven zal naast het ongerief vaak gepaard gaan met aantasting van de integriteit van het dier. Beschrijf op welke wijze er sprake is van aantasting van integriteit.
De integriteit van de dieren wordt aangetast door het instrumentele gebruik en experimentele procedures en de effecten daarvan. De gevolgen daarvan op de sociale context, lichamelijke integriteit en zelfredzaamheid van het dier blijven beperkt tot de duur van het experiment. Een deel van de dieren zal worden gedood voor nader onderzoek (wetenschappelijke uitkomst), dit impliceert niet zozeer aantasting van het welzijn maar wel van de integriteit.

13. Beoordeel of de criteria voor humane eindpunten goed zijn gedefinieerd en of goed is ingeschat welk percentage dieren naar verwachting een humaan eindpunt zal bereiken. Licht uw beoordeling toe.
Het bereiken van een humaan eindpunt is goed gedefinieerd en toegespitst op de aard van het onderzoek. Dieren zullen niet onnodig lijden omdat de dieren direct uit de proef genomen zullen worden en adequaat behandeld zullen worden mochten er complicaties optreden. Het instituut heeft veel ervaring op het gebied van het testen van vaccins voor luchtweg virussen en de commissie acht het waarschijnlijk dat bij geen van de dieren het humane eindpunt bereikt zal worden.

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte

vervangingsalternatieven zijn.

De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. De immuunrespons op een vaccin in een zoogdier is te complex om die in vitro of in silico te modelleren. Het is essentieel dat een vaccin zal worden getest op effectiviteit en veiligheid in een hiervoor relevante diersoort (zie ook C9).

15. Beoordeel of het aantal te gebruiken dieren realistisch is ingeschat en of er een heldere strategie is om ervoor te zorgen dat tijdens het project met zo min mogelijk dieren wordt gewerkt waarmee een betrouwbaar resultaat kan worden verkregen. Licht uw beoordeling toe.

Er zullen in de loop van het project maximaal 3 vaccin kandidaten worden getest op mogelijke bijwerkingen, immunogeniciteit en bescherming tegen infectie. Daarvoor is ingeschat dat er per vaccintest maximaal 30 dieren zullen worden ingezet. De precieze groepsgroottes zijn op dit moment nog niet bekend en worden ingeschat op maximaal 10 dieren per groep. Op basis van power analyses zullen de uiteindelijke groepsgroottes worden bepaald waarmee statistisch significante en relevante resultaten kunnen worden behaald. Deze marges en daarmee het maximaal aantal te gebruiken dieren is op basis van de resultaten uit het voorafgaande onderzoek ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de aangevraagde looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee wetenschappelijk betrouwbare en verantwoorde resultaten kunnen worden verkregen (proefopzet, statistische power). Vanwege de op dit moment inherente onzekerheid over de variatie in de uitleesparameters is er door de indieners voor wat betreft het aantal dieren uitgegaan van een maximum scenario. Maximaal 3 nieuwe virusvarianten zullen worden gebruikt om vast te stellen welke virus dosis gebruikt moet worden voor de besmettingsproeven. Hiervoor zullen 4 dieren per dosisgroep, 2 dosisgroepen per virus en in totaal maximaal 24 dieren worden gebruikt. De commissie weegt mee dat hierbij ook het aantal vaccinkandidaten en aantal experimenten kader stellend zijn.

16. Beoordeel of het project in overeenstemming is met de vereiste van verfijning van dierproeven en het project zodanig is opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Licht uw beoordeling toe.

De uitvoering is verfijnd door gebruik te maken van goed geadapteerde en sociaal gehuisveste dieren. Ook het gebruik van telemetrie om het verloop van de lichaamstemperatuur (en eventueel activiteit) te volgen is een noemenswaardige verfijning. Er zal sedatie worden toegepast tijdens de onderzoeksprocedures, en anesthesie waar nodig. Dieren worden gedurende de gehele studie gemonitord door ervaren dierverzorgers en een klinische scorelijst wordt bijgehouden. De dieren staan onder veterinaire begeleiding. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Beoordeel, indien het wettelijk vereist onderzoek betreft, of voldoende aannemelijk is gemaakt dat er geen duplicatie plaats zal vinden en of de aanvrager beschikt over voldoende expertise en informatie om tijdens de uitvoering van het project te voorkomen dat onnodige duplicatie plaatsvindt. Licht uw beoordeling toe.
N.v.t.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. Geef aan of dieren van beide geslachten in gelijke mate ingezet zullen worden. Indien alleen dieren van één geslacht gebruikt worden, beoordeel of de aanvrager dat in voldoende mate wetenschappelijk heeft onderbouwd.
In onderhavige projectaanvraag worden per studie dieren van één geslacht gebruikt. De immuunrespons van mannen en vrouwen is verschillend. Door dieren van hetzelfde geslacht te nemen zal de variatie in de proefuitkomsten zo klein mogelijk zijn en dit zal de variatie in de uitkomst parameters verkleinen. Daardoor zullen minder proefdieren nodig zijn dan wanneer er met groepen van gemengde geslachten wordt gewerkt. Er is geen sprake van overschotten.

19. Geef aan of dieren gedood worden in kader van het project (tijdens of na afloop van de dierproef). Indien dieren gedood worden, geef aan of en waarom dit noodzakelijk is voor het behalen van de doelstellingen van het project. Indien dieren gedood worden, geef aan of er een voor de diersoort passende dodingsmethode gebruikt wordt die vermeld staat in bijlage IV van richtlijn 2010/63/EU. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht uw beoordeling toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd.
De dieren zullen niet worden gedood aan het einde van de studie tenzij er aanleiding is tot nader pathologisch onderzoek (wetenschappelijke uitkomst). De DEC is het eens met het eventuele hergebruik van de dieren mits er aan de kaders gesteld in de Wet op de Dierproeven wordt voldaan.

20. Indien niet-humane primaten, honden, katten of landbouwhuisdieren worden gedood om niet-wetenschappelijke redenen, is herplaatsing of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.
De dieren zullen niet worden gedood aan het einde van de experimenten tenzij er bijwerkingen zijn die het noodzakelijk maken de dieren te doden (humaan eindpunt, wetenschappelijke analyse van oorzaak bijverschijnselen). De dieren kunnen eerder in onderzoek zijn gebruikt en kunnen na afloop voor hergebruik bestemd worden, dit alles met inachtneming van overwegingen rond geschiktheid en dierenwelzijn.

NTS

21. Is de niet-technische samenvatting een evenwichtige weergave van het project en begrijpelijk geformuleerd?

De niet-technische samenvatting is een evenwichtige weergave van het project.

D. Ethische afweging

1. Benoem de centrale morele vraag. Rechtvaardigt het belang van het ontwikkelen van nieuwe types vaccinkandidaten met een beoogde brede en langdurige werking tegen influenza het testen op bijwerkingen, immunogeniciteit, en op bescherming in apen, het ongerief dat de dieren daardoor wordt aangedaan en is bij de uitvoering van deze experimenten aan alle zorgvuldigheidseisen (3V's) voldaan? Is het gebruik van niet-humane primaten in dit geval gerechtvaardigd of kan de gewenste informatie ook verkregen worden door het inzetten van andersoortige proefdieren, andere onderzoek modellen of patiënten?

2. Weeg voor de verschillende belanghebbenden, zoals beschreven onder C5, de sociale en morele waarden waaraan tegemoet gekomen wordt of die juist in het geding zijn, ten opzichte van elkaar af. Om dit proces te vergemakkelijken, kunt u de belangrijkste belanghebbenden en de belangrijkste waarden die in het geding zijn waarderen. U kunt dit verwoorden in termen van gering, matig of veel/ernstig voordeel of nadeel. Geef aan waarom de DEC bevordering van waarden (baten) voor de ene belanghebbende prevaleert boven de aantasting van waarden (kosten) voor de andere belanghebbende.

Alle 114 dieren (resus of Java apen) zullen hoogstens matig ongerief ondergaan. Voor de proefdieren zijn integriteit, welzijn en de autonomie in het geding, door de dieren handelingen met een wetenschappelijk doel te laten ondergaan. Het betreft experimenten waarbij de dieren geïmmuniseerd worden met een experimenteel influenzavaccin en/of zullen worden besmet met influenzavirus. De welzijnsaantasting kan resulteren in ongerief na het plaatsen van een datalogger, het afnemen van bloed of slijmvlies monsters, en de klinische verschijnselen van de influenza infectie. Alle handelingen zullen onder sedatie of volledige narcose gebeuren. Het ongerief is dan veelal het bijkomen uit de verdoving/narcose. Ongerief en lijden zal zo veel mogelijk worden beperkt. Daarmee resulteert dit in maximaal matig ongerief voor de dieren.

De waarde voor de patiënten, de zorginstellingen en de samenleving is het op termijn beschikbaar komen

van een werkzaam universeel vaccin voor influenzavaccin dat een brede bescherming biedt tegen meerdere varianten van het virus en dat slechts een keer in de circa 5 jaar gegeven hoeft te worden. Op dit moment is er nog geen universeel influenza vaccin beschikbaar. Hierdoor is het noodzakelijk jaarlijks de samenstelling van het influenza vaccin aan te passen aan de op dat moment circulerende varianten. Echter deze zullen geen bescherming bieden tegen nieuwe varianten, waardoor er altijd een dreiging is op grote influenza uitbraken met veel patiënten die intensieve zorg nodig hebben. Het krijgen van beschikking over een universeel vaccin zal het aantal patiënten met ernstige verschijnselen ten gevolge van nieuwe varianten reduceren, zal de belasting van de zorg door de jaarlijkse vaccinaties verminderen en zullen nadelige economische en financiële effecten voor de maatschappij afnemen.
Voor de producent van het vaccin is het op de markt brengen van een vaccin van economisch belang. Echter het belang voor de maatschappij is aanzienlijk groter.

3. Beantwoord de centrale morele vraag. Maak voor het beantwoorden van deze vraag gebruik van bovenstaande afweging van morele waarden. Maak daarnaast gebruik van de volgende moreel relevante feiten: belang onderzoek (C4), kennis en kunde van betrokkenen (C7), haalbaarheid doelstellingen (C8), categorieën en herkomst dieren (C9), 3V's (C14-C18), ongerief (C10-13 en C19) en relevante wet en regelgeving (C2). Onderbouw hoe al deze elementen zijn meegewogen bij de beantwoording van de centrale morele vraag, zodanig dat het navolgbaar is zonder gedetailleerde kennis te hebben van het projectvoorstel *(Zie Praktische handreiking ETK: Stap 3.C; zie bijlage I voor voorbeeld)*.

De DEC is overtuigd van het belang van de doelstelling: het testen van nieuwe types vaccinkandidaten voor influenza op bijwerkingen, immunogeniciteit, en op bescherming in niet-humane primaten. Het uiteindelijke doel is het op de markt brengen van een werkzaam universeel influenza vaccin. Deze doelstelling vertegenwoordigt een essentieel belang voor de maatschappij omdat de huidige vaccins alleen beschermen tegen het beperkte aantal varianten waartegen het vaccin is gericht, het virus veelvuldig muteert waardoor jaarlijks nieuwe vaccins nodig zijn. Met de ontwikkeling van een universeel influenzavaccin zal er voor langere tijd bescherming zijn tegen meerdere varianten van het virus, inclusief toekomstige varianten. De DEC is van mening dat het gebruik van niet-humane primaten voor dit onderzoek gerechtvaardigd is. Het gaat in dit onderzoek om nieuwe vaccinatie strategieën die pas klinisch kunnen worden getest als (ook) in niet-humane primaten is aangetoond dat de vaccins beschermen tegen een influenzavirus infectie.
De commissie is overtuigd van de kwaliteit van het onderzoek en de uitvoering hiervan. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat voorkomen wordt dat mens, dier en milieu onbedoelde negatieve effecten zullen ondervinden als gevolg van de dierproeven.

De DEC is samenvattend van mening dat aan alle zorgvuldigheidseisen omtrent de 3 V's en de kwaliteit van het onderzoek is voldaan en dat het hierboven genoemde belang voor de samenleving als geheel het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van welzijn en integriteit) rechtvaardigt.

E. Advies

1. Advies aan de CCD

   X De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden

   X Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD. Het betreft hier niet-humane primaten.

   Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten: geen

2. Het uitgebrachte advies kan unaniem tot stand zijn gekomen dan wel gebaseerd zijn op een meerderheidsstandpunt in de DEC. Indien gebaseerd op een meerderheidsstandpunt, specificeer het minderheidsstandpunt op het niveau van verschillende belanghebbenden en de waarden die in het geding zijn.

   Het uitgebrachte advies is gebaseerd op consensus.

3. Omschrijf de knelpunten/dilemma's die naar voren zijn gekomen tijdens het beoordelen van de aanvraag en het opstellen van het advies zowel binnen als buiten de context van het project.

   Er zijn bij de beoordeling van dit project geen knelpunten ondervonden; de inherente ethische dilemma's zijn hierboven uitgebreid uiteengezet.

NIET-TECHNISCHE PROJECTSAMENVATTING

Naam van het project	Onderzoek naar de beschermende werking van nieuwe griepvaccins.
NTS-identificatiecode	NTS-NL-512304 v.1
Nationale identificatiecode van de NTS *Veld wordt niet gepubliceerd.*	
Land	Nederland
Taal	nl
Indiening bij EU *Veld wordt niet gepubliceerd.*	ja
Duur van het project, uitgedrukt in maanden.	60
Trefwoorden	Influenza Vaccin Effectiviteit Infectie non-humane primaten
Doel(en) van het project	Omzettinggericht en toegepast onderzoek: Besmettelijke ziekten van de mens

DOELSTELLINGEN EN VERWACHTE VOORDELEN VAN HET PROJECT

Beschrijf de doelstellingen van het project (bijvoorbeeld het aanpakken van bepaalde wetenschappelijke onduidelijkheden, of wetenschappelijke of klinische behoeften).	Griep wordt veroorzaakt door het influenzavirus. Jaarlijks krijgen tussen 2,5 en 10% van de mensen de griep. Meestal volgt na infectie een spoedig herstel, maar met name bij jonge kinderen, ouderen of bij mensen met longproblemen kan de infectie ernstig verlopen. Naar schatting overlijden jaarlijks tussen de 250.000 en 500.000 mensen wereldwijd aan de griep. Daarom krijgen kwetsbare groepen mensen, waaronder ouderen, jaarlijks een vaccin; de griepprik. Het probleem bij de griep is dat het virus dat de griep veroorzaakt constant verandert. Doordat het maken van een nieuw vaccin minimaal een half jaar duurt kan het gebeuren dat het griepvirus in de tussentijd veranderd is en het vaccin niet goed meer beschermt. Bovendien kunnen ook heel nieuwe varianten ontstaan door overdracht van griepvirussen tussen vogels, varkens en de mens. Omdat mensen nog niet eerder met dergelijke nieuwe virussen in aanraking zijn gekomen hebben ze er geen afweer tegen en verspreidt het virus zich wereldwijd en veroorzaakt een zogenaamde pandemie. Tijdens een pandemie kunnen miljoenen mensen overlijden, zoals bijvoorbeeld tijdens de Spaanse griep in 1918-1919. De huidige griepvaccins geven geen bescherming tegen dergelijke nieuwe varianten en ook niet tegen vogelgriep. Het is daarom nodig om zowel betere vaccins te maken die tegen vele griepvarianten, nieuwe griepvirussen en vogelgriep beschermen en om betere methodes te ontwikkelen voor een snelle productie van het vaccin. Omdat hiervoor geheel nieuwe methodes nodig zijn, waarvan nog niet goed voorspeld kan worden hoe goed ze zullen werken, is uitgebreid testen zowel in het laboratorium als in proefdieren noodzakelijk, voordat deze vaccins bij de mens getest kunnen worden. Het directe doel van dit project is om deze nieuwe griepvaccins in apen te testen om vast te stellen of ze geen onverwachte bijwerkingen geven, een goede afweerreactie tegen griep opwekken en of het vaccin bescherming biedt tegen griepinfectie.
Welke potentiële voordelen kan dit project opleveren? Leg uit hoe de wetenschap vooruit kan worden geholpen of mensen, dieren of het milieu uiteindelijk voordeel kunnen hebben bij het project. Maak, waar van toepassing, een onderscheid	Het uiteindelijke doel van de experimenten die in dit project worden uitgevoerd is om een vaccin te verkrijgen dat kan beschermen tegen een groot aantal griepvarianten en indien mogelijk ook tegen vogelgriep. Een dergelijk vaccin kan veel levens redden bij een nieuwe pandemie en biedt een betere bescherming van kwetsbare groepen in de samenleving. Bovendien is de jaarlijkse griepprik dan niet meer nodig en kan volstaan worden met een griepprik eens in de 5 jaar.

tussen voordelen op korte termijn (binnen de looptijd van het project) en voordelen op lange termijn (die mogelijk pas worden bereikt nadat het project is afgerond).	

VOORSPELDE SCHADE

In welke procedures worden de dieren gewoonlijk gebruikt (bijvoorbeeld injecties, chirurgische procedures)? Vermeld het aantal en de duur van deze procedures.	Sedatie, bij iedere handeling (gedurende 15-60 minuten). Implanteren/verwijderen van implantaat voor meten temperatuur, chirurgisch 1x in 1 x uit (± 60 minuten). Vaccinatie (maximaal 6x, 10 minuten). Bloedafnames (maximaal 4x na iedere vaccinatie en 10x na elke virusinfectie, 10 minuten). Bronchoalveolaire lavage (maximaal 3x na iedere vaccinatie en 5 x na elke virusinfectie, 30 minuten). CT scan (maximaal 5x na elke virusinfectie, 15min). Virusinfectie (maximaal 2x, 10 minuten). Bepaling virusload (neus, keel swabs maximaal 10x na infectie).
Wat zijn de verwachte gevolgen/nadelige effecten voor de dieren, bijvoorbeeld pijn, gewichtsverlies, inactiviteit/verminderde mobiliteit, stress, abnormaal gedrag, en wat is de duur van die effecten?	De dieren zullen stress ondervinden ten gevolge van de biotechnische handelingen. Ook kunnen de dieren pijn ondervinden door de biotechnische handelingen. Deze handelingen worden echter onder verdoving uitgevoerd. Ook kunnen de dieren ziek worden door de experimentele infectie met het griepvirus. Het nadelig gevolg van de biotechnische handelingen duurt over het algemeen 1 dag, met uitzondering van de chirurgische ingreep, waarvan het effect maximaal 1 week kan duren.
Welke soorten en aantallen dieren zullen naar verwachting worden gebruikt? Wat zijn de verwachte ernstgraden en de aantallen dieren in elke ernstcategorie (per soort)?	

Soort:	*Totaal aantal*	*Geraamde aantallen naar ernstgraad*			
		Terminaal	*Licht*	*Matig*	*Ernstig*
Java-apen (Macaca fascicularis)	90	0	0	90	0
Rhesusapen (Macaca mulatta)	24	0	0	24	0

Wat gebeurt er met de dieren die aan het einde van de procedure in leven worden gehouden?	

Soort:	*Geraamd aantal te hergebruiken, in het habitat-/houderijsysteem terug te plaatsen of voor adoptie vrij te geven dieren*		
	Hergebruikt	*Teruggeplaatst*	*Geadopteerd*
Java-apen (Macaca fascicularis)	50	0	0
Rhesusapen (Macaca mulatta)	12	0	0

Geef de redenen voor het geplande lot van de dieren na de procedure.	Na afloop van het experiment zullen de dieren over het algemeen hersteld zijn van de infectie en kunnen dan deel blijven uitmaken van de experimentele dieren op het instituut en worden hergebruikt voor niet griep-gerelateerde studies. Echter, als moet worden nagegaan of het vaccin eventueel schadelijke effecten op het lichaam kan hebben of indien de dieren ten gevolge van de griepinfectie blijvende schade aan de longen krijgen, dan zullen ze op een humane wijze worden gedood.

TOEPASSING VAN DE DRIE V'S

1. Vervanging Beschrijf welke diervrije alternatieven op dit gebied voorhanden zijn en waarom zij niet voor het project kunnen worden gebruikt.	Het is nog niet mogelijk om de beschermende werking van vaccins zonder gebruik van proefdieren te bepalen. Het afweersysteem is dermate ingewikkeld dat dit nog niet in het laboratorium kan worden nagebootst. Ook de beschermende werking van het vaccin tegen virusinfectie is complex en wordt bepaald door hoe de diverse componenten van het afweersysteem op lokaal niveau in de long de juiste type cellen kunnen beschermen tegen infectie en het individu kunnen beschermen tegen griepverschijnselen.
2. Vermindering Leg uit hoe de aantallen dieren voor dit project zijn bepaald. Beschrijf de stappen die zijn genomen om het aantal te gebruiken dieren te verminderen en de beginselen die zijn gebruikt bij het opzetten van de studies. Beschrijf, waar van toepassing, de praktijken die gedurende het hele project zullen worden toegepast om het aantal dieren die in overeenstemming met de wetenschappelijke doelstellingen werden gebruikt, tot een minimum te beperken. Deze praktijken kunnen bijvoorbeeld bestaan uit proefprojecten, computermodellen, het delen van weefsel en hergebruik.	Voordat een griepvaccin in apen wordt getest is het al uitgebreid getest in het laboratorium en in andere diersoorten, bijvoorbeeld in muizen. Uit dit eerdere onderzoek moet zijn gebleken dat het vaccin veilig is en dat het vaccin voldoende werkzaam is om in een afweerreactie op te roepen na injectie. Hierna worden alleen de meest belovende vaccinkandidaten in apen getest. Het aantal benodigde dieren wordt per experiment bepaald aan de hand van statische analyses. Dit aantal zal afhangen van de eigenschappen van het vaccin en van het te gebruiken testvirus. Waar mogelijk zullen meerdere vaccins tegelijk getest worden, waardoor maar één controlegroep nodig is. Het aantal dieren in de controlegroep zal zoveel mogelijk worden beperkt door gegevens te gebruiken van dieren die reeds eerder geïnfecteerd zijn bij het opzetten van de virusinfectie modellen.
3. Verfijning Geef voorbeelden van de specifieke maatregelen (bv. verscherpte monitoring, postoperatieve behandeling, pijnbestrijding, training van dieren) die in verband met de procedures moeten worden genomen om de welzijnskosten (schade) voor de dieren tot een minimum te beperken. Beschrijf de mechanismen om gedurende de looptijd van het project nieuwe verfijningstechnieken in gebruik te nemen.	Alle handelingen worden uitgevoerd onder verdoving. Waar nodig wordt bovendien pijnstilling gegeven. De dieren worden getraind om zoveel mogelijk vrijwillig mee te werken aan de verdoving. Tijdens de studie worden de dieren dagelijks geobserveerd en tijdens de griepinfectie tweemaal daags. De ziekteverschijnselen worden genoteerd op een scorelijst. Wanneer ernstige ziekteverschijnselen optreden of wanneer een bepaalde score overschreden wordt, worden de dieren direct op een humane wijze gedood om verder ongerief te voorkomen.
Licht de keuze van de soorten en de bijbehorende levensstadia toe	Onderzoek naar de werkzaamheid van griepvaccins kan in diverse dieren worden uitgevoerd. Alleen in de laatste fase van de vaccinontwikkeling is testen in apen nodig, omdat deze dieren wat betreft de anatomie van de luchtwegen, het afweersysteem en vatbaarheid voor influenza het meest op de mens lijken. Andere proefdieren, zoals muizen zijn in deze fase van het onderzoek niet geschikt omdat humane griepvirussen in muizen vaak niet een goede infectie geven en zowel muizen als fretten wat betreft hun afweersysteem op diverse punten afwijken van de mens. Daarom is in apen de kans het grootst dat eventuele onverwachte nadelige effecten alsnog opgespoord kunnen worden en een goede voorspelling gedaan kan worden wat betreft werkzaamheid bij de mens. Dit geldt met name voor de nieuwe methodes voor het maken van een griepvaccin die hier getest worden, aangezien die nog nooit bij de mens getest zijn en het dus niet voldoende duidelijk is of ze veilig en werkzaam zijn.

VOOR EEN BEOORDELING ACHTERAF GESELECTEERD PROJECT

Project geselecteerd voor BA?	ja
Termijn voor BA	31-05-2027
Reden voor de beoordeling achteraf	
Bevat ernstige procedures	
Maakt gebruik van niet-menselijke primaten	ja
Andere reden	
Toelichting van de andere reden voor de beoordeling achteraf	

AANVULLENDE VELDEN

Nationaal veld 1 *Veld wordt niet gepubliceerd.*	
Nationaal veld 2 *Veld wordt niet gepubliceerd.*	
Nationaal veld 3 *Veld wordt niet gepubliceerd.*	
Nationaal veld 4 *Veld wordt niet gepubliceerd.*	
Nationaal veld 5 *Veld wordt niet gepubliceerd.*	
Startdatum project *Veld wordt niet gepubliceerd.*	
Einddatum project *Veld wordt niet gepubliceerd.*	
Goedkeuringsdatum project *Veld wordt niet gepubliceerd.*	
ICD-code 1 *Veld wordt niet gepubliceerd.*	
ICD-code 2 *Veld wordt niet gepubliceerd.*	
ICD-code 3 *Veld wordt niet gepubliceerd.*	
Link naar de eerdere versie van de NTS buiten het EC-systeem	

Centrale Commissie Dierproeven

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information on the project proposal, see the Guidelines to the project licence application form for animal procedures on 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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure *Use the numbers provided at 3.4.3 of the project proposal.*	Serial number: 1 — Type of animal procedure: Influenza vaccine evaluation in macaques

2 Description of animal procedures

A. Experimental approach and primary outcome parameters
Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

We have developed a general study protocol for the evaluation of influenza vaccines in macaques. Temperature and potentially movement and heart rate will be measured by telemetry. A device that records and transmits these parameters is surgically placed in the abdominal cavity. When all mentioned parameters need to be monitored during the immunization as well as the infection phase then a relatively large device is needed that is capable of performing all these measurements and can be used during a long period. This larger device has the advantage that it can be temporarily put on stand-by to safe battery life and then be reactivated when needed, so that it will last through the entire study period. Such devices will be placed at least 4 weeks before the first immunization. In case only the temperature needs to be monitored during the influenza virus infection phase then a small device suffices. Such a device has however a shorter life-span (28 weeks) and has to be placed in the abdominal cavity four weeks before infection. Subsequently the animals are immunized either once or they receive a number of immunizations over a certain period of time. Although the vaccines that are used in these studies have already been extensively evaluated in other animals and are expected to give no or only very limited adverse effects, macaques will be monitored for possible changes in general behaviour and health. The site of immunization will be checked for local reactions and blood will be drawn to measure clinical chemistry and haematology parameters. Before, between and after immunizations, blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. Both T-cell and antibody responses will be measured against several influenza viruses in order to establish the strength and breadth of the immune response. When adequate immune responses are induced that indicate that protection against infection might be achieved, the efficacy against infection will be tested by experimental infection with influenza virus. A group of non-vaccinated animals will be included as infection controls. Usually only one virus will be used for experimental infection. The choice of the virus strain to be used will depend on the outcome of the evaluation of the immune responses and the stage of development of the vaccine. An entirely new vaccine concept may require that in first instance protection against infection by a homologous virus strain is tested. However, for most vaccine candidates protection against infection with a heterologous virus will be tested. All animals in a specific vaccine group will be challenged with the same virus. If protection against two different viruses needs to be established than two groups of animals are immunized with the same vaccine and subsequently group 1 will be challenged with virus 1 and group 2 with virus 2. If animals are protected against infection, then a second challenge with a different virus may be performed. In this case an additional challenge control group is needed. In this manner the breadth of the immune response is both measured *in vitro;* via the evaluation of the induced immune response and *in vivo* via experimental infection with different virus strains. The infection will be performed as described in appendix 2.

The primary outcome parameters are:

- Absence of unexpected reactogenicity of the vaccine: effects of the vaccine on general behaviour, health, local reactions and blood parameters.
- Immunogenicity: Induction of cellular and humoral immune responses. The type and strength of the induced responses will determine if the objectives of the vaccine strategy are achieved and whether immune responses are sufficiently strong to proceed with viral challenge.
- Efficacy: Capacity to protect against viral challenge will be established in terms of: reduction in clinical symptoms, fever, virus multiplication and changes in blood parameters.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

A telemetry device is surgically placed in the abdominal cavity at least 4 weeks either before the first immunization or before the infection. This time frame is necessary for full recovery of the animals and to allow adequate recording of body temperature and/or heart rate and activity during a two to three-week period to establish normal values before immunizations start. Animals will receive one or more immunizations. If multiple immunizations are given then typically a 4 to 8-week time interval is used, although occasionally a longer time frame is needed between immunizations when different vaccine modalities are used for priming and boosting of the immune response. In rare occasions these limits may have to be exceeded, with a maximum of 6 immunizations. Specific rationale will then be presented to the animal welfare body (AWB). Immunizations will be done either by intradermal injection, intramuscularly, subcutaneously, intravenously, intranasally, intra-tracheally or via aerosol using a nebulizer. All vaccines have to be sterile and have to be given under aseptic conditions. At regular time intervals, usually two and four weeks after every immunization, blood is drawn to measure systemic adverse effects, which includes measurement of clinical chemistry and haematology, and to measure induction of cellular and humoral immune responses. The two-week interval after immunization is chosen, because this is optimal for measuring cellular immune responses, while four weeks is optimal for measuring humoral immune responses. In some studies, nasal washes and lung lavages (BAL) are taken after immunization in order to measure induction of local immune responses. BAL will be maximally collected three times after each immunization, resulting in total in 18 BAL collections if animals are immunized six times. Immune responses recorded after the final immunization will be used to decide whether protection against viral challenge can be reasonably expected. If these responses are inadequate then the study will be stopped and animals may be re-used in other non-influenza virus related experiments. Otherwise, experimental challenge will usually take place between 4 and 8 weeks after the last immunization. This time period is needed to allow immunological memory to form after the last immunization. Influenza infection may be done by intra-bronchial or intra-tracheal inoculation alone or in combination with oral, intranasal and intraocular inoculation or via aerosol using a nebulizer (as described in appendix 2). Clinical symptoms will be monitored twice daily during the infection phase. Nasal and tracheal swabs will be taken before infection, frequently during the first days after infection (daily and then every other day) and subsequently less frequently until day 21, to measure virus multiplication in the upper airways. Lung lavages may be taken at selected time points (max 5 times) to measure virus multiplication in the lungs. Blood is taken simultaneously with the swabs, to monitor changes in clinical chemistry and haematology parameters, leucocyte subsets and cytokine production. At the same time points body weight and physiological parameters (for instance pulse rate, blood pressure) are recorded and imaging (for instance CT or PET-CT) is performed to measure lung infiltration. After the virus is cleared (usually at day 21 after infection) animals may be exposed to a second challenge virus. All handling will then be repeated. However, this will be rarely the case and specific argumentation will be presented to the AWB. In general animals are either returned to the experimental stock or they are euthanized and a full necropsy is performed in order to establish lung pathology. Euthanasia is only performed when assessment of lung pathology is required to establish vaccine safety and in case pathogenic influenza viruses are used that are known to cause persistent lung pathology. Some telemetric devices, that have been designed to measure multiple parameters, can be quite large and require a substantial operation. These will not be removed and the animal will be euthanized instead. A small telemetric device can be surgically removed and animals may be re-used. A specific rational has to be provided to the AWB for using these devices. In case an animal should reach the humane endpoint during the study it will be immediately euthanized and a full necropsy will be performed to establish lung pathology and virus multiplication in the respiratory tract.

The details of each study, regarding the interval between the immunizations, the number and time points of sampling, the specific criteria to proceed with a viral challenge, the time interval between the last immunization and viral challenge will depend on the actual type of vaccine that is being tested and this will be submitted to the AWB.

Table. Maximum number of repeats per procedure. Indicated is which procedure is performed under sedation and which procedure under deep anesthesia.

Procedure	Maximum	Duration	sedation	anesthesia
Recorder in/out	2	60 min		X
Vaccination	6	10 min	X	
Blood sample	44	10 min	X	
Bronchoalveola lavage (BAL)	28	30 min		X
Infection	2	10 min		X
Swabs	20	10 min	X	
CT-scan	10	15 min		X

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

The number of animals will be based on statistical power analysis. Calculations take into account the number of animals needed to measure statistically significant induction of immune responses in relation to unvaccinated controls or, in case different vaccines are compared, whether significant differences between the vaccine groups can be obtained. In addition, calculations are performed to establish the number of animals needed to obtain a significant reduction in virus load in the trachea between the vaccine groups and the challenge control group. Experience in previous influenza vaccine evaluation studies performed at our institute indicates that statistically significant reductions in virus multiplication can be obtained with six animals per group. However, in order to be able to measure differences between the vaccine groups themselves, more animals per group may be needed. Only the minimum number of animals required will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the non-vaccinated challenge control group than in the vaccine groups.

B. The animals

Specify the species, origin, life stages, estimated numbers, gender, genetic alterations and, if important for achieving the immediate goal, the strain.

Serial number	Species	Origin	Life stages	Number	Gender	Genetically altered	Strain
1	Rhesus or cynomolgus macaque	Purpose bred	adult	90	M / F	no	Not applicable

Provide justifications for these choices

Species	Macaque species have been used in several influenza vaccine studies (1-6). The most frequently used species are the rhesus- (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*). Both species are semi-permissive to influenza infection. Therefore, both rhesus and cynomolgus macaques can be used for influenza vaccine evaluation studies.
Origin	All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier.
Life stages	Adult animals will be used because these allow larger volumes of blood to be collected.
Number	90 macaques in total (either cynomolgus or rhesus). The number of animals requested is based on the assumption that each study will contain two vaccine groups and 1 control group, with 10 animals per group. The group size will be determined per experiment, based on power calculation specific for the experiment. As stated above, probably fewer animals will be needed in the non-vaccinated challenge control groups and also in the vaccine groups in some of the studies. In all, we anticipate performing 3 such studies over a 5-year period.
Gender	Adult male and female animals can be used. There are immunological differences between males and females (7, 8). However, for influenza vaccine induced responses these differences are only modest (9) and not observed in all reports (10) and are unlikely to affect study outcome in pre-clinical studies with relatively low number of animals. Therefore, both male and female animals can be used.
Genetic alterations	Not applicable
Strain	Not applicable

C. Accommodation and care

Is the housing and care of the animals used in experimental procedures in accordance with Annex III of the Directive 2010/63/EU?

☒ Yes

☐ No > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices

D. Pain and compromised animal welfare

Will the animals experience pain during or after the procedures?

☐ No

☒ Yes > Will anaesthesia, analgesia or other pain relieving methods be used?

☐ No > Justify why pain relieving methods will not be used.

☒ Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

After placement of the recording device in the abdomen and after removal, which is needed in case the animal will not be euthanized after completion of the experiment, animals will receive analgesics for as long as necessary, typically 3 days. In previous studies we have observed that animals can experience some body temperature elevation during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation. Pain relieve will also be applied when substantial induration is seen at the site of vaccine injection. In case of the latter, analgesics known not to interfere with the induction of the vaccine response will be used.

Describe which other adverse effects on the animals' welfare may be expected?

1. Discomfort because of insertion or removal of the temperature recording device
2. Discomfort due to injection
3. Discomfort due to lung lavages
4. Discomfort due to virus installation
5. Discomfort due to PET-CTs
6. Stress because of sedation and recovery
7. Reduced food intake during the first days after infection
8. Disease symptoms due to the infection

Explain why these effects may emerge.

1. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
2. When vaccines are given by injection, this can cause local pain and irritation.
3. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
4. When virus is given intra-bronchially a bronchoscope is used and this combined with the inoculum volume will cause irritation. Aerosol application implies that animals have to be sedated and forced to breathe via a nebulizer device and this can give irritation.
5. For the PET-CT animals are sedated, intubated and mechanical ventilation with a forced breathing pattern is applied. No adverse effects are expected from the scan itself
6. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs, CTs and lung lavages. Nausea can sometimes be observed during recovery from the sedation.
7. Especially during daily sedation during the first 2 days after infection food intake might be reduced.
8. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, increased breathing rate, loss of appetite, inactivity.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

1. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
2. Animals will be sedated for vaccine delivery. Only rarely are strong adverse effects seen. Should granuloma formation be observed, then the animal will be sedated, the wound will be cleaned and analgesics are applied if necessary following veterinary consultation.
3. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
4. The same procedure as described under 3 will be followed
5. The animals are first deeply sedated and then receive a local mucosal relaxant before intubation. The mechanical ventilation & forced breathing patterns will be monitored and adapted on each animals characteristics.
6. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
7. Animals will receive supportive feeding with dense "brokkenballen".
8. Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms. When a certain pre-determined clinical score is reached then the animal will be humanely euthanized and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory tract.

E. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

☐ No > Continue with question F

☒ Yes > Describe the criteria that will be used to identify the humane endpoints.

Animals are monitored twice daily and a weighed clinical scoring list is used to record the clinical symptoms (11). When a clinical score of 35 is reached this indicates that the maximum duration of severity is reached then the animal will be euthanized. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort. Symptoms associated with score 35 that lead to an immediate endpoint are: open mouth breathing or cyanosis, lethargy as defined by minimal response to human approach.

Indicate the likely incidence.

The likely incidence for reaching a clinical endpoint depends on the virus strain that is used for infection. Human influenza viruses only rarely cause severe disease (<1% of the animals). Highly pathogenic avian influenza viruses can cause lethal disease in up to 75% of the non-vaccinated control animals.

F. Classification of severity of procedures

Provide information on the experimental factors contributing to the discomfort of the animals and indicate to which category these factors are assigned ('non-recovery', 'mild', 'moderate', 'severe'). In addition, provide for each species and treatment group information on the expected levels of cumulative discomfort (in percentages).

The total amount of discomfort is estimated as moderate. This is mainly caused by the surgical implantation and removal of the recording device and development of disease due to infection and the high maximum number of sedations.

G. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement	The immune system is very complex and the *in vivo* interactions between virus and/or vaccine and host are not completely understood. At present there is no *in vitro* model available that can mimic the human immune system sufficiently to study the potential of a vaccine to induce protective immune responses. Due to the complex interaction of influenza virus with different tissues and the role of local immunity in eradication of the virus, the efficacy of an influenza vaccine to protect against infection can only be adequately established in an animal model. Several animal species have been used as a model for human influenza virus infection (12). However, mice are not naturally susceptible to influenza and usually lethal infection models are used, that are based on extremely adapted human viruses. Ferrets are naturally susceptible to most human influenza viruses and recapitulate the natural course of infection. However, infection is often restricted to the upper airways and this model has the disadvantage that limited immunological tools are available, especially to study innate and cellular adaptive immune responses, which are especially important in the evaluation of "universal" vaccine strategies. NHP have an immune system that most closely resembles that of humans. Also the availability of many cross reactive reagents makes it possible to study in detail the contribution of the innate immune system and to analyse vaccine induced immune responses and evaluate their role in control of infection. In addition, vaccine strategies that aim to trigger immune responses through targeting of specific cell surface molecules or innate immune receptors have either limited cross reactivity to other animal species or trigger different cell types or different responses because of differences in receptor expression pattern or cell signalling pathways. These aspects are essential for the evaluation of so called "universal" influenza vaccines. For this type of vaccines new vaccination strategies as well as vaccine modalities are used that aim for the induction of cross protective cellular immune responses or induction of broadly cross neutralizing antibody responses or non-neutralizing antibody responses that become effective through interaction with innate immune cells. Here, the close homology between the immune system in NHP and humans is essential for proper induction and adequate analysis of these responses, allowing a better extrapolation to the human situation. Vaccines that will be tested in NHP have to be in the final stages of preclinical development and require this last validation step in order to assure that there are no adverse effects that were missed in the preliminary studies in other species and that they are effective in an animal species that has an immune system closely related to humans.
Reduction	The number of animals needed per experiment will be based on statistical power calculation for achieving statistically significant induction of immune responses and a significant reduction in virus load in the respiratory tract between the vaccine groups and the challenge control group. Only the minimum number of animals needed will be used. Since historical data are available on infection in non-vaccinated animals (appendix 2), usually less animals can be used in the challenge control group than in the vaccine groups. Furthermore, we aim to evaluate multiple vaccine candidates in a single experiment, so that a single challenge control group can be used. This has to be weighed against the fact that in order to obtain significant differences in immune response between the vaccine groups, more animals per vaccine group may occasionally be required.

Refinement	The use of recording devices enables the monitoring of body temperature, heart- and breathing-rate every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by influenza infection (4). With this method we have observed a significant reduction in fever by influenza vaccine candidates (1). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgical procedure, which will be done under anaesthesia. Subsequently, animals will receive analgesics as long as required. Animals are trained to cooperate as much as possible for the invasive handlings, such as receiving the sedation. Animals will be socially housed with a socially compatible animal. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food. During the study animals will be observed daily by qualified and competent animal caretakers. Should changes occur in behaviour, appetite or stool then a veterinarian will be informed and measures will be discussed with the investigator and implemented. Possible local reactions on the injection site of the vaccine will be recorded at multiple time points using a scoring system that includes redness, swelling and induration. In case substantial induration is seen, then the wound will be treated and analgesics will be applied. During the infection phase, animals will be observed twice daily and clinical symptoms will be scored using a well-established clinical scoring list adapted from Brining et al. (11). On the basis of the scoring system a humane endpoint is defined. In addition, a sudden strong decrease in body temperature is taken as a humane endpoint. When this endpoint is reached the animal will be immediately euthanized and a full necropsy will be performed to determine the cause of clinical disease. All handlings will be performed under sedation. On every time point where a handling is performed the animal will be weighed and closely examined. During the first 2 days of the infection the animal will receive supportive feeding with dense "brokkenballen". This is necessary, because animals have to fast the night before the sedation and since animals will be sedated daily during the first days after infection the food intake during this period would otherwise be very limited.

Are adverse environmental effects expected? Explain what measures will be taken to minimise these effects.

☒ No

☐ Yes > Describe the environmental effects and explain what measures will be taken to minimise these effects.

H. Re-use

Will animals be used that have already been used in other animal procedures ?

☐ No > Continue with question I.

☒ Yes > Explain why re-use is considered acceptable for this animal procedure.

Animals that will be used in these experiments, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infection studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life-span of the animals of this species re-use of animals will take place within the limitations described in art 1e of the Wet op de Dierproeven.

Are the previous or proposed animal procedures classified as 'severe'?

☒ No

☐ Yes > Provide specific justifications for the re-use of these animals during the procedures.

I. Repetition

Explain for legally required animal procedures what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, describe why duplication is required.

Not applicable

J. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

☒ No > Continue with question K.

☐ Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

End of experiment

K. Destination of the animals

Will the animals be killed during or after the procedures?

☐ No > Provide information on the destination of the animals.

☒ Yes > Explain why it is necessary to kill the animals during or after the procedures.

In most studies the animals will be re-used after the virus is cleared (usually at day 21 after infection). However, in cases where possible adverse effects of the vaccine have to be studied animals are euthanized and a full necropsy is performed.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

☐ No > Describe the method of killing that will be used and provide justifications for this choice.

☒ Yes > Will a method of killing be used for which specific requirements apply?

☒ No > Describe the method of killing.

Euthanasia is done by injection of an anaesthetic dose of ketamine followed by an overdose of barbiturate intravenously

☐ Yes > Describe the method of killing that will be used and provide justifications for this choice.

If animals are killed for non-scientific reasons, justify why it is not feasible to rehome the animals.

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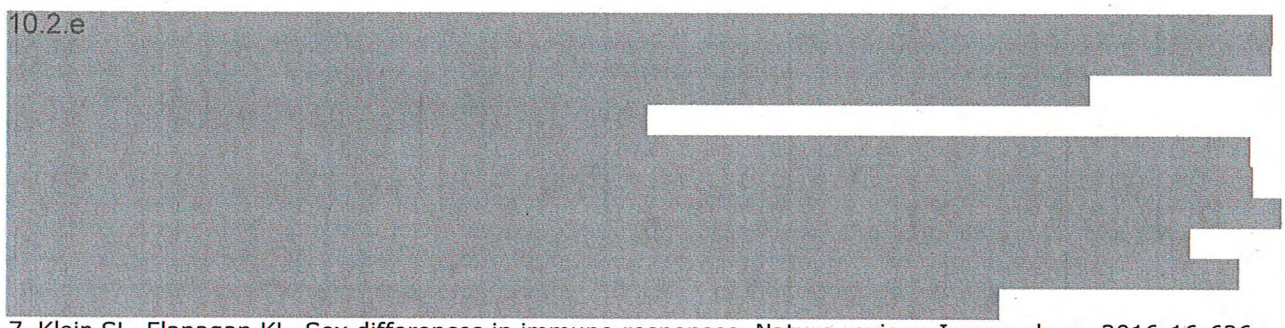
10.2.e



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