| | Inventaris verzoek W21-04 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | wordt verstrekt | | | weigerings gronden | | | |
| nr. | document NTS2016704 | reeds openbaar | niet | geheel | deels | 5.1, lid 1c | 5.2, lid 2e | 5.1, lid 2h | 5.2, lid 1 |
| 1 | Aanvraagformulier | | | | x | | x | x | |
| 2 | Projectvoorstel oud | | | | x | | x | | |
| 3 | Niet-technische samenvatting | x | | | | | | | |
| 4 | Bijlage beschrijving dierproeven 1 oud | | | | x | | x | | |
| 5 | Bijlage beschrijving dierproeven 2 oud | | | | x | | x | | |
| 6 | DEC-advies oud | | | | x | | x | x | |
| 7 | Ontvangstbevestiging | | | | x | | x | x | |
| 8 | Verzoek aanvulling aanvraag | | | | x | | x | | |
| 9 | Projectvoorstel nieuw | | | | x | | x | | |
| 10 | Bijlage beschrijving dierproeven 1 nieuw | | | | x | | x | | |
| 11 | Bijlage beschrijving dierproeven 2 nieuw | | | | x | | x | | |
| 12 | DEC-advies nieuw | | | | x | | x | x | |
| 13 | Reactie verzoek aanvulling | | | | x | | x | | |
| 14 | Adviesnota | | | | x | | x | | |
| 15 | Beschikking en vergunning | | | | x | | x | x | |

Centrale Commissie Dierproeven

# Aanvraag
## Projectvergunning Dierproeven
*Administratieve gegevens*

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.centralecommissiedierproeven.nl. of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

## 1 Gegevens aanvrager

**1.1** Heeft u een deelnemernummer van de NVWA?
*Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.*

X Ja > *Vul uw deelnemernummer in* 50200
☐ Nee > *U kunt geen aanvraag doen*

**1.2** Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

| | |
|---|---|
| Naam instelling of organisatie | Biomedical Primate Research Centre |
| Naam van de portefeuillehouder of diens gemachtigde | 10.2.e |
| KvK-nummer | 41146967 |

**1.3** Vul de gegevens van het postadres in.
*Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.*

| | |
|---|---|
| Straat en huisnummer | Lange Kleiweg 161 |
| Postbus | 3306 |
| Postcode en plaats | 2288 GJ Rijswijk |
| IBAN | 10.2.g |
| Tenaamstelling van het rekeningnummer | Stichting Biomedical Primate Research Centre |

**1.4** Vul de gegevens in van de verantwoordelijke onderzoeker.

| | |
|---|---|
| (Titel) Naam en voorletters | 10.2.e ☐ Dhr. ☒ Mw. |
| Functie | 10.2.g |
| Afdeling | 10.2.g |
| Telefoonnummer | 10.2.e |
| E-mailadres | |

**1.5** *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

| | |
|---|---|
| (Titel) Naam en voorletters | ☒ Dhr. ☐ Mw. |
| Functie | 10.2.g |
| Afdeling | |
| Telefoonnummer | 10.2.e |
| E-mailadres | |

| | | | |
|---|---|---|---|
| 1.6 | *(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning. | (Titel) Naam en voorletters | ☐ Dhr. ☐ Mw. |
| | | Functie | |
| | | Afdeling | |
| | | Telefoonnummer | |
| | | E-mailadres | |
| 1.7 | Is er voor deze projectaanvraag een gemachtigde? | ☒ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag* | |
| | | ☐ Nee | |

## 2 Over uw aanvraag

| | | |
|---|---|---|
| 2.1 | Wat voor aanvraag doet u? | ☒ Nieuwe aanvraag > Ga verder met vraag 3 |
| | | ☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn<br>Vul uw vergunde projectnummer in en ga verder met vraag 2.2 |
| | | ☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn<br>Vul uw vergunde projectnummer in en ga verder met vraag 2.3 |
| 2.2 | Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is? | ☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier |
| | | ☐ Nee > Ga verder met vraag 3 |
| 2.3 | Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend? | ☐ Nee > Ga verder met vraag 3 |
| | | ☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6 |

## 3 Over uw project

| | | | |
|---|---|---|---|
| 3.1 | Wat is de geplande start- en einddatum van het project? | Startdatum | 01 - 06 - 2017 |
| | | Einddatum | 01 - 06 - 2022 |
| 3.2 | Wat is de titel van het project? | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques. | |
| 3.3 | Wat is de titel van de niet-technische samenvatting? | Onderzoek naar de beschermende werking van nieuwe griep vaccins. | |
| 3.4 | Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt? | Naam DEC | 10.2.g |
| | | Postadres | |
| | | E-mailadres | 10.2.e |

# 4 Betaalgegevens

| | | |
|---|---|---|
| 4.1 | Om welk type aanvraag gaat het? | ☒ Nieuwe aanvraag Projectvergunning € 1187 Lege<br>☐ Wijziging € Lege |
| 4.2 | Op welke wijze wilt u dit bedrag aan de CCD voldoen.<br>*Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.* | ☐ Via een eenmalige incasso<br>X Na ontvangst van de factuur |

# 5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

☒ Projectvoorstel

☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

☒ Melding Machtiging

☒ 2 bijlages beschrijving dierproeven

# 6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.7). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

| | |
|---|---|
| Naam | 10.2.e |
| Functie | 10.2.g |
| Plaats | Rijswijk |
| Datum | 27 - 10 - 2016 |
| Handtekening | 10.2.e en 10.2.g |

10.2.g

Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Date November 17, 2016 Our ref. 1902/AM Your letter

☎ 10.2.e ✉ 10.2.e

Subject aanvraag projectvergunning dierproeven AVD502002016704

Geachte heer/mevrouw,

Hierbij stuur ik u de aanvraag projectvergunning dierproeven AVD502002016704, getiteld: "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques", alsmede het DEC advies betreffende deze aanvraag.

Met vriendelijke groeten,

10.2.e

Bijlagen:
1. DEC advies
2. Aanvraag projectvergunning dierproeven
3. Niet technische samenvatting
4. Projectvoorstel dierproeven
5. Bijlage 1
6. Bijlage 2

Centrale Commissie Dierproeven

# Form
## Project proposal

- This form should be used to write the project proposal for animal procedures.
- The appendix 'description animal procedures' is an appendix t this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed.
- For more information on the project proposal, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 50200 |
| 1.2 | Provide the name of the licenced establishment. | Biomedical Primate Research Centre |
| 1.3 | Provide the title of the project. | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques. |

## 2 Categories

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

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

## 3 General description of the project

**3.1 Background**

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

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

Influenza epidemics are estimated to result in infection of 2,5-10% of the world population every year, causing 2-5 million cases of severe illness and 250.000-500.000 deaths (http://www.who.inf). Especially vaccination is considered the most effective measure against the influenza disease and, as such, it is

recommended by the European Council (http://eur-ex.europe.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:348:0072:EN:PDF) and implemented in all EU/European Economic Area member states. The main problems of the current influenza vaccines are; a) they are not very effective in the elderly, b) they only protect against highly homologous strains, while circulating influenza virus strains constantly evolve as a result of antigenic drift, c) they do not protect against new pandemic strains that emerge as a result of recombination between different viral strains found in animal reservoirs, d) they do not protect against highly pathogenic avian influenza virus (1-3). These problems are amplified by the cumbersome current methods of vaccine production, which involves either growing the virus on eggs and/or preparing live attenuated virus. The 6 months time needed for vaccine preparation means that the vaccines have to be based on predictions about which virus strains will circulate during the next influenza season. A mismatch between the vaccine and the actually circulating influenza strain(s) however, results in lower vaccine effectiveness as shown for the 2014-15 influenza season with regard to the H3N2 strain (4). New vaccine strategies that can provide broader protection and cover a range of seasonal influenza strains as well as pandemic and avian influenza virus strains are urgently needed. These so called "universal" influenza vaccines are directed at either a) inducing broadly neutralizing antibodies by targeting the relatively conserved stem region of the haemagglutinin (HA) subunit, which is responsible for virus entry into the target cell, b) inducing protective T-cell responses that are usually directed against more conserved proteins of the virus and show more broad recognition (5, 6). Retrospective epidemiological studies as well as studies in experimentally infected volunteers indicate that in the absence of antibodies, cellular immune responses can have a protective effect (7-9). Their role in achieving cross protection was recently demonstrated in a H1N1 vaccine evaluation study in non-human primates (NHP) (10). More recently the appreciation of the importance of non-neutralizing anti-influenza antibodies in conferring a broad protection against variant strains, especially in the case of avian influenza viruses, has prompted research into their mechanism of action (via antibody dependent cellular cytotoxicity (ADCC), antibody dependent phagocytosis (ADP) or complement activation (11-13)) and vaccine strategies to induce these antibodies. New methods for faster vaccine production, the induction of T-cell responses and improvement of vaccine responses in the elderly have involved application of DNA, virus like particles (VLP), recombinant viral vectors and strategies to target vaccines to the appropriate antigen presenting cells (3, 14-18). Evaluation of the immunogenicity of these vaccines requires additional methods, besides the standard antibody ELISA, micro-neutralization and haemagglutination inhibition assays. Especially, proper assessment of adaptive cellular immune responses and function of the innate immune system in relation to non-neutralizing antibody effector function and induction of immune responses by the new vaccine modalities is needed.

Animal models have played an important role in preclinical evaluation of candidate influenza vaccines (19, 20). While a number of species have been used, the most commonly used models to assess immunogenicity and efficacy against influenza virus infection are the mouse, ferret and non-human primate (NHP) models. There are important differences between these species in immune function and susceptibility to influenza virus infection. 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 play an important role in influenza virus research and have been used to study pathogenesis as well as efficacy of preventive and therapeutic intervention strategies (21). Of the different animals models used in influenza virus research, NHP have a unique close homology to humans in most components of their immune system (22-24). For instance, similar T and B-cell subsets have been described in NHP (25). Moreover, the immunoglobulin gene germline repertoire is highly conserved between macaques and humans, which is important when induction of broadly neutralizing antibodies by new "universal" influenza vaccine strategies is studied (26). In addition, structure and function of Fc receptors, which are essential for the function of non-neutralizing antibodies, show many homologies between macaques and humans (27). Only very limited information is available on Fc receptors in ferrets and only reagents to detect the IgA receptor are available. Finally in NHP the innate immune system, including molecular pathways and antigen presenting cell subsets, are much more homologous to humans than what is seen in mice (23). NHP not only most closely reflect the human physiology, but also resemble humans in their clinical

virus multiplication is (bijlage 2). Virus infection is routinely performed by inoculating the animals via a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular, using a standard dose. For refinement, we will investigate another mode of virus delivery, namely as an aerosol. The rational for setting up this model of infection is that the virus is given in the form of small droplets that better reflect the natural mode of exposure. Furthermore, previous studies in ferrets (35) have shown that a lower dose of virus may suffice, which may also better reflect the natural situation. At present it is still unknown whether such an infection model will be sufficiently robust to allow proper vaccine evaluation and therefore this model can still be considered as experimental. Once this method is established, it may be applied in subsequent vaccine evaluation studies, for instance in cases where less stringent criteria of protection against infection are needed (in case it is difficult to make a protective vaccine and it is necessary to establish relatively modest improvements in vaccine development that can only be measured when a low dose of virus is used).

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

Vaccine evaluation in macaques. For this type of experiment animals will be immunized either once or they will receive a number of immunizations over a certain time period. During the study animals will be monitored for adverse effects of the vaccine, including monitoring of general behaviour and health. Blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. 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.
Influenza virus infection in macaques. In order to establish infectivity and pathogenicity of a new virus that has not been tested previously in NHP at our institute, 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 what the amount of virus multiplication is. To evaluate a new virus, the virus is inoculated via a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular using a standard dose. Proper application in vaccine evaluation requires that in these infection studies > 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 humane endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust.

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

Vaccine candidates that fulfil the criteria for evaluation in NHP may be directly tested in a vaccine evaluation study (bijlage 1), if the influenza virus that is to be used for establishing capacity of the vaccine to protect against infection has already been used in NHP at our institute. If this is not the case then the virus has to be tested first in an influenza virus infection study (bijlage 2). Also when efficacy against low dose aerosol infection has to be tested, a preceding influenza virus infection study (bijlage 2) is necessary. Requirement to proceed from influenza virus infection study to vaccine evaluation study are: a) more than 80% of the animals have to become infected, b) variation between animals has to be such that in a vaccine evaluation study less than 10 animals per test group suffice to obtain statistically significant results, c) no animals reach the humane endpoint within 4 days after infection.


1. **de Vries RD, Altenburg AF, Rimmelzwaan GF.** 2015. Universal influenza vaccines, science fiction or soon reality? Expert Rev Vaccines **14:**1299-1301.
2. **Osterhaus A, Fouchier R, Rimmelzwaan G.** 2011. Towards universal influenza vaccines? Philos Trans R Soc Lond B Biol Sci **366:**2766-2773.
3. **Krammer F, Palese P.** 2015. Advances in the development of influenza virus vaccines. Nat Rev Drug Discov **14:**167-182.

4. **Valenciano M, Kissling E, Reuss A, Rizzo C, Gherasim A, Horvath JK, Domegan L, Pitigoi D, Machado A, Paradowska-Stankiewicz IA, Bella A, Larrauri A, Ferenczi A, Joan OD, Lazar M, Pechirra P, Korczynska MR, Pozo F, Moren A, team IMmc-c.** 2016. Vaccine effectiveness in preventing laboratory-confirmed influenza in primary care patients in a season of co-circulation of influenza A(H1N1)pdm09, B and drifted A(H3N2), I-MOVE Multicentre Case-Control Study, Europe 2014/15. Euro Surveill **21:**pii=30139.
5. **Sridhar S, Begom S, Bermingham A, Hoschler K, Adamson W, Carman W, Bean T, Barclay W, Deeks JJ, Lalvani A.** 2013. Cellular immune correlates of protection against symptomatic pandemic influenza. Nat Med **19:**1305-1312.
6. **Wilkinson TM, Li CK, Chui CS, Huang AK, Perkins M, Liebner JC, Lambkin-Williams R, Gilbert A, Oxford J, Nicholas B, Staples KJ, Dong T, Douek DC, McMichael AJ, Xu XN.** 2012. Preexisting influenza-specific CD4+ T cells correlate with disease protection against influenza challenge in humans. Nat Med **18:**274-280.
7. **Epstein SL.** 2006. Prior H1N1 influenza infection and susceptibility of Cleveland Family Study participants during the H2N2 pandemic of 1957: an experiment of nature. J Infect Dis **193:**49-53.
8. **McMichael AJ, Gotch FM, Noble GR, Beare PA.** 1983. Cytotoxic T-cell immunity to influenza. N Engl J Med **309:**13-17.
9. **McElhaney JE, Xie D, Hager WD, Barry MB, Wang Y, Kleppinger A, Ewen C, Kane KP, Bleackley RC.** 2006. T cell responses are better correlates of vaccine protection in the elderly. J Immunol **176:**6333-6339.
10. **Weinfurter JT, Brunner K, Capuano SV, 3rd, Li C, Broman KW, Kawaoka Y, Friedrich TC.** 2011. Cross-reactive T cells are involved in rapid clearance of 2009 pandemic H1N1 influenza virus in nonhuman primates. PLoS Pathog **7:**e1002381.
11. **Jegaskanda S, Reading PC, Kent SJ.** 2014. Influenza-specific antibody-dependent cellular cytotoxicity: toward a universal influenza vaccine. J Immunol **193:**469-475.
12. **Jegaskanda S, Amarasena TH, Laurie KL, Tan HX, Butler J, Parsons MS, Alcantara S, Petravic J, Davenport MP, Hurt AC, Reading PC, Kent SJ.** 2013. Standard trivalent influenza virus protein vaccination does not prime antibody-dependent cellular cytotoxicity in macaques. J Virol **87:**13706-13718.
13. **Henry Dunand CJ, Leon PE, Huang M, Choi A, Chromikova V, Ho IY, Tan GS, Cruz J, Hirsh A, Zheng NY, Mullarkey CE, Ennis FA, Terajima M, Treanor JJ, Topham DJ, Subbarao K, Palese P, Krammer F, Wilson PC.** 2016. Both Neutralizing and Non-Neutralizing Human H7N9 Influenza Vaccine-Induced Monoclonal Antibodies Confer Protection. Cell Host Microbe **19:**800-813.
14. **Florek NW, Weinfurter JT, Jegaskanda S, Brewoo JN, Powell TD, Young GR, Das SC, Hatta M, Broman KW, Hungnes O, Dudman SG, Kawaoka Y, Kent SJ, Stinchcomb DT, Osorio JE, Friedrich TC.** 2014. Modified vaccinia virus Ankara encoding influenza virus hemagglutinin induces heterosubtypic immunity in macaques. J Virol **88:**13418-13428.
15. **Deliyannis G, Boyle JS, Brady JL, Brown LE, Lew AM.** 2000. A fusion DNA vaccine that targets antigen-presenting cells increases protection from viral challenge. Proc Natl Acad Sci U S A **97:**6676-6680.
16. **Fossum E, Grodeland G, Terhorst D, Tveita AA, Vikse E, Mjaaland S, Henri S, Malissen B, Bogen B.** 2015. Vaccine molecules targeting Xcr1 on cross-presenting DCs induce protective CD8+ T-cell responses against influenza virus. Eur J Immunol **45:**624-635.
17. **Grodeland G, Mjaaland S, Roux KH, Fredriksen AB, Bogen B.** 2013. DNA vaccine that targets hemagglutinin to MHC class II molecules rapidly induces antibody-mediated protection against influenza. J Immunol **191:**3221-3231.
18. **Laddy DJ, Yan J, Khan AS, Andersen H, Cohn A, Greenhouse J, Lewis M, Manischewitz J, King LR, Golding H, Draghia-Akli R, Weiner DB.** 2009. Electroporation of synthetic DNA antigens offers protection in nonhuman primates challenged with highly pathogenic avian influenza virus. J Virol **83:**4624-4630.
19. **Bodewes R, Rimmelzwaan GF, Osterhaus AD.** 2010. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines **9:**59-72.
20. **Bouvier NM, Lowen AC.** 2010. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses **2:**1530-1563.

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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | | |
|---|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 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. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number | Type of animal procedure |
| | | 1 | 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. Typically a recording device is surgically placed in the abdominal cavity before the start of the study to retrospectively evaluate body temperature (measured every 15 minutes) and/or heart rate and activity. Subsequently the animals are immunized either once or they receive a number of immunizations over a certain time period. 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 that is 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 bijlage 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 recording device is surgically placed in the abdominal cavity at least 4 weeks before the first immunization takes place. 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, typically at 4 to 8 week time intervals, although occasionally a longer time frame is needed between immunizations when different vaccine modalities are used for priming and boosting of the immune response. Usually 3 immunizations suffice over a period of 20 weeks. However, in rare occasions these limits may have to be exceeded. 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, intra-bronchially using a bronchoscope 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. Occasionally, usually before the start of the study and after the last immunisation, a nasal wash and lung lavage is taken in order to measure induction of local immune responses. 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 bijlage 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 6 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 (X-ray or 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. 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. 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 will not be euthanized, the recording devices are surgically removed and body temperature and/or heart rate and activity data are analysed, upon which, the animals may be re-used (within the limitations described in art 1e of the Wet op de Dierproeven).

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.

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 needed will be used. Since historical data are available on infection in unvaccinated animals (bijlage 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, estimated numbers, and life stages. Provide justifications for these choices.

The experiment will be performed in macaques, adult, M/F, n=150.
All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier. Adult male and female animals can be used. However, since there are immunological differences between males and females we prefer that for each individual experiment either all animals are male or all are female. 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 5 to 6 such studies over a 5-year period.

**C. Re-use**

Will the animals be re-used?

☐ No, continue with question D.

☒ 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 of the animals of this species reuse 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.

**D. 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 (1). 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 these 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 trachea 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 unvaccinated animals (bijlage 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 be needed.

**Refinement**

The use of temperature recording devices makes it possible to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection (2). With this method we have observed a significant reduction in fever by some of the vaccine candidates (3). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgery, 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.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be socially housed with a socially compatible animal, whenever possible. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food (http://www.bprc.nl/en/welfare/).
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. (4). On the basis of the scoring system a humane endpoint is defined. 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 tube feeding. 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.
The "Flora and Fauna wet" and "wet dieren" do not pose additional requirements that are needed for the type of studies proposed in this application.

## Repetition and duplication

**E. Repetition**

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

## Accommodation and care

**F. Accommodation and care**

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

**G. 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 H.

☐ 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.

## Classification of discomfort/humane endpoints

**H. Pain and pain relief**

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ 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 placing the recording device in the abdomen or 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 fever 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.

**I. Other aspects compromising the welfare of the animals**

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

1. Stress because of change in housing
2. Discomfort because of insertion or removal of the temperature recording device
3. Discomfort due to injection
4. Discomfort due to lung lavages
5. Discomfort due to virus installation
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. Animals have to be moved to DM-III facilities because of the experimental infection with influenza virus.
2. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
3. When vaccines are given by injection, this can cause local pain and irritation.
4. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
5. When virus is given intra-bronchially a bronchoscope is used and this 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.
6. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs 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 will be reduced.
8. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, loss of appetite, inactivity.

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

1. Animals will be socially housed en enrichment is provided.
2. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
3. 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.
4. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
5. The same procedure as described under 4 will be followed.
6. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
7. Animals will receive tube feeding. This is applied during sedation.
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.

**J. 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 K.

☒ 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. When a certain pre-determined clinical score is reached that indicates that the maximum duration of severity is reached then the animal will be humanely euthanized. Individual scores are added and the decision is based on the total daily score. 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.

The percentage of animals reaching the end point will depend on the virus being used. Seasonal influenza viruses (H1N1 and H3N2) as well as pandemic H1N1 will only cause minimal disease and typically resolve within 14-21 days. Highly pathogenic influenza virus has been described to be able to cause severe disease in macaques, requiring euthanasia of the animal. Depending on the virus, this was observed in 25% of the animals at most and later than day 4 after infection. At our institute these viruses will first be evaluated in a small number of animals (see bijlage 2). If any of the animals reach the clinical end point before day 4 then this virus will not be used at the dose that was given. It may be tested at a 10-100 times lower dose in a new small group of animals. Also then the same criteria will be used and the virus will not be used if any of the animals reach the clinical end point before day 4. Weight will be recorded during the study, but a weight loss of 10% because of the infection has not been observed in any study described thus far and hence does not serve as a suitable end point.

**K. Classification of severity of procedures**

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

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.

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

☒ 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 or when influenza viruses are used that are known to cause persistent lung pathology, animals are humanely euthanized and a full necropsy is performed.


1. **Bodewes R, Rimmelzwaan GF, Osterhaus AD.** 2010. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines **9:**59-72.
2. [redacted]
3. **Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, van Meersbergen R, Huizingh J, Wanningen P, Verspuij J, de Man M, Ding Z, Apetri A, Kukrer B, Sneekes-Vriese E, Tomkiewicz D, Laursen NS, Lee PS, Zakrzewska A, Dekking L, Tolboom J, Tettero L, van Meerten S, Yu W, Koudstaal W, Goudsmit J, Ward AB, Meijberg W, Wilson IA, Radosevic K.** 2015. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science **349:**1301-1306.
4. **Brining DL, Mattoon JS, Kercher L, LaCasse RA, Safronetz D, Feldmann H, Parnell MJ.** 2010. Thoracic radiography as a refinement methodology for the study of H1N1 influenza in cynomologus macaques (Macaca fascicularis). Comp Med **60:**389-395.


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

Centrale Commissie Dierproeven

5

# 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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 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>*Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number: 2<br>Type of animal procedure: Influenza virus infection 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 viruses (1, 2). 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 what the amount of virus production is. To evaluate a new virus, the virus is inoculated by a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular 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. In order to further improve the model, we will investigate another mode of virus delivery, namely as an aerosol. The rational for setting up this mode of infection is that the virus is given in the form of small droplets that better reflects the natural mode of exposure. Furthermore, previous studies in ferrets (3, 4) have shown that a lower dose of virus may

suffice, which may also better reflect the natural situation. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust. Three doses, covering a total range in amount of virus of $10^4$ (50% tissue culture infectious dose ($TCID_{50}$)) will be tested to establish the lowest dose that fulfils these statistical criteria.

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.

A recording device 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 6 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 (X-ray or 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 know 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.

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 virus is inoculated by a standard combination of routes at a standard dose, will be performed in four animals. Experience in the pandemic H1N1 infection model has 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. For virus given by aerosol delivery it is not yet possible to predict the minimal group size. An initial experiment performed by Marriott et al showed that three out of four animals that were exposed to the virus did become infected (5). This implies that there may be considerable variation in this model. With 6 animals per group it would be acceptable if one of the animals does not become infected, because then still statistically significant results can be obtained in a vaccine evaluation studies with 10 vaccinated animals, even if one of the vaccinated animals happens to

be unprotected (Chi-squared test, power is 0.835). Therefore, in a first experiment we would like to use six animals. In subsequent experiments the number of animals per group may be reduced. Whether aerosol challenge will provide a robust infection model, will be determined on the basis of power calculations, as outlined above. In addition, in this model when more than 4 animals are used per group, at least 80% of the animals have to become infected.

**B. The animals**

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The experiment will be performed in macaques, adult, M/F, n=56.
All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier. Adult male and female animals can be used. When combined routes of inoculation are used; i.e. intra-bronchial, oral, intranasal and intraocular, a single dose usually suffices to establish the infection model. In the literature almost all viruses have been used at a dose of $10^6$ to $10^7$ $TCID_{50}$ in NHP (6-9). However, it may be necessary to evaluate an extra dose in some cases. Assuming 4 animals per groups, evaluation of 3 new viral stains, from which 2 have to be tested at two doses, the total number of animals needed will be maximum 20.
For aerosol delivery we expect to need 6 animals per dose. Assuming maximum 3 doses to be tested for 2 viruses, the total number of animals needed is 36.
In total 56 animals are the maximum needed for setting up infection models for new influenza viruses in a period of 5 years.

**C. Re-use**

Will the animals be re-used?

☐ No, continue with question D.

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

Animals that will be used in these experiements, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infections studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life of the animals of this species reuse 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.

**D. 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 (10, 11). 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 bijlage 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 bijlage 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. Because of the limited experience with aerosol delivery in NHP and the results obtained so far (5) six animals per group are expected to be sufficient for the first experiment. On the basis of the outcome of the first study the number of animals needed in follow up experiment can be calculated and less animals may be needed. Only the minimum number of animals needed, will be used.

**Refinement**

The use of temperature recording devices makes it possible to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection (1). With this method we have observed a significant reduction in fever by some of the vaccine candidates (12). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the recording devices will require a small surgery, 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.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

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. (13). On the basis of the scoring system a humane endpoint is defined. 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 days of the infection the animal will receive tube feeding. 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.
There are no other aspects in this proposal that are not in agreement with the Dutch law other than the Wod.

## Repetition and duplication

**E. Repetition**

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

## Accommodation and care

**F. Accommodation and care**

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

**G. 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 H.

☐ Yes > Describe this establishment.

21. **Davis AS, Taubenberger JK, Bray M.** 2015. The use of nonhuman primates in research on seasonal, pandemic and avian influenza, 1893-2014. Antiviral Res **117:**75-98.
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3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

| Serial number | Type of animal procedure |
|---|---|
| 1 | Vaccine evaluation in macaques |
| 2 | Influenza virus infection in macaques |

| | |
|---|---|
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |

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

## Classification of discomfort/humane endpoints

**H. Pain and pain relief**

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ 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 placing the recording device in the abdomen or after removal, which is needed in case the animal will return to the experimental stock 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 fever during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation.

**I. Other aspects compromising the welfare of the animals**

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

1. Stress because of change in housing
2. Discomfort because of insertion or removal of the recording device.
3. Discomfort due to lung lavages
4. Discomfort due to virus installation
5. Stress because of sedation
6. Reduced food intake during the first days after infection
7. Disease symptoms due to the infection

Explain why these effects may emerge.

1. Animals have to be moved to DM-III facilities because of the experimental infection with influenza virus.
2. The surgery needed for insertion and removal of the recording device will cause pain and some local inflammation.
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 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. 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.
6. Especially during daily sedation during the first 2 days after infection food intake will be reduced.
7. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, loss of appetite, inactivity.

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

1. Animals will be socially housed en enrichment is provided.
2. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
3. For the lung lavages animals are first deeply sedated and receive a muscle relaxant.
4. The same procedure are described under 3 will be followed.
5. Recovery of the animals is monitored and the veterinarian will intervene if animals do not recover fast enough.

6. Animals will receive tube feeding. This is applied during sedation.
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 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 organs.

**J. 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 K.

☒ 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. When a certain pre-determined clinical score is reached, indicating the maximum duration of severity, then the animal will be humanely euthanized. Individual scores are added and the decision is based on the total daily score. 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.

The percentage of animals reaching the end point will depend on the virus being used. Seasonal influenza viruses (H1N1 and H3N2) as well as pandemic H1N1 will only cause minimal disease and typically resolve within 14-21 days. Highly pathogenic influenza virus has been described to be able to cause severe disease in macaques requiring euthanasia of the animal. Depending on the virus this was observed in 25% of the animals at most and later than day 4 after infection. If any of the animals reach the humane end point before day 4 then this virus will not be used at the dose that was given. It may be tested at a 10-100 times lower dose in a new group of four animals. Also then the same criteria will be used and the virus will not be used if any of the animals reach the humane end point before day 4. Weight will be recorded during the study, but a weight loss of 10% because of the infection has not been observed in any study described thus far and hence does not serve as a suitable end point.

**K. Classification of severity of procedures**

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

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.

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

☒ 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 influenza viruses are used that are known to cause persistent lung pathology, animals are humanely euthanized and a full necropsy is performed.

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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

# DEC-advies

6

*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

1. Aanvraagnummer AVD502002016704
2. Titel van het project: Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques
3. Titel van de NTS: Onderzoek naar beschermende werking van nieuwe griep vaccins
4. Type aanvraag:
   - ☐ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer
5. Contactgegevens DEC:
   - naam DEC: 10.2.g
   - telefoonnummer contactpersoon: 10.2.e
   - mailadres contactpersoon: 10.2.e en 10.2.g
6. Adviestraject (data dd-mm-jjjj):
   - ☑ ontvangen door DEC: 01-11-2016
   - ☑ aanvraag compleet: 01-11-2016
   - ☑ in vergadering besproken: 07-11-2016
   - ☐ anderszins behandeld:
   - ☑ termijnonderbreking(en) van: 08-11-2016 tot 15-11-2016
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   - ☐ aanpassing aanvraag
   - ☑ advies aan CCD 17-11-2016
7. Eventueel horen van aanvrager - n.v.t.
8. Correspondentie met de aanvrager
   - Datum: 08-11-2016
   - Strekking van de vraag / vragen: enige tekstuele aanpassingen en verduidelijking inzake de keuze van de groepsgrootte
   - Datum antwoord: 15-11-2016

- Strekking van het (de) antwoord(en): tekstuele aanpassingen zijn doorgevoerd en de keuze van de groepsgrootte is verduidelijkt.
- De antwoorden hebben geleid tot aanpassing van de aanvraag:.

9. Eventuele adviezen door experts (niet lid van de DEC) - nvt

## B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (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 de virologie, vaccinologie, immunologie, onderzoek naar niet-humane primaten, en toepassing van alternatieven op deze gebieden. Ook is er voldoende expertise op gebied van ontwerp van dierproeven, proefdiergeneeskundige praktijk, het houden en verzorgen van dieren, ethiek en proefdieren en hun bescherming. De DEC heeft ruime ervaring met het beoordelen van onderzoek naar vaccins tegen virale infecties in niet-humane primaten.
4. Vanwege betrokkenheid bij het betreffende project is een aantal DEC-leden, met het oog op onafhankelijkheid en onpartijdigheid, niet betrokken bij de advisering. Een van de DEC leden was betrokken bij de aanvraag. Deze persoon heeft zich teruggetrokken van de vergadering bij de bespreking van de aanvraag.

## C. Beoordeling (inhoud):

1. Het project is:

   ☐ uit wetenschappelijk oogpunt verantwoord

2. De in de aanvraag aangekruiste doelcategorie(ën) is / zijn in overeenstemming met de hoofddoelstelling(en). Het betreft toegepast onderzoek naar nieuwe vaccin-concepten ter bescherming van mensen tegen influenza virus infectie.
3. De DEC onderschrijft het belang van de doelstelling. Het wordt ingeschat als een essentieel belang: Jaarlijks overlijden wereldwijd meer dan een kwart miljoen mensen (vooral ouderen en kinderen) ten gevolge van een griepinfectie. Daarnaast heeft de griepgolf een grote socio-economische impact. Aangezien een griepvaccin dat beschermt tegen alle verschillende varianten van het influenza virus niet beschikbaar is, wordt er jaarlijks een nieuw vaccin aangeboden. Omdat het productieproces (op bevruchte kippeneieren of in celkweek) en de daaraan gekoppelde kwaliteitscontroles langdurige processen zijn, wordt de keuze van de vaccin-stammen minstens een half jaar voor de eventuele griepuitbraken gemaakt. Hierdoor loopt men het

risico dat het geproduceerde vaccin geen volledige bescherming biedt tijdens het griepseizoen (zoals in het seizoen 2014-2015). Ook is gebleken dat de immuunrespons tegen de huidige vaccins in oudere mensen niet zo sterk is, waardoor juist de doelgroep die bescherming tegen griep hard nodig heeft, toch vaak ziek wordt ondanks de vaccinatie. Er is dus behoefte aan een breed (universeel) vaccin dat langdurige, sterke bescherming biedt tegen infecties met een grote verscheidenheid aan influenza virussen. De onderzoekers willen de veiligheid en effectiviteit van dergelijke vaccin kandidaten testen in niet-humane primaten als laatste stadium voor toepassing in de mens.

4. De gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. Het voorgestelde onderzoek bestaat uit twee fasen, te weten 1) evaluatie van de effectiviteit en veiligheid van kandidaat vaccins tegen influenza virus in non humane primaten en 2) ontwikkeling van een influenza virus infectiemodel indien nieuwe virusstammen gebruikt moeten worden ten behoeve van de evaluatie van vaccins. Vaccins die getest gaan worden in dit model zijn innovatief en nog niet eerder getest in niet-humane primaten. Eerder zijn binnen het onderzoeksinstituut de immunogeniciteit, veiligheid en effectiviteit van vaccins tegen onder andere HIV, Hepatitis B, Hepatitis C, malaria en tuberculose in niet-humane primaten onderzocht. Sinds 2012 wordt er in de instelling onderzoek verricht aan influenza virus-infecties en vaccins tegen influenza. De gekozen strategie voor infectie en vaccinatie is mede gebaseerd op ervaringen uit deze eerdere studies. De expertise voor het verrichten van deze experimenten is beschikbaar binnen de instelling en bij de betrokken onderzoekers.
5. Er is sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren. De keuze hiervoor is voldoende wetenschappelijk onderbouwd. Het experiment wordt uitgevoerd met niet-humane primaten. De keuze voor deze diersoort is gebaseerd op bewezen gevoeligheid voor influenza virus infecties en de zeer grote fysiologische en immunologische overeenkomsten met de mens. De kandidaat vaccins zijn tevoren gekarakteriseerd in *in vitro* en *in vivo* (veelal knaagdieren) modellen. In de laatste fase van de preklinische ontwikkeling van kandidaat vaccins is het echter noodzakelijk om de effectiviteit en veiligheid van de kandidaat vaccins te onderzoeken in een diermodel waarin de aard en verloop van de ziekte grote overeenkomsten vertonen met de mens. Vaccin kandidaten kunnen niet in andere diermodellen of alternatieve modellen worden getest op bescherming tegen infectie in de mens. De resultaten verkregen in dit proefdiermodel kunnen de sterkte van de immuunrespons van de kandidaat vaccins in de mens voorspellen. De eventuele nadelige effecten (bijvoorbeeld als gevolg van de complexiteit van immuunresponsen en hun interactie met het infectieus agents) van de kandidaat vaccins kunnen in dit diermodel worden vastgesteld.

6. Het ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief is correct als matig ingeschat, op basis van ervaring met influenza-infecties in niet-humane primaten. Ook de toegepaste experimentele technieken en de vaccinaties zijn terecht als matig ongerief ingeschat. De dieren die gebruikt zullen worden voor de experimenten zijn speciaal voor dit doel gefokt, en zullen als duo worden gehuisvest in een BSL3-faciliteit. Alles betreffende deze dierproef is in overeenstemming met de nieuwe directive.
7. Er zijn geen methoden die de voorgestelde dierproeven geheel of gedeeltelijk zouden kunnen **vervangen.** Er is geen *in vitro* model beschikbaar waarin een immuunrespons kan worden opgewekt, of dat de beschermende werking van vaccins kan meten. Er kan veel informatie betreffende specificiteit en hoogte van de immuunrespons worden verkregen uit *in vitro* assays die zullen worden uitgevoerd met het materiaal afkomstig van de dieren, maar het gebruik van proefdieren kan nog niet worden overgeslagen. Wereldwijd worden verschillende diersoorten gebruikt in influenza onderzoek, ook voor vaccin–evaluatie studies. Echter voor het testen van 'universele' influenza-vaccins is het noodzakelijk een dier te gebruiken met een immuunsysteem dat zoveel mogelijk gelijkenis vertoont met dat van de mens. Voorwaarde is wel dat producten die in deze dieren getest gaan worden in het eindstadium van de ontwikkeling zijn. In de experimenten in niet-humane primaten kunnen mogelijke bijwerkingen naar voren komen die in andere diersoorten gemist zouden zijn. Direct onderzoek met menselijke vrijwilligers is in dit stadium van de vaccin ontwikkeling daarom dan ook niet verantwoord.
8. In het project wordt optimaal tegemoet gekomen aan de vereiste van de **vermindering** van dierproeven. Het aantal te gebruiken dieren per behandelgroep wordt bepaald met behulp van statische powerberekeningen op basis van de verwachte effect grootte. Omdat historische data worden meegenomen in de uiteindelijke statistische analyse zal het aantal dieren in de controlegroep lager zijn dan in de gevaccineerde groepen. Door het onderzoeken van meerdere vaccin kandidaten binnen een experiment zal reductie van het aantal controlegroepen worden verkregen.
9. Het project is in overeenstemming met de vereiste van de **verfijning** van dierproeven en het project is zo opgezet dat de dierproeven zo humaan mogelijk kunnen worden uitgevoerd. Er is geen sprake van belangwekkende milieueffecten. De uitvoering is verfijnd door gebruik te maken van sociaal gehuisveste dieren die goed aan mensen gewend zijn, de dieren zijn bovendien getraind om mee te werken aan bepaalde diertechnische handelingen, waardoor ze minder stress ervaren. Sedatie en pijnbestrijding zullen worden toegepast wanneer geïndiceerd, doorlopende (temperatuur-)metingen en waarnemingen zullen worden gedaan zonder de dieren te hoeven hanteren. Het gebruik van temperatuurloggers maakt een zeer nauwkeurige temperatuurregistratie gedurende langere tijd mogelijk zonder dat de dieren onder narcose gaan. Uiteraard zullen bij onverwacht

grotere welzijnsaantasting dan voorzien in het protocol humane eindpunten worden toegepast. Er is geen sprake van belangwekkende milieueffecten.

10. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd. De niet-technische samenvatting is een zelfstandig leesbare en evenwichtige weergave van het project en is begrijpelijk geformuleerd.

## D. Ethische afweging

De doeleinden van het project rechtvaardigen het voorgestelde gebruik van dieren (niet), de schade in de vorm van lijden, pijn en angst bij dit aantal dieren wordt (niet) gerechtvaardigd door het verwachte resultaat. Het is uit wetenschappelijk oogpunt verantwoord en het is waarschijnlijk dat de doeleinden worden gehaald. Op termijn kan het project (geen) voordelen opleveren voor mens, dier of milieu.

Het belang van de doelstelling wordt door de DEC onderschreven, waarbij het (risico op) ongerief voor de dieren (resusapen) is afgewogen ten opzichte van de potentiële positieve effecten van resultaten van het onderzoek naar nieuwe griepvaccins voor de mens. De huidige griepvaccins zijn ontoereikend wat betreft effectiviteit en productiemethode. Aangezien griepvaccins er juist op gericht zijn kwetsbare mensen met een verlaagde immuniteit te beschermen tegen influenza virusinfecties is het des te belangrijker dat er een goed en veilig vaccin op de markt komt dat een hoge mate van bescherming biedt tegen verschillende soorten griepvirussen. Met een dergelijk vaccin zullen vele levens worden gered. De haalbaarheid van de doelstellingen van dit project wordt als hoog ingeschat. Binnen de instelling en bij de onderzoeker is veel ervaring beschikbaar met het uitvoeren van het voorgestelde onderzoek met dit type virussen. Daardoor is het mogelijk de dierstudies met de grootst mogelijke zorgvuldigheid uit te voeren, waarbij significante uitkomsten verkregen worden met een zo beperkt mogelijk aantal dieren, tesamen met zo min mogelijk aantasting van hun welzijn. Tenslotte zijn de dieren in leven aan het einde van het experiment.
De principes van vervanging, vermindering en/of verfijning van dierproeven zijn toegepast voor zover mogelijk. Bij de proefdieren en hun verzorging, behandeling en huisvesting wordt adequaat invulling gegeven aan de vereisten op het gebied van de specifieke eigenschappen en sociaal gedrag van deze diersoort en wordt in ruime mate aandacht besteed aan verrijking, monitoring van ziekteverschijnselen en mogelijk afwijkend gedrag ten gevolge van de infectie. Dit zijn behalve wetenschappelijke uitkomsten ook belangrijke parameters om het welzijn van de dieren te waarborgen.

Voor het voorgestelde onderzoek is dit gebruik van niet-humane primaten naar het oordeel van de commissie essentieel en ethisch aanvaardbaar.

## E. Advies

1. Advies aan de CCD

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

   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.

2. Het uitgebrachte advies is gebaseerd op consensus

Centrale Commissie Dierproeven

7

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**Onze referentie**
Aanvraagnummer
AVD502002016704
**Bijlagen**
2

Datum 18 november 2016
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte 10.2.e

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 18 november 2016. Het gaat om uw project "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD502002016704. Gebruik dit nummer wanneer u contact met de CCD opneemt.

**Wacht met de uitvoering van uw project**
Als wij nog informatie van u nodig hebben dan ontvangt u daarover bericht. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn bijgeschreven op de rekening van de CCD. U ontvangt binnen veertig werkdagen een beslissing op uw aanvraag. Als wij nog informatie van u nodig hebben, wordt deze termijn opgeschort. In geval van een complexe aanvraag kan deze termijn met maximaal vijftien werkdagen verlengd worden. U krijgt bericht als de beslisperiode van uw aanvraag vanwege complexiteit wordt verlengd. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

**Factuur**
Bijgaand treft u de factuur aan voor de betaling van de leges. Wij verzoeken u de leges zo spoedig mogelijk te voldoen, zodat we uw aanvraag in behandeling kunnen nemen. Is uw betaling niet binnen dertig dagen ontvangen, dan kan uw aanvraag buiten behandeling worden gesteld. Dit betekent dat uw aanvraag niet beoordeeld wordt en u uw project niet mag starten.

**Meer informatie**
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

**Gegevens aanvrager**

Uw gegevens

| | |
|---|---|
| Deelnemersnummer NVWA: | 50200 |
| Naam instelling of organisatie: | Biomedical Primate Research Centre |
| Naam portefeuillehouder of diens gemachtigde: | 10.2.e |
| KvK-nummer: | 41146967 |
| Straat en huisnummer: | Lange Kleiweg 161 |
| Postbus: | 3306 |
| Postcode en plaats: | 2288 GJ RIJSWIJK |
| IBAN: | 10.2.g |
| Tenaamstelling van het rekeningnummer: | Stichting Biomedical Primate Research Centre |

Gegevens verantwoordelijke onderzoeker

| | |
|---|---|
| Naam: | 10.2.e |
| Functie: | 10.2.g |
| Afdeling: | 10.2.g |
| Telefoonnummer: | 10.2.e |
| E-mailadres: | 10.2.e |

Gegevens plaatsvervangende verantwoordelijke onderzoeker

| | |
|---|---|
| Naam: | 10.2.e |
| Functie: | 10.2.g |
| Afdeling: | 10.2.g |
| Telefoonnummer: | 10.2.e |
| E-mailadres: | 10.2.e |

Gegevens gemachtigde

| | |
|---|---|
| Naam: | 10.2.e |
| Postbus: | 3306 |
| Postcode en plaats: | 2288 GJ RIJSWIJK |
| Wilt u een nieuwe machtiging afgeven? | Nee |
| Wat mag de gemachtigde doen? | [ ] Een projectvergunning aanvragen<br>[ ] Een wijziging op een verleende projectvergunning aanvragen<br>[ ] Een melding doorgeven op een verleende projectvergunning<br>[ ] Een bezwaarschrift indienen en daarover communiceren met de Centrale Commissie Dierproeven en alle andere handelingen verrichten die nodig zijn voor een goede afwikkeling van het bezwaarschrift<br>[x] Alle bovenstaande opties |

**Over uw aanvraag**

| | |
|---|---|
| Wat voor aanvraag doet u? | [x] Nieuwe aanvraag<br>[ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn<br>[ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn |

**Over uw project**

| | |
|---|---|
| Geplande startdatum: | 1 juni 2017 |
| Geplande einddatum: | 1 juni 2022 |
| Titel project: | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques |
| Titel niet-technische samenvatting: | Onderzoek naar de beschermende werking van nieuwe griep vaccins |
| Naam DEC: | 10.2.g |
| Postadres DEC: | 10.2.g |
| E-mailadres DEC: | 10.2.e |

**Betaalgegevens**

| | |
|---|---|
| De leges bedragen: | € 1.187,- |
| De leges voldoet u: | na ontvangst van de factuur |

**Checklist bijlagen**

| | |
|---|---|
| Verplichte bijlagen: | [x] Projectvoorstel<br>[x] Beschrijving Dierproeven<br>[x] Niet-technische samenvatting |
| Overige bijlagen: | [x] Melding Machtiging<br>[x] DEC-advies |

**Ondertekening**

| | |
|---|---|
| Naam: | 10.2.e |
| Functie: | 10.2.g |
| Plaats: | Rijswijk |
| Datum: | 27 oktober 2016 |

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Biomedical Primate Research Centre
10.2.e
Postbus 3306
2288 GJ RIJSWIJK
10.2.g

**Centrale Commissie Dierproeven**
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

**Onze referentie**
Aanvraagnummer
AVD502002016704
**Bijlagen**
2

Datum 18 november 2016
Betreft Factuur aanvraag projectvergunning Dierproeven

**Factuur**
Factuurdatum: 18 november 2016
Vervaldatum: 18 december 2016
Factuurnummer: 16700704

| **Omschrijving** | **Bedrag** |
|---|---|
| Betaling leges projectvergunning dierproeven<br>Betreft aanvraag AVD502002016704 | € 1.187,00 |

Wij verzoeken u het totaalbedrag vóór de gestelde vervaldatum over te maken op rekening NL29INGB 070.500.1512 onder vermelding van het factuurnummer en aanvraagnummer, ten name van Centrale Commissie Dierproeven, Postbus 93144, 2509 AC te 's Gravenhage.

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

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info@zbo-ccd.nl

**Onze referentie**
Aanvraagnummer
AVD502002016704

Datum 1 december 2016
Betreft Aanvulling aanvraag projectvergunning Dierproeven

Geachte 10.2.e ,

Op 18 november 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques" met aanvraagnummer AVD502002016704. In uw aanvraag zitten voor ons nog enkele onduidelijkheden. In deze brief leest u wat wij nog nodig hebben en wanneer u een beslissing kunt verwachten.

**Welke informatie nog nodig**
Wij hebben de volgende informatie van u nodig om uw aanvraag verder te kunnen beoordelen:

**Onduidelijkheden**
- U beschrijft in uw aanvraag het gebruik van makaken. Kunt u aangeven van welke soort u gebruik maakt?
- U beschrijft in uw aanvraag het gebruik van slechts 1 geslacht per experiment. Kunt u aangeven waarom u hiervoor kiest, gezien het feit dat de vaccins bij de mens in zowel mannen als vrouwen zullen worden toegediend?
- U beschrijft ook vaccins te testen waarbij immunogeniciteit in andere diersoorten niet getest is (beschreven bij uw criteria for vaccin evaluation punt 'e'). Kunt u aangeven op basis waarvan u voor deze vaccins baseert dat immunogeniciteit in de NHP wel wordt verwacht?

**Leges**
De leges die u verschuldigd bent zijn nog niet door ons ontvangen of de betaling is nog niet verwerkt. Uw aanvraag is niet compleet als de leges niet zijn ontvangen.

Zonder deze aanvullende informatie kan de beslissing nadelig voor u uitvallen omdat de gegevens onvolledig of onduidelijk zijn.

**Datum:**
1 december 2016
**Aanvraagnummer:**
AVD502002016704

**Opsturen binnen veertien dagen**
Stuur de ontbrekende informatie binnen veertien dagen na de datum van deze brief op. U kunt dit aanleveren via NetFTP. Stuurt u het per post op, gebruik dan het formulier dat u bij deze brief krijgt.

**Wanneer een beslissing**
De behandeling van uw aanvraag wordt opgeschort tot het moment dat wij de aanvullende informatie hebben ontvangen. Uw aanvraag is in ieder geval niet compleet als de leges niet zijn ontvangen. Als u goedkeuring krijgt op uw aanvraag, kunt u daarna beginnen met het project.

**Meer informatie**
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Centrale Commissie Dierproeven

# Melding bijlagen

U wilt één of meerdere bijlagen naar ons versturen? Voeg altijd deze Melding Bijlagen toe.
Wij weten dan welke documenten van u zijn en hoeveel documenten u opstuurt.
Meer informatie vindt u op www.centralecommissiedierproeven.nl
Of bel met ons: 0900 28 000 28 (10 ct/min).

**1 Uw Gegevens**

Naam instelling: Biomedical Primate Research Centre

Adres: .....

Postcode en plaats: .....

Aanvraagnummer: AVD502002016704

**2 Bijlagen**
Welke bijlagen stuurt u mee?
Vink de bijlagen aan of vul de naam of omschrijving in.

O Projectvoorstel

O Beschrijving Dierproeven

O Niet-technische samenvatting

O Melding Machtiging

O Aanvraagformulier

O .....

O .....

O .....

**Datum:**
1 december 2016
**Aanvraagnummer:**
AVD502002016704

## 3 Ondertekening

Naam: .....

Datum: ..... - ..... - .....

Handtekening: .....

Onderteken het formulier en stuur het met alle bijlagen op naar:
Centrale Commissie Dierproeven
Postbus 20401
2500 EK Den Haag

Centrale Commissie Dierproeven

# Form
## Project proposal

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

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 50200 |
| 1.2 | Provide the name of the licenced establishment. | Biomedical Primate Research Centre |
| 1.3 | Provide the title of the project. | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques. |

## 2 Categories

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

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

## 3 General description of the project

### 3.1 Background

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

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

Influenza epidemics are estimated to result in infection of 2,5-10% of the world population every year, causing 2-5 million cases of severe illness and 250.000-500.000 deaths (http://www.who.inf). Especially vaccination is considered the most effective measure against the influenza disease and, as such, it is

recommended by the European Council (http://eur-ex.europe.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:348:0072:EN:PDF) and implemented in all EU/European Economic Area member states. The main problems of the current influenza vaccines are; a) they are not very effective in the elderly, b) they only protect against highly homologous strains, while circulating influenza virus strains constantly evolve as a result of antigenic drift, c) they do not protect against new pandemic strains that emerge as a result of recombination between different viral strains found in animal reservoirs, d) they do not protect against highly pathogenic avian influenza virus (1-3). These problems are amplified by the cumbersome current methods of vaccine production, which involves either growing the virus on eggs and/or preparing live attenuated virus. The 6 months time needed for vaccine preparation means that the vaccines have to be based on predictions about which virus strains will circulate during the next influenza season. A mismatch between the vaccine and the actually circulating influenza strain(s) however, results in lower vaccine effectiveness as shown for the 2014-15 influenza season with regard to the H3N2 strain (4). New vaccine strategies that can provide broader protection and cover a range of seasonal influenza strains as well as pandemic and avian influenza virus strains are urgently needed. These so called "universal" influenza vaccines are directed at either a) inducing broadly neutralizing antibodies by targeting the relatively conserved stem region of the haemagglutinin (HA) subunit, which is responsible for virus entry into the target cell, b) inducing protective T-cell responses that are usually directed against more conserved proteins of the virus and show more broad recognition (5, 6). Retrospective epidemiological studies as well as studies in experimentally infected volunteers indicate that in the absence of antibodies, cellular immune responses can have a protective effect (7-9). Their role in achieving cross protection was recently demonstrated in a H1N1 vaccine evaluation study in non-human primates (NHP) (10). More recently the appreciation of the importance of non-neutralizing anti-influenza antibodies in conferring a broad protection against variant strains, especially in the case of avian influenza viruses, has prompted research into their mechanism of action (via antibody dependent cellular cytotoxicity (ADCC), antibody dependent phagocytosis (ADP) or complement activation (11-13)) and vaccine strategies to induce these antibodies. New methods for faster vaccine production, the induction of T-cell responses and improvement of vaccine responses in the elderly have involved application of DNA, virus like particles (VLP), recombinant viral vectors and strategies to target vaccines to the appropriate antigen presenting cells (3, 14-18). Evaluation of the immunogenicity of these vaccines requires additional methods, besides the standard antibody ELISA, micro-neutralization and haemagglutination inhibition assays. Especially, proper assessment of adaptive cellular immune responses and function of the innate immune system in relation to non-neutralizing antibody effector function and induction of immune responses by the new vaccine modalities is needed.

Animal models have played an important role in preclinical evaluation of candidate influenza vaccines (19, 20). While a number of species have been used, the most commonly used models to assess immunogenicity and efficacy against influenza virus infection are the mouse, ferret and non-human primate (NHP) models. There are important differences between these species in immune function and susceptibility to influenza virus infection. 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 play an important role in influenza virus research and have been used to study pathogenesis as well as efficacy of preventive and therapeutic intervention strategies (21). Of the different animals models used in influenza virus research, NHP have a unique close homology to humans in most components of their immune system (22-24). For instance, similar T and B-cell subsets have been described in NHP (25). Moreover, the immunoglobulin gene germline repertoire is highly conserved between macaques and humans, which is important when induction of broadly neutralizing antibodies by new "universal" influenza vaccine strategies is studied (26). In addition, structure and function of Fc receptors, which are essential for the function of non-neutralizing antibodies, show many homologies between macaques and humans (27). Only very limited information is available on Fc receptors in ferrets and only reagents to detect the IgA receptor are available. Finally in NHP the innate immune system, including molecular pathways and antigen presenting cell subsets, are much more homologous to humans than what is seen in mice (23). NHP not only most closely reflect the human physiology, but also resemble humans in their clinical

symptoms, limited pathology, pattern of viral replication, fever and cytokine and chemokine responses following influenza virus infection (28).
In conclusion, the strong immunological and physiological resemblances to humans make NHP a unique model in pre-clinical safety, immunogenicity and efficacy evaluation, particularly in relation to the new influenza virus vaccine delivery platforms being developed and for the evaluation of the important broadly neutralizing antibody, non-neutralizing antibody and cellular broadly protective immune responses. Evaluation in NHP is essential before the new "universal" influenza candidates can be evaluated in humans. Moreover, although challenge studies have been performed in humans (29), these are limited to the milder influenza strains and intranasal application of the virus (30) limiting the value of the vaccine efficacy data that can be obtained.

### 3.2 Purpose

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

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

The goal of this project is to evaluate novel influenza virus vaccine candidates for occurrence of adverse effects, immunogenicity and capacity to protect against influenza virus infection in macaques. Both the capacity of new vaccine candidates to elicit a broad immune response, that not only protects against a homologous virus that is similar to the vaccine but also against heterologous viruses, as well as the immunogenicity of new influenza vaccine delivery platforms will be evaluated under this project application. The ultimate goal is to develop an influenza vaccine that can induce an immune response that is sufficiently broad to provide protection against seasonal influenza virus variants over a 5 year period (to obviate the need to vaccinate every year), is effective in elderly and can provide a degree of heterogeneous protection that would lead to reduced morbidity and mortality caused by pandemic as well as highly pathogenic avian influenza viruses. 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. Additional criteria for vaccine evaluation are: a) the vaccine strategy must be novel, for instance with regards to choice of antigen, formulation, route of application, that have not been tested before in similar NHP studies, b) demonstration that the vaccine or vaccine components are non-toxic, c) when specific host molecules are targeted then cross recognition of macaque homologues must have been demonstrated, d) the vaccine cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action or the type of immunological assessment needed, e) preferably immunogenicity of vaccine candidates should have been proven in other species, unless this is not possible because the specific vaccine modality used does not work in other species. This concerns only vaccines for which it is not possible to directly evaluate them in other species because interaction with specific host molecules is required that are only present in humans and in NHP, but not in other species. In this case, we would like to add the additional requirement that for this type of vaccine a similar vaccine strategy that targets slightly different molecules but uses the same mode of action has been evaluated and found to be immunogenic in other species.
The main goal can be divided in 2 sub-goals:

1. Vaccine evaluation. Immunogenicity and efficacy to protect against infection will be evaluated using an appropriate influenza virus challenge strain in relation to the type of vaccine being used.
2. Set-up infection model for influenza viruses that have not yet been used in NHP at our institute and that are needed for vaccine evaluation (sub-goal 1) and refinement of influenza virus infection models. For refinement the optimal dose and aerosol delivery will be investigated in order to improve the assessment of vaccine efficacy.

In case a vaccine has to be evaluated against an influenza virus strain that has been used before at our institute, then this can be directly performed under sub-goal 1. However, in case a new influenza virus strain has to be used then two stages are necessary; first to set-up the infection model (sub-goal 2) and then use this information in the model for vaccine evaluation (sub-goal 1). Two types of experiments are needed to achieve these sub-goals, namely a vaccine evaluation model (described in bijlage 1) and an

influenza virus infection model (bijlage 2).
At our institute we have been performing vaccine evaluation studies in NHP for over 20 years. Most vaccine candidates were directed against human immunodeficiency virus, hepatitis B virus and hepatitis C virus and tuberculosis. Since 2012 we have been working on influenza virus infection in macaques and the evaluation of vaccines against influenza 10.2.g ). We have the appropriate facilities and experience to work with pathogenic viruses, including influenza virus, at DM-3 and ML-3 biosafety conditions. In addition, we have the appropriate immunological assays for assessment of cellular, humoral and innate immune responses against influenza. Our long-standing experience with pathogenic viruses, including influenza, and with vaccine evaluation guarantees that these animal studies will be adequately performed.

**3.3 Relevance**

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

The annual influenza virus epidemics cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with pulmonary disease. In addition, there is the continuous threat of the emergence of new viral recombinants that would lead to a pandemic. Previous pandemics, especially the 1918 pandemic, have caused millions of deaths. Finally, avian influenza viruses are widely spread and can occasionally infect humans. Mutations that lead to a strong increase in transmission have been described (34), indicating that also these viruses pose a continuous threat to the human population. Current influenza vaccine strategies and vaccine production methods are not adequate to deal with such emergencies. Even for protection against the current seasonal influenza viruses, yearly vaccination of risk groups is necessary. Hence a vaccine that could offer protection against a broader range of viruses, including as yet unknown recombinants and avian influenza would be of great benefit to the community. In addition, the yearly vaccination would no longer be necessary since a broadly protective vaccine would be effective over a period of at least five years against newly emerging variants. This has led the EU to invest in the development of so called "universal" influenza vaccines that would fulfil these criteria. Both the application of new delivery methods, for instance in the form of DNA or viral vectors, as well as new vaccine modalities require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established, before these new vaccines can be tested in clinical studies.

**3.4 Research strategy**

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

In order to evaluate that a new influenza virus vaccine candidate is immunogenic, has the capacity to protect against infection and that no adverse effects occur, a vaccine evaluation experiment will be performed according to well established procedures, as described in bijlage 1. Typically one or a number of immunizations are given over a certain time period. After immunization the induction of T-cell and antibody immune responses is measured. The strength of these responses as well their breadth, i.e. the capacity to recognize not only homologous viruses that are similar to the vaccine but also heterologous viruses, is determined. Subsequently, the capacity of the vaccine to protect against infection is tested by experimental infection of the animals with influenza virus. The choice of the virus strain that is to be used for experimental infection 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. Experimental infection will only be performed when the immunization has induced clearly measurable immune responses against the virus that is to be used for experimental infection so that protection against infection is possible. Whether protection is actually achieved depends on local interaction between cells of the immune system and local anti-viral antibodies with the virus and virus infected cells in the respiratory tract. This cannot be adequately modelled in an *in vitro* system and requires experimental infection of an animal. Ideally the vaccine should provide a robust level of protection and be able to reduce disease and virus multiplication in animals that receive a standard virus dose via delivery to the upper respiratory tract and lungs. However, since most people become infected via exposure to small droplets containing a limited amount

of virus, a less stringent infection model; i.e. using a low dose of virus given via aerosol delivery, may sometimes be chosen.
In case proper evaluation of the capacity of a vaccine to protect against infection requires that a virus has to be used that has not been tested before in macaques at our institute then this virus will first be tested in a small number of animals to determine if all animals become infected and what the amount of virus multiplication is (bijlage 2). Virus infection is routinely performed by inoculating the animals via a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular, using a standard dose. For refinement, we will investigate another mode of virus delivery, namely as an aerosol. The rational for setting up this model of infection is that the virus is given in the form of small droplets that better reflect the natural mode of exposure. Furthermore, previous studies in ferrets (35) have shown that a lower dose of virus may suffice, which may also better reflect the natural situation. At present it is still unknown whether such an infection model will be sufficiently robust to allow proper vaccine evaluation and therefore this model can still be considered as experimental. Once this method is established, it may be applied in subsequent vaccine evaluation studies, for instance in cases where less stringent criteria of protection against infection are needed (in case it is difficult to make a protective vaccine and it is necessary to establish relatively modest improvements in vaccine development that can only be measured when a low dose of virus is used).

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

Vaccine evaluation in macaques. For this type of experiment animals will be immunized either once or they will receive a number of immunizations over a certain time period. During the study animals will be monitored for adverse effects of the vaccine, including monitoring of general behaviour and health. Blood and occasionally nasal washes and lung lavages will be taken to measure induction of systemic as well as local immune responses. 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.
Influenza virus infection in macaques. In order to establish infectivity and pathogenicity of a new virus that has not been tested previously in NHP at our institute, 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 what the amount of virus multiplication is. To evaluate a new virus, the virus is inoculated via a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular using a standard dose. Proper application in vaccine evaluation requires that in these infection studies > 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 humane endpoint within the first four days after infection then a 10-100 times lower virus dose will be evaluated. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust.

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

Vaccine candidates that fulfil the criteria for evaluation in NHP may be directly tested in a vaccine evaluation study (bijlage 1), if the influenza virus that is to be used for establishing capacity of the vaccine to protect against infection has already been used in NHP at our institute. If this is not the case then the virus has to be tested first in an influenza virus infection study (bijlage 2). Also when efficacy against low dose aerosol infection has to be tested, a preceding influenza virus infection study (bijlage 2) is necessary. Requirement to proceed from influenza virus infection study to vaccine evaluation study are: a) more than 80% of the animals have to become infected, b) variation between animals has to be such that in a vaccine evaluation study less than 10 animals per test group suffice to obtain statistically significant results, c) no animals reach the humane endpoint within 4 days after infection.

1. **de Vries RD, Altenburg AF, Rimmelzwaan GF.** 2015. Universal influenza vaccines, science fiction or soon reality? Expert Rev Vaccines **14:**1299-1301.

2. **Osterhaus A, Fouchier R, Rimmelzwaan G.** 2011. Towards universal influenza vaccines? Philos Trans R Soc Lond B Biol Sci **366:**2766-2773.
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3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

| Serial number | Type of animal procedure |
|---|---|
| 1 | Vaccine evaluation in macaques |
| 2 | Influenza virus infection in macaques |
| 3 | |
| 4 | |
| 5 | |
| 6 | |
| 7 | |
| 8 | |
| 9 | |
| 10 | |

# 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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 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>*Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number: 1<br>Type of animal procedure: 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. Typically a recording device is surgically placed in the abdominal cavity before the start of the study to retrospectively evaluate body temperature (measured every 15 minutes) and/or heart rate and activity. Subsequently the animals are immunized either once or they receive a number of immunizations over a certain time period. 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 that is 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 bijlage 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 recording device is surgically placed in the abdominal cavity at least 4 weeks before the first immunization takes place. 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, typically at 4 to 8 week time intervals, although occasionally a longer time frame is needed between immunizations when different vaccine modalities are used for priming and boosting of the immune response. Usually 3 immunizations suffice over a period of 20 weeks. However, in rare occasions these limits may have to be exceeded. 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, intra-bronchially using a bronchoscope 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. Occasionally, usually before the start of the study and after the last immunisation, a nasal wash and lung lavage is taken in order to measure induction of local immune responses. 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 bijlage 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 6 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 (X-ray or 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. 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. 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 will not be euthanized, the recording devices are surgically removed and body temperature and/or heart rate and activity data are analysed, upon which, the animals may be re-used (within the limitations described in art 1e of the Wet op de Dierproeven).

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.

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 needed will be used. Since historical data are available on infection in unvaccinated animals (bijlage 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, estimated numbers, and life stages. Provide justifications for these choices.

The experiment will be performed in macaques, adult, M/F, n=150.
Macaque species have been extensively used in influenza virus vaccine research (1-3)The most often used species are the rhesus macaque (*Macaca mulatta*) and cynomolgus macaque (*Macaca fascicularis*). Both species are susceptible to an array of human and avian influenza viruses. We have recently shown that cynomolgus macaques are more susceptible for pandemic H1N1 (pH1N1) virus than rhesus macaques (4). Therefore, cynomolgus macaques are the preferred species for vaccine research against pH1N1. All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier. Adult male and female animals can be used. However, since there are immunological differences between males and females (5) 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, and thereby enhance the probability of finding statistically significant differences between the test groups, we prefer that for each individual experiment either all animals are male or all are female. This choice is also important with regard to the amount of blood needed to perform all assays. Male animals are bigger 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 above females.
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 5 to 6 such studies over a 5-year period.

**C. Re-use**

Will the animals be re-used?

☐ No, continue with question D.

☒ 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 of the animals of this species reuse 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.

**D. 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 (1). 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 these 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 trachea 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 unvaccinated animals (bijlage 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 be needed.

**Refinement**

The use of temperature recording devices makes it possible to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection (4). With this method we have observed a significant reduction in fever by some of the 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 surgery, 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.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

Animals will be socially housed with a socially compatible animal, whenever possible. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food (http://www.bprc.nl/en/welfare/).
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. (7). On the basis of the scoring system a humane endpoint is defined. 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 tube feeding. 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.
The "Flora and Fauna wet" and "wet dieren" do not pose additional requirements that are needed for the type of studies proposed in this application.

## Repetition and duplication

**E. Repetition**

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

## Accommodation and care

**F. Accommodation and care**

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

**G. 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 H.

☐ 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.

## Classification of discomfort/humane endpoints

**H. Pain and pain relief**

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ 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 placing the recording device in the abdomen or 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 fever 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.

**I. Other aspects compromising the welfare of the animals**

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

1. Stress because of change in housing
2. Discomfort because of insertion or removal of the temperature recording device
3. Discomfort due to injection
4. Discomfort due to lung lavages
5. Discomfort due to virus installation
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. Animals have to be moved to DM-III facilities because of the experimental infection with influenza virus.
2. The surgery needed for insertion and removal of the temperature recording device will cause pain and some local inflammation.
3. When vaccines are given by injection, this can cause local pain and irritation.
4. For the lung lavages a bronchoscope is used. Insertion will cause irritation.
5. When virus is given intra-bronchially a bronchoscope is used and this 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.
6. Animals will be repeatedly sedated for vaccine delivery, blood sampling, virus infection, collection of swabs 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 will be reduced.
8. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, loss of appetite, inactivity.

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

1. Animals will be socially housed en enrichment is provided.
2. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
3. 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.
4. For the lung lavages animals are first deeply sedated and then receive a local muscle relaxant.
5. The same procedure as described under 4 will be followed.
6. Recovery of the animals will be monitored and the veterinarian will be consulted in case of problems.
7. Animals will receive tube feeding. This is applied during sedation.

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.

**J. 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 K.

☒ 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. When a certain pre-determined clinical score is reached that indicates that the maximum duration of severity is reached then the animal will be humanely euthanized. Individual scores are added and the decision is based on the total daily score. 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.

The percentage of animals reaching the end point will depend on the virus being used. Seasonal influenza viruses (H1N1 and H3N2) as well as pandemic H1N1 will only cause minimal disease and typically resolve within 14-21 days. Highly pathogenic influenza virus has been described to be able to cause severe disease in macaques, requiring euthanasia of the animal. Depending on the virus, this was observed in 25% of the animals at most and later than day 4 after infection. At our institute these viruses will first be evaluated in a small number of animals (see bijlage 2). If any of the animals reach the clinical end point before day 4 then this virus will not be used at the dose that was given. It may be tested at a 10-100 times lower dose in a new small group of animals. Also then the same criteria will be used and the virus will not be used if any of the animals reach the clinical end point before day 4. Weight will be recorded during the study, but a weight loss of 10% because of the infection has not been observed in any study described thus far and hence does not serve as a suitable end point.

**K. Classification of severity of procedures**

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

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.

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

☒ 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 or when influenza viruses are used that are known to cause persistent lung pathology, animals are humanely euthanized and a full necropsy is performed.


1. **Bodewes R, Rimmelzwaan GF, Osterhaus AD.** 2010. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines **9:**59-72.
2. **Davis AS, Taubenberger JK, Bray M.** 2015. The use of nonhuman primates in research on seasonal, pandemic and avian influenza, 1893-2014. Antiviral Res **117:**75-98.
3. [redacted]
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10.2.g


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6. **Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, van Meersbergen R, Huizingh J, Wanningen P, Verspuij J, de Man M, Ding Z, Apetri A, Kukrer B, Sneekes-Vriese E, Tomkiewicz D, Laursen NS, Lee PS, Zakrzewska A, Dekking L, Tolboom J, Tettero L, van Meerten S, Yu W, Koudstaal W, Goudsmit J, Ward AB, Meijberg W, Wilson IA, Radosevic K.** 2015. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science **349:**1301-1306.
7. **Brining DL, Mattoon JS, Kercher L, LaCasse RA, Safronetz D, Feldmann H, Parnell MJ.** 2010. Thoracic radiography as a refinement methodology for the study of H1N1 influenza in cynomologus macaques (Macaca fascicularis). Comp Med **60:**389-395.


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

# 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, see our website (www.centralecommissiedierproeven.nl).
- Or contact us by phone (0900-2800028).

## 1 General information

| | | |
|---|---|---|
| 1.1 | Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'. | 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>*Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.* | Serial number: 2<br>Type of animal procedure: Influenza virus infection 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 viruses (1, 2). 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 what the amount of virus production is. To evaluate a new virus, the virus is inoculated by a combination of routes; i.e. intra-bronchial, oral, intranasal and intraocular 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. In order to further improve the model, we will investigate another mode of virus delivery, namely as an aerosol. The rational for setting up this mode of infection is that the virus is given in the form of small droplets that better reflects the natural mode of exposure. Furthermore, previous studies in ferrets (3, 4) have shown that a lower dose of virus may

suffice, which may also better reflect the natural situation. The same criteria will be used to determine whether the aerosol infection model is sufficiently robust. Three doses, covering a total range in amount of virus of $10^4$ (50% tissue culture infectious dose ($TCID_{50}$)) will be tested to establish the lowest dose that fulfils these statistical criteria. Although cynomolgous macaques have been proved to be more susceptible to infection with pandemic H1N1 viruses than rhesus macaques (1), this may be different for other influenza viruses. It may therefore be necessary to evaluate a new virus in both species.

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.

A recording device 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 6 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 (X-ray or 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 know 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.

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 virus is inoculated by a standard combination of routes at a standard dose, will be performed in four animals. Experience in the pandemic H1N1 infection model has 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. For virus given by aerosol delivery it is not yet possible to predict the minimal group size. An initial experiment performed by Marriott et al showed that three out of four animals that were exposed to the virus did become infected (5). This implies that there may be considerable variation in this model. With 6 animals per group it would be acceptable if one of the

animals does not become infected, because then still statistically significant results can be obtained in a vaccine evaluation studies with 10 vaccinated animals, even if one of the vaccinated animals happens to be unprotected (Chi-squared test, power is 0.835). Therefore, in a first experiment we would like to use six animals. In subsequent experiments the number of animals per group may be reduced. Whether aerosol challenge will provide a robust infection model, will be determined on the basis of power calculations, as outlined above. In addition, in this model when more than 4 animals are used per group, at least 80% of the animals have to become infected.

**B. The animals**

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

The experiment will be performed in macaques, adult, M/F, n=56.
All animals are purpose bred. They are either bred at our institute or obtained from a certified supplier. Adult male and female animals can be used. When combined routes of inoculation are used; i.e. intra-bronchial, oral, intranasal and intraocular, a single dose usually suffices to establish the infection model. In the literature almost all viruses have been used at a dose of $10^6$ to $10^7$ $TCID_{50}$ in NHP (6-9). However, it may be necessary to evaluate an extra dose in some cases. Assuming 4 animals per groups, evaluation of 3 new viral stains, from which 2 have to be tested at two doses, the total number of animals needed will be maximum 20.
For aerosol delivery we expect to need 6 animals per dose. Assuming maximum 3 doses to be tested for 2 viruses, the total number of animals needed is 36.
In total 56 animals are the maximum needed for setting up infection models for new influenza viruses in a period of 5 years.

**C. Re-use**

Will the animals be re-used?

☐ No, continue with question D.

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

Animals that will be used in these experiements, have possibly been used in previous experiments. Animals that have been involved in previous influenza vaccine or influenza virus infections studies or that have pre-existing antibodies against influenza are not suitable. In view of the long life of the animals of this species reuse 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.

**D. 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 (10, 11). 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 bijlage 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 bijlage 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. Because of the limited experience with aerosol delivery in NHP and the results obtained so far (5) six animals per group are expected to be sufficient for the first experiment. On the basis of the outcome of the first study the number of animals needed in follow up experiment can be calculated and less animals may be needed. Only the minimum number of animals needed, will be used.

**Refinement**

The use of temperature recording devices makes it possible to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection (1). With this method we have observed a significant reduction in fever by some of the vaccine candidates (12). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the recording devices will require a small surgery, 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.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

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. (13). On the basis of the scoring system a humane endpoint is defined. 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 days of the infection the animal will receive tube feeding. 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.
There are no other aspects in this proposal that are not in agreement with the Dutch law other than the Wod.

## Repetition and duplication

**E. Repetition**

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable.

## Accommodation and care

**F. Accommodation and care**

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

☒ No

☐ Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

**G. 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 H.

☐ 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.

## Classification of discomfort/humane endpoints

**H. Pain and pain relief**

Will the animals experience pain during or after the procedures?

☐ No > Continue with question I.

☒ 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 placing the recording device in the abdomen or after removal, which is needed in case the animal will return to the experimental stock 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 fever during the first days after insertion of the recording device, but have recovered very well within 1 week after the operation.

**I. Other aspects compromising the welfare of the animals**

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

1. Stress because of change in housing
2. Discomfort because of insertion or removal of the recording device.
3. Discomfort due to lung lavages
4. Discomfort due to virus installation
5. Stress because of sedation
6. Reduced food intake during the first days after infection
7. Disease symptoms due to the infection

Explain why these effects may emerge.

1. Animals have to be moved to DM-III facilities because of the experimental infection with influenza virus.
2. The surgery needed for insertion and removal of the recording device will cause pain and some local inflammation.
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 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. 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.
6. Especially during daily sedation during the first 2 days after infection food intake will be reduced.
7. Influenza infection can cause fever, coughing, sneezing, nose discharge, laboured breathing, loss of appetite, inactivity.

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

1. Animals will be socially housed en enrichment is provided.
2. Surgery will be done under anaesthesia and after surgery analgesics will be applied.
3. For the lung lavages animals are first deeply sedated and receive a muscle relaxant.
4. The same procedure are described under 3 will be followed.

5. Recovery of the animals is monitored and the veterinarian will intervene if animals do not recover fast enough.
6. Animals will receive tube feeding. This is applied during sedation.
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 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 organs.

**J. 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 K.

☒ 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. When a certain pre-determined clinical score is reached, indicating the maximum duration of severity, then the animal will be humanely euthanized. Individual scores are added and the decision is based on the total daily score. 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.

The percentage of animals reaching the end point will depend on the virus being used. Seasonal influenza viruses (H1N1 and H3N2) as well as pandemic H1N1 will only cause minimal disease and typically resolve within 14-21 days. Highly pathogenic influenza virus has been described to be able to cause severe disease in macaques requiring euthanasia of the animal. Depending on the virus this was observed in 25% of the animals at most and later than day 4 after infection. If any of the animals reach the humane end point before day 4 then this virus will not be used at the dose that was given. It may be tested at a 10-100 times lower dose in a new group of four animals. Also then the same criteria will be used and the virus will not be used if any of the animals reach the humane end point before day 4. Weight will be recorded during the study, but a weight loss of 10% because of the infection has not been observed in any study described thus far and hence does not serve as a suitable end point.

**K. Classification of severity of procedures**

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned ('non-recovery', 'mild', 'moderate', 'severe').

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.

## End of experiment

**L. Method of killing**

Will the animals be killed during or after the procedures?

☐ No

☒ 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 influenza viruses are used that are known to cause persistent lung pathology, animals are humanely euthanized and a full necropsy is performed.

1. 10.2.g [redacted]
2. [redacted]

3. **Gustin KM, Belser JA, Wadford DA, Pearce MB, Katz JM, Tumpey TM, Maines TR.** 2011. Influenza virus aerosol exposure and analytical system for ferrets. Proc Natl Acad Sci U S A **108:**8432-8437.
4. **Marriott AC, Dove BK, Whittaker CJ, Bruce C, Ryan KA, Bean TJ, Rayner E, Pearson G, Taylor I, Dowall S, Plank J, Newman E, Barclay WS, Dimmock NJ, Easton AJ, Hallis B, Silman NJ, Carroll MW.** 2014. Low dose influenza virus challenge in the ferret leads to increased virus shedding and greater sensitivity to oseltamivir. PLoS One **9:**e94090.
5. **Marriott AC, Dennis M, Kane JA, Gooch KE, Hatch G, Sharpe S, Prevosto C, Leeming G, Zekeng EG, Staples KJ, Hall G, Ryan KA, Bate S, Moyo N, Whittaker CJ, Hallis B, Silman NJ, Lalvani A, Wilkinson TM, Hiscox JA, Stewart JP, Carroll MW.** 2016. Influenza A Virus Challenge Models in Cynomolgus Macaques Using the Authentic Inhaled Aerosol and Intra-Nasal Routes of Infection. PLoS One **11:**e0157887.
6. **Laddy DJ, Yan J, Khan AS, Andersen H, Cohn A, Greenhouse J, Lewis M, Manischewitz J, King LR, Golding H, Draghia-Akli R, Weiner DB.** 2009. Electroporation of synthetic DNA antigens offers protection in nonhuman primates challenged with highly pathogenic avian influenza virus. J Virol **83:**4624-4630.
7. **Fan S, Gao Y, Shinya K, Li CK, Li Y, Shi J, Jiang Y, Suo Y, Tong T, Zhong G, Song J, Zhang Y, Tian G, Guan Y, Xu XN, Bu Z, Kawaoka Y, Chen H.** 2009. Immunogenicity and protective efficacy of a live attenuated H5N1 vaccine in nonhuman primates. PLoS Pathog **5:**e1000409.
8. **Kreijtz JH, Suezer Y, de Mutsert G, van den Brand JM, van Amerongen G, Schnierle BS, Kuiken T, Fouchier RA, Lower J, Osterhaus AD, Sutter G, Rimmelzwaan GF.** 2009. Recombinant modified vaccinia virus Ankara expressing the hemagglutinin gene confers protection against homologous and heterologous H5N1 influenza virus infections in macaques. J Infect Dis **199:**405-413.
9. **Rimmelzwaan GF, Baars M, van Amerongen G, van Beek R, Osterhaus AD.** 2001. A single dose of an ISCOM influenza vaccine induces long-lasting protective immunity against homologous challenge infection but fails to protect Cynomolgus macaques against distant drift variants of influenza A (H3N2) viruses. Vaccine **20:**158-163.
10. **Bodewes R, Rimmelzwaan GF, Osterhaus AD.** 2010. Animal models for the preclinical evaluation of candidate influenza vaccines. Expert Rev Vaccines **9:**59-72.
11. **Bouvier NM, Lowen AC.** 2010. Animal Models for Influenza Virus Pathogenesis and Transmission. Viruses **2:**1530-1563.
12. **Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, van Meersbergen R, Huizingh J, Wanningen P, Verspuij J, de Man M, Ding Z, Apetri A, Kukrer B, Sneekes-Vriese E, Tomkiewicz D, Laursen NS, Lee PS, Zakrzewska A, Dekking L, Tolboom J, Tettero L, van Meerten S, Yu W, Koudstaal W, Goudsmit J, Ward AB, Meijberg W, Wilson IA, Radosevic K.** 2015. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. Science **349:**1301-1306.
13. **Brining DL, Mattoon JS, Kercher L, LaCasse RA, Safronetz D, Feldmann H, Parnell MJ.** 2010. Thoracic radiography as a refinement methodology for the study of H1N1 influenza in cynomologus macaques (Macaca fascicularis). Comp Med **60:**389-395.


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

06-12-2016

## Format DEC-advies

*Maak bij de toepassing van dit format gebruik van de Praktische Handreiking: Ethisch Toetsingskader voor proefdiergebruik. Voor voorbeelden, zie bijlage I.*

### A. Algemene gegevens over de procedure

*Bij de punten 1 t/m 7 dienen altijd de gevraagde gegevens te worden ingevuld.*

1. Aanvraagnummer AVD502002016704

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

3. Titel van de NTS Onderzoek naar beschermende werking van nieuwe griep vaccins

4. Type aanvraag:
   - ✓ nieuwe aanvraag projectvergunning
   - ☐ wijziging van vergunning met nummer

5. Contactgegevens DEC:
   - naam DEC: 10.2.g
   - telefoonnummer contactpersoon: 10.2.e
   - e-mailadres contactpersoon: 10.2.e

6. Adviestraject (data dd-mm-jjjj):
   - ☑ ontvangen door DEC: 01-11-2016
   - ☑ aanvraag compleet: 01-11-2016
   - ☑ in vergadering besproken: 07-11-2016
   - ☐ anderszins behandeld:
   - ☑ termijnonderbreking(en) van: 08-11-2016 tot 15-11-2016
   - ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   - ☐ aanpassing aanvraag
   - ☑ advies aan CCD 17-11-2016
   - ☑ advies nieuw format aan CCD 16-12-2016

7. Afstemming IvD
   - De aanvrager heeft het projectvoorstel afgestemd met de IvD alvorens het voor te leggen aan de DEC.

8. Eventueel horen van aanvrager : NVT

9. Correspondentie met de aanvrager
   - Datum: 08-11-2016
   - Gestelde vraag/vragen: enige tekstuele aanpassingen en verduidelijking inzake de bepaling van proefopzet en groepsgrootte
   - Datum antwoord: 15-11-2016
   - Verstrekt(e) antwoord(en) : tekstuele aanpassingen zijn doorgevoerd en de bepaling van de proefopzet (m.i.v. de groepsgrootte) is verduidelijkt.
   - De antwoorden hebben **wel** geleid tot aanpassing van de aanvraag

10. Eventuele adviezen door experts (niet lid van de DEC): NVT.

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag
3. Is de DEC competent om hierover te adviseren? De commissie heeft voldoende expertise, inbegrepen de virologie, vaccinologie, immunologie, onderzoek met niet-humane primaten, en toepassing van alternatieven op deze gebieden. Ook is er voldoende expertise op gebied van ontwerp van dierproeven, proefdiergeneeskundige praktijk, het houden en verzorgen van dieren, ethiek en proefdieren en hun bescherming. De DEC heeft ruime ervaring met het beoordelen van onderzoek naar vaccins tegen virale infecties in niet-humane primaten.

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 DEC leden was betrokken bij de aanvraag. Deze persoon heeft zich teruggetrokken van de vergadering bij de bespreking van de aanvraag en overigens geen aandeel gehad in de afweging en advisering.

**C. Beoordeling (inhoud)**

1. Beoordeel of de aanvraag toetsbaar is en voldoende samenhang heeft *(Zie handreiking 'Invulling definitie project'; zie bijlage I voor toelichting en voorbeeld).*
   Het voorgestelde onderzoek bestaat uit twee fasen, te weten 1) evaluatie van de effectiviteit en veiligheid van kandidaat vaccins tegen influenza virus in niet-humane primaten en 2) ontwikkeling van een influenza virus infectiemodel indien nieuwe virusstammen gebruikt moeten worden ten behoeve van de evaluatie van vaccins. Vaccins die getest gaan worden in dit model zijn innovatief en nog niet eerder getest in niet-humane primaten. De subdoelen sluiten logisch aan bij het hoofddoel en samen vormen met elkaar een samenhangend geheel.
   Het verwachte ongerief voor de dieren is duidelijk omschreven en de uitkomsten uit dit experiment zijn helder en meetbaar. Het aantal dieren dat

gebruikt zal worden is realistisch ingeschat en statistisch onderbouwd. De klinische eindpunten zijn duidelijk gedefinieerd.

2. Geef aan of er aspecten in deze aanvraag zijn die niet in overeenstemming zijn met wet- en regelgeving anders dan de Wod? De aanvraag is, zover de DEC kan overzien, in overeenstemming met alle toepasselijke regelgeving.

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 doelcategorie, te weten 'translationeel of toegepast onderzoek', sluit aan bij het projectvoorstel. In deze projectaanvraag worden nieuwe typen griep vaccins getest op hun vermogen om een goede immuunrespons te induceren die bescherming biedt tegen griepinfectie en op veiligheid. Het nieuwe van deze vaccins bestaat er uit dat ze gericht zijn op het induceren van een brede bescherming tegen diverse griepvarianten bij de mens en/of de mogelijkheid om het vaccin snel in grote hoeveelheden te produceren.

*Belangen en waarden*

4. Benoem zowel het directe doel als het uiteindelijke doel en geef aan of er een reële relatie is tussen beide doelstellingen *(Zie Praktische handreiking ETK: Stap 1.C4; zie bijlage I voor voorbeeld)*. Het directe doel van het project is het testen van nieuwe anti influenza vaccins op veiligheid en effectiviteit in niet-humane primaten. Het uiteindelijke doel is de implementatie van nieuwe vaccin-concepten ter bescherming van mensen tegen influenza virus infectie. Jaarlijks overlijden wereldwijd meer dan een kwart miljoen mensen (vooral ouderen en kinderen) ten gevolge van een griepinfectie. Daarnaast heeft de griepgolf een grote socio-economische impact. Aangezien een griepvaccin dat beschermt tegen alle verschillende varianten van het influenza virus niet beschikbaar is, wordt er jaarlijks een nieuw vaccin aangeboden. Omdat het productieproces (op bevruchte kippeneieren of in celkweek) en de daaraan gekoppelde kwaliteitscontroles langdurige processen zijn, wordt de keuze van de vaccin-stammen minstens een half jaar voor de eventuele griepuitbraken gemaakt. Hierdoor loopt men het risico dat het geproduceerde vaccin geen volledige bescherming biedt tijdens het griepseizoen (zoals in het seizoen 2014-2015). Ook is gebleken dat de immuunrespons tegen de huidige vaccins in oudere mensen niet zo sterk is, waardoor juist de doelgroep die bescherming tegen griep hard nodig heeft, toch vaak ziek wordt ondanks de vaccinatie. De onderzoekers willen de veiligheid en effectiviteit van dergelijke vaccin kandidaten testen in niet-humane primaten als laatste stadium voor toepassing in de mens. Het betreft hier een *pre-klinisch* project. Er is binnen dit project 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 dit translationele project dat gericht is op de ontwikkeling van een nieuw beter vaccin tegen influenza zijn de proefdieren, het onderzoeksveld en de te beschermen personen.

Waarden die voor proefdieren in het geding zijn: De integriteit van de dieren zal tijdelijk worden aangetast, de dieren zullen tijdelijk beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden, ziek worden en soms enige mate van pijn ondervinden.

Het onderzoeksveld krijgt nieuwe informatie die wordt gedeeld d.m.v. publicatie(s).

Het belang voor de samenleving is dat mensen individueel beschermd worden tegen meerdere griepvarianten en dat een hoge vaccinatiegraad onder de bevolking de kans op epidemieën reduceert. Bij kwetsbare doelgroepen kan sterfte worden voorkomen. De beschikbaarheid en kwaliteit van griepvaccins zal verbeteren. Daardoor zullen minder mensen ziek worden of overlijden ten gevolge van griep.

6. Geef aan of er sprake kan zijn van substantiële milieueffecten. Zo ja, benoem deze, leg uit waarom daar sprake van kan zijn en of geef aan of deze effecten afgedekt worden door specifieke wetgeving.
   Er zijn geen substantiële milieueffecten te verwachten binnen de kaders of ten gevolge van dit project.

*Proefopzet en haalbaarheid*

7. Beoordeel of de kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven voldoende gewaarborgd zijn. Licht uw antwoord toe. *(Zie Praktische handreiking ETK: Stap 1.C5).*
   De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn boven iedere twijfel verheven gezien de wetenschappelijke output, de verworven interne- en externe financiering alsmede de aandacht voor de drie V's.

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 antwoord toe. *Zie Praktische handreiking ETK: Stap 1.C6).*

   De DEC is er van overtuigd dat het projectvoorstel aansluit bij recente wetenschappelijke inzichten en geen hiaten bevat die de bruikbaarheid van de resultaten in de weg zullen staan.
   De voorgestelde experimentele opzet en uitkomstparameters zijn logisch en helder gekozen en sluiten aan bij de aangegeven doelstellingen en de gekozen strategie en experimentele aanpak kunnen naar de mening van de DEC leiden tot het behalen van de doelstelling in het kader van het project.

*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 de aanvrager voldoet aan de in de Wod voor de desbetreffende categorie genoemde beperkende voorwaarden. Licht uw antwoord 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)

   Het experiment wordt uitgevoerd met niet-humane primaten. De keuze voor deze diersoort is gebaseerd op bewezen gevoeligheid voor influenza virus infecties en de zeer grote fysiologische en immunologische overeenkomsten met de mens. De kandidaat vaccins zijn tevoren gekarakteriseerd in *in vitro* en *in vivo* (veelal knaagdieren) modellen. In de laatste fase van de preklinische ontwikkeling van kandidaat vaccins is het echter noodzakelijk om de effectiviteit en veiligheid van de kandidaat vaccins te onderzoeken in een diermodel waarin de aard en verloop van de ziekte grote overeenkomsten vertonen met de mens. Vaccin kandidaten kunnen niet afdoende in andere diermodellen of alternatieve modellen worden getest met oog op bescherming tegen infectie in de mens. De resultaten verkregen in dit proefdiermodel kunnen de sterkte van de immuunrespons van de kandidaat griepvaccins in de mens voorspellen. De eventuele nadelige effecten (bijvoorbeeld als gevolg van de complexiteit van immuunresponsen en hun interactie met het infectieus agens) van de kandidaat vaccins kunnen in dit diermodel worden bepaald.

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 de richtlijn. Indien niet aan deze minimale eisen kan worden voldaan omdat het om wetenschappelijke redenen noodzakelijk is hiervan af te wijken, beoordeel of dit in voldoende mate is onderbouwd. Licht toe waarom wel/niet.

    De huisvesting en verzorging voldoet ten volle aan de vereisten in bijlage III.

11. Beoordeel of het ongerief als gevolg van de dierproeven realistisch is ingeschat en geclassificeerd, waarbij uitgegaan wordt van de kans op angst, pijn, stress en/of ziekte bij individuele dieren *(Zie Praktische handreiking ETK: Stap 1.C2).*

    Het ongerief is correct als matig ingeschat, op basis van ervaring met influenza-infecties in niet-humane primaten, de experimentele technieken en

de vaccinaties. In combinatie zijn de gevolgen van deze handelingen terecht als matig ongerief ingeschat. De dieren die gebruikt zullen worden voor de experimenten zijn speciaal voor dit doel gefokt, en zullen als sociaal compatibel duo worden gehuisvest in een BSL3-faciliteit. Alles betreffende deze dierproef is in overeenstemming met de nieuwe Richtlijn.

12. Geef aan op welke wijze de integriteit van de dieren wordt aangetast *(Zie Praktische handreiking ETK: Stap 1.C2). (zie bijlage I voor voorbeeld).*

    De integriteit van de dieren wordt aangetast door de dieren te vaccineren en te infecteren met influenza virussen. Deze aantasting is van voorbijgaande aard, de dieren keren naderhand geheel terug naar hun oorspronkelijke toestand, qua gezondheid en welzijn.

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 antwoord toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.

    Naar de mening van de DEC zijn de humane eindpunten zorgvuldig beschreven (gebaseerd op algemene en specifieke criteria) en is de inschatting van de kans dat dieren een humaan eindpunt zullen bereiken adequaat ingeschat.

14. Beoordeel of de aanvrager voldoende aannemelijk heeft gemaakt dat er geen geschikte vervangingsalternatieven zijn? Onderbouw uw antwoord *(Zie Praktische handreiking ETK: Stap 1.C3)*.

    Er is geen *in vitro* model beschikbaar waarin een immuunrespons kan worden opgewekt, of dat de beschermende werking van vaccins kan meten. Er kan veel informatie betreffende specificiteit en hoogte van de immuunrespons worden verkregen uit *in vitro* assays die zullen worden uitgevoerd met het materiaal afkomstig van de dieren, maar het gebruik van proefdieren kan nog niet worden overgeslagen. Wereldwijd worden verschillende diersoorten gebruikt in influenza onderzoek, ook voor vaccin–evaluatie studies. Echter voor het testen van 'universele' influenza-vaccins is het noodzakelijk een dier te gebruiken waarvan het immuunsysteem zoveel mogelijk gelijkenis vertoont met dat van de mens. Voorwaarde is wel dat producten die in deze dieren getest gaan worden in het eindstadium van de ontwikkeling zijn. In de experimenten in niet-humane primaten kunnen inzichten in effectiviteit en mogelijke bijwerkingen naar voren komen die bij onderzoek met andere diersoorten gemist zouden zijn. Deze stap over te slaan en direct onderzoek met menselijke vrijwilligers te verrichten is in dit stadium van de vaccin ontwikkeling daarom dan ook niet verantwoord.

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. Onderbouw uw antwoord *(Zie Praktische handreiking ETK: Stap 1.C3)*.

    De proefopzet wordt telkens gebaseerd op de concrete experimentele

vraagstelling. Het aantal te gebruiken dieren per behandelgroep wordt bepaald met behulp van statische powerberekeningen op basis van de verwachte effect grootte en spreiding van de data. Omdat historische data worden meegenomen in de uiteindelijke statistische analyse zal het aantal dieren in de controlegroep lager zijn dan in de gevaccineerde groepen. Bij het onderzoeken van meerdere vaccin kandidaten binnen een proefopzet zal het aantal controlegroepen beperkt worden.

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 antwoord toe *(Zie Praktische handreiking ETK: Stap 1.C3)*.

    De uitvoering is verfijnd door gebruik te maken van sociaal gehuisveste dieren die goed aan mensen gewend zijn, de dieren zijn bovendien getraind om mee te werken aan bepaalde diertechnische handelingen, waardoor ze minder stress ervaren. Sedatie en pijnbestrijding zullen worden toegepast wanneer geïndiceerd, doorlopende (temperatuur-)metingen en waarnemingen zullen worden gedaan zonder de dieren te hoeven hanteren. Het gebruik van temperatuurloggers maakt een zeer nauwkeurige temperatuurregistratie gedurende langere tijd mogelijk. Bij onverwacht grotere welzijnsaantasting dan voorzien zal een humaan eindpunt worden toegepast. Er is geen sprake van belangwekkende milieueffecten.

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. Onderbouw uw antwoord.

    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? Geef ook aan welke maatregelen verder zijn getroffen om bij fok of aankoop van dieren het aantal in voorraad gedood te beperken *(Zie Praktische handreiking ETK: Stap 1.C3; zie bijlage I voor voorbeeld).*

    In onderhavige projectaanvraag worden dieren van hetzelfde geslacht gebruikt. Uit de projectaanvraag blijkt dat dit weloverwogen gebeurt met oog op het minimaliseren van het aantal te gebruiken dieren en de onderzoeker heeft in de projectaanvraag naar de mening van de DEC dit voldoende onderbouwd. In het geval van niet-humane primaten leidt dit niet tot een 'fokoverschot'.

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 de richtlijn. Zo niet, beoordeel of dit in voldoende mate is onderbouwd. Licht dit toe. Indien van toepassing, geeft ook aan of er door de aanvrager ontheffing is aangevraagd *(Zie Praktische handreiking ETK: Stap 1.C3).*

    De meeste dieren zullen in leven blijven aan het einde van het project. Alleen in het geval van onderzoek zeer virulente of nieuwe virussen zal tot euthanasie worden overgegaan om de orgaanpathologie te onderzoeken. Daarnaast worden dieren ge-euthanaseerd wanneer humane eindpuntcriteria worden bereikt, dit om verder ongerief te voorkomen (zoals gedefinieerd in de projectaanvraag). Er wordt een passende dodingsmethode gebruikt (conform bijlage IV van de richtlijn).

20. Indien dieren worden gedood, is adoptie of hergebruik overwogen? Licht toe waarom dit wel/niet mogelijk is.

    Hergebruik wordt altijd overwogen en ook nagestreefd (binnen de kaders omtrent dierenwelzijn en wetenschappelijke kwaliteit).

    *NTS*

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

    Naar de mening van de DEC beschrijft de niet-technische samenvatting het project inhoudelijk correct en in begrijpelijke taal.

## D. Ethische afweging

1. Benoem de centrale morele vraag *(Zie Praktische handreiking ETK: Stap 3.A).*

Rechtvaardigt het testen van nieuwe anti-influenza vaccins die uiteindelijk in de mens moeten worden toegepast het ongerief dat niet-humane primaten wordt aangedaan? Is het gebruik van niet-humane primaten in dit geval gerechtvaardigd of kan de gewenste informatie ook verkregen worden door het inzetten van anderssoortige proefdieren?

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 *(Zie Praktische handreiking ETK: Stap 3.B; zie bijlage I voor voorbeelden).*

Voor de proefdieren zijn integriteit, welzijn en de autonomie in het geding, door de dieren handelingen met een wetenschappelijk doel te laten ondergaan. Ze kunnen hierdoor ziek worden en enige pijn ondervinden door bloedafnames en injecties. De dieren ondervinden hiervan matig nadeel.

De waarden voor het onderzoeksveld zijn toenemend wetenschappelijk inzicht in de werking van potentiële nieuwe griepvaccins en de respons van het immuunsysteem hierop, en in voorkomende gevallen ook de agens-gastheer interactie. Dit is een voordeel voor het onderzoeksveld omdat niet-humane primaten goed modelleren voor deze fenomenen bij de mens en dit onderzoek ook bijdraagt aan extrapolatie naar de mens. Daarmee is dit onderzoek informatief en het is de uitdrukkelijke bedoeling om dit te publiceren.

De waarden die voor de samenleving zijn gelegen in het ontwikkelen van betere vaccins met een betere beschikbaarheid (een brede werking betekent dat er niet jaarlijks nieuw vaccin hoeft te worden ontwikkeld op basis van een onzekere prognose welke virusvariant zal gaan circuleren). Naar verwachting leidt dit tot verlaging van het aantal griepgevallen en minder doden ten gevolge hiervan, deze leveren groot voordeel voor de samenleving op. De voordelen zijn zowel economisch (minder ziekteverzuim, minder uitval van zorgenden inclusief mantelzorgers, minder zorgkosten) als bevorderlijk voor het algemene welzijn van de samenleving (minder doden, minder zieken en minder blijvend letsel als gevolg van griepinfectie).
Indien de doelstellingen bereikt worden, zal dit werk leiden tot de ontwikkeling van nieuwe vaccins die beter en langduriger beschermen tegen griep. Vooral voor kleine kinderen en oudere mensen is dit van belang omdat in die groepen door influenza infecties de meeste dodelijke slachtoffers vallen (geschat wordt een kwart miljoen doden per jaar). Het verwachte ongerief voor de dieren valt daardoor moreel te verantwoorden, bovendien zijn de dieren over het algemeen in leven aan het einde van het experiment.

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 concludeert dat de belangen van de samenleving die worden nagestreefd in dit project zwaarder wegen dan de belangen/waarden van de betrokken proefdieren.
Het onderzoek heeft zowel belang voor een groot deel van de mensheid als voor dieren. Het belang voor de mens is hierboven al beschreven. Het belang

dieren is het volgende: als een nieuw griepvaccin wordt gevonden dat veilig en effectief is betekent dat een andere productiemethode voor het vaccin en langdurige bescherming van de gevaccineerde populatie tegen infectie met influenzavirus. Hierdoor zal op termijn het aantal dieren dat gebruikt wordt voor onderzoek en testen kunnen afnemen, aangezien het nu nog nodig is om via dierproeven te testen of nieuwe vaccin batches effectief en veilig zijn. Dit levert voor de dieren in dit project geen voordeel op, maar draagt wel bij aan de vermindering van proefdiergebruik op de lange termijn.
De expertise voor het verrichten van deze experimenten is beschikbaar binnen de instelling en bij de betrokken onderzoekers. De kennis en kunde van de aanvragers wordt onderbouwd door eerder onderzoek naar de immunogeniciteit, veiligheid en effectiviteit van vaccins tegen infectieziekten, en de vereiste expertise en voorzieningen voor dergelijk onderzoek met niet-humane primaten. Reeds enkele jaren wordt er in de instelling onderzoek verricht aan influenza virus-infecties en vaccins tegen influenza. De gekozen strategie voor infectie en vaccinatie, alsmede het management van dierenwelzijn, is mede gebaseerd op ervaringen uit deze eerdere studies. De keuze voor deze diersoort is gebaseerd op bewezen gevoeligheid voor influenza virus infecties en de zeer grote fysiologische en immunologische overeenkomsten met de mens.
De haalbaarheid van de doelstellingen van dit project wordt als hoog ingeschat. De te onderzoeken vaccins zijn in gevorderde stadia van ontwikkeling. De kandidaat vaccins zijn tevoren gekarakteriseerd in *in vitro* en *in vivo* (veelal knaagdieren) modellen. In de laatste fase van de preklinische ontwikkeling van kandidaat vaccins is het echter noodzakelijk om de effectiviteit en veiligheid van de kandidaat vaccins te onderzoeken in een diermodel waarin de aard en verloop van de ziekte, alsmede de immuunresponsen, grote overeenkomsten vertonen met de mens. Vaccin kandidaten kunnen niet in andere diermodellen of alternatieve modellen worden getest op bescherming tegen infectie in de mens. De effecten (bijvoorbeeld als gevolg van de complexiteit van immuunresponsen en hun interactie met het infectieus agens) van de kandidaat vaccins kunnen in dit diermodel goed worden onderzocht en ook kunnen eventuele nadelige effecten aan het licht komen. Gezien het bovenstaande is de DEC van mening dat dit project het gebruik van deze proefdieren rechtvaardigt.

## E. Advies

1. Advies aan de CCD
   - ☐ De DEC adviseert de vergunning te verlenen.
   - ☑De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden
     - ☑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.
     - ☐ Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
     - ☐ Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...
   - ☐ De DEC adviseert de vergunning niet te verlenen vanwege:
     - ☐ De vaststelling dat het project niet vergunningplichtig is om de

volgende redenen
☐ De volgende doorslaggevende ethische bezwaren:...
☐ De volgende tekortkomingen in de aanvraag:...

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 *(Zie Praktische handreiking ETK: Stap 4.A; zie bijlage I voor voorbeeld).*

   Het uitgebrachte advies is gebaseerd op consensus. De belanghebbende die lid is van de DEC heeft geen aandeel gehad in de totstandkoming van dit advies.

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 *(Zie Praktische handreiking ETK: Stap 4.B)*.

   Er zijn geen knelpunten ondervonden; de inherente ethische dilemma's zijn hierboven uitgebreid uiteengezet.

Reply to questions CCD regarding project proposal AVD502002016704. Modifications in the project proposal are indicated in yellow.

- U beschrijft in uw aanvraag het gebruik van makaken. Kunt u aangeven van welke soort u gebruik maakt?

The most often used species are the rhesus macaque (*Macaca mulatta*) and cynomolgus macaque (*Macaca fascicularis*). Both species are susceptible to an array of human and avian influenza viruses. We have recently shown that cynomolgus macaques are more susceptible for pandemic H1N1 (pH1N1) virus than rhesus macaques (10.2.e en 10.2.g ). Therefore, cynomolgus macaques are the preferred species for vaccine research against pH1N1. However, for other influenza viruses it may turn out that rhesus macaques are more susceptible. We therefore request permission for using either cynomolgus or rhesus macaques. *This information has been added to appendix 1 under B.* If a virus has to be used that has not been tested before at our institute it will be evaluated for infectivity as described in bijlage 2. In this case the virus will first be evaluated in cynomolgus macaques. Only in case it is not possible to obtain a good infection model in cynomolgus macaques will the virus be tested in rhesus macaques. *This information has been added to appendix 2 under A.*

- U beschrijft in uw aanvraag het gebruik van slechts 1 geslacht per experiment. Kunt u aangeven waarom u hiervoor kiest, gezien het feit dat de vaccins bij de mens in zowel mannen als vrouwen zullen worden toegediend?

We agree that a vaccine should work in males as well as females. Both male and female animal macaques are susceptible for influenza virus infection and have been used in vaccine evaluation studies against influenza. Therefore both males and females can be used in our studies. However, there are immunological differences between males and females (Klein et al 2015 TRSTMH; 109; 9). In order to minimize the variation within the experimental test group, and thereby enhance the probability of finding statistically significant differences between the test groups, we prefer that for each individual experiment either all animals are male or all are female. This choice is also important with regard to the amount of blood needed to perform all assays. Male animals are bigger 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 above females. *This information has been added to appendix 1 under B.*

- U beschrijft ook vaccins te testen waarbij immunogeniciteit in andere diersoorten niet getest is (beschreven bij uw criteria for vaccin evaluation punt 'e'). Kunt u aangeven op basis waarvan u voor deze vaccins baseert dat immunogeniciteit in de NHP wel wordt verwacht?

This concerns only vaccines for which it is not possible to directly evaluate them in other species because interaction with specific host molecules is required that are present in humans and in NHP, but not in other species. In this case, we would like to add the additional requirement that for this type of vaccines a similar vaccine strategy that targets slightly different molecules but uses the same mode of action has been evaluated and found to be immunogenic in other species. *This information is now added to the Project proposal under 3.2.*

Centrale Commissie Dierproeven

14

Datum 15-12-2016
Betreft Advies Secretariaat over Aanvraag projectvergunning Dierproeven AVD2016704

# Advies aan CCD

B

| | |
|---|---|
| Instelling: | Biomedical Primate Research Centre |
| Onderzoeker: | 10.2.e |
| Project: | Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques |
| Aanvraagnummer: | AVD2016704 |
| Betreft: | Nieuwe aanvraag |
| Categoriën: | Translationeel of toegepast onderzoek |

**1 Inzicht in aanvraag en de eventuele knelpunten en risico's**

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| **Proces** | De DEC is gevraagd om een advies in het nieuwe format aan te leveren. De aanvrager is nog de volgende vragen gesteld:<br>- U beschrijft in uw aanvraag het gebruik van makaken. Kunt u aangeven van welke soort u gebruik maakt?<br>- U beschrijft in uw aanvraag het gebruik van slechts 1 geslacht per experiment. Kunt u aangeven waarom u hiervoor kiest, gezien het feit dat de vaccins bij de mens in zowel mannen als vrouwen zullen worden toegediend?<br>- U beschrijft ook vaccins te testen waarbij immunogeniciteit in andere diersoorten niet getest is (beschreven bij uw criteria for vaccin evaluation punt 'e'). Kunt u aangeven op basis waarvan u voor deze vaccins baseert dat immunogeniciteit in de NHP wel wordt verwacht? | | | |
| **Naam proef** | **Diersoort** | **Stam** | **Aantal dieren** | **Herkomst** |
| **3.4.4.1 Vaccine evaluation in macaques** | | | | |
| | Rhesusapen (Macaca mulatta) | | 75 | Dieren die voor onderzoek gefokt zijn **Niet menselijke primaten** |
| | Java-apen (Macaca fascicularis) | | 75 | Dieren die voor onderzoek gefokt zijn **Niet menselijke primaten** |
| **3.4.4.2 Influenza virus infection in macaques** | | | | |
| | Rhesusapen (Macaca mulatta) | | 28 | Dieren die voor onderzoek gefokt zijn **Niet menselijke primaten** |
| | Java-apen (Macaca fascicularis) | | 28 | Dieren die voor onderzoek gefokt zijn **Niet menselijke primaten** |

Het gebruik van NHP is voldoende onderbouwd. De aanvrager beschrijft het gebruik van makaken, maar specificeert niet de soort. De aanvrager is gevraagd dit nog te benoemen.

**Gebruik van mannelijke en vrouwelijke dieren**

- 3.4.4.1 Vaccine evaluation in macaques / Rhesusapen (Macaca mulatta): Er worden zowel mannelijke als vrouwelijke dieren gebruikt. Adult male and female animals can be used. However, since there are immunological

differences between males and females we prefer that for each individual experiment either all animals are male or all are female.

- 3.4.4.2 Influenza virus infection in macaques / Rhesusapen (Macaca mulatta): Er worden zowel mannelijke als vrouwelijke dieren gebruikt. Adult male and female animals can be used.
- 3.4.4.1 Vaccine evaluation in macaques / Java-apen (Macaca fascicularis): Er worden zowel mannelijke als vrouwelijke dieren gebruikt. goed onderbouwd
- 3.4.4.2 Influenza virus infection in macaques / Java-apen (Macaca fascicularis): Er worden zowel mannelijke als vrouwelijke dieren gebruikt. zie resusaap

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| **Locatie uitvoering experimenten** | - Alle proeven vinden plaats in een instelling van een vergunninghouder.<br>- Er zijn geen problemen bekend met de vergunninghouder. |
| **Maatschappij** | Er wordt verwacht dat het onderwerp in die mate politiek of maatschappelijk gevoelig is, dat eventuele extra communicatie uitingen nodig zijn. Wegens het gebruik van NHP kan maatschappelijke onrust ontstaan. Het handelt echter om een ziekte waar veel mensen jaarlijks aan overlijden. |

**2 DEC advies**

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| **DEC-advies** | De DEC heeft de onderzoeker vragen gesteld over: enige tekstuele aanpassingen en verduidelijking inzake de keuze van de groepsgrootte. Deze aanpassingen zijn doorgevoerd en de keuze van de groepsgrootte is verhelderd.<br><br>Het experiment wordt uitgevoerd met niet-humane primaten. De keuze voor deze diersoort is gebaseerd op bewezen gevoeligheid voor influenza virus infecties en de zeer grote fysiologische en immunologische overeenkomsten met de mens. De kandidaat vaccins zijn tevoren gekarakteriseerd in in vitro en in vivo (veelal knaagdieren) modellen. In de laatste fase van de preklinische ontwikkeling van kandidaat vaccins is het echter noodzakelijk om de effectiviteit en veiligheid van de kandidaat vaccins te onderzoeken in een diermodel waarin de aard en verloop van de ziekte grote overeenkomsten vertonen met de mens. Vaccin kandidaten kunnen niet in andere diermodellen of alternatieve modellen worden getest op bescherming tegen infectie in de mens. De resultaten verkregen in dit proefdiermodel kunnen de sterkte van de immuunrespons van de kandidaat vaccins in de mens voorspellen. De eventuele nadelige effecten (bijvoorbeeld als gevolg van de complexiteit van immuunresponsen en hun interactie met het infectieus agents) van de kandidaat vaccins kunnen in dit diermodel worden vastgesteld.<br><br>Wereldwijd worden verschillende diersoorten gebruikt in influenza onderzoek, ook voor vaccin–evaluatie studies. Echter voor het testen van |

'universele' influenza-vaccins is het noodzakelijk een dier te gebruiken met een immuunsysteem dat zoveel mogelijk gelijkenis vertoont met dat van de mens. Voorwaarde is wel dat producten die in deze dieren getest gaan worden in het eindstadium van de ontwikkeling zijn. In de experimenten in niet-humane primaten kunnen mogelijke bijwerkingen naar voren komen die in andere diersoorten gemist zouden zijn. Direct onderzoek met menselijke vrijwilligers is in dit stadium van de vaccin ontwikkeling daarom dan ook niet verantwoord.

Omdat historische data worden meegenomen in de uiteindelijke statistische analyse zal het aantal
dieren in de controlegroep lager zijn dan in de gevaccineerde groepen.

Ethische afweging van de DEC:
Het belang van de doelstelling wordt door de DEC onderschreven, waarbij het (risico op) ongerief voor de dieren (resusapen) is afgewogen ten opzichte van de potentiële positieve effecten van resultaten van het onderzoek naar nieuwe griepvaccins voor de mens. De huidige griepvaccins zijn ontoereikend wat betreft effectiviteit en productiemethode. Aangezien griepvaccins er juist op gericht zijn kwetsbare mensen met een verlaagde immuniteit te beschermen tegen influenza virusinfecties is het des te belangrijker dat er een goed en veilig vaccin op de markt komt dat een hoge mate van bescherming biedt tegen verschillende soorten griepvirussen. Met een dergelijk vaccin zullen vele levens worden gered. De haalbaarheid van de doelstellingen van dit project wordt als hoog ingeschat.
Binnen de instelling en bij de onderzoeker is veel ervaring beschikbaar met het uitvoeren van het voorgestelde onderzoek met dit type virussen. Daardoor is het mogelijk de dierstudies met de grootst mogelijke zorgvuldigheid uit te voeren, waarbij significante uitkomsten verkregen worden met een zo beperkt mogelijk aantal dieren, tesamen met zo min mogelijk aantasting van hun welzijn. Tenslotte zijn de dieren in leven aan het einde van het experiment.
De principes van vervanging, vermindering en/of verfijning van dierproeven zijn toegepast voor zover mogelijk. Bij de proefdieren en hun verzorging, behandeling en huisvesting wordt adequaat invulling gegeven aan de vereisten op het gebied van de specifieke eigenschappen en sociaal gedrag van deze diersoort en wordt in ruime mate aandacht besteed aan verrijking, monitoring van ziekteverschijnselen en mogelijk afwijkend gedrag ten gevolge van de infectie. Dit zijn behalve wetenschappelijke uitkomsten ook belangrijke parameters om het welzijn van de dieren te waarborgen.

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| | Voor het voorgestelde onderzoek is dit gebruik van niet-humane primaten naar het oordeel van de commissie essentieel en ethisch aanvaardbaar.<br><br>De DEC heeft extern advies ingewonnen bij<br>- de aanvrager is om aanvullingen gevraagd<br><br>Het DEC advies is Positief<br><br>Het uitgebrachte advies is gebaseerd op consensus. |

**3 Kwaliteit DEC advies**

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| **Kwaliteit DEC-advies** | Het DEC-advies is volledig. |
| | Er zijn DEC leden uitgesloten van de behandeling van de aanvraag vanwege onafhankelijkheid of onpartijdigheid.<br>Een van de DEC leden was betrokken bij de aanvraag. Deze persoon heeft zich teruggetrokken van de vergadering bij de bespreking van de aanvraag. |
| Deze DEC heeft gebruik gemaakt van het oude format. Het DEC advies is daarom niet compleet. Ook verzuimt de DEC om hergebruik van de dieren te benoemen. De DEC is gevraagd een compleet advies in de nieuwe format aan te leveren. | |

**4 Inhoudelijke beoordeling**

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| **Belangen-verstrengeling** | Geen van de medewerkers van het Secretariaat heeft een arbeidsrelatie gehad met de aanvrager die minder dan 5 jaar geleden is beëindigd of heeft andere belangen die mogelijk zouden kunnen leiden tot oneigenlijke beïnvloeding. |

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| **Doelstelling**<br>Doelstelling | citaat: The goal of this project is to evaluate novel Influenza virus vaccine candidates for occurrence of adverse effects, immunogenicity and capacity to protect against influenza virus infection in macaques. Both the capacity of new vaccine candidates to elicit a broad immune response, that not only protects against a homologous virus that is similar to the vaccine but also against heterologous viruses, as well as the immunogenicity of new influenza vaccine delivery platforms will be evaluated under this project application. The ultimate goal is to develop an influenza vaccine that can induce an immune response that is sufficiently broad to provide protection against seasonal influenza virus variants over a 5 year period (to obviate the need to vaccinate every year), is effective in elderly and can provide a degree of heterogeneous protection that would lead to reduced morbidity and mortality caused by pandemie as well as highly pathogenic avian influenza viruses. 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. Additional criteria for vaccine evaluation are: a) the vaccine strategy must be novel, for instance with regards to choice of antigen, formulatlon, route of application, that have not been tested before in slmilar NHP studies, b) demonstratien that the vaccine or vaccine components are non-toxic, c) when specific host molecules are targeted then cross recognition of macaque homologues must have been demonstrated, d) the vaccine cannot be adequately tested in other than NHP animal models, for instance due to the mechanism of action or the type of immunological assessment needed, e) preferably immunogenicity of vaccine candidates should have been proven in other species, unless this is not possible because the specific vaccine modality used does not work in other species.<br><br>The main goal can be divided in 2 sub-goals:<br>1. Vaccine evaluation. Immunogenicity and efficacy to protect against infection will be evaluated using an appropriate influenza virus challenge strain in relation to the type of vaccine being used.<br>2. Set-up infection model for influenza viruses that have not yet been used in NHP at our institute and that are needed for vaccine evaluation (sub-goal 1) and refinement of influenza virus infection models. For refinement the optimal dose and aerosol delivery will be investigated in order to improve the assessment of vaccine efficacy.<br><br>De doelstelling is helder |

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| Wetenschappelijk en maatschappelijk belang | Citaat: The annual influenza virus epidermes cause considerable morbidity and mortality world-wide and especially affect more vulnerable groups like young children, the elderly and people with pulmonary disease. In addition, there is the continuous threat of the emergence of new viral recombinants that would lead to a pandemie. Previous pandemics, especially the 1918 pandemie, have caused millions of deaths. Finally, avian influenza viruses are widely spread and can occasionally infect humans. Mutations that lead to a strong increase in transmission have been described {34}, indicating that also these viruses pose a continuous threat to the human population. Current influenza vaccine strategies and vaccine production methods are not adequate to deal with such emergencies. Even for protection against the current seasonal influenza viruses, yearly vaccination of risk groups is necessary. Hence a vaccine that could offer protection against a broader range of viruses, including as yet unknown recombinants and avian influenza would be of great benefit to the community. In addition, the yearly vaccination would no longer be necessary since a broadly protective vaccine would be effective over a period of at least five years against newly emerging variants. This has led the EU to invest in the development of so called "universal" influenza vaccines that would fulfil these criteria. Both the application of new delivery methods, for instance in the term of DNA or viral vectors, as well as new vaccine modalities require evaluation in appropriate animal models so that the level as well as the mechanism of protection can be adequately established before these new vaccines can be tested in clinical studies. |
| Onderbouwing wetenschappelijk en maatschappelijk belang | Het belang van de doelstelling is helder. |
| **Wetenschappelijke kwaliteit**<br>Kwaliteit aanvrager/ onderzoeksgroep en onderzoek | Citaat uit DEC advies: Eerder zijn binnen het onderzoeksinstituut de immunogeniciteit, veiligheid en effectiviteit van vaccins tegen onder andere HIV, Hepatitis B, Hepatitis C, malaria en tuberculose in niet-humane primaten onderzocht. Sinds 2012 wordt er in de instelling onderzoek verricht aan influenza virus-infecties en vaccins tegen influenza. De gekozen strategie voor infectie en vaccinatie is mede gebaseerd op ervaringen uit deze eerdere studies. De expertise voor het verrichten van deze experimenten is beschikbaar binnen de instelling en bij de betrokken onderzoekers.<br><br>Het secretariaat heeft geen reden om te twijfelen aan de kwaliteit van het onderzoek of de aanvragers. |

| **3V's**<br>Vervanging | |
|---|---|
| | **3.4.4.1 Vaccine evaluation in macaques**: Citaat: 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 eradicatlon of the virus, the efficacy of an influenza vaccine to proteet against Infection can only be adequately establlshed In an animal model.<br>Several animal species have been used as a model for human influenza virus infection (1). However, mice are not naturally susceptlble to influenza and usually lethal infection models are used, that are based on extremely adapted human vlruses. Ferrets are naturally susceptlble to most human Influenza vlruses and recapltulate the natura! course of Infectlon. However, infection is often restrlcted to the upper airways and this model has the disadvantage that limited lmmunologlcal tools are available, especially to study innate and cellular adaptive Immune responses, whlch are especially important in the evaluation of "universa!" vaccine strategles. NHP have an Immune system that most closely resembles that of humans. Also the avallablllty of many cross reactive reagents makes lt posslble to study In detail the contrlbution of the innate Immune system and to analyse vaccine lnduced 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 lnnate Immune receptors have either limited cross reactivity to other animal species or trigger different cell types or different responses because of dlfferences in receptor expression pattern or cell signalling pathways. These aspects are essential for the evaluatlon of so called "universal" Influenza vaccines. For these 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.<br>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. |

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| | **3.4.4.2 Influenza virus infection in macaques**: citaat: 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.<br>Several animal species have been used to study influenza virus infection (...). However, of these different species NHP have the advantage that they physiologically and immunologlcally most closely resemble humans. This has important implications, both for vaccine evaluation (explained In bijlage 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 bijlage 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. |
| Verminderen | |
| | **3.4.4.1 Vaccine evaluation in macaques**: citaat: 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 trachea 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 unvaccinated animals (bijlage 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 I 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 be needed. |
| | **3.4.4.2 Influenza virus infection in macaques**: citaat: 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 sufflcient 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. Because of the limited experience with aerosol delivery in NHP and the results obtained so far (5) six animals per group are expected to be sufficient for the first experiment. On the basis of the outcome of the first study the number of animals needed in follow up experiment can be calculated and less animals may be needed. Only the minimum number of animals needed, will be used. |

| Verfijnen | |
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| | **3.4.4.1 Vaccine evaluation in macaques**: citaten: The use of temperature recordlng devices makes it posslble to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection. With this method we have observed a significant reduction in fever by some of the vaccine candidates. Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the temperature responders will require a small surgery, which will be done under anaesthesia. Subsequently, animals will recelve analgesics as long as required. Animals are trained to cooperate as much as posslble for the invasive handlings, such as receiving the sedation.<br><br>Animals will be socially housed with a socially compatible animal, whenever possible. There is an extensive program for enrichment in our institute that consists of playing material and methods to present food (...).<br>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 wil 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. (4). On the basis of the scoring system a humane endpoint is defined. 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.<br>All handlings will be performed under sedatlon. 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 tube feeding. 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 otherwlse be very limited. |

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| | **3.4.4.2 Influenza virus infection in macaques**: citaten: The use of temperature recording devices makes it possible to record the temperature every 15 minutes. We have designed a method that allows very precise calculation of fever induction caused by the infection (1). With this method we have observed a significant reduction in fever by some of the vaccine candidates (12). Such precise measurements are not possible with the traditional rectal temperature measurement. Placement and removal of the recording devices will require a small surgery, which will be done under anaesthesia. Subsequently animals will receive analgesics as long as required. Animals are<br>trained to cooperate as much as possible for the invasive handlings, such as receivlng the sedation.<br><br>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.<br>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. (13). On the basis of the scoring system a humane endpoint is defined. 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 days of the infection the animal wil receive tube feeding. 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. |
| **3.4.4.1 Vaccine evaluation in macaques**: voldoende onderbouwd | |
| **3.4.4.2 Influenza virus infection in macaques**: voldoende onderbouwd | |
| **Wettelijk vereist onderzoek**<br>Indien ja, is er sprake van herhaling? | Er is geen sprake van wettelijk vereist onderzoek. |
| **Hergebruik** | Er is sprake van hergebruik van dieren. |

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| 3.4.4.1 Vaccine evaluation in macaques: Mogelijk hergebruik. Dieren die eerder zijn gebruikt in influenza vaccin of influenza infectie studies of die pre-existing antilichamen tegen influenza hebben, zijn niet bruikbaar. Hergebruik zal plaatsvinden binnen de limitaties beschreven in artikel 1e van de Wod.<br><br>Citaat: In most studies the animals will be re-used after the virus is cleared (...). However, in cases where possible adverse effects of the vaccine have to be studied or when influenza viruses are used that are known to cause persistent lung pathology, animals are humanely euthanized and a full necropsy is performed. |
| 3.4.4.2 Influenza virus infection in macaques: zie bijlage 3.4.4.1 |

| **Naam proef** | **Worden de dieren gedood?** | **Doden volgens richtlijn?** |
|---|---|---|
| 3.4.4.1 Vaccine evaluation in macaques | Ja | volgens de richtlijn. |
| 3.4.4.2 Influenza virus infection in macaques | Ja | volgens de richtlijn. |

| Naam proef | | |
|---|---|---|
| **3.4.4.1 Vaccine evaluation in macaques** | HEP: afhankelijk van het virus, maximaal 25% | citaat: 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 that indicates that the maximum duration of severity is reached then the animal will be humanely euthanlzed. Individual scores are added and the decision is based on the total daily score. Symptoms that lead to an immediate endpoint are: open mouth breathing or cyanosis, lethargy as defined by minimal response to human approach. |
| Rhesusapen (Macaca mulatta) | Ongerief:<br><br>100% Matig | ongerief met name door operatieve implantatie en verwijdering van het recording device en de ontwikkeling van ziekte door de infectie. |
| Java-apen (Macaca fascicularis) | Ongerief:<br><br>100% Matig | |
| **3.4.4.2 Influenza virus infection in macaques** | HEP: zie bijlage 3.4.4.1 | zie bijlage 3.4.4.1 |
| Rhesusapen (Macaca mulatta) | Ongerief:<br><br>100% Matig | ongerief met name door operatieve implantatie en verwijdering van de recording device en ontwikkeling van ziekte door de infectie. |
| Java-apen (Macaca fascicularis) | Ongerief:<br><br>100% Matig | |

Opmerkingen over de dierproeven

| Naam proef | Opmerkingen |
|---|---|
| 3.4.4.1 Vaccine evaluation in macaques | Verdeling tussen aantallen Macaca mulatta/Macaca fascicularis afhankelijk van virusstam. Totale aantal 150 dieren. |
| 3.4.4.2 Influenza virus infection in macaques | Verdeling tussen aantallen Macaca mulatta/Macaca fascicularis afhankelijk van virusstam. Totale aantal 56 dieren. |

**5 Samenvatting**

De aanvraag bevat voldoende informatie over de doelstelling, het belang, de 3V's en de strategie om tot een oordeel te komen. Op dit moment is het niet aannemelijk dat er dierproefvrije modellen of andere diermodellen beschikbaar zijn die als volledig alternatief voor het gebruik van NHP in aanmerking komen.

Het onderzoek wordt uitgevoerd op makaken. De keuze voor het gebruik van NHP is voldoende onderbouwd:

Veelgebruikte modellen in influenza-onderzoek zijn muizen, fretten en NHP. Voor gebruik van muizen zijn vaak geadapteerde humane virussen nodig aangezien muizen van nature niet vatbaar zijn voor influenzastammen die mensen infecteren.
Fretten kunnen wel geinfecteerd worden door de meeste humane influenzavirussen en het infectieverloop is er gelijkend op dat bij de mens. Echter, infectie vindt meestal alleen in de bovenste luchtwegen plaats, en minder immunologische tools zijn beschikbaar voor de fret.
NHP zijn uniek in hun nauwe homologie met de mens in de meeste componenten van hun immuunsysteem. De NHP is het nauwst verwant met de humane fysiologie, maar lijken ook op mensen in hun klinische symptomen, pathologie, patroon van virusreplicatie, koorts en cytokine/chemokine responsen.

De vaccins die in dit model getest zullen worden zijn al in de laatste fase van de pre-clinische ontwikkeling en zijn nodig voor de laatste validatiestap om te verzekeren dat er geen nadelige effecten van de vaccins zijn, die gemist zijn in andere studies met andere diersoorten, en om te verzekeren dat ze ook effectief zijn in een diersoort met een immuunsysteem dat erg lijkt op dat van de mens. Er is voldoende beschreven dat veel informatie al bekend is van de vaccins alvorens ze in dit NHP model getest zullen worden. Er is nog wel een vraag gesteld aan de aanvrager over vaccins waarvan immunogeniciteit in andere diersoorten niet bepaald kan worden.

Beide geslachten kunnen gebruikt worden, maar de aanvrager geeft aan per experiment alleen mannen of alleen vrouwen te willen gebruiken vanwege immunologische verschillen tussen mannen en vrouwen. Er wordt niet verwacht dat bij apen een fokoverschot van een bepaald geslacht is. Wel is de aanvrager gevraagd te onderbouwen waarom slechts 1 geslacht wordt gebruikt, aangezien vaccins bij de mens in zowel mannen als vrouwen toegepast zullen worden.

De dieren worden mogelijk hergebruikt voor andere studies en zijn mogelijk ook al voor andere studies gebruikt. Dit is voldoende onderbouwd.

Vanwege het gebruik van NHP is een beoordeling achteraf vereist.

Het DEC advies is niet volledig (oude format gebruikt). De DEC is gevraagd een advies in het correcte format aan te leveren. Het besluit van de CCD kan pas definitief worden gemaakt nadat het volledige DEC advies is aangeleverd en voldoende is bevonden.

**6 Voorstel besluit incl. voorstel geldigheidsduur van de vergunning**
Het Secretariaat volgt het DEC-advies, maar stelt voor aanvullende algemene voorwaarde(n) te stellen.

Het Secretariaat adviseert dit project toe te wijzen.
Voorstel is om de vergunning te verlenen van 1 juni 2017 tot en met 31 mei 2022.
Hierbij stelt het Secretariaat voor de volgende voorwaarden te stellen:

**Voorwaarden**

*Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen*
Dit project wordt voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk mei 2023 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

Indien blijkt dat de aerosol methode voor virale infectie tot verfijning leidt, wordt deze methode waar mogelijk toegepast. Dit moet worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

De ingangsdatum van de vergunning kan niet voor de verzenddatum van de beschikking zijn en zal indien van toepassing aangepast worden. Dit is ook het geval bij een voorgenomen besluit.

## 7 Concept beschikking voor akkoord CCD

15

Centrale Commissie Dierproeven

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Biomedical Primate Research Centre
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Postbus 3306
2288 GJ RIJSWIJK
10.2.g

**Centrale Commissie Dierproeven**
Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

**Onze referentie**
Aanvraagnummer
AVD502002016704
**Bijlagen**
1

Datum 19 december 2016
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte 10.2.e

Op 18 november 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques" met aanvraagnummer AVD502002016704. Wij hebben uw aanvraag beoordeeld.

Op 9 december 2016 heeft u uw aanvraag aangevuld. Op ons verzoek heeft u de te gebruiken diersoort verder gespecificeerd, de keuze voor het gebruik van 1 geslacht verder onderbouwd en de criteria voor testen van vaccins in de NHP uitgebreid. Deze informatie was voldoende.

**Beslissing**
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, en artikel 10 lid 1 sub a, zijn er algemene voorwaarden gesteld.

U kunt met uw project "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques" starten. De vergunning wordt afgegeven van 1 juni 2017 tot en met 31 mei 2022. Deze termijn is anders dan in uw aanvraag, omdat een vergunning een maximale looptijd van vijf jaar kan hebben.

Overige wettelijke bepalingen blijven van kracht.

**Beoordeling achteraf**
Na afloop van het project zal er een beoordeling plaatsvinden, zoals bedoeld in artikel 10a1, lid 1d en lid 3, in de wet. Meer informatie over de eisen bij een beoordeling achteraf vindt u in de bijlage.
Een beoordeling achteraf dient plaats te vinden vanwege het gebruik van niet-humane primaten.

**Procedure**
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie DEC-BPRC gevoegd. Dit advies is opgesteld op 17 november 2016. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet. Wij hebben de DEC om aanvullende informatie gevraagd. Op 16 december 2016 heeft de DEC gereageerd op onze vragen. Bij indiening was het DEC advies onvolledig (het oude format was gebruikt, waardoor niet een volledig advies was ingediend). Op ons verzoek heeft de DEC een volledig advies ingediend.
Wij kunnen ons vinden in de inhoud van het advies van de Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

**Bezwaar**
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

**Meer informatie**
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

**Datum:**
19 december 2016
**Aanvraagnummer:**
AVD502002016704

Centrale Commissie Dierproeven

10.2.e

Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

# Projectvergunning

**gelet op artikel 10a van de Wet op de Dierproeven**

Verleent de Centrale Commissie Dierproeven aan

| | |
|---|---|
| Naam: | Biomedical Primate Research Centre |
| Adres: | Postbus 3306 |
| Postcode en plaats: | 2288 GJ RIJSWIJK |
| Deelnemersnummer: | 50200 |

deze projectvergunning voor het tijdvak 1 juni 2017 tot en met 31 mei 2022, voor het project "Evaluation of novel influenza vaccine candidates for immunogenicity and capacity to protect against influenza virus infection in macaques" met aanvraagnummer AVD502002016704, volgens advies van Dierexperimentencommissie 10.2.g Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is Section head Cell Mediated Immunity.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 18 november 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 9 december 2016;
b Niet-technische Samenvatting van het project, zoals ontvangen per brief op 18 november 2016;
c Advies van dierexperimentencommissie d.d. 17 november 2016, ontvangen op 18 november 2016.
d De aanvullingen op uw aanvraag, ontvangen op 9 december 2016

**Aanvraagnummer:**
AVD502002016704

| Naam proef | Diersoort/ Stam | Aantal dieren | Ernst | Opmerkingen |
|---|---|---|---|---|
| **3.4.4.1 Vaccine evaluation in macaques** | | | | Verdeling tussen aantallen Macaca mulatta/Macaca fascicularis afhankelijk van virusstam. Totale aantal 150 dieren. |
| | Rhesusapen (Macaca mulatta) / | 75 | 100% Matig | |
| | Java-apen (Macaca fascicularis) / | 75 | 100% Matig | |
| **3.4.4.2 Influenza virus infection in macaques** | | | | Verdeling tussen aantallen Macaca mulatta/Macaca fascicularis afhankelijk van virusstam. Totale aantal 56 dieren. |
| | Rhesusapen (Macaca mulatta) / | 28 | 100% Matig | |
| | Java-apen (Macaca fascicularis) / | 28 | 100% Matig | |

**Voorwaarden**

*Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen*
Dit project wordt voorzien van beoordeling achteraf.Deze beoordeling zal uiterlijk mei 2023 plaatsvinden. Er zal dan beoordeeld worden of de doelstellingen van het project werden bereikt. Daarnaast wordt bekeken of de schade die de dieren hebben ondervonden, het aantal en soorten proefdieren en de ernst de dierproeven conform de vergunning waren.

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

Indien blijkt dat de aerosol methode voor virale infectie tot verfijning leidt, wordt deze methode waar mogelijk toegepast. Dit moet worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

**Aanvraagnummer:**
AVD502002016704

# Weergave wet- en regelgeving

**Dit project en wijzigingen**
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

**Verzorging**
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

**Pijnbestrijding en verdoving**
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

**Einde van een dierproef**
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.

**Beoordeling achteraf**
Volgens artikel 10a1, lid 1d en lid 3 van de wet worden projecten waarbij niet-menselijke primaten worden gebruikt, projecten die als ernstig ingedeelde dierproeven omvatten of een dierproef die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, achteraf beoordeeld worden.