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 (0800-7890789).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 50200 |
| 1.2 | Provide the name of the licenced establishment. | Biomedical Primate Research Centre |
| 1.3 | List the serial number and type of animal procedure<br><br>*Use the numbers provided at 3.4.3 of the project proposal.* | Serial number: 1 — Type of animal procedure: Development of a coronavirus infection model in NHP |

## 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.

To establish the capacity of a vaccine to protect against coronavirus infection, or to determine the therapeutic efficacy of an antiviral compound, it is necessary to have a well-defined coronavirus infection model. For new emerging coronaviruses, or viral strains that have not yet been used in NHP, it is necessary to establish infectivity and pathogenicity in NHP before they can be applied in vaccine efficacy or antiviral therapeutic evaluation studies. The main objective is to obtain an infection model that is sufficiently robust to allow adequate evaluation of vaccine or therapeutic efficacy in terms of reduction in clinical symptoms and/or virus replication.
Typically, the study set-up is as follows: a small number of animals will be infected and monitored for clinical symptoms, changes in body temperature, changes in body weight and in blood parameters. Nasal and tracheal swabs as well as bronchoalveolar lavages (BAL) will be collected to determine if the animals have become infected and what the amount of virus production is. To evaluate a new virus, the virus can be inoculated by various (and combinations of) routes, like intravenous, intratracheal, oral, intranasal, ocular, and via aerosol. Proper application for vaccine evaluation requires that: 1) all animals become infected, 2) that the amount of virus produced in the respiratory tract over the infection period is clearly measurable, 3) that the variation between the animals is sufficiently low to allow measurement of reduction in virus load in vaccinated animals with preferably less than 10 animals per group. In case these parameters are not achieved the experiment will be repeated with a higher dose. In case any of the animals reaches the clinical endpoint within the first four days after infection a lower virus dose will be evaluated.
The **primary outcome parameters for virus infection is**:
1. Virus replication and virus load. Clinical symptoms and fever are considered as secondary outcome measures. .

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

At least four weeks before infection, and depending on the protocol, a telemetric temperature sensor will be implanted in the abdominal cavity of the animals that will allow continuous monitoring of body temperature. This time frame is necessary for full recovery of the animals and to allow adequate temperature recording during a two to three-weeks period to establish baseline values before infection.
Then, the animals will be infected intravenous, intradermal, orbital, intranasally, intra-tracheally, intra-bronchially using a bronchoscope, via aerosol, or via a combination of these routes. At the same time, blood is collected for a baseline-value determination. The animals will be monitored daily during the study period for general behaviour, appetite, faeces, breathing frequency, etc., and at each time-point when the animals are sedated, body weight will be measured. Typically, shortly before, and after infection of the animals, nasal and tracheal swabs and/or Bronchoalveolar Lavages (BAL) will be collected to measure virus replication in the (upper) airways. Blood is taken simultaneously with the swabs, to monitor changes in clinical chemistry and haematology parameters and leucocyte subsets, as well as to monitor the development of plasma viraemia. At the same time points body weight is recorded and imaging (CT or PET-CT scan) may be performed to measure lung infiltration. After the animals have become virus-negative in the PCR on swab/wash samples for the first time, they may be followed for 4 weeks to confirm absence of the virus and to monitor for potential re-activations of virus replication. Thus, the length of an infection study will typically be 6 to 8 weeks, but longer follow-up periods may be required (e.g. long Covid). At the end of the study, the animals will be killed and necropsy will be performed for the collection of tissue samples for histopathological and virological tests. The latter will be done to investigate tissue and organ distribution of the virus, and to identify potential viral reservoirs. Viral reservoirs may contribute to virus re-activation and are therefore of particular interest.
The details of each future study, regarding the NHP species used, route of infection, dose used, follow-up duration, etc., will be submitted for approval to the Animal Welfare Body (Instantie voor Dierenwelzijn; IvD).

Table. Maximum number of repeats per procedure.

| Procedure | Maximum | Duration |
|---|---|---|
| Sedation | 18 | 15-60 min |
| Recorder in / out | 2 | 60 min |
| Blood sample | 16 | 30 min |
| Challenge | 1 | 30 min |
| (PET-) CT scan | 16 | 60 min |
| BAL | 16 | 30 min |
| Swabs | 16 | 30 min |
| Killing | 1 | 15 min |

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, in which the NHPs are inoculated with a coronavirus virus, will be performed in four to six animals. For a reliable estimate of the standard deviation, a number of 4 animals is the absolute minimum. In addition, with 4 animals infected out of 4 the chance is 100% with a 95% confidence interval of 39.8 to 100% and with 6 out of 6 animals infected this is 100% with a 95% confidence interval of 54.1 to 100%. In-house experience with SARS-CoV-2 NHP infection models has shown that with this number of animals an adequate assessment can be made on the reproducibility of infection.
All animals need to show virus replication in the (upper) respiratory tract. If infection success is not 100%, the experiment will be repeated with a higher virus challenge dose. Alternatively, multiple virus challenge doses may be tested in a single experiment.

**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 | 60 | M/F | Not applicable | Not applicable |

Provide justifications for these choices

| | |
|---|---|
| Species | Several research groups, including BPRC, have established nonhuman primate (NHP) models for infection with coronaviruses like SARS-CoV-1, SARS-CoV-2 and MERS CoV (1-21). Mostly widely used in CoV research are rhesus macaques (*Macaca mulatta*) and cynomolgus macaques (*Macaca fascicularis*) and their susceptibility for infection with coronaviruses is well established. |
| 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 |
| Number | Assuming group sizes of six animals, evaluation of 5 coronaviruses to be tested at two doses, the total number of animals required is 60 over a period of five years. |
| Gender | Adult 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 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 are expected not to experience pain 1 week after the operation.

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

1. Discomfort because of insertion of the telemetric temperature sensor.
2. Discomfort due to lung lavages
3. Discomfort due to virus installation
4. Stress because of sedation
5. Reduced food intake during the first days after infection
6. Disease symptoms due to the infection

Explain why these effects may emerge.

1. The surgery needed for insertion of telemetric temperature sensor 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 will cause irritation.
4. Animals will be repeatedly sedated for blood sampling, virus infection, collection of swabs and lung lavages. Nausea can sometimes be observed during recovery from the sedation.
5. Especially during daily sedation during the first days after infection, food intake will be reduced.
6. Coronavirus infections can cause fever, coughing, sneezing, nose discharge, laboured breathing, loss of appetite, loss of weight, 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 receive a muscle relaxant.
3. The same procedure as described under 2 will be followed.
4. Recovery of the animals is monitored by the animal caretakers, and the veterinarian will intervene if animals do not recover fast enough.
5. Animals will receive an adapted calorie rich diet, or tube feeding (which is applied during sedation).
6. Animals are monitored twice daily (and 24/7 by camera), and a weighed clinical scoring list is used to record the clinical symptoms (22). When a clinical score of 35 is reached this indicates that the maximum duration of severity is reached then the animal will be killed and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory organs. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort.

**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.

When a clinical score of 35 is reached (22), this indicates that the maximum duration of severity is reached then the animal will be killed and a full necropsy will be performed to establish the cause of the disease and viral distribution over the respiratory organs. Individual scores are added and the decision is based on the total daily score and veterinarian assessment of discomfort. Symptoms 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.

Maximally 35%

**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 symptoms 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 | Several animal species have been used to study coronavirus infection (2, 19, 23-26). However, of these different species, NHP have the advantage that they physiologically, anatomically and immunologically most closely resemble humans. This has important implications, both for vaccine evaluation and antiviral therapeutics (Appendices 2, 3 and 5), as well as for the interaction with coronaviruses since this is affected both by physiology and by the reaction of the innate and adaptive immune system. These aspects are important for the evaluation of vaccines and antiviral therapeutics. The proper evaluation of these vaccines and antiviral therapeutics requires adequate infection models in NHP, which is the purpose of the studies proposed here. |

| | |
|---|---|
| Reduction | Based on the extensive experience with other viral infection models within the institute where this research will be performed, it is expected that four to six animals per test group are sufficient 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 or antiviral compound 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. On the basis of the outcome of the first study the number of animals needed in follow up experiments can be calculated and less animals may be needed. Only the minimum number of animals needed, will be used. |
| Refinement | Animals will be socially housed with a socially compatible cage mate. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food as per guidelines for macaques (27).The use of telemetric temperature sensor makes it possible to continuously record the temperature during the study-period. For our studies with the H1N1 influenza virus, we have used a method that allows very precise calculation of fever induction caused by the infection using this method (28).This method will also be applied in this project. Such precise measurements are not possible with the traditional rectal temperature measurement. Placement of the telemetric temperature sensor for body temperature measurement will require surgery, which will be done under anaesthesia. Subsequently animals will receive analgesics if required. Animals are trained to cooperate as much as possible for the invasive procedures, such as receiving the sedation. The application of CT or PET-CT scanning to measure lung infiltration will give us insight in the disease progression of the CoV infection. CT or PET-CT scanning will be performed when animals are already sedated for sampling of blood and swabs and will thus not cause additional discomfort. During the study animals will be observed daily by qualified animal caretakers (and 24/7 by camera). 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. (22). On the basis of the scoring system a clinical endpoint is defined. When this endpoint is reached the animal will be humanely killed immediately and a necropsy will be performed to determine the cause of disease. All procedures 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 days of the infection the animal will receive tube feeding or an adapted calorie rich diet. This is necessary, because the daily sedations of the animals may cause reduced appetite and weight loss. Regular analysis of haematological and clinical chemistry parameters is part of the experiment. During these experiments, the virus load in plasma will also be analysed as an indicator of infection. These data will also be consulted to determine if changes in behaviour, appetite or stool are clinically relevant. If necessary, judged by the veterinarian, measures will then be taken to treat the animal. |

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 procedures. Animals that have pre-existing antibodies against recently emerged coronaviruses are not suitable. In view of the long life 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.

Animals will be humanely killed in case they show serious signs of disease to avoid severe discomfort. To investigate the presence of virus in tissues and organs, and for the investigation of possible tissue damage caused by CoV, it is necessary to kill the animals at the end of the study.

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.

Killing is done by injecting an anaesthetic dose of ketamine followed by intravenous overdose of barbiturate.

☐ 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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