	Inventaris Wob-verzoek W20-03								
		wordt verstrekt				weigeringsgronden			
nr.	document NTS20209944	reeds openbaar	niet	geheel	deels	10.1.c	10.2.e	10.2.g	11.1
1	Begeleidende e-mail bij spoedaanvraag, d.d. 14 mei 2020				x		x	x	
2	Aanvraagformulier, d.d. 14 mei 2020				x		x	x	
3	Ontvangstbevestiging, d.d. 18 mei 2020				x		x	x	
4	Begeleidende brief bij aanvraag, d.d. 14 mei 2020				x	x	x	x	
5	NTS (versie 1)			x					
6	Projectvoorstel (versie 1)				x	x		x	
7	Bijlage dierproeven 1 (versie 1)				x	x		x	
8	Bijlage dierproeven 2 (versie 1)				x	x		x	
9	Bijlage dierproeven 3 (versie 1)				x	x		x	
10	Bijlage dierproeven 4 (versie 1)				x	x		x	
11	Bijlage dierproeven 5 (versie 1				x	x		x	
12	Bijlage dierproeven 6 (versie 1)				x	x		x	
13	Wetenschappelijk artikel Fuk Woo Chan behorend bij onderbouwing aanvraag: https://academic.oup.com/cid/article/71/9/2428/5811871?login=true	x							
14	Wetenschappelijk artikel Richard behorend bij onderbouwing aanvraag: https://www.nature.com/articles/s41467-020-17367-2	x							
15	Wetenschappelijk artikel Shi et al. behorend bij onderbouwing aanvraag: https://science.sciencemag.org/content/368/6494/1016	x							
16	Wetenschappelijk artikel Zhang behorend bij onderbouwing aanvraag: https://www.biorxiv.org/content/10.1101/2020.04.01.021196v1	x							
17	E-mail van CCD met ontvangstbevestiging en informatie over behandeling aanvraag, d.d. 15 mei 2020				x		x	x	

18	E-mail van CCD aan DEC met aankondiging verzoek om advies, d.d. 15 mei 2020				x		x	x	
19	E-mail van DEC aan CCD met reactie op aankondiging verzoek om advies, d.d. 15 mei 2020				x		x		
20	E-mail van vergunninghouder aan CCD met reactie op ontvangstbevestiging, d.d. 18 mei 2020				x		x	x	
21	Interne e-mail vraag status aanvraag projectvergunning, d.d. 18 mei 2020				x		x	x	
22	Interne e-mail antwoord status aanvraag projectvergunning, d.d. 18 mei 2020				x		x	x	
23	E-mail van CCD aan vergunninghouder met verzoek om factuurinformatie, d.d. 18 mei 2020				x		x	x	
24	E-mail van vergunninghouder aan CCD over factuurinformatie, d.d. 18 mei 2020				x		x	x	
25	Factuurinformatie				x		x	x	
26	Interne e-mail betaalgegevens, d.d. 18 mei 2020				x		x	x	
27	Verzoek om advies van CCD aan DEC, d.d. 18 mei 2020				x			x	
28	E-mail van CCD aan vergunninghouder met kennisgeving van verzenden adviesvraag aan DEC, 18 mei 2020				x			x	
29	Ontvangstbevestiging van verzoek om advies, d.d. 19 mei 2020				x		x	x	
30	E-mail van CCD aan DEC met vraag naar stand van zaken, d.d. 2 juni 2020				x		x	x	
31	E-mail van DEC aan CCD over stand van zaken, d.d. 2 juni 2020				x		x	x	
32	E-mail van DEC aan CCD over stand va nzaken, d.d. 3 juni 2020				x		x	x	
33	Begeleidende e-mail van DEC bij DEC-advies, d.d. 4 juni 2020				x		x	x	

34	Tweede begeleidende e-mail van DEC bij DEC-advies, d.d. 4 juni 2020				x			x	
35	DEC-advies, d.d. 4 juni 2020				x		x	x	
36	E-mail van DEC-secretaris aan DEC-voorzitter en CCD met verzoek aan voorzitter om aangepaste stukken aan CCD te sturen, d.d. 4 juni 2020				x		x	x	
37	E-mail van CCD aan DEC met verzoek om aangepaste stukken, d.d. 5 juni 2020				x		x	x	
38	E-mail van DEC aan CCD over aangepaste stukken, d.d. 5 juni 2020				x		x	x	
39	Begeleidende e-mail van DEC bij aangepaste stukken, d.d. 5 juni 2020				x		x	x	
40	Aangepaste stukken bij DEC-advies, d.d. 5 juni 2020: bevat versie 2 van de NTS, het projectvoorstel en de zes bijlagen dierproeven				x	x		x	
41	E-mail van DEC aan CCD met mededeling dat aangepaste stukken zijn verstuurd, d.d. 5 juni 2020				x		x	x	
42	Verzoek om aanvullende informatie van CCD aan vergunninghouder, d.d. 10 juni 2020				x		x	x	
43	Kennisgeving van verzoek om aanvullingen aan DEC, d.d. 10 juni 2020				x		x	x	
44	E-mail van CCD aan vergunninghouder met herinnering aan verzoek om aanvullende informatie, d.d. 19 juni 2020				x		x	x	
45	E-mail van vergunninghouder aan CCD met reactie op aanvullende vragen, d.d. 19 juni 2020				x		x	x	
46	E-mail van CCD aan vergunninghouder over verzoek om aanvullende informatie, d.d. 19 juni 2020				x		x	x	

47	Begeleidende e-mail van vergunninghouder aan CCD bij antwoorden op verzoek om aanvullende informatie, d.d. 21 juni 2020				x		x	x	
48	Reactie op verzoek om aanvullende informatie, d.d. 21 juni 2020				x	x	x	x	
49	Intern: adviesnota CCD, d.d. 23 juni 2020				x	x	x	x	x
50	NTS (versie 3)				x			x	
51	Bijlage dierproeven 1 (versie 4)				x	x		x	
52	Bijlage dierproeven 2 (versie 3)				x	x		x	
53	Bijlage dierproeven 3 (versie 3)				x	x		x	
54	Bijlage dierproeven 5 (versie 3)				x	x		x	
55	Tweede verzoek om aanvullende informatie, d.d. 29 juni 2020				x		x	x	
56	Begeleidende e-mail van vergunninghouder aan CCD bij reactie op tweede verzoek om aanvullende informatie, d.d. 2 juli 2020				x		x	x	
57	Reactie op tweede verzoek om aanvullingen, d.d. 2 juli 2020				x	x	x	x	
58	NTS (versie 4)				x			x	
59	Bijllage dierproeven 5 (versie 4)				x	x		x	
60	Interne e-mail controle beschikkingsbrief, d.d. 13 juli 2020				x		x		
61	Interne e-mail controle beschikkingsbrief 2, d.d. 13 juli 2020				x		x	x	x
62	Interne e-mail controle beschikkingsbrief 3, d.d. 13 juli 2020				x		x		
63	Interne e-mail controle beschikkingsbrief 4, d.d. 14 juli 2020				x		x		x
64	Interne e-mail controle beschikkingsbrief 5, d.d. 14 juli 2020				x		x		
65	Interne e-mail gespreksnotitie, d.d. 15 juli 2020				x		x	x	x
66	Interne e-mail gespreksnotitie antwoord, d.d. 15 juli 2020				x		x		

67	Intern: juridische opmerkingen concept beschikking, d.d. 15 juli 2020				x		x	x	x
68	Interne e-mail controle beschikkingsbrief 6, d.d. 16 juli 2020				x		x	x	
69	Interne e-mail: beschikking klaar voor ondertekening, d.d. 16 juli 2020				x		x	x	
70	Interne e-mail: beschikking ondertekend, d.d. 16 juli 2020				x		x	x	
71	Begeleidende e-mail bij beschikking, d.d. 16 juli 2020				x		x	x	
72	Beschikking, d.d. 16 juli 2020				x		x	x	
73	NTS (versie 5)	x							
74	E-mail van CCD aan DEC met terugkoppeling over DEC-advies, d.d. 21 juli 2020				x		x	x	
75	E-mail van vergunninghouder met reactie op begeleidende e-mail bij beschikking, met aangepast NTS, d.d. 29 juli 2020								
76	NTS (versie 6)				x			x	
77	E-mail van CCD aan vergunninghouder met reactie op aangepaste NTS, d.d. 6 augustus 2020				x		x	x	

Van: 10.2.e
Aan: Info-zbo
Onderwerp: Aanvraag projectvergunning Covid-19_AVD10.2.g - spoed [Vertrouwelijk-Confidential] 10.2.e
Datum: donderdag 14 mei 2020 17:17:52
Bijlagen: image001.png
Prioriteit: Hoog

Informatie opgevraagd over Factuurgegevens. 10.2.e 18-5-2020

Vertrouwelijk-Confidential

Geachte CCD,

Hierbij een spoedaanvraag voor een CCD projectvergunning voor ontwikkeling voor COVID-19 vaccin voor katten en fretten.

In de recente weken is duidelijk geworden dat dieren, met name katten maar ook hamsters en marterachtigen zoals fretten en nertsen geïnfecteerd kunnen worden door het SARS-CoV-2. Mogelijk kunnen ook konijnen het virus doorgeven. 10.2.g om de gezondheid van dieren èn mensen te kunnen waarborgen een vaccin ontwikkelen om dieren effectief te kunnen beschermen tegen COVID-19, de ziekte die door SARS-CoV-2 veroorzaakt wordt. Daarnaast is nadrukkelijk het streven om door vaccinatie een rol van dieren als reservoir voor SARS-CoV-2 infectie bij mensen uit te sluiten of te minimaliseren.

Graag verzoeken wij de CCD dit projectvoorstel te beoordelen. Wij vertrouwen erop dat u gezien de urgentie van vaccinontwikkeling deze aanvraag hoge prioriteit geeft.

Vriendelijke groeten,

10.2.e en 10.2.g

Centrale Commissie Dierproeven 2

19 MEI 2020

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.

☒ Ja > *Vul uw deelnemernummer in* 10.2.g

☐ Nee > *U kunt geen aanvraag doen*

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

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.

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

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

Naam instelling of organisatie
Naam van de portefeuillehouder of diens gemachtigde
KvK-nummer
Straat en huisnummer
Postbus
Postcode en plaats
IBAN
Tenaamstelling van het rekeningnummer
(Titel) Naam en voorletters
Functie
Afdeling
Telefoonnummer
E-mailadres
(Titel) Naam en voorletters
Functie
Afdeling
Telefoonnummer
E-mailadres

10.2.e en 10.2.g

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
		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
		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 - juni - 2020
		Einddatum	31 - mei - 2025
3.2	Wat is de titel van het project?	Development of a SARS-CoV-2 vaccine for cats and ferrets	
3.3	Wat is de titel van de niet-technische samenvatting?	Ontwikkeling van een SARS-CoV-2 vaccin voor katten en fretten	
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	

4 Betaalgegevens

4.1	Om welk type aanvraag gaat het?	☒ Nieuwe aanvraag Projectvergunning € 2.402
		☐ Wijziging Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
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
- ☒ Na ontvangst van de factuur (zie bijlage Factuurinformatie)

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- ☒ Projectvoorstel
- ☒ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- ☐ Melding Machtiging
- ☐ DEC-Advies

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	
Plaats	
Datum	14 mei 2020
Handtekening	10.2.e en 10.2.g

3

Centrale Commissie Dierproeven

> Retouradres Postbus 93118 2509 AC Den Haag

10.2.e en 10.2.g

Centrale Commissie Dierproeven
Postbus 93118
2509 AC Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD10.2.g 0209944
Bijlagen
2

Datum 18 mei 2020
Betreft Ontvangstbevestiging aanvraag projectvergunning Dierproeven

Geachte 10.2.e

Wij hebben uw aanvraag voor een projectvergunning dierproeven ontvangen op 14 mei 2020. Het gaat om uw project "Development of a SARS-CoV-2 vaccine for cats and ferrets". Het aanvraagnummer dat wij aan deze aanvraag hebben toegekend is AVD10.2.g 20209944. 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, stuur een e-mail naar info@zbo-ccd.nl of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Datum:
18 mei 2020
Aanvraagnummer:
AVD10 2 g 20209944

Met vriendelijke groet,

Centrale Commissie Dierproeven

Deze brief is automatisch aangemaakt en daarom niet ondertekend.

Bijlagen:
- Gegevens aanvraagformulier
- Factuur

Centrale Commissie Dierproeven

Gegevens aanvrager

Uw gegevens

Deelnemersnummer NVWA: 10.2.g

Naam instelling of organisatie: 10.2.g

Naam portefeuillehouder of diens gemachtigde: 10.2.e

Straat en huisnummer:

Postbus: 10.2.g

Postcode en plaats:

Gegevens verantwoordelijke onderzoeker

Naam:

Functie:

Afdeling: 10.2.e

Telefoonnummer:

E-mailadres:

Gegevens plaatsvervangende verantwoordelijke onderzoeker

Naam:

Functie:

Afdeling: 10.2.e

Telefoonnummer:

E-mailadres:

Over uw aanvraag

Wat voor aanvraag doet u?

[x] Nieuwe aanvraag

[ ] Wijziging op een (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn

[ ] Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Over uw project

Geplande startdatum:	1 juni 2020
Geplande einddatum:	31 mei 2025
Titel project:	Development of a SARS-CoV-2 vaccine for cats and ferrets
Titel niet-technische samenvatting:	Ontwikkeling van een SARS-CoV-2 vaccin voor katten en fretten
Naam DEC:	10.2.g
Postadres DEC:	
E-mailadres DEC:	

Betaalgegevens

De leges bedragen:	€ 2.402,-
De leges voldoet u:	na ontvangst van de factuur

Checklist bijlagen

Verplichte bijlagen:

[x] Projectvoorstel
[x] Beschrijving Dierproeven
[x] Niet-technische samenvatting

Ondertekening

Naam:	10.2.e en 10.2.g
Functie:	
Plaats:	

Centrale Commissie Dierproeven

> Retouradres Postbus 93118 2509 AC Den Haag

10.2.e en 10.2.g

Centrale Commissie Dierproeven
Postbus 93118
2509 AC Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD10.2.g 20209944
Bijlagen
2

Datum 18 mei 2020
Betreft Factuur aanvraag projectvergunning Dierproeven

Factuur
Factuurdatum: 18 mei 2020
Vervaldatum: 17 juni 2020
Factuurnummer: 209944

Omschrijving	**Bedrag**
Betaling leges projectvergunning dierproeven Betreft aanvraag AVD10.2.g 20209944	€ 2.402,00

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.

4 10.2.g

10.2.g 14 mei 2020

Aan: CCD
10.2.g

Van: 10.2.e

Betreft: Aanvraag nieuwe CCD vergunning

Geachte leden van de CCD/DEC

Hierbij 10.2.g aanvraag voor een nieuwe CCD Project vergunning getiteld "Development of a SARS-CoV-2 vaccine for cats and ferrets".
In de recente weken is duidelijk geworden dat dieren, met name katten maar ook hamsters en marterachtigen zoals fretten en nertsen geïnfecteerd kunnen worden door het SARS-CoV-2. Mogelijk kunnen ook konijnen het virus doorgeven. 10.2.g om de gezondheid van dieren èn mensen te kunnen waarborgen een vaccin ontwikkelen om dieren effectief te kunnen beschermen tegen COVID-19, de ziekte die door SARS-CoV-2 veroorzaakt wordt. Daarnaast is nadrukkelijk het streven om door vaccinatie een rol van dieren als reservoir voor SARS-CoV-2 infectie bij mensen uit te sluiten of te minimaliseren.

In de project omschrijving is aangegeven dat de postulaten van Koch voor SARS-CoV-2 vervuld zijn. 10.1.c in Appendix 1 hebben we de keuze tussen cavia's, hamsters en konijnen nog open gelaten.

De ontwikkeling van een klinisch model voor dieren is opgenomen in het voorstel onder Appendix 2. 10.1.c model zal voorafgaand aan klinische studies getoetst moeten worden, vandaar dat de ontwikkeling en verbetering van een model opgenomen is in de aanvraag.

De aanvraag omvat de wettelijk vereiste dierstudies die nodig zijn om een stand-alone vaccine 10.1.c om zo snel mogelijk op de markt te kunnen brengen. Wanneer het traject richting een full license gaat zullen te zijner tijd mogelijk nog extra studies (en extra dieren) nodig zijn, waarvoor aanvragen op dat moment zullen volgen. De Europese Farmacopee bevat op dit moment nog geen richtlijnen voor de ontwikkeling van een SARS-CoV-2 vaccin, vandaar dat zulke studies op dit moment nog niet gedefinieerd zijn.

10.2.g

10.2.g

De infectieusiteit van SARS-CoV-2 bij dieren, de (sub)klinische gevolgen van infectie en de rol van dieren in de verspreiding van het virus zijn op dit moment nog niet volledig opgehelderd. Bij de ontwikkeling van een vaccin zullen de voortschrijdende inzichten hierover nadrukkelijk meegewogen worden bij beslissingen om het traject van ontwikkeling wel of niet voort te zetten. Zulke beslissingsmomenten zijn in de projectaanvraag verwerkt.

Graag verzoeken wij de DEC en de CCD dit projectvoorstel te beoordelen. Wij vertrouwen erop dat u gezien de urgentie van vaccinontwikkeling deze aanvraag hoge prioriteit geeft.

Met vriendelijke groet,

10.2.e

Centrale Commissie Dierproeven **5**

Format
Niet-technische samenvatting

- Dit format gebruikt u om uw niet-technische samenvatting te schrijven
- Meer informatie over de niet-technische samenvatting vindt u op de website www.centralecommissiedierproeven.nl.
- Of neem telefonisch contact op. (0900-2800028).

1 Algemene gegevens

1.1	Titel van het project	Ontwikkeling van een SARS-CoV-2 vaccin voor katten en fretten
1.2	Looptijd van het project	5 jaar
1.3	Trefwoorden (maximaal 5)	Vaccin, coronavirus, kat, fret, immuniteit

2 Categorie van het project

2.1 In welke categorie valt het project.

U kunt meerdere mogelijkheden kiezen.

- ☐ Fundamenteel onderzoek
- ☒ Translationeel of toegepast onderzoek
- ☒ Wettelijk vereist onderzoek of routinematige productie
- ☐ Onderzoek ter bescherming van het milieu in het belang van de gezondheid of het welzijn van mens of dier
- ☐ Onderzoek gericht op het behoud van de diersoort
- ☐ Hoger onderwijs of opleiding
- ☐ Forensisch onderzoek
- ☐ Instandhouding van kolonies van genetisch gemodificeerde dieren, niet gebruikt in andere dierproeven

3 Projectbeschrijving

3.1	Beschrijf de doelstellingen van het project (bv de wetenschappelijke vraagstelling of het wetenschappelijk en/of maatschappelijke belang)	Dit project betreft de ontwikkeling van een nieuw vaccin tegen SARS-CoV2 voor katten en fretten. Deze dieren kunnen net als mensen het SARS-CoV2 bij zich dragen en verspreiden. In het project worden studies beschreven die noodzakelijk zijn om aan eisen voor Europese en internationale productregistratie te voldoen. Hierbij worden dierexperimenten uitgevoerd om de juiste vaccin kandidaat te selecteren en te testen op bescherming en veiligheid. Bij de ontwikkeling worden experimenten uitgevoerd in diersoorten die in contact kunnen komen met katten en fretten om veiligheid te garanderen.

3.2	Welke opbrengsten worden van dit project verwacht en hoe dragen deze bij aan het wetenschappelijke en/of maatschappelijke belang?	Vaccinatie is de meest effectieve methode voor preventie en eliminatie van infectieziekten in dieren en mensen, zeker als het ziekteverwekker betreft die zowel mensen als dieren ziek maakt. Een vaccin voor (huis)dieren draagt bij aan verdere vermindering van de virusdruk bij mensen, omdat overbrenging door deze dieren verminderd of uitgesloten kan worden.
3.3	Welke diersoorten en geschatte aantallen zullen worden gebruikt?	Onderzoeksfase: Hamsters, cavia's of konijnen: 180 Testen in doeldieren: Katten: 371 Fretten: 371 Hamsters: 171* *Hamsters zijn geen doeldieren maar het infectiemodel kan ook in hamsters getest worden om het aantal fretten en katten te beperken. Testen in andere dieren: Muizen: 65 Konijnen: 57 Kippen: 45 Hamster: 45 Honden: 45
3.4	Wat zijn bij dit project de verwachte negatieve gevolgen voor het welzijn van de proefdieren?	De dieren ondervinden licht ongerief van de entingen en bemonsteringen (b.v. bij bloedafname, neusswab). Bij herhaalde bemonstering voor enkele specifieke procedures wordt het ongerief als matig ingeschaald. Voor het testen van werkzaamheid van het (kandidaat) vaccin wordt gebruik gemaakt van verplichte infectiestudies waarin gevaccineerde dieren SARS-CoV2 krijgen toegediend. In deze studies moeten ook een minimaal aantal niet-gevaccineerde dieren deze ziekteverwekkers toegediend krijgen waardoor ze ziek worden. Hierbij zullen de ongevaccineerde dieren voor een korte periode licht, matig of in enkele gevallen ernstig ongerief kunnen ondervinden. Net als bij mensen geldt dat veruit de meeste dieren slechts milde of matige symptomen laten zien bij een SARS-CoV2 infectie.
3.5	Hoe worden de dierproeven in het project ingedeeld naar de verwachte ernst?	Onderzoeksfase: Cavia's of hamsters of konijnen Aantal: 180 Licht: 100% indien voor hamsters of konijnen gekozen wordt Matig: 100% indien voor Cavia's gekozen wordt Ernstig: 0% Testen in doeldieren:* Katten: Aantal: 321 Licht: ≥69% Matig: ≤27% Ernstig: ≤4% Fretten: Aantal: 321 Licht: ≥69% Matig: ≤27% Ernstig: ≤4%

Hamsters*
Aantal: 171
Licht: ≥35%
Matig: ≤57%
Ernstig: ≤8%
*Hamsters zijn geen doeldieren maar het infectiemodel kan ook in hamsters getest worden om het aantal fretten en katten te beperken.

Testen in contactdieren + werving biomaterialen:
Muis:
Aantal: 65
Licht: 100%

Konijn:
Aantal: 57
Licht: 100%

Kip:
Aantal: 45
Licht: 100%

Hamster
Aantal: 45
Licht: 100%

Hond
Aantal: 45
Licht: 100%

3.6 Wat is de bestemming van de dieren na afloop?

Waar mogelijk worden katten, honden, fretten en konijnen na beëindiging van een studie hergebruikt of ter adoptie aangeboden. Voor cavia's, hamsters, muizen en kippen is dit niet het geval, deze zullen aan het einde van een studie worden gedood. Ernstig zieke dieren of dieren waarbij het welzijn onverwacht is aangetast als ook dieren die om wettelijke dan wel wetenschappelijke redenen geëuthanaseerd dienen te worden, worden op een humane wijze geëuthanaseerd volgens geaccepteerde en wettelijk toegestane methoden.

4 Drie V's

4.1 **Vervanging**
Geef aan waarom het gebruik van dieren nodig is voor de beschreven doelstelling en waarom proefdiervrije alternatieven niet gebruikt kunnen worden.

De werkzaamheid hangt af van de afweerontwikkeling die in reactie op het vaccin door het immuunsysteem van het dier gemaakt wordt. Deze bepaalt het vermogen om later in de tijd een infectie met de ziekteverwekker, SARS-CoV2, te overwinnen. Dit is een dermate complex systeem dat er geen betrouwbare vervangende in vitro test voor is.

Als de productie van antilichamen voldoende correleert met bescherming en de regelgevende instanties deze in vitro test accepteren, hoeven er geen of minder infectiestudies te worden uitgevoerd.

4.2 **Vermindering**
Leg uit hoe kan worden verzekerd dat een zo

Voordat vaccins worden getest in dieren, worden ze eerst uitvoerig getest in vitro in het laboratorium en alleen de meest veelbelovende vaccinkandidaten zullen worden getest in dieren. Testen die dienen te worden uitgevoerd in dit project, alsmede het aantal daarvoor te gebruiken

gering mogelijk aantal dieren wordt gebruikt.	dieren, worden in overleg met (Europese) overheden bepaald Daarnaast worden dieren, indien mogelijk, opnieuw ingezet met in acht neming van dierenwelzijn.
4.3 **Verfijning** Verklaar de keuze voor de diersoort(en). Verklaar waarom de gekozen diermodel(len) de meest verfijnde zijn, gelet op de doelstellingen van het project.	Bij vaccinontwikkeling wordt veiligheid en werkzaamheid van een product aangetoond in het dier waar het vaccin voor bedoeld is. Daarnaast moet in het geval van genetisch gemodificeerde organismen-gebaseerde vaccins de veiligheid worden aangetoond in diersoorten waarmee het doeldier in aanraking kan komen (hond, muis, kip, konijn). Indien toepassen van veterinaire behandeling (bijvoorbeeld pijnstilling) niet interfereert met het experiment zal adequate veterinaire behandeling worden toegepast. Daarnaast worden er bij alle dierproeven vooraf vastgestelde humane eindpunten gehanteerd om het ongerief en lijden van dieren zo veel mogelijk te beperken.
Vermeld welke algemene maatregelen genomen worden om de negatieve (schadelijke) gevolgen voor het welzijn van de proefdieren zo beperkt mogelijk te houden.	De instelling beschikt over adequate gebouwen en voorzieningen om in de huisvestingsbehoefte van betreffende diersoorten te voorzien en om de procedures efficiënt uit te voeren met zo min mogelijk stress bij de dieren. Alle dieren worden in groepen gehuisvest en beschikken over afleidingmateriaal passend bij de diersoort zodat de dieren soort-specifiek gedrag kunnen uitvoeren. Alle biotechnische handelingen en de dagelijkse verzorging van de dieren worden gedaan door gediplomeerde en ervaren medewerkers. Voor de controle en monitoring van het dierenwelzijn beschikt de instelling over een Instantie voor Dierenwelzijn en gekwalificeerde dierenartsen waardoor passende veterinaire zorg altijd beschikbaar is.

5 In te vullen door de CCD

Publicatie datum	
Beoordeling achteraf	
Andere opmerkingen	

Centrale Commissie Dierproeven 6

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	Provide the title of the project.	Development of a SARS-CoV-2 vaccine for cats and ferrets

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 animal health or welfare
- ☐ 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.

Motivation, background and context

10.2.g

The SARS-CoV-2 pandemic, which causes the disease that WHO named COVID-19, has an unprecedented world-wide impact. Rapid spread of the virus has caused millions of infections in humans, of which a significant percentage required hospitalization because of severe bilateral pneumonia. Despite drastic control measures, our sophisticated medical institutions reached the limits of their abilities to hospitalize, treat and cure patients.

The virus presumably originated from bats and may have jumped to humans via intermediate animal hosts. Recent experimental studies have shown that SARS-CoV-2 replicates efficiently in cats and ferrets causing pathology in the upper respiratory tract and spreading to in-contact animals. SARS-CoV-2 is transmitted efficiently via direct contact and via the air via respiratory droplets and/or aerosols (Shi et al., 2020; Richard et al., 2020). Veterinarians have reported clinical cases that were confirmed positive for SARS-CoV-2 in which cats were diagnosed with diarrhea, vomiting, difficulty in breathing and other mild respiratory symptoms, such as sneezing and ocular discharge (https://promedmail.org/promed-post/?id=7151215; https://content.govdelivery.com/accounts/USDAAPHIS/bulletins/287d9a0). A serology study from domestic cats in the Wuhan area (China, original area where the virus emerged) demonstrated seroconversion in 14.7% of cats in shelters and pet hospitals (Zhang et al., 2020).
A couple of official reports document infected cats of COVID-19 patients that were infected at the same time as their human pet-owner, or cats from a household in a COVID-19 affected neighborhood that were allowed to go outdoors. Other species now diagnosed with SARS-CoV-2 are lions (United States, India) and minks (The Netherlands; https://www.rijksoverheid.nl/onderwerpen/coronavirus-covid-19/nieuws/2020/04/26/covid-19-geconstateerd-op-twee-nertsenbedrijven) and presumably rabbits.

Effective countermeasures against further expansion of the virus and assumed seasonal return of the virus as a part of the respiratory complex that causes flu-like symptoms in autumn and winter require rapid development of vaccination strategies for humans. Although their role in the epidemiology of this disease remains unclear at this stage, in which there is a general lack of scientific data on the biology of this novel virus, cats and ferrets may contribute to outbreaks and spread of the virus as hosts or as reservoir hosts, apart from the pathology that the viruses causes in these species themselves. Vaccination of these animals may therefore become critical to control virus spread in both animals and humans.

Of note, there is increasing evidence that the virus is infectious before onset of clinical symptoms, and the virus may infect human hosts without causing recognizable clinical symptoms in up to 80% of the infections. An infection of a pet may go unrecognized, and a subsequent infection of a human owner by his or her pet may also go unrecognized at least for a while, thereby causing a cascade of infections.

It is therefore essential that vaccine development for cats and ferrets is initiated.

Estimates put cat (Felis catus) pet ownership in the EU at approximately 74 million cats. Worldwide estimates are more than 250 million cats. Ferret (Mustela putorius furo) is a minor pet species in the US and Europe, with a US population estimated at 300,000-500,000. Although a minor pet species, ferrets are handled intensively, similar as cats, by their owners and therefore spread of the virus from animal to human and vice versa is a realistic scenario, especially now similar routes of spread have been confirmed. Vaccination is the single most important measure that can be taken to ensure these animals do not contribute to further spread of the SARS-CoV-2, the causative agent of COVID-19. 10.1.c

The two key requirements of any vaccine are (i) that it is safe, and (ii) that it is efficacious. Vaccination is a medical treatment that is administered to healthy individuals. Therefore, apart from perhaps some transient minor discomfort, it is important that no harm is done. With regard to efficacy, the use of a vaccine can only be justified when significant protection from disease and/or infection and/or shedding is shown.

10.1.c

When a conditional license is obtained, complementary experiments have to be performed to proceed towards full licensing.

10.2.g

SARS-CoV-2 Vaccine Research & development

The current project proposal covers both the research and development phase for a cat and ferret vaccine against SARS-CoV-2. Koch's postulates have been fulfilled for SARS-CoV-2 in cats and ferrets, which means that based on the pathogen information, vaccine candidates can be designed and tested for an initial selection based on serological response (although not necessarily in the target animal). Next step is to show safety and efficacy of the selected vaccine candidate in the target animals. Subsequent animal studies are performed to prepare the registration dossier that is submitted to the regulatory authorities. This dossier includes target animal for efficacy and safety studies as well as non-target animal for safety studies. The requirements for these studies are laid down in EU directives, the Pharmacopoeia Europaea (Ph.Eur) and guidelines and regulations of the European Medicines Agency and other international regulatory bodies. However, a specific monograph for SARS-CoV-2 is not available at this stage. In the absence of a monograph, requirement for a SARS-CoV-2 emergency vaccine will be discussed directly with authorities or based on comparable dossiers.

Once the registration dossier has been submitted for a conditional licence, additional studies are requested by the regulatory authorities during the licensing procedure for a full license. It is important to state here that there are not sufficient scientific data at this point in time to determine the possible contribution of animal species in general and cats and ferrets specifically to SARS-CoV-2 virus spread. Such data will be obtained while the project is ongoing and will lead to a go/no go decision for commercial vaccine development. 10.1.c

Regulatory Requirements

The requirements for the development of specific veterinary vaccines (i.e., per pathogen and animal species) are set out in a series of monographs published in the European Pharmacopoeia. There are general monographs outlining the studies required to demonstrate safety and efficacy of veterinary vaccines (5.2.6 and 5.2.7). The vaccine will likely be produced on cell lines, of which safety is addressed in monograph 5.2.4.

For the purpose of this application, relevant information on vaccine parameters, safety and minimum group sizes for studies is taken from historic projects, as no monograph for SARS-CoV-2 is available and feedback on the question to agencies on requested studies for a conditional license has not yet been received (pending).

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 the project is the development of an emergency vaccine and later a fully licensed vaccine that fulfils the important unmet need for SARS-CoV-2 disease control in both cats and ferrets, 10.1.c

We aim to generate the serological, efficacy and safety data to be included in marketing authorization applications (registration dossiers) for this vaccine. This includes proof of concept (i.e. induction of protection combined with an acceptable safety profile), and the efficacy and safety studies to be undertaken as described in this proposal to fulfil the regulatory requirements.

3.3 Relevance

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

Importantly, the new vaccine will reduce animal suffering from SARS-CoV-2 infection (respiratory symptoms, pathology in the upper respiratory tract). Consequently, vaccination will benefit animal welfare and healthcare for cats and ferrets in general.

10.2.g

The new vaccine should also reduce or ablate shedding of SARS-CoV-2 by cats and ferrets, so that these animals (and related mustelids like minks) do not contribute to further spread of the virus in the human population, with devastating consequences. Furthermore, these species then cannot longer act as a reservoir for SARS-CoV-2. The vaccine thus contributes to control of a zoonotic virus infection.
Vaccines are the most effective method for control, prevention or eradication of infectious diseases. The SARS-CoV-2 pandemic shows that in the absence of vaccination, control of respiratory viruses is complicated if not impossible in our current society. The prospects are that yet to be developed human and animal vaccines enable disease control so that other control measures such as social distancing, home isolation and the use of surgical face masks are no longer necessary, and society can return to normal functioning.

3.4 Research strategy

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

The project includes research activities (testing candidate vaccines for serological response in non-target animals, validation of an infection/ vaccination-challenge model for efficacy studies in target animals) and development studies such as efficacy studies, safety studies in the target animals, and safety studies in non-target animals.
For licensure of the candidate vaccine for emergency vaccination, specific regulatory studies will be required and discussed with authorities, as a monograph is lacking.

General description of the studies to be performed:
Serological studies: Candidate vaccines will be tested for serological response, and based on the outcome of the study, the best candidate will be used for vaccine development.
Challenge model optimization: Existing experimental infection models for SARS-CoV-1 and SARS-CoV2 will be tested and optimized to allow efficacy studies on the novel vaccine.
Efficacy studies: Determination of vaccine efficacy requires that cats/ferrets are 'challenged', that is to say that they are exposed to the virulent organism against which the vaccine is targeted. When performing efficacy studies, it is attempted to find an immunological correlate of protection, so that in further studies efficacy can be evaluated on the basis of the serological response after vaccination instead of challenge.
Safety studies: Safety of vaccine candidates has also to be evaluated in cats/ferrets to show that systemic and local (injection site) reactions after vaccination, if any, are acceptable.

It is to be decided based on feedback of regulatory agencies which specific studies have to be performed to obtain a conditional and later a full licence. This will determine in detail which types of studies have to be performed to comply with regulatory requirements. Also, at that time the minimum vaccination age, the vaccination schedule and the intended label claims are set, which will further determine the types of studies that have to be performed.

Registration dossiers need to contain a comprehensive set of efficacy and safety studies in the target animals to allow regulatory bodies to make a sound risk-benefit analysis that is the basis for their decision on the marketing approval of a new product. Therefore, all studies have to be performed with the formulation to be marketed and all production and quality controls methods should be finalized before start of the studies. In addition, results of laboratory safety and efficacy studies will have to be provided to authorities in order to be able to obtain a permit for the field studies (which are outside the scope of this project) that are to be included in the dossier. The ecotoxicological effects that the vaccine candidate may have on the environment need to be assessed. In terms of in vivo work, this includes the potential for recombination with other vaccines/vectors and the potential to persist in the environment. During the evaluation of the registration dossier, additional animal studies may be requested by the regulatory authorities. For safety studies, also non-target animals are used, 10.1.c [redacted] for which genomic stability, dissemination and spread need to be assessed. Additionally, safety in non-target animals will have to be assessed for all newly developed vaccines in case of accidental improper administration of vaccines to other species.

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.

10.2.g [redacted]

The generation of a(n) (emergency) vaccine against SARS-CoV-2 for cats and ferrets will consist of the following types of animal experiments (described in detail in Appendices 1 through 6).

1. Selection candidate vaccines (Appendix 1):
Initial studies are required to determine which antigen of the virus and which confirmation of such antigen potentially evokes an efficacious immune response. Also, the best performing viral vector vaccine platform will be assessed. This can be tested by in vivo serology in non-target animals.

2. Optimization of challenge model (Appendix 2):
Infection/disease (challenge) models for SARS-CoV-2 will be developed based on the scientific literature 10.1.c . In such models, it will be attempted to reproduce the clinical signs that are associated with SARS-CoV-2 in cats and ferrets. A challenge model will allow assessment of the efficacy of vaccine candidates under controlled laboratory circumstances.

Studies to refine a challenge model will have the following set-up:
- Infection/Challenge = Administration of a SARS-CoV-2 (dose/route to be determined)
- Observation of clinical signs post infection/sampling (e.g. for shedding of the pathogen)
- Necropsy to investigate (histo)pathological changes

The degree of discomfort generated for the animals in a SARS-CoV-2 infection model is supposed to mimic the natural disease as much as possible and includes mild respiratory symptoms, and possibly vomiting and diarrhoea in a percentage of cases. Initial estimates of severe disease in humans are around 7%. There are not sufficient data on disease in cats and ferrets yet, but from the limited studies a picture emerges that the development of severe respiratory disease is not observed in cats and ferrets.

3. Efficacy studies using target animals (Appendix 3):
Once a vaccine candidate has been selected and a challenge model has been established, the efficacy of the candidate vaccine against SARS-CoV-2 can be evaluated. The efficacy of vaccines (of the final product/formulation) should be shown under controlled laboratory conditions as described in Ph.Eur 5.2.7 (Evaluation of efficacy of veterinary vaccines and immunosera) and national guidelines and regulations outside the EU. For all efficacy claims (e.g. protection against infection/disease, reduction of clinical signs, reduction of shedding, onset and duration of immunity) to be made for this new vaccine, proof should be provided by showing a meaningful and statistically significant difference between vaccinated animals and unvaccinated controls in a challenge model (vaccination-challenge studies). Also, claims regarding onset and duration of immunity have to be substantiated by vaccination-challenge studies. By studying the immune response after vaccination, it will be attempted to find a correlation between the height of the immune response (e.g. as measured in in vitro virus-neutralization tests) and protection in the cat or ferret. However, this correlation cannot always be established, despite good protection against the disease in question.

Studies will be performed according to the following basic set-up (challenge will not be performed in case an immunological marker for protection can be monitored):
- Administration of a candidate vaccine
- Inoculation with a pathogen (challenge)
- Observation of clinical signs post-challenge
- Sampling (e.g. for shedding of the pathogen in blood or excreta)
- Necropsy to investigate (histo)pathological changes

4. Safety studies using target and non-target animals (Appendices 4 and 5):
To be able to make a proper risk-benefit analysis for a new product, all vaccines have to be tested in safety studies in the target animal (Ph.Eur 5.2.6 (Evaluation of safety of veterinary vaccines and immunosera), and national guidelines and regulations outside the EU. This generally means observation for abnormal systemic (clinical signs, body temperature etc.) and local (injection site) reactions after administration.
Inactivated and subunit vaccines usually contain an adjuvant that enhances the immune response to the antigen(s) in the vaccine. 10.1.c nfortunately, although the adjuvant preparations themselves can be considered safe, the combination of antigen and adjuvant sometimes results in unwanted systemic and/or local reactions after vaccination. Therefore, for each new adjuvated vaccine the effect on the animals' general health, determined by observing clinical signs (e.g. general demeanour, body temperature, appetite etc.) and injection site reactions has to be determined.

10.2.g

Live vaccines have to be shown not to induce disease. Therefore, live vaccines, particularly genetically modified vaccine candidates, will have to be evaluated for persistence and dissemination in vaccinated animals and their ability to spread to unvaccinated sentinel animals. Live vaccines will also be tested for reversion to virulence, i.e. re-isolation of the vaccine from vaccinated animals followed by administration back into other animals up to five times (five subsequent passages in naive animals) in total. For live vaccines that are derived from a pathogen with a broad host range and/or zoonotic potential, it is necessary to also investigate the safety for non-target species that may come into contact with the vaccine according to Ph.Eur 5.2.6 (Evaluation of safety of veterinary vaccines and immunosera), national guidelines and regulations outside the EU and GMO guidelines.
For vaccines that are intended to be given to pregnant animals, also the effect on reproductive performance must be investigated. Also, possible influence of maternally derived antibodies (MDAs) has to be studied if relevant.

Studies will be performed according to the following basic set-up, but will only be performed if required by authorities:

- Administering the candidate vaccine
- Observation of systemic and local reactions post vaccination
- Monitoring of persistence and excretion of the vaccine by sampling of blood, excretions and mucosal swabs (live vaccines only)
- Necropsy to investigate (histo)pathological changes at the injection site and re-isolation of the vaccine
- Vaccine overdose and repeated dose

5. Preparation of biomaterials for assay development (Appendix 6):
To set-up assays that can help to detect and/or quantify antigens/pathogens (e.g. ELISAs, , immunohistochemistry, virus neutralization tests) or to reduce and/or replace challenge studies (serology as possible correlate of protection) it may be necessary to use laboratory animals for the preparation of antisera or monoclonal and/or polyclonal antibodies if the required reagents are not available.
For each new vaccine, batch tests for the quantification and identification of the active ingredients are required to verify the consistency of the manufacturing process and the quality and stability of the final product. Preferably, in vitro tests are used for batch testing, but in case an in vitro test is not possible (e.g. correlation between vaccine dose and protection/response is (still) unknown), a serological assay in laboratory animals will have to be set up. 10.1.c

Immunization experiments of laboratory animals will generally be as follows:

- Administration of antigen/pathogen
- Collection of blood

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.

10.1.c . Only vaccine candidates for which the safety and efficacy data obtained indicate a high probability of success are approved for further development.

With regard to SARS-CoV-2 vaccine development, the first milestones are (1) selection of the most efficacious antigen and (2) establishment of a challenge model. Based on the outcome of these studies, and epidemiological information on SARS-CoV-2 infection in animals and their owners, feasibility of a rapid production platform for the antigen will be evaluated for the conditional license (go/no go).

A challenge model for ferrets is described in literature and should be evaluated for dose-response and clinical signs with the specific SARS-CoV-2 isolate available. 10.1.c

At the start of the registration studies, the project team agrees on the types of studies required and the sequence of these studies, with input of regulatory affairs and the European Medicines Agency.

Typically, the vaccine dose, route and schedule are first determined in efficacy study(ies) in naïve animals, free of antibodies against the respective antigen. In these basic efficacy studies, the challenge

infection is administered 78 hours to 4 weeks after the vaccination. In a later stage of the project, the efficacy is tested for a longer interval between vaccination and challenge to demonstrate the duration of immunity (DOI). For the conditional license, studies in animals with maternally derived antibodies are not planned with priority but may be performed later according to regulatory guidelines to obtain a full license.

The safety of the vaccine is investigated in parallel, but in separate studies, as the dose levels to be tested are different from the levels in the efficacy studies. For live vaccines, the following safety characteristics must be investigated: i) dissemination of the strain in the vaccinated animal, ii) shedding of the strain, iii) the potential to spread to in-contact animals and iv) the potential to revert to virulence by animal-to-animal passage.

Once these criteria are met, the safety of the final product composition (including any excipients such as freeze-dry stabilizers and adjuvant) has to be demonstrated in animals of the most susceptible categories: animals of the youngest age group intended for vaccination. If a vaccine will be licenced for use during pregnancy and / or lactation, the safety has also to be demonstrated in these categories.

It is important to realize that some of these experiments may not be required for a conditional license, as 10.1.c The experiments are however required in a later stage for full licensing.

10.1.c In the case of cats, non-target species are often dogs, poultry, rodents and rabbits. Other non-target species may be requested by the authorities during the licensing procedure, including other non-target species specifically for ferrets such as minks.

All of the above-mentioned studies are mandatory for the regulatory approval of companion animal vaccines.

10.1.c Proof of concept for the selected antigen is provided in the first phase of the project and leads to selection of antigen and vaccine platform. In the next phase, efficacy and safety of the candidate vaccine are investigated thoroughly and further decision points are more refined during the development phase (e.g. 6 months or 1 year DOI, clinical protection and/ or accompanied with shedding claims)

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	Selection of candidate vaccines
2	Optimization of the challenge model
3	Efficacy studies in cats and ferrets
4	Safety studies in cats and ferrets
5	Safety studies in non-target animals
6	Preparation of biomaterials for assay development

10.2.g

Centrale Commissie Dierproeven

7

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 1 — Type of animal procedure: Selection of candidate vaccines

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.

10.1.c . In this way, a pre-selection of candidate vaccines can be made 10.1.c For this purpose, **serology** models are set up that help to evaluate candidate vaccines. The study will normally include one or more of the following procedures:

- Application of the antigen, and booster(s)
- Monitoring of changes in general health (no clinical signs expected because all vaccine platforms should be safe. Can be made part of the non-target animal safety studies required).
- Confirmation of absence of vaccine shedding or dissemination (swabbing of mucosal surfaces (including nose swabs), testing of faecal/urinal samples, in case non-replicating viruses or non-pathogenic viruses are used)
- Blood sampling for serology
- Post-mortem examination (in case non-replicating viruses or non-pathogenic viruses are used as platform technology)

A necropsy may be performed to determine the presence of gross or histologic lesions and/or take samples to detect the presence of the vaccine. The severity of discomfort will be limited because no clinical disease is expected, discomfort is caused by repeated blood sampling and swabbing.

10.2.g

will be set up 10.1.c

As it is important to select the antigen with the optimal serological response (indications are that serology correlates with protection to SARS-CoV-2), and at the same time to work with the lowest number of animals possible in these experiments, we base our serological test on present knowledge 10.1.c The present tests are to be performed in guinea pigs or Syrian hamsters or rabbits (one model to be chosen).

Once an antigen has been selected based on serology results obtained, a **vaccine dose – response** study will be initiated in the target animals (cats, ferrets). Such vaccine dose-response study can be combined with a challenge experiment as described in Appendix 3.

10.1.c Parameters to be measured are serological response, but this type of experiments can simultaneously be used to determine safety in non-target animals of the SARS-CoV-2 vaccine candidate.

Animals will be euthanized at the end of the experiment for serum collection and if deemed necessary post mortem examination. This serum can be used in diagnostic assays and potentially limits the use of animals as described in Appendix 6.

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

Two or more of the following procedures will be employed for study of **serological response** to SARS-CoV-2 antigens in guinea pigs or Syrian hamsters or rabbits.

1. Application of the antigen, booster(s) (intramuscular, subcutaneous, oral, nasal, intravenous, ocular, intra-peritoneal *(1 – 5 x)*
2. Daily observation *(min 1x – daily)*
3. Weighing *(1 – 5 x)*
4. Blood sampling (including anaesthesia) to determine immunological parameters and/or to determine the presence of the vaccine antigen in the blood *(1 – 6 x)*
5. Swabbing of (mucosal) surfaces (oral/nasal/ocular/rectal/vaginal) to determine excretion of the vaccine strain *(1 – 6 x)*
6. Euthanasia

The duration of all procedures described above will only be minutes and will be applied in accordance with "handboek proefdierkunde", van Zutphen et al.,2016.
The length of the observation period after application will be 9 weeks.

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 used in this type of study will be the smallest group size possible (6) given the fact that statistically relevant differences between the serological titers have to be determined. At present, no data are available of SARS-CoV-2 antibody titers and variation thereof in animal species, and for that reason no power calculations can be made for group sizes. 10.1.c In the serology experiment, knowledge of the variance will be obtained and used for sample size calculation for future experiments, including the vaccine dose-response study, possibly leading to adjustment in group size.

B. The animals

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

Serology/antigen selection:
Guinea pigs or Syrian hamsters or Rabbits: only one of these species to be chosen for studies

10.2.g

Purchase of animals:
SPF guinea pigs, Syrian hamsters and rabbits () will be purchased from licensed commercial vendors. Experiments to be conducted with females but we will look at the possibility to include males in the experiments as well (based on age at start and duration of the experiment).

Age of animals:
Guinea pigs: >4 weeks / 250-350 g body weight
Syrian hamster: > 4 weeks
Rabbit: > 6 weeks

Numbers:
Guinea pigs or Syrian hamsters or Rabbits:

- 6 animals per antigen candidate tested
- 2 variants 10.1.c used
- 10 antigens to be tested (10 different antigens and 2 different platforms → max 120 animals)
- A negative control group has to be included per 5 antigen/platform combinations tested (→ max 24 animals)
- Repetition of an experiment in case of unexpected events during the test that invalidate results: 5 groups plus negative control (→ max 36 animals)

Total max 180.

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.

Guinea pigs and rabbits originating from a previous test for a different antigen is possible.
Age may be a factor in qualifying animals for re-use

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:
Guinea pigs/Syrian hamsters/rabbits are used for serology studies because there are no suitable in vitro alternatives or models for testing antigenicity. The vaccine is developed for cats and ferrets but use of a less sentient species is suited for the purpose of serology/candidate selection.

Reduction:
The numbers of animals used have been reduced to six per group based on the outcome of similar experiments, without endangering the scientific integrity of the work. This will be evaluated in each study. The number of animals per study will be substantiated in each study protocol. 10.1.c

Refinement:
Regulations and guidelines determine to a large extent what kind of data must be generated and, to a large extent, this determines what form of models and methods should be employed.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test.
However, the immune responses measured in non-target less sentient vaccine recipients will be of value

10.2.g

in the follow up efficacy studies for which antigens need to be selected. In general, animals will be closely monitored.

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

Guinea pigs, Syrian hamsters and rabbits are always housed socially. Furthermore, to enhance animal welfare three major criteria are taken into account: 1) social interaction, 2) access to food and 3) the possibility of moving freely. Programs are in place for the housing and caring of animals exposed experimentally to pathogens, with emphasis on management and safety practices for containment (according to the regulations of Biosafety level 1 – 3).

An active approach to providing environmental enrichment will be taken. This will enable animals to express their species-specific behavioural repertoire. Appropriate and multiple objects that allow comfortable resting and play materials will be provided, and new ideas for improving the general wellbeing of the animals encouraged and implemented when and where appropriate.

The procedures being carried out are routine vaccinations, minimally invasive swabbing and the taking of small blood samples. In order to prevent undue stress during blood sampling in guinea pigs, sedation will be used. Sedation will be carried out using licensed agents and dosing regimens developed under guidance of a veterinarian.

For monitoring of the clinical health status, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed by an experienced veterinarian.

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.

For novel pathogens like SARS-CoV-2, serology and vaccine dose-response studies are essential for vaccine development. 10.1.c

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 animal 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.

10.2.g

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.

Blood sampling and mucosal swabbing are part of normal veterinary care and will induce only mild discomfort. 10.1.c [redacted] Blood sampling in guinea pigs is carried out under anaesthesia.

Vaccination can result in a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity and appetite, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks. Vaccination with a live attenuated vaccine could induce mild disease symptoms specific for the pathogen used as vaccine, but this is very unlikely.

For monitoring of the clinical health status of animals, all study animals will be checked by a certified person once a day or multiple times a day in case clinical symptoms/disease becomes apparent or is expected. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed by a veterinarian.

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

In consultation with the veterinarian and study director it will be decided whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (If treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in an SOP referred to in each study protocol.
Pain relief may be used, if it does not interfere with the test results. A decision to apply adequate veterinary care to alleviate pain and/or distress will be decided in consultation with the veterinarian and study director. Possible options for analgesia: local analgesia, NSAIDs and opioids.
10.1.c [redacted] The number of samplings will be done in accordance with the applicable guidelines or if no requirements are given, the number of samplings is reduced to a minimum number required for a valid evaluation of results.

I. Other aspects compromising the welfare of the animals

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

None

Explain why these effects may emerge.

Not applicable

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

J. Humane endpoints

10.2.g [redacted]

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.

Indicate the likely incidence.

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 following overview shows the maximal discomfort caused by the serology and vaccine dose-response studies.

For procedures described in section A the cumulative discomfort score for the sampling may be moderate due to repeated sampling.

Pathogen	**Animal category**	**Discomfort of disease (% of animals with highest score)**	**Duration of discomfort**
Candidate vaccines	Guinea pigs (> 4 weeks) **or**	Moderate (100%)	Max 1 day
Candidate vaccines	Rabbits (>7 weeks) **or**	Mild (100%)	Max 1 day
Candidate vaccines	Syrian hamsters (>4 weeks)	Mild (100%)	Max 1 day

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.

Guinea pigs / Syrian hamsters / rabbits will be humanely euthanized at the end of the study. Blood will be collected (serum to be used in in vitro experiments) and post mortem examination will be carried out to determine the safety profile of the vaccine used (if deemed necessary).

10.1.c

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

10.2.g

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 2 — Type of animal procedure: Optimization of the challenge model

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

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

To determine the efficacy of a vaccine, vaccinated animals are challenged with the pathogenic organism or disease agent (in this case SARS-CoV-2) to demonstrate that vaccinated animals do not show any clinical signs of disease or at most, mild and transient clinical signs after challenge, and reduced shedding of the virus. For this purpose, challenge models are set up that try to mimic the natural disease as much as possible.
In these procedures, it is useful to include non-infected / non-vaccinated animals (sentinel animals) for confirmation that a pathogenic organism effectively spreads between individuals.
In general, application of the pathogen or disease agent will be done via the natural route of infection, but if the natural route does not induce all presentations of a disease under laboratory conditions, it might be necessary to use another route (e.g. parenteral injection to induce a systemic infection).
Parameters to be measured depend on the pathogen or disease agent used, in this case SARS-CoV-2, but will normally include one or more of the following procedures:

- Inoculation of challenge virus
- Monitoring of clinical signs (e.g. changes in general health)
- Measuring of body temperature (rectal temperature or via transponder)
- Determination of pathogen shedding or dissemination (swabbing of mucosal surfaces (nasal swabs/washes), testing of faecal/urinal samples)
- Serological response (amnestic response), viraemia, or haematological changes (blood sampling)
- X-ray analysis (pneumonia), or ultrasound if possible
- Post-mortem examination (pathology in the respiratory tract and other organs)

Necropsy will be performed to determine the presence of gross or histologic lesions and/or take samples to detect the presence of the challenge pathogen or disease agent in internal organs and tissues. The severity of discomfort is depending on the nature of the pathogen or disease agent. However, the duration of severe suffering will be limited due to the application of a humane endpoint. For SARS-CoV-2, no challenge model is described in a specific Ph.Eur monograph. 10.1.c [redacted] to ensure a suitable challenge model taking efficacy and discomfort into account.

Challenge models will be optimized to enable vaccine development for SARS-CoV-2. The setup of a challenge model for such a novel pathogen requires that the basic characteristics of the pathogen have to be identified, and that experimental confirmation of Koch's postulates has been obtained, which is the case for SARS-CoV-2 (see for example Fuk-Woo Chan et al., 2020). As it is important to have the smallest possible variation in the level of disease/clinical signs between animals to be able to work with the lowest number of animals possible in challenge experiments, improvement/refinement of the challenge model (e.g. change in the route of inoculation, apply a dose-response with regard to challenge dose) will need to be undertaken. Also, when a new challenge inoculum is prepared, suitability for use in challenge studies will have to be evaluated in the model.

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

Two or more of the following procedures will be employed for study of the SARS-CoV-2 in infected/challenged Syrian hamsters, cats and ferrets (in italics the frequency of the procedures):

1. Daily observation / scoring clinical signs *(1x – daily)*
2. Measurement of body temperature (via intra-peritoneal transponder placed prior to the study under sedation, rectal measurement as backup) *(1x- daily)*
3. Weighing *(1 – 5 x)*
4. Blood sampling to determine immunological parameters and/ / or to determine the presence of the challenge strain in the blood *(1 – 10 x)*
5. Challenge administration (intravenous / subcutaneous / intramuscular / intradermal / ocular / intranasal (dropwise or nebulisation) / oral / intratracheally / rectal) *(1 – 2 x)*
6. Swabbing of (mucosal) surfaces (oral/nasal/ocular/rectal/vaginal), nasal washes to determine excretion of the vaccine (up to 14 days) and / or excretion of the challenge strain (up to 28 days) *(1x-daily)*
7. X-ray (1 – 3x) or ultrasound if possible
8. Sedation (transponder placement, challenge administration, blood sampling, X-ray *(1- 10x)*
9. Euthanasia and necropsy

The duration of all procedures described above will only be minutes. The length of the observation period after challenge will be 1 day to 4 weeks. To rule out that clinical signs are caused by (co-) infection with another pathogen or disease agent, non-infected control animals (sentinels) will be included in a study.

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 used in this type of study will be relatively small (6-8) as they are only intended to get an indication of the ability of a (potential) pathogen or disease agent or live vaccine candidate to induce disease and the variation within an infected group. Knowledge of the variance in an unvaccinated and vaccinated group is needed for sample size calculation for future vaccination-challenge studies.

B. The animals

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

Cats (target species, both sexes) and ferrets (target species, males) will be used for this type of animal experiment. Furthermore, Syrian hamsters (females, males if possible) can be used as a less sentient species for model optimization or confirmation of challenge batch infectivity in animals.

Purchase of animals:
Syrian hamsters, cats and ferrets (SPF) will be purchased from licensed commercial vendors.

10.2.g

Age of animals:
The age of Syrian hamsters is 4 weeks to adult. The age of cats and ferrets varies from 8 weeks old to adult. The age of the cats and ferrets to be used is representative of the age for which the actual vaccine will be developed. Since vaccines are intended of usage in every life stage of the animal the effect of the pathogen or disease agent and/or vaccine on pregnancy needs to be studied, however not for conditional licensing. Therefore, it will be necessary to purchase pregnant animals in a later stage of the project.
Numbers:
10.1.c [redacted] These can be taken as a starting point to develop challenge models for SARS-CoV-2. This will include an infectious dose - response study.

Syrian hamsters:
Maximum of 6 directly challenged and 2 sentinel animals per group (plus 1 untreated per group)
- Route of infection and dose finding: 2 possible routes, 3 challenge doses (→ max 48 hamsters)
- Validation with optimal dose and route (8 cats per experiment) if new batch of challenge virus is prepared (3 batches, → max 24 hamsters)
- Repetition of an experiment in case of unexpected events during the test that invalidate results (→ max 32 hamsters)
- Untreated hamsters kept for comparison (→ max 13 hamsters)

Total max 117.

Cats:
Maximum of 6 directly challenged and 2 sentinel animals per group (plus 1 untreated per group)
- Route of infection: 2 possible routes, 3 challenge doses (→ max 48 cats)
- Repetition of an experiment in case of unexpected events during the test that invalidate results (→ max 24 cats)
- Untreated cats kept for comparison (→ max 9 cats)

Total max 81.

Ferrets:
Maximum of 6 directly challenged and 2 sentinel animals per group (plus 1 untreated per group)
- Route of infection: 2 possible routes, 3 challenge doses (→ max 48 ferrets)
- Repetition of an experiment in case of unexpected events during the test that invalidate results (→ max 24 ferrets)
- Untreated ferrets kept for comparison (→ max 9 ferrets)

Total max 81.

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.

For research on novel pathogens such as SARS-CoV-2, animals from previous studies can be re-used if sero-negative (highly likely)

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.

10.2.g

Replacement:
Cats and ferrets must be used for these studies because ultimately these are the species for which the vaccines are tended to induce protection. Therefore, infection models have to be developed in the target animals. Syrian hamsters are qualified as less sentient model species.

Reduction:
The numbers of animals used will be reduced wherever possible without endangering the scientific integrity of the work. This will be achieved through an on-going evaluation of the observations in each study. The number of animals per study will be substantiated in each study protocol. 10.1.c

Refinement:
Cats and ferrets are the animal species that the vaccine is intended to be ultimately applied to, Syrian hamsters can be regarded sentient model species for the cat or ferret disease. Regulations and guidelines determine to a large extent what sort of data must be generated and, to a large extent, this determines what form of models and methods can be employed.
The classic method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, the immune responses measured in the vaccine recipients will be assessed in each orientating efficacy study. If immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge tests in further studies. When a challenge model has to be used, humane end-points will be employed and staff will be fully trained to recognize animals that experience discomfort. Animals will be closely monitored, and extra checks will be made to ensure that no animal is left suffering. The intratracheal route is specifically relevant in the study of respiratory viruses. It will be assessed if nebulization can be used as an alternative for the intratracheal inoculation. After animals have arrived from the suppliers, handling of the animals for sampling procedures specific for the study to be performed is trained by qualified personnel.

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

In general Syrian hamsters, cats and ferrets are always housed socially. Furthermore, to enhance animal welfare three major criteria are taken into account: 1) social interaction, 2) access to food and 3) the possibility of moving freely. Programs are in place for the housing and caring of animals exposed experimentally to pathogens, with emphasis on management and safety practices for containment (according to the regulations of Biosafety level 1 – 3).

An active approach to providing environmental enrichment will be taken. This will enable animals to express their species-specific behavioural repertoire. Appropriate and multiple objects that allow comfortable resting and play materials will be provided, and new ideas for improving the general wellbeing of the animals encouraged and implemented when and where appropriate.

The procedures being carried out are routine vaccinations, minimally invasive swabbing and the taking of small blood samples. In order to prevent undue stress during blood sampling in ferrets and cats, sedation will be used. Sedation will be carried out using licensed agents and dosing regimens developed under guidance of a veterinarian.

For monitoring of the clinical health status of Syrian hamsters, cats and ferrets, all study animals will be checked at least once a day by a certified person. The frequency of observation will be increased after challenge in anticipation of possible clinical symptoms of disease. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed by a veterinarian.

Infection studies using a challenge model will require that all animals are exposed to SARS-CoV-2. This likely results in clear clinical symptoms in unvaccinated control animals. When it is clear that the clinical picture of the disease agent has been established, the scientific endpoint is reached and subsequently these animals will be euthanized. In some cases, the scientific and the humane endpoint are reached simultaneously at which point these animals will be euthanized. Consequently, the level of severity for

10.2.g

the challenge studies will be mild to severe. When it is clear that continuation would not provide any additional scientific data, every effort will be made to euthanise the animal before the humane endpoint is reached. The challenge with virus takes place in DM-III facilities to prevent spread of the virus into the environment.

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.

For novel pathogens like SARS-CoV-2, infection (challenge) model setup is essential for vaccine development. The model will be set up according to information already obtained in experimental infection models for SARS-CoV-2 in Syrian hamsters, cats and ferrets. Until the moment a serological correlate of protection is identified, challenge models have to be used to test vaccine efficacy. Challenge models will be improved and refined on the basis of the current scientific literature 10.1.c

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 animal 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.

Blood sampling, administration of a transponder, nasal washes and mucosal swabbing are part of normal veterinary care and will induce only mild discomfort. 10.1.c

The administration of the infection/challenge inoculum will only induce short term mild to moderate discomfort but depending on the nature of the subsequent infection the discomfort of the challenge can range from moderate to severe.

10.2.g

For monitoring of the clinical health status of animals, all study animals will be checked by a certified person once a day or multiple times a day in case clinical symptoms/disease becomes apparent or is expected. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed by an experienced veterinarian.

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

In consultation with the veterinarian and study director it will be decided whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (If treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. General humane endpoints are described in a SOP and test specific humane endpoints are given in each study protocol. In case of blood sampling (ferret, cat) or challenge via the intratracheal route (all species), animals will first be sedated to minimize discomfort.
X-ray analysis will be conducted after sedation as indicated above.
These procedures are part of the study design to monitor the course of the infection model and the health and welfare of the animals or to facilitate smooth administration of challenge material (intratracheal).
The number of samplings will be done in accordance with the applicable guidelines or if no requirements are given, the number of samplings is reduced to a minimum number required to for a valid evaluation of results.

I. Other aspects compromising the welfare of the animals

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

Explain why these effects may emerge.

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

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.

To determine the efficacy of a vaccine it is necessary to challenge animals with SARS-CoV-2. Symptoms to be expected are mild respiratory symptoms such as difficulty breathing, sneezing, ocular discharge, but possibly also vomiting and diarrhea. Such symptoms typically last for 4-5 days. Fever seems mild if detected at all. The duration of discomfort will be limited due to the application of a test- and pathogen specific humane endpoint described in the corresponding study protocol. These contain information about the clinical signs that can be expected and describe when a humane endpoint has been reached for (a combination of) the respective clinical signs. It also describes the scientific endpoint; The humane endpoints are always leading.
In addition, general humane endpoints (e.g. the condition of the animal prevents it from eating and drinking regularly, severe loss of body weight, pain) are applicable to all animals, irrespectively of the type of experiment.

The description and improvement of specific humane endpoints is an ongoing process 10.1.c An active approach will be taken to improve and adjust humane endpoints based on experience during the studies.

Indicate the likely incidence.

Considering the expected number of studies with SARS-CoV-2, the expected number of animals included in the different treatment groups (i.e. experimentally infected, sentinel vs control group) and the

10.2.g

expected severity, a maximum of 10% of the animals is expected to have severe discomfort that would require euthanasia. In the published ferret model (Richard et al., 2020), all inoculated and the majority of sentinel ferrets acquired the infection and showed no or mild symptoms as described above. The cat model of Shi et al., 2020 showed similar results for cats. The Syrian hamster model of Fuk-Woo Chan et al. (2020) showed moderate symptoms in inoculated animals, including respiratory symptoms and weight loss, from which the animals recovered.

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').

Similar to natural field infections they may cause mild to sometimes severe pain, distress or suffering. There are not sufficient studies published at the moment to get a full picture of clinical symptoms, which may for example depend on route of challenge and challenge dose. We estimate that a large subset of animals, primarily sentinels, undergo mild infections with hardly any clinical symptoms, and that the other subset of the animals experiences moderate discomfort due to clinical symptoms including an upper respiratory tract infection. Only very few animals will show severe symptoms. In Syrian hamsters, it is likely that more inoculated animals show moderate symptoms

The following overview shows the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category over the next 5 years:
For procedures described in section A the cumulative discomfort score for the sampling may be moderate due to repeated sampling.

Pathogen	**Animal category**	**Discomfort of disease (% of animals with highest score)**	**Duration of discomfort**
SARS-CoV-2	Syrian hamster >4 weeks old	Mild (39/117= 33%) Moderate (65/117= 56%) Severe (13/117= 11%)	Max 1 week Max 1 week Max 1 day
SARS-CoV-2	Cats >8 weeks old	Mild (52/117= 44.5%) Moderate (52/117= 44.5%) Severe (13/117= 11%)	Max 1 week Max 1 week Max 1 day
SARS-CoV-2	Ferrets >8 weeks old	Mild (52/117= 44.5%) Moderate (52/117= 44.5%) Severe (13/117= 11%)	Max 1 week Max 1 week Max 1 day

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.

Animals will be humanely euthanized at the end of a study. In addition, necropsy may be required at the end of the study for scientific reasons. .

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 3 — Type of animal procedure: Efficacy studies in cats and ferrets

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.

The aim of the work to be carried out under this Appendix is to test efficacy of a cat/ferret vaccine that should prevent clinical symptoms of the COVID-19 disease caused by the SARS-CoV-2 in these species, and a significant reduction in virus shedding. This Appendix builds on the challenge model established in Appendix 2.
The efficacy of the vaccine is tested based on clinical parameters or relevant legislation, such as general Ph.Eur guidelines (pathogen-specific monograph not available for this emerging pathogen). Clinical parameters as read-out may be replaced by a serological marker if a correlation is established.

Cats/ferrets that have received the recommended vaccination schedule and unvaccinated control animals will be challenged after a specific interval. A label claim can only be obtained for those aspects of a disease that are measured in the respective challenge model and for which a statistically significant difference between vaccinates and controls are shown. In general, efficacy of a vaccine has to be demonstrated for each of the vaccination routes and schedules and for each category of target animal (e.g. age, biological status) to substantiate the claims made for the product in the registration dossier. When testing compatibility of vaccines (by either mixing of vaccines or concurrent separate administration), EU guidelines dictate that in principle for all efficacy claims made for the products protection should be proven by challenge for all components, unless an immunological correlate for protection has been established.

The types of studies in the target animal that in general need to be undertaken are the following:

1. A vaccine dose-response study followed by SARS-CoV-2 challenge (vaccination-challenge) to test efficacy of the vaccine. In general, application of the antigen will be done via the intramuscular route or subcutaneous route, but it might necessary to use another route (e.g. nasal, mucosal). Parameters to be

measured are serological response, determine necessity of booster vaccinations, but this type of experiments can simultaneously be used to confirm safety of the SARS-CoV-2 vaccine candidate chosen. The challenge model described in Appendix 2 will be applied to test efficacy.
2. Determination of the minimum interval between completion of basic vaccination and challenge after which protection can be observed (Onset of immunity study, critical study according to Ph.Eur. 0062, 5.2.7)
3. Determination of the maximum interval between completion of basic vaccination and challenge after which protection can be observed (Duration of immunity study)

4. Determination of the effect of maternally-derived antibody status on the level of protection (MDA study; this study may include vaccination of the pregnant female; for full license, animals to be requested later)

5. Determination of the effect of a single (yearly, two-yearly or three-yearly) re-vaccination, using the desired interval after the basic vaccination(s). (Full license)
6. In case of more than one vaccination in the basic vaccination schedule: determination of the minimum and maximum interval between the vaccinations (Full licence)

When challenge (administration of disease agent, i.e. SARS-CoV-2) is required, one or more of the following parameters will be evaluated:

- Monitoring of clinical signs (e.g. changes in general health)
- Measuring of body temperature
- Determination of pathogen shedding or dissemination (swabbing of mucosal surfaces, nasal washes, testing of faecal/urinal samples)
- Serological response (amnestic response), viraemia, or haematological changes (blood sampling)
- X-ray analysis (pneumonia) (or ultrasound)
- Post-mortem examination (pathology in the respiratory tract and other organs)

If an immunological correlate has been established, the evaluation of the immune response (antibody levels) will suffice (blood sampling) and no challenge will be needed.

From a regulatory standpoint, animals must be monitored for differing lengths of time following challenge. In a successful challenge study, control animals generally develop disease well within the stated time period. Virus spread to sentinel animals that are placed in the group after experimental challenge may be part of the model.

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

Depending on the study to be performed, two or more of the following treatments for study of the SARS-CoV-2 in cats and ferrets will be employed (in italics the frequency of the treatments) and will be applied in accordance with 'Handboek proefdierkunde' (van Zutphen et al., 2016).

1. Daily observation / scoring clinical signs *(1x – daily)*
2. Application of the antigen, booster(s) (intramuscular, subcutaneous, oral, nasal, intravenous, ocular, intra-peritoneal *(1 – 5 x)*
3. *Measurement of body temperature (via intra-peritoneal transponder placed prior to the study under sedation, rectal measurement as backup) (1x- daily)*
4. Weighing *(1 – 5 x)*
5. Blood sampling to determine immunological parameters and/ / or to determine the presence of the challenge strain in the blood *(1 – 15 x)*
6. Challenge administration (intravenous / subcutaneous / intramuscular / intradermal / ocular / intranasal (dropwise or nebulisation) / oral / intratracheally / rectal) *(1 – 2 x)*
7. Swabbing of (mucosal) surfaces (oral/nasal/ocular/rectal/vaginal), nasal washes to determine excretion of the vaccine (up to 14 days) and / or excretion of the challenge strain (up to 28 days) *(1x-daily)*
8. X-ray (1 – 3x) or ultrasound if possible
9. Sedation (transponder placement, challenge administration, blood sampling, X-ray *(1- 10x)*
10. Euthanasia and necropsy

The duration of all procedures described above are expected to be carried out within a matter of minutes. The dose-response study length is 9 weeks, followed by a challenge study. The length of the observation period after challenge depends on the incubation period of the pathogen, for SARS-CoV-2 (short incubation time of days) it is estimated at approximately 4-5 weeks. To rule out that clinical signs are caused by (co-)infection with another disease agent, or to monitor for shed and spread post infection, non-infected control 'sentinel' animals will be included in a study.

The studies described in Appendix 1 (serology study) may involve Syrian hamsters, and the setup of a challenge model for this less sentient species is described in Appendix 2. Experimental challenge of vaccinated Syrian hamsters described in Appendix 1 is therefore also requested in this Appendix, with only the aim of confirming efficacy of vaccine candidates.

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

When animal numbers are not specified in a Ph.Eur. monograph, such as is the case for SARS-CoV-2, the minimum number of animals required to give a sufficient likelihood of a statistically significant result will be used. In this way it can be demonstrated that the test vaccine is efficacious in comparison with the control group. In particular, the variance in the groups together with the magnitude of effect will be used in power calculations to achieve 80% power at the 95% confidence level (regarded by regulatory authorities as the standard by which such experiments should be designed). For respiratory viruses in general, group sizes of 10-15 animals are typically required to reach statistical significance.

For Syrian hamsters, group sizes in Appendix 1 are set at 6. Challenge experiments are conducted with 6 vaccinated animals, 2 sentinels and negative control groups.

B. The animals

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

Cats of both sexes will be used for this type of animal experiment. Ferrets of both sexes will be used for this type of animal experiment.

Sourcing of animals:
Cats and ferrets (SPF) will be purchased from licensed commercial vendors.

Numbers:
Numbers are based on the set of experiments to be completed for registration. We base the numbers on the following studies to be completed:

- Dose-finding (2 vaccine candidates)
- Efficacy for conditional licensing (2 vaccine candidates)
- Onset of immunity at 2, 3, 4 weeks after vaccination (1 vaccine candidate, full license)
- Duration of immunity 6-12 months (1 vaccine candidate, full license)
- Repetition of an experiment plus control group in case of unexpected events during the test that invalidate results

Additional animals for studies that are required by Regulatory agencies at the time of full licensing will be requested once regulatory guidelines have been obtained.

The estimated group size is 15 cats/ferrets, including sentinel animals for challenge studies. Total of 10 groups (maximum 150 cats/150 ferrets).

Age of animals:
The age of cats and ferrets used for vaccine development varies from 8 weeks old to adult. The age of the animals to be used should be representative of the age for which the vaccine will be developed. As a general rule, the vaccination schedule begins at 8-9 weeks of age.

The following overview shows the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category over the next 5 years:
For procedures described in section A the cumulative discomfort score for the sampling may be moderate due to repeated sampling.
Vaccination is supposed to reduce clinical symptoms in the vaccinates and therefore there is an estimated shift towards mild symptoms compared to discomfort scores in Appendix 2.

Pathogen	**Animal category**	**Discomfort of disease (% of animals with highest score)**	**Duration of discomfort**
SARS-CoV-2	Cats >8 weeks old	Mild: 95/150= 63% Moderate: 50/150= 33% Severe: 5/150= 4%	Max 1 week Max 1 week Max 1 day
SARS-CoV-2	Ferrets >8 weeks old	Mild: 95/150= 63% Moderate: 50/150= 33% Severe: 5/150= 4%	Max 1 week Max 1 week Max 1 day

From a regulatory standpoint, kittens are considered the most sensitive age and must therefore be used for basic efficacy studies. Therefore, the above table is based on the expected discomfort scores in kittens. So far, challenge studies have provided data that support the view that kittens are slightly more sensitive, in contrast to humans.

Syrian hamsters will be obtained from studies described in Appendix 1, and necessary extra sentinel and challenge control and negative control animals are purchased from licensed commercial vendors.

Age of the Syrian hamsters is > 4 weeks. Experiments to be conducted with females but we will look at the possibility to include males in the experiments as well (based on age at start and duration of the experiment).

A maximum of 4 vaccine candidates will be tested, requiring 24 animals from Appendix 1 plus in addition 8 sentinels, 16 challenge-control (including sentinels for these groups) plus 6 negative control animals (maximum 54 animals).

The following overview shows the maximal discomfort caused by the disease and the maximum number of animals expected to reach the highest discomfort category over the next 5 years:
For procedures described in section A the cumulative discomfort score for the sampling may be moderate due to repeated sampling.
Vaccination is supposed to reduce clinical symptoms in the vaccinates and therefore there is an estimated shift towards mild symptoms compared to discomfort scores in Appendix 2.

Pathogen	**Animal category**	**Discomfort of disease (% of animals with highest score)**	**Duration of discomfort**
SARS-CoV-2	Syrian hamster >4 weeks old	Mild (30/54= 55%) Moderate (22/54= 41%) Severe (2/54= 4%)	Max 1 week Max 1 week Max 1 day

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.

For research on novel pathogens, animals from previous studies can theoretically be re-used if seronegative, but given the preference for kittens this may not be pursued. Re-use of animals from Appendix 1 is indicated above.

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:
Cats and ferrets must be used for these studies because there are no suitable alternatives or models for the induction of immunity in a whole organism or for the colonization of living tissues as complex as those found in the whole animal for which these vaccines are intended to induce protection. The use of the target animal is specified in the Ph.Eur. or other legislations. It is possible to test vaccine candidates in Syrian hamsters as a less sentient species for initial selection based on efficacy.

Reduction:
The number of animals used will be reduced wherever possible without endangering the scientific integrity of the work. Study protocols will be designed to combine the collection of data on as many different parameters as possible within a study in order to minimise the total number of animals used. Where possible the same control group will be used for multiple comparisons in order to reduce the number of animals required. The minimum numbers of animals required in safety and efficacy studies are set out in the European Pharmacopoeia and EMA guidelines. In many cases, the number of animals stipulated by the guidelines is small. From earlier vaccine research work, challenge models have been refined and are robust enough to allow the use of the minimal numbers of animals, and at least solid guidelines are now available through literature for challenge models for cats and ferrets and Syrian hamsters. 10.1.c

As indicated above animals will be re-used where possible, keeping animal welfare in mind without endangering the scientific integrity of the work.

Refinement:
Regulations and guidelines determine to a large extent what kind of data must be generated and, to a large extent, this determines what form of models and methods should be employed.

The classical method to prove protection of a new vaccine is efficacy in a vaccination-challenge test. However, if immunological correlates of protection (e.g. a serological response) can be used to prove efficacy this will be used rather than challenge tests. When a challenge model is mandated, clearly defined humane endpoints will be applied and staff will be fully trained to recognize animals that are experiencing discomfort. Animals will be closely monitored and frequent checks will be made to ensure that no animal is left suffering.

Where challenge efficacy studies are mandated, clearly defined humane endpoints specific to the pathogen will be described and the frequency of observation will be increased during any anticipated critical period. Throughout all challenge phases, the welfare of the animals will be monitored by experienced animal technicians under the care of the attending veterinarian. Animals will be euthanised before reaching the humane endpoint when it is clear that continuation would not provide any additional scientific data. After animals have arrived from the suppliers, handling of the animals for sampling procedures specific for the study to be performed is trained by qualified personnel.

An ongoing assessment of the challenge models used will be undertaken. We will try to find a correlation between changes in relevant parameters and the (start of) clinical signs. If the results are acceptable for regulatory submission, a validated change in biochemical/haematological parameter(s) can be implemented so that an earlier scientific endpoint can be determined with fewer clinical signs (and

10.2.g

therefore discomfort). X-ray examination/ultrasound if possible, if available at the facilities, is another example of refinement with regard to determining the scientific end point earlier.

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

In general cats and ferrets are always housed socially. Furthermore, to enhance animal welfare three major criteria are taken into account: 1) social interaction, 2) access to food and 3) the possibility of moving freely. Programs are in place for the housing and caring of animals exposed experimentally to pathogens, with emphasis on management and safety practices for containment (according to the regulations of Biosafety level 1 – 3).

An active approach to providing environmental enrichment will be taken. This will enable animals to express their species-specific behavioural repertoire. Appropriate and multiple objects that allow comfortable resting and play materials will be provided, and new ideas for improving the general wellbeing of the animals encouraged and implemented when and where appropriate.

The procedures being carried out are routine vaccinations, minimally invasive swabbing and the taking of small blood samples. In order to prevent undue stress during blood sampling in ferrets and cats sedation will be used. Sedation will be carried out using licensed agents and dosing regimens developed under guidance of a veterinarian. Experience has shown that for certain procedures such as intranasal challenge, sedation provides a significant increase in consistency of dosing. Therefore, in certain circumstances, it will be advantageous to both animal welfare and scientific consistency to make the decision to use sedation for certain procedures.

For monitoring of the clinical health status of animals, all study animals will be checked once daily by a certified person. The frequency of observation will be increased after challenge in anticipation of possible clinical symptoms of disease. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any unexpected event, a veterinarian will be consulted to ensure appropriate veterinary care.
Few, if any, adverse reactions are expected in the majority of animals during vaccinations, and small volumes (keeping the age of the animal in consideration) of blood will be sampled. These procedures are minimally invasive. Efficacy studies using a challenge model however will require that all animals are exposed to the disease agent SARS-CoV-2. This may result in clear clinical symptoms in unprotected animals. When it is clear that continuation would not provide any additional scientific data, every effort will be made to euthanise the animal before the humane endpoint is reached.

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.

10.1.c [redacted] a number of the safety and efficacy studies done with the individual products then have to be repeated with the vaccines administered together according to international regulations and guidelines.

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?

10.2.g

☒ 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 animal 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.

Blood sampling, swabbing, rectal temperature measurements, administration of the vaccine and weighing are procedures which may cause mild to moderate discomfort. 10.1.c

Vaccination can result in a transient increase in rectal temperature, sometimes accompanied with a reduced level of activity and appetite, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks. Vaccination with a live attenuated vaccine could induce mild disease symptoms specific for the pathogen used as vaccine, but this is very unlikely.

The administration of the challenge inoculum will only induce short term mild to moderate discomfort but depending on the nature of the subsequent challenge the discomfort of the challenge can range from mild in the absence of any clinical signs to moderate, and even severe. At present, the full clinical presentation of SARS-CoV-2 in cats and ferrets is not known, but in humans such variety in clinical symptoms has been observed. Vaccination is expected to result in a significant reduction of clinical abnormalities and virus shedding after challenge compared to the unvaccinated control group.

For monitoring of the clinical health status of animals, all study animals will be checked. Animals will be checked at least once daily, or more frequently as required, by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded

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

In consultation with the veterinarian and study director it will be decided whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (if treatment does not interfere with the test results). Sedation will be applied when placing the transponder and performing X-rays. Furthermore sedation will be applied during challenge and blood sampling of ferrets and if deemed necessary challenge and blood sampling of cats.

I. Other aspects compromising the welfare of the animals

10.2.g

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

Transport
10.1.c
transport and an acclimatisation period will be required before challenge.

Explain why these effects may emerge.

There are few laboratories that have BSL3 facilities to work with SARS-CoV-2

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

Acclimatisation will extended to ensure animals are healthy and appear settled showing no signs of stress.

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.

To determine the efficacy of a vaccine, it is necessary to challenge animals with SARS-CoV-2. However, the duration of severe discomfort will be limited with the application of a humane endpoint.

For each type of experiment test-specific humane endpoints will be described in the corresponding study protocol. Animals will be checked at least once daily, or more frequently as required, for general health by a certified animal technician so that any welfare concerns are detected quickly. All daily observations are recorded. 10.1.c
The description and improvement of specific humane endpoints is an ongoing process 10.1.c
These will be used during future vaccine development and improved/adjusted when needed based on findings during the studies.

At present it is not possible to define humane end points for SARS-CoV-2 in cats, as much remains unknown in this stage about clinical effects

In the event that animals do display unexpected clinical signs whether or not these are believed to be study related, the veterinarian will be consulted and the necessary treatment will be given or the animal will be euthanised.

Indicate the likely incidence.

In this type of experiment, 4% of the animals are estimated to experience severe discomfort and may reach the humane endpoint.

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').

For studies or study subjects (animals) without challenge, discomfort will be mild. For vaccination challenge studies, the type and severity of the clinical signs are depending on the presence of protective immunity. Similar to natural field SARS-CoV-2 infections, symptoms may cause mild to severe pain, distress and suffering. Vaccination is expected to reduce the level of discomfort after challenge, but the non-vaccinated control group will experience the symptoms of the natural infection.

The discomfort score is an estimate based the limited experience with the above infection models for the species. See Tables under B.

The cumulative discomfort score for the sampling may be moderate due to repeated sampling, which would decrease the percentage of mild an increase the percentage of moderate discomfort.

End of experiment

L. Method of killing

10.2.g

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.

Animals will be humanely euthanized at the end of a study. 10.2.g In addition, necropsy may be required at the end of the study for scientific reasons.

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

10.2.g

Centrale Commissie Dierproeven

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'.	10.2.g
1.2	Provide the name of the licenced establishment.	10.2.g
1.3	List the serial number and type of animal procedure. *Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.*	Serial number: 4 — Type of animal procedure: Safety studies in cats and ferrets

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.

The following parameters must be assessed in order to confirm the safety of live and inactivated vaccines:

1. <u>The effect on general health</u>

With live vaccines, particular attention should be paid to those signs typical of infection with the virulent organism. General safety parameters are assessed after a 10x overdose and repeat vaccination. After vaccination, one or more of the following parameters will be evaluated:

- Clinical signs (e.g. changes in general health)
- Haematological changes (blood samples)
- Body Weight
- Injection site reactions (measurement and palpation)
- Body temperature (subcutaneous transponder), or rectal temperature
- Post-mortem examination

For live, particularly genetically modified vaccines, a number of additional studies are required:

2. <u>Spreading and dissemination of the vaccine strain</u>

Spread from the vaccinated animal is determined by looking for the vaccine strain in bodily secretions. Consequently, swabs from for example oral, rectal, nasal and ocular mucosa are taken. Unvaccinated sentinel animals that are also being sampled are placed in contact with vaccinated animals to evaluate animal-to-animal transmission. Also, the dissemination of the vaccine strain in the vaccinated animal, with particular attention paid to the site(s) of replication and the injection site in case of a vaccine strain based on a zoonotic pathogen should be investigated.

3. <u>Reversion to virulence</u>

To test for reversion to virulence the vaccine should be given by the route most likely to make the vaccine strain revert. It should then be re-isolated from the animal and passaged by administration back into subsequent animals up to a total of five times. The safety profile of the passaged material should then be compared with the starting material.

4. Ecotoxicity
The ecotoxicological effects that the vaccine candidate may have on the environment need to be assessed. In terms of in vivo work, this includes the potential for recombination with other vaccines/vectors and the potential to persist in the environment. Methods of disinfection that prevent the spread of the vaccine candidate from contaminated kennels to naive animals need to be investigated in studies involving vaccinates and sentinels.

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

Depending on the safety study to be performed, two or more of the following procedures will be employed depending on the characteristics of the pathogen/disease involved *(in italics the frequency of the treatments)* and will be applied in accordance with 'Handboek proefdierkunde' (van Zutphen et al., 2016).

1. Application of a subcutaneous transponder for body temperature *(1x)*
2. Blood sampling *(1 – 7x)*, for serology, haematology and biochemical parameters.
   Blood samples may be taken up to every other day in order to measure haematological changes.
3. Administration of vaccine (intramuscular, subcutaneous, oral, nasal, intravenous, ocular, intra-peritoneal) *(1 – 5 x)*.
4. Measurement of body temperature (rectally or through a transponder) *(2x – daily)* to measure body temperature.
5. Palpation of the injection site *(1x – daily)*, to measure local safety reaction.
6. Weighing *(1x – daily)*.
7. Swabbing of mucosal surfaces (oral/nasal/ocular/rectal) *(1x each – daily)*, to measure the presence of viruses, bacteria or antibodies.
8. Euthanasia and necropsy

The duration of all procedures described above are expected to be carried out within a matter of minutes. The length of the observation period after vaccination is in principle 14 days after each vaccination. Also, in case of injection site reactions that have not resolved after 14 days or a persistent live vaccine strain a longer observation period will be needed.

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 to be used is specified or indicated in Ph.Eur monographs or national regulations and in these instances all measures will be taken to meet the mandatory requirements of the regulatory authorities while using the minimum possible number of animals. In absence of such monograph for SARS-CoV-2, numbers are taken from comparable studies in cats.

B. The animals

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

Cats (European short hair) of both sexes will be used for this type of animal experiment. Ferrets of both sexes will be used for this type of animal experiment.

Sourcing of animals:
Cats and ferrets will be purchased from licensed suppliers of SPF laboratory cats and ferrets.

Age of animals:
The age of cats and ferrets used for vaccine development varies from 8 weeks old to adult. The age of the animals to be used should be representative of the age for which the actual vaccine will be developed. As a general rule, the vaccination schedule begins at 8 weeks of age.

Numbers:

The following studies are in scope for safety:

- single dose
- repeated dose
- 10x overdose
- spread/dissemination
- reversion to virulence
- ecotoxicity

This equals 6 studies with a mean groups size of 15 animals per experiment.
90 cats
90 ferrets

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.

Re-use is acceptable if the immunological status of the animals allows.

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:
Cats and ferrets must be used for these studies in accordance with international regulations. For these safety investigations, the number of animals to be used are mostly specified and all measures will be taken to meet the mandatory requirements of the regulatory authorities.

Reduction:
If the number of animals required is not specified in the relevant legislation, they will be reduced wherever possible without endangering the scientific integrity of the work. This will be achieved through an on-going evaluation of the observations in each study. For these studies, the number of animals used will be the minimum number required to demonstrate a statistical difference between the vaccine and control groups. Usually, this will be circa 10 vaccinates and 5 controls. The number of animals per study will be detailed in each study protocol. 10.1.c

Animals will be re-used where possible, keeping animal welfare in mind without endangering the scientific integrity of the work.

Refinement:
International regulations determine to a large extent what sort of data must be generated and this determines which methods have to be employed

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

In general cats and ferrets are always housed socially. Furthermore, to enhance animal welfare three major criteria are taken into account: 1) social interaction, 2) access to food and 3) the possibility of moving freely. Programs are in place for the housing and caring of animals exposed experimentally to pathogens, with emphasis on management and safety practices for containment (according to the regulations of Biosafety level 1 – 3).

10.2.g

An active approach to providing environmental enrichment will be taken. This will enable cats and ferrets to express their species-specific behavioural repertoire. Appropriate and multiple objects that allow comfortable resting and play materials will be provided, and new ideas for improving the general wellbeing of the animals encouraged and implemented when and where appropriate.

The procedures being carried out are routine vaccinations, minimally invasive swabbing and the taking of small blood samples. In order to prevent undue stress during blood sampling in ferrets and cats sedation will be used. Sedation will be carried out using licensed agents and dosing regimens developed under guidance of a veterinarian. Experience has shown that for certain procedures such as intranasal challenge, sedation provides a significant increase in consistency of dosing. Therefore, in certain circumstances, it will be advantageous to both animal welfare and scientific consistency to make the decision to use sedation for certain procedures.

For monitoring of the clinical health status of animals, all study animals will be checked once daily by a certified person, and more frequently when required. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any unexpected event, a veterinarian will be consulted to ensure appropriate veterinary care.

Few adverse reactions are expected in the majority of animals since the procedures that are being carried
out are routine vaccinations and small volumes (keeping the age of the animal in consideration) of blood will be sampled. These procedures are minimally invasive. Consequently, the level of severity for the safety studies will be **mild** (100%).

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.

10.1.c [redacted] a number of the safety and efficacy studies done with the individual products then have to be repeated with the vaccines administered together according to international regulations and guidelines.

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 animal 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.

10.2.g [redacted]

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.

Blood sampling, rectal temperature measurement, swabbing and weighing are part of normal veterinary care and will induce only mild to moderate (only when sedation is required for e.g. blood sampling) discomfort. 10.1.c [redacted]

Vaccination can result in a transient increase in body temperature, sometimes accompanied with a reduced level of activity and appetite, and a transient vaccination site reaction. Systemic reactions will generally disappear within 24 hours, but local reactions (that are generally painless) can persist for several days and even weeks. Vaccination with a live attenuated vaccine could induce mild disease symptoms specific for the pathogen used as vaccine, but this is very unlikely. Palpation of local reactions will only give mild discomfort.

For monitoring of the clinical health status of animals, all study animals will be checked at least once a day by a certified person. Special attention will be paid to the general health of the animals as well as feed and water consumption. All daily observations are recorded. In case of any abnormalities, a clinical examination of the respective animal will be performed by a veterinarian.

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

In consultation with the veterinarian and study director it will be decided whether to apply adequate veterinary care to alleviate unexpected pain and/or distress (If treatment does not interfere with the test results). In case of severe suffering, humane endpoints are applicable. However, severe discomfort in safety studies is not experiment related.
Sedation will be applied for blood sampling of ferrets, and for cats if deemed necessary.

I. Other aspects compromising the welfare of the animals

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

None

Explain why these effects may emerge.

Not applicable

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

Not applicable

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.

Indicate the likely incidence.

10.2.g

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').

Mild: 100%

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 many cases, it will not possible to re-use or re-home animals used in safety studies. 10.1.c In addition, necropsy may be required at the end of the study for scientific reasons. These animals will be humanely euthanized by experienced personal at the end of the study.

The following animals may be considered for re-homing:
- Animals that have not been vaccinated during their role as a sentinel
- 10.1.c

Re-homing will be in accordance with Directive 2010/63/EU. An animal will only be considered for re-homing if it has been assessed by a veterinarian with knowledge of the lifetime experience of the animal and it is considered to be healthy and not likely to suffer future adverse effects as a result of the regulated procedures. All animals will be suitably socialised for everyday life outside of the facility and fully vaccinated before release.

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

10.2.g