Netherlands National Committee
for the protection of animals
used for scientific purposes

Learning from COVID-19

An initial exploration of the landscape and the research methods used.

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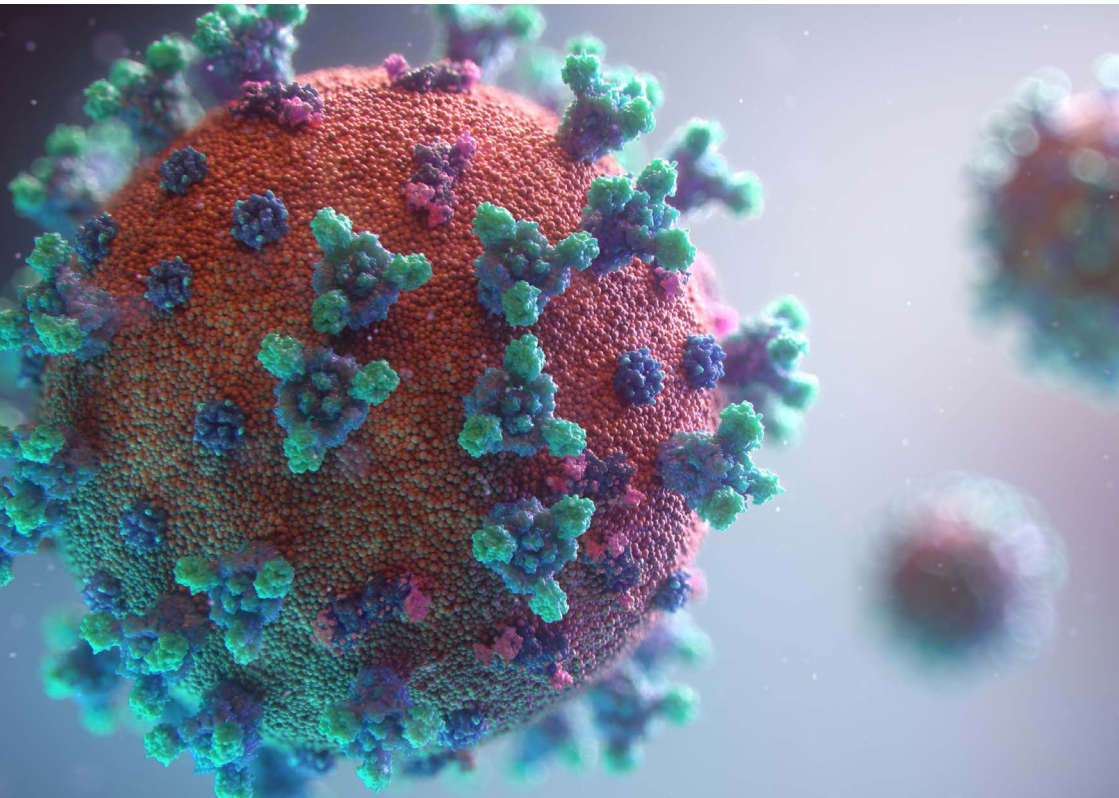


Introduction and background

In December 2019, the first patient with COVID-19 symptoms was hospitalised in Wuhan, China. After this, the contours of a global pandemic emerged, which is still ongoing. Worldwide, the number of confirmed cases has risen to more than 114 million and deaths from COVID-19 have increased to around 2.5 million (data from early March 2021).
The pandemic has not only affected public health, but has also had a major impact on society as a whole: on the economy, culture and social interactions. It has also impacted the direct and indirect use of laboratory animals. Many animal studies have been initiated for research into the virus, its transmission and the possibilities for prevention and infection control. On the other hand, due to the restrictions in place to contain the virus, animal studies in other research areas have been postponed and/or breeding colonies have been scaled back. This merits the question what influence the pandemic has had and – as it has certainly not ended – will continue to have.

On 27 October 2020, the Minister of Agriculture, Nature and Food Quality requested the Netherlands National Committee for the protection of animals used for scientific purposes (NCad) to provide an opinion on the effects of COVID-19 on the transition to innovation without laboratory animals and the possibilities for parallel studies.
We were asked to answer the following questions:

a. Which animal models have been used around the world in COVID-19 research? What has been the influence of COVID-19 research around the world on the use of laboratory animals?
b. Which alternative (non-animal) methods have been used around the world in COVID-19 research? What was the effect?
c. Which changes have occurred in the prescribed procedures for vaccine and/or medicine-development or in the international adherence to these procedures?
d. With regard to a., b. and c.: Which lessons or advice can be distilled for the future of the transition to non-animal innovation?

The Minister requested this opinion to gain an overview of and insight into the use of laboratory animals and non-animal methods for research into vaccines and methods for treatment of COVID-19 worldwide. And to map the (possible) implications for the transition to non-animal innovation *(transitie naar proefdiervrije innovaties*, TPI) and formulate lessons for the future of TPI based on the findings of this opinion.

The NCad was requested to share its initial impressions in March 2021 in a concise interim report. The final report is scheduled for December 2021. In the coming months, a number of findings from the interim report will be further substantiated and elaborated. This applies, for example, to the use of non-human primates (NHPs), One Health and COVID-19 and the acceleration of vaccine development through the use of non-animal methods. We will also address aspects such as the funding of research into non-animal methods in the Netherlands, including funding provided by the European Commission, such as through the Netherlands Organisation for Health Research and Development (ZonMw) and current European research programmes.[1] In addition, additional interviews with experts will be conducted. Two literature reviews into the possibilities for in vitro models and analytical models in infectious disease research will be conducted. Conclusions and recommendations will be developed.

Approach of the NCad for an initial exploration

Based on the literature reviews and information gathered through various channels, an effort was made to gain an initial picture of the COVID-19 research landscape. In addition to literature research, interviews were conducted with various experts. To date, interviews have been held with experts involved in or working in:

- human and veterinary vaccine development
- giving vaccination advice to the Dutch government
- the WHO Blue Print Group
- the Dutch Medicines Evaluation Board (CBG), preclinical area
- CBG batch release of vaccines
- the US Food and Drug Administration (FDA)
- contract research organisations (CROs)
- the Central Committee on Research Involving Human Subjects (CCMO)
- the Royal Society for the Prevention of Cruelty to Animals (RSPCA)
- the department of virology at Erasmus University Medical Center
- research institutes using primates

The experts interviewed are all directly or indirectly involved in the issue of COVID-19.

[1] https://ec.europa.eu/info/research-and-innovation/research-area/health-research-and-innovation/coronavirus-research-and-innovation_en

Research landscape

On 7 January 2020, Chinese researchers isolated the virus that causes COVID-19 from a patient. The virus, which belongs to a class of coronaviruses, was named SARS-CoV-2 because it is related to the SARS-CoV virus. Following the isolation of the virus, there was a cascade of studies into specific aspects of the virus and its treatment, each with its own dynamics. Through this exploration, we aim to provide an insight into the COVID-19 research landscape, with a special focus on the aspects of the use of laboratory animals and non-animal alternatives and the regulations on the authorisation of vaccines and medicines.
This research landscape breaks down into a number of areas on which research has successively and also simultaneously focused:

- isolation and characterisation of the virus, including the cause and development (aetiology and pathogenesis) of the infection
- selection and validation of research models
- development of prophylactics (vaccines) and therapeutics (pharmaceuticals, antibodies, etc.)
- assessment of safety and efficacy of prophylactics and therapeutics
- marketing authorisation procedure for vaccines and medicines
- quality control of vaccines and medicines
- related COVID-19 research, such as research into the spread of the virus within or between populations (epidemiology) or research into side effects of COVID-19, such as cardiovascular and neural complications
- non-animal methods: uses and needs

Each of these focus areas is briefly explained below.

isolation and characterisation of the virus (exploratory research)

In general, the process applied to isolate and characterise viruses still follows the rules laid down by the physician and microbiologist Robert Koch in 1884, known as Koch's Postulates. In a nutshell, Koch's first two postulates are that the potential pathogen (microorganism) must be isolated from a patient with the unknown disease. After the microorganism has been grown in pure culture, a susceptible species must be infected with it. If these animals then show symptoms characteristic of the disease in the patient, the link between the microorganism and the infectious disease has been established. This is also the approach applied for the virus that causes COVID-19. In the case of COVID-19, the virus belongs to a class of coronaviruses and the symptoms are similar to those of previous diseases caused by

coronaviruses, such as Severe Acute Respiratory Syndrome (SARS) from 2002. For this reason, the virus was named SARS-CoV-2. But today we also have molecular biology research, which did not exist in Koch's lifetime. This has enabled us to map the genetic profile of the virus after it was isolated, and to characterise the domains of the virus for cell binding and uptake, for replication and excretion and for cell pathogenicity. Much of this research has been carried out using sensitive cell culture methods. Scientists managed to gather a lot of information in a short time, which served as a basis for the development of vaccines and medicines. In characterising the virus, the question was whether animals could be a reservoir for the virus. From the perspective of the One Health[2] principle, it is important to know to what extent the virus can spread from animals to humans and vice versa. Various animal species were used for this epidemiological research, including bats, pigs and cats.

Selection and validation of research models (exploratory research)

COVID-19 has developed into a pandemic at a very fast pace. Unlike with previous infections, where there was sufficient time to set up a research strategy and develop models, in this pandemic scientists have relied on existing models developed and available for other respiratory diseases, such as SARS and Middle East Respiratory Syndrome (MERS) (both from the family of coronaviruses) and influenza. These are mainly animal models, which after infection show a clinical picture (phenotypic and mechanistic characteristics) which (partly) resembles that in humans, mice, hamsters and ferrets (SARS) and in rabbits (MERS) and non-human primates. Each of these models has possibilities and limitations, such as in terms of sensitivity to the virus, course of the infection, excretion of the virus and disease process. The availability of some reagents has also played a role in the choice of model.
In the mouse model, for example, mice had a low sensitivity to the SARS-CoV-2 virus, with the exception of hACE2 transgenic mice. These are a genetically modified mice that have the human receptor for the coronavirus on the walls of the epithelial cells in their respiratory tract. However, many reagents are available for mice that allow further research into immunological processes. Hamsters and non-human primates (macaques and African green monkeys) are susceptible to the coronavirus and show a clinical picture and histopathology similar to a coronavirus infection in humans; ferrets are particularly important for research into transmission of the virus. In addition, the choice of model is also impacted by the availability of and societal views on laboratory animals.

[2] One Health involves interdisciplinary collaboration to improve the health of people, animals and the environment. https://www.rivm.nl/one-health

In addition to the exploratory research, studies with animals were carried out into the course of the infection, the infectious dose to be used, the peak in infection and the recovery and pathology after infection.

Particularly in the early phase of the pandemic, significant international coordination of research took place through the online meetings of the WHO Blue Print Group (held two to four times a month). This helped to clarify the focus areas of the different research groups, which contributed to limiting the duplication of research. Researchers, mostly from public organisations (universities and research institutes), but also from the pharmaceutical industry and NGOs, were able to present and discuss their research findings at the meetings, often before the findings were published. However, as the pandemic unfolded, the meetings were mainly used to present findings that had already been published. With the emergence of new virus mutants, more presentations of as yet unpublished findings are being given. One of the members of the Blue Print Group has stated that in his opinion, the Blue Print consultation has led to a reduction in the duplication of research. According to attendees of the Blue Print Group meetings, non-animal methods played a minor role in the presentations and were often based on individual initiatives. This was partly due to the urgency of the moment, which meant that scientists mostly relied on animal models that are widely known and accepted. But the argument that many research questions require a complex functioning immune system, and that in vitro equivalents are not yet available for this, was also frequently mentioned.

Development of therapeutics, such as pharmaceuticals, antibodies, etc., and prophylactics, such as vaccines

Therapeutics

The pandemic has put great pressure on the development of therapeutics and prophylactics. When it comes to therapeutics, the first step taken was a targeted search in databases of existing and authorised medicines to look for medicines that could be repurposed for COVID-19. This mainly concerned antiviral drugs, drugs with a regulating effect on the immune system and treatments for complications from COVID-19, such as cardiovascular problems. As the various national authorities already had marketing authorisation dossiers on these medicines considered for repurposing, extensive research into their safety and mode of action was not necessary. As a result, the efficacy and effectiveness of many of these medicines could be clinically assessed after approval by a Medical Research Ethics Committee (*Medisch-Ethische Toetsingscommissie*, MREC). The situation was different for new medicines and antisera. The application of such new medicinal products is subject to legal requirements whereby every new product must be assessed for safety, efficacy and mode of

action (dosages, route of administration, etc.).[3] This requires research in laboratory animals. For example, the safety studies for antisera for immunotherapy were carried out in dogs.[4]

Prophylactics

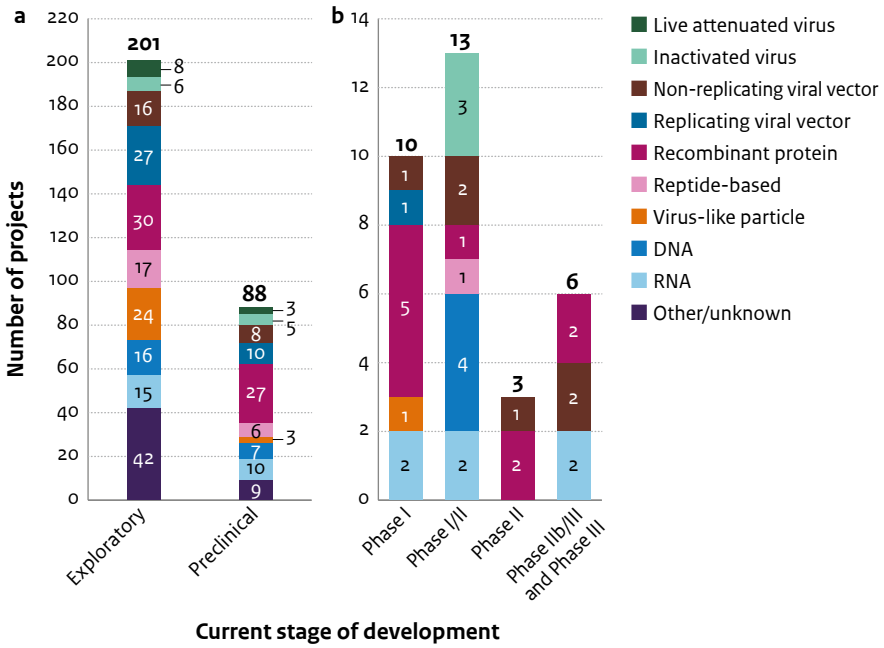
Prophylactics concern vaccines. The technology for vaccine development has improved greatly in recent decades. Whereas earlier vaccines were based on the injection of killed or weakened pathogens, new generations of vaccines often contain only the part of the pathogen that is able to elicit protective immunity against the pathogen. As a result, this new generation of vaccines can be better characterised and has fewer side effects. In addition, in recent years, molecular biology techniques are often used to efficiently deliver the protective part of the pathogen to the immune system. In recent years, including during the SARS, MERS and EBOLA outbreaks, knowledge has been gained about the safety and effectiveness of these technologies. This meant they could be used for the development of trial vaccines at an early stage of the COVID-19 pandemic. For example, the manufacturers BioNTech and Moderna have used RNA platforms that were developed to facilitate the development of vaccines for respiratory syncytial virus (RSV). The Janssen vaccine and the AstraZeneca/Oxford vaccine were developed using an adenoviral vector.

The COVID-19 pandemic has triggered a global search for a vaccine. A 2020 Nature Reviews article shows that at the time of publication (September 2020), there were 321 vaccines in various stages of development. Some of these vaccines have since received marketing authorisation, while others have been dropped because they failed to pass some of the selection criteria.
The COVID-19 vaccines (already authorised or still in development) break down into four categories:

- inactivated vaccines and attenuated vaccines
- replicating and non-replicating viral vector vaccines
- DNA/RNA vaccines
- protein subunit vaccines and virus-like particle vaccines.

[3] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community Code relating to medicinal products for human use

[4] The safety studies are required by law and dogs are the most relevant animal model in this case.



Bron: Nature Reviews | Drug Discovery

Figure 1: COVID-19 vaccines in development (information from September 2020) (*https://www.nature.com/articles/d41573-020-00151-8*)

The development of a vaccine consists of a number of steps, which are described below. In the exploratory stage, broad research takes place. This depends on the type of vaccine to be developed. This stage involves examining how the vaccine candidate behaves in the body (the kinetics of the vaccine candidate), whether it elicits an immune response, determining the optimal route of administration, dose and formulation, whether an immune-potentiating substance (adjuvant) will need to be added to the vaccine candidate, etc. This safety and efficacy of the vaccine candidate is then assessed in the preclinical stage. Research in the preclinical stage is based to a large extent on animal testing, which (at least in Europe) is subject to prior ethical review by a competent authority (in the Netherlands, an Animal Tests Committee (DEC) and the Central Authority for Scientific Procedures on Animals (CCD)). With respect to safety, this research concerns determining whether there are acute and/or

chronic side effects, including on reproduction and foetuses.[5] For inactivated vaccines, research into complete inactivation is required, and for attenuated vaccines, research into residual virulence. Efficacy research focuses on inducing an immune response (antibodies and cellular immunity) and involves challenge tests to determine whether the immune response protects against infection with virulent virus (and for how long). For COVID-19, this efficacy research is usually carried out using rodent models, especially hamsters. But before a decision is made to enter the vaccine candidate into a clinical trial, a challenge study may be conducted. The animal model chosen depends on the pathogenesis caused by the disease agent in the model. Based on the data from the preclinical stage, the CCMO or Medical Ethics Review Board (*Medisch-Ethische Toetsingscommissie*, METC) decides whether a vaccine candidate may be tested in humans (the clinical trials, phases I to III).

To what extent the results of preclinical research can be translated to humans is a matter of debate. In none of the animal models used, including the primates, did post-infection pathology fully resemble that in humans; that is, the severity of respiratory symptoms was often limited. That said, extensive lung histopathology was found and replication and excretion of the virus were similar to that in humans. However, it should be noted that most of the animals used in the studies were young adults, which are not the most susceptible age group in humans. Using adult or old animals is difficult due to limited availability of animals in these age groups. In addition, it should be noted that risk factors known in humans, such as obesity, were not incorporated in the animal models used.

Marketing authorisation procedure for vaccines and medicines

The applicable legislation[6] requires that every medicine and vaccine on the market is safe and effective. In the European Union, the European Medicines Agency (EMA) is responsible for the authorisation of new active substances for use in humans and animals. Each EU Member State has a national authority responsible for the marketing authorisation of medicines (in the Netherlands this is the Dutch Medicines Evaluation Board (*College ter Beoordeling* van Geneesmiddelen, CBG), which is involved in the assessment of the submitted dossier. For new medicinal products,[7] it is mandatory to follow the centralised procedure for

[5] Results of these studies have not been included in the marketing authorisation dossier yet and will be added at a later stage (following marketing authorisation).

[6] Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community Code relating to medicinal products for human use

[7] It is mandatory to follow the centralised marketing authorisation procedure for biotechnological medicines, as well as new medicines for the treatment of certain diseases, including cancer, AIDS, neurodegenerative diseases and diabetes. For other innovative products, firms can choose between centralised or national marketing authorisation.

authorisation. The assessment of new medicinal products is carried out by EMA's Committee for Medicinal Products for Human Use (CHMP), which is supported in its duties by the EMA Secretariat. Each Member State is represented by two members in the CHMP. A medicinal product is initially assessed by the regulatory authorities of two different Member States which have been appointed as rapporteur or co-rapporteur. Their reports are shared and discussed with the other Member States and decided upon in the CHMP.
In addition, for vaccines and blood products, every batch must be released in Europe (under the responsibility of the EDQM). To this end, each Member State has an Official Medicines Control Laboratory. In the Netherlands, this is the National Institute for Public Health and the Environment (*Rijksinstituut voor Volksgezondheid en Milieu*, RIVM).
Many other countries around the world also have national authorities for the marketing authorisation of medicines. In the US, the Food and Drug Agency (FDA) is responsible for the marketing authorisation of human vaccines and medicines. To apply for marketing authorisation, manufacturers must submit a dossier describing the results of their preclinical studies and clinical trials, as well as the production process and the quality controls in this process. Producers must submit their dossier as soon as the results of all their studies are available. With the exception of three commercially manufactured batches used for the clinical trials, production may only start after obtaining marketing authorisation.
The successive steps in the development process and marketing authorisation procedure are shown in Figure 2. This is a highly schematic representation: in practice, several processes run in parallel and objectives are exchanged between the various sub-steps.

Figure 2: Steps in the development and marketing authorisation process for a medicine/vaccine

As it can take a lot of time to get from the start of vaccine development to completion of the clinical trials, and the assessment of the submitted dossier is also time-consuming, the development and authorisation process may take up to 10 years. At the beginning of the COVID-19 pandemic, the International Coalition of Medicines Regulatory Authorities (ICMRA)[8] set up in 2012, in which regulatory authorities from many countries worldwide

[8] ICMRA: www.icmra.info

participate, adopted a statement[9] on which preclinical data are required before first-in-human clinical trials can be started, and on sharing information between developers and regulatory authorities during the development process. The position adopted by the ICMRA is an important initiative that has accelerated the marketing authorisation process.
For COVID-19 vaccines, EMA's centralised marketing authorisation procedure has been fast-tracked by means of 'rolling reviews' conducted by the CHMP.[10] With a rolling review, data for an application are assessed as soon as they become available. To this end, the procedure is shortened by splitting it up: the manufacturer first submits the preclinical dossier for assessment by a competent body, such as the Dutch CBG (on behalf of the CHMP), followed by the clinical part of the dossier. Pursuant to the Dutch Medical Research (Human Subjects) Act (*Wet medisch-wetenschappelijke onderzoek met mensen*, WMO), clinical research is subject to an ethics review. For research into therapeutics, this is conducted by a Medical Research Ethics Committee (METC), and for research into prophylactics by the Central Committee on Research Involving Human Subjects (CCMO). For ethics reviews relating to COVID-19, the CCMO or METC applies a fast-track procedure, whereby submitted COVID-19 protocols receive priority and parts of the dossier are assessed as soon as they become available. Fast-tracking of the procedures of the CBG and CCMO/METC has helped to shorten the time from development to marketing authorisation. This acceleration has also been helped by other factors at the level of vaccine developers, marketing authorisation authorities and policy. The table below summarises the conventional steps in the development and marketing authorisation of a new vaccine and the factors that have accelerated this process for COVID-19 marketing authorisations. It should be emphasised that all interviewees stated that this fast-tracking has not and will not come at the expense of the safety and efficacy of the products. However, this fast-tracking has led to delays in other dossiers that do not relate to COVID-19.

[9] For all SARS-CoV-2 vaccine candidates it is necessary to obtain data in animals and to characterize the immune response induced by a SARS-CoV-2 vaccine candidate. It is not required to demonstrate the efficacy of the SARS-CoV-2 vaccine candidate in animal challenge models prior to proceeding to FIH (First In Human) clinical trials.

[10] https://www.ntvg.nl/artikelen/versnelde-beoordeling-van-covid-19-vaccins/volledig

	Conventional process from development to authorisation	Accelerated corona vaccine development and authorisation
Factors		
Technology	Conventional vaccines based on inactivation/attenuation of the microorganism, often with the addition of an adjuvant to strengthen the immune response.	Recently developed vaccine technologies were used. These had already been used for the development of other vaccines, e.g. for Ebola. So, information on the efficacy and safety of these technologies was available at the start of development, enabling a flying start.
Vaccine manufacturers	Often, the market for a new vaccine is limited and the decision to invest in development requires an in-depth cost-benefit analysis.	In the COVID-19 pandemic, however, marketing a vaccine is a blockbuster for the manufacturer, and also boosts their reputation.
	Vaccine development is costly. Numerous go/no-go steps have been built into the development to keep the financial risks manageable.	Manufacturers made agreements with governments on purchase volumes during the development phase. Financial risks were therefore less of an issue. This meant manufactures could accelerate the development of promising products. The process was accelerated by carrying out phase 1 and phase 2 trials partly in parallel with animal studies (e.g. in primates). Vaccine production started before marketing authorisation was obtained.
	Vaccine development is only one of the activities of manufacturers and always competes with other priorities	Absolute priority was given to the development of a COVID-19 vaccine. Academic and other research institutions and governments worldwide provided financial and political support.
Preclinical research	Preclinical studies require prior approval from the CCD (after obtaining an opinion from the Animal Tests Committee (DEC)) and each individual animal study requires prior approval from the Animal Welfare Body (IvD)	Procedures and ethics review processes have not changed. However, priority has been given to COVID-19 related research and, where possible, the CCD has handled dossiers entirely in writing instead of discussing them in meetings held every three weeks.[11]

[11] To date, the CCD has reviewed 20 applications relating to COVID-19.

	Conventional process from development to authorisation	Accelerated corona vaccine development and authorisation
Clinical research	By law, clinical trials require a prior ethics review by a competent authority (CCMO or METC). There is no prioritisation of dossiers.	Situation in the Netherlands: fast-track procedure for assessment by CCMO (vaccines) and METCs (therapeutics). No difference in assessment process between COVID-19 research and other research.
	Clinical trials require sufficiently large cohorts of people to demonstrate the significance of treatment effects. The level of protection is a key criterion for vaccines for infectious diseases. Many diseases have a low infection rate in a population, so it takes a long time to demonstrate a significant level of protection.	With COVID-19, the infection rate was high, so sufficient information about control and vaccine groups became available in a short period to demonstrate statistical significance for the protective effect. People's willingness to participate in the trials was also high.
Marketing authorisation authorities	Assessment of the full marketing authorisation dossier takes place after the manufacturer has completed all studies (preclinical and clinical).	Priority was given to COVID-19 protocols, for which separate assessment sessions were scheduled. A rolling review procedure was applied, in which interim opinions were given and parts of the dossier were assessed as soon as they were received by the authorities. As a result, clinical trials could be started on the basis of demonstrated safety in preclinical studies.
	The dossier is only assessed when **all** results are available, including the results of longevity studies (to determine how long a vaccine provides protection) and reproductive toxicity studies; studies that take longer than six months. Upon submission, three batches of the product have already been commercially manufactured and tested to confirm the manufacturing process.	Results of these studies have not been included in the marketing authorisation dossier and will be added at a later stage (after marketing authorisation). Upon marketing authorisation, three batches confirming the commercial manufacturing process were not available yet, but a protocol was in place to which the batches had to adhere.

The significantly shorter time between the development and marketing of COVID-19 vaccines is therefore not attributable to a single factor or a single stakeholder, but to a complex and coordinated set of measures.

Despite the acceleration of the development and marketing authorisation process, there are two steps in the process that are difficult to speed up: the clinical studies and preclinical trials. It takes time to conduct animal studies. Efficacy studies, for example, take around one to two months, plus the time needed for preparations and processing of the results. For some studies, such as monitoring immune status until long after vaccination, the results of animal studies will not be available until after vaccine marketing authorisation. This time factor also applies to clinical trials.
Replacing time-consuming animal studies with short-running in vitro studies and immunochemical and physicochemical studies could further reduce the time needed for the development and marketing of a vaccine. This will have to be clarified through the ongoing synthesis of evidence.

Quality control of vaccine batches

Vaccines fall in the category of biological products. They are manufactured from living materials by means of batch production. This has consequences for the quality of the end product, which can vary from batch to batch. This applies in particular to vaccines produced on the basis of conventional principles, i.e. by inactivating the pathogen (e.g. rabies vaccine), by detoxifying the toxin of the pathogen (e.g. tetanus and diphtheria vaccine) or by weakening (attenuating) the pathogen (e.g. measles vaccine).

For a COVID-19 vaccine produced through one of these conventional techniques, each batch may need to be checked for safety and efficacy. If animal models are used for this purpose, the number of animals required annually for the recurrent batch quality controls will be considerable.

By contrast, vaccine types produced on the basis of new technologies have already been extensively characterised in the development stage. This, combined with a standardised and controlled production process, makes the successive batches very consistent in composition, which eliminates the need for laboratory animals for routine batch controls. For these products, quality control is carried out by analytical methods (physical/immunological/biochemical), supplemented, if necessary, by in vitro methods for monitoring immunogenic parameters.

It should be noted, however, that new-generation vaccines may be less effective against escape mutants of the SARS-CoV-2 virus. Because new-generation vaccines are based on expression of a small part of the virus, the elicited immunity will be more specific. As a result, there is a risk that when a vaccinated person is infected with a new variant of the virus, their immune system will not recognise the virus and they still fall ill. By contrast, a conventional vaccine based on the whole virus elicits a very broad immune response directed against multiple parts of the virus. Even if the immune system does not recognise the mutated part, it will still recognise other parts of the virus, so the vaccine may still provide at least partial protection. Incidentally, the relevant part of the SARS CoV-2 virus (the spike protein) is a large protein and therefore also elicits a broader immune response.

COVID-19-related research

COVID-19 may trigger complications. Confirmed complications include cardiovascular complications (atrial fibrillation, heart failure, blood clots), complications of the central nervous system (brain cell damage, possible link to Parkinson's disease) or kidney complications (chronic kidney damage, kidney failure). Much of the research into this is done with patients, for which university hospitals have often obtained permission from the Central Committee on Research Involving Human Subjects (CCMO). For experimental research, relevant in vitro models, where available, can be used, such as organoids or organs-on-a-chip. When research requires a complex model, such as with research into cause and effect, currently animals are often used for this.

Non-animal methods

The previous sections have outlined the COVID-19 research landscape. Animal testing has played a major role from the start of pandemic until now, especially in the development of vaccines. This is partly due to the fact that the models used at the start of the pandemic were those with which experience had been gained from the SARS and MERS outbreaks and which did not require major investments in development. However, it has been found that animal models have limitations (see above under '*Selection and validation of research models (exploratory research)*'). This is evident, for example, from a statement by the International Coalition of Medicines Regulatory Authorities (ICMRA) that animal models are of value but to what extent they can be translated to humans is open to debate. Animal testing accounts for a large part of the research effort. This is partly because the application of non-animal models, such as organs-on-a-chip and organoids, is complicated due to the difficulty of integrating elements of the functional immune system, especially when the immune response is the subject of research. Despite this limitation, non-animal methods have certainly made an important contribution to COVID-19 research, both directly and indirectly. In discussions on non-animal methods, the emphasis is on cell culture techniques. Other

non-animal methods, including physicochemical methods, such as HPLC,[12] and immunochemical methods (such immunoelectrophoresis) or molecular biological techniques.[13] When it comes to the latter non-animal methods, in the early stage of vaccine development the focus was very much on analysing the virus and characterising potential vaccine candidates. This focus on characterisation was combined with platform techniques to bring about good presentation of characterised parts of the virus in the body to obtain an optimal immune response. While this did not lead to a reduction in the number of animals used for testing, it has ensured that new-generation vaccines have been characterised so well that the use of animals for the release of vaccine batches is no longer necessary. It is sufficient to demonstrate consistency in production based on non-animal methods (controls using physicochemical and immunochemical techniques). This will indirectly lead to a significant reduction in the use of laboratory animals. As an illustration, European figures on the use of laboratory animals (2017) shows that around 10% of the use in Europe was for routine testing of old-generation vaccines.
Non-animal alternatives include research with human volunteers. This does not apply to most of the clinical studies, but human challenge studies have been and are being conducted in some locations, although not in the Netherlands. In these studies, human volunteers are vaccinated and then infected with SARS-CoV-2. Clearly, such studies can only be conducted with the highest possible safety measures (approved by an ethics committee, with a strict selection that admits only healthy young adults, a low virus dose of a well-characterised virus and close monitoring). Human research has also been carried out in a clinical setting, for example into complications of COVID-19. The CCMO website with summaries of clinical research involving human subjects shows that 177 applications for COVID-19- related studies have been submitted up to March 2021 (which did not include any challenge studies).

The process from the start of vaccine development to marketing authorisation of the first COVID-19 vaccines took just one year. A substantial part of that year was spent conducting animal studies. This vaccine development period could be shortened further by replacing animal studies with short-running non-animal studies and/or by integrating and combining animal and human studies for various research questions. There are opportunities in this area, but we are not there yet. A significant investment, preferably internationally coordinated, will be required to develop these alternative models and/or research strategies and to make them operational.

[12] High-performance liquid chromatography (HPLC), sometimes also referred to as high-pressure liquid chromatography, is a separation method that involves pumping a pressured liquid containing the sample mixture through a tightly packed column.

[13] Molecular biology techniques make it possible to study processes at cell level.

In the follow-up phase to its opinion, the NCad will focus on mapping new developments in non-animal research methods and/or research strategies and present a vision for the future. To support this effort, the NCad has commissioned the University of Amsterdam to conduct a literature review of innovative developments in the field of cell culture techniques in relation to infectious disease research. The NCad will also approach research groups and experts for the other issues mentioned.

Provisional findings

The scale and impact of the COVID-19 pandemic placed great pressure, both in terms of capacity and resources, on research into the pathogenesis and the subsequent development of vaccines and drugs. This development has mostly relied on known research models, mainly animal models.

None of the models used is ideal. Each model offers possibilities and has limitations in terms of translating it to people. It is the scientific question that determines which model is preferable. In some cases this is a non-animal method, in other cases an animal model.

Particularly in the early phase of the pandemic, the WHO took up a coordinating role in the research effort by setting up the Blue Print Group, which had online meetings twice a month. These meetings focused on animal research.

The impact of COVID-19 on the use of laboratory animals can only be assessed after the data on their use for the year 2020 becomes available. It is likely that the pandemic has led to an increase in the number of animals used in the 'infectious disease research' category, especially rodents (mice, hamsters), ferrets and non-human primates. On the other hand, it is to be expected that the number of animal tests used for other purposes was lower, as some of the research was terminated or delayed due to the coronavirus restrictions.

Non-animal methods are used to a limited extent in COVID-19 research. This is partly due to the urgency of the research and the immediate need for known models, and partly due to the fact that many of the non-animal in vitro models have limitations in terms of integrating the 'immune system' into these models. Non-animal methods appear to have greater usefulness in questions where the immune system does not play a role (virus characterisation, pathogenesis research).

The acceleration in the development and marketing authorisation of COVID-19 vaccines and medicines is the result of adjustments made by all parties involved in development and marketing authorisation, such as manufacturers, marketing authorisation authorities, the CCMO and others, including by making available considerable resources and capacity. This was made possible by the urgency of the pandemic.

A substantial part of the time between the start of vaccine development and marketing authorisation is taken up by preclinical research (which currently often involves animal research) and clinical trials. A key question to consider in further detailing the opinion will be whether we can shorten the preclinical phase and possibly increase the quality of research by using existing methods or new methods developed without using animals.

Although using non-animal methods has not been a priority in COVID-19 research, there are great opportunities for non-animal research in the future. These include not only innovative in vitro models, such as organoids, but also non-animal methods in the fields of molecular biology, biochemistry, immunochemistry, physicochemistry and controlled human infection models.

Glossary and abbreviations

CBG:	Medicines Evaluation Board (*College ter Beoordeling van Geneesmiddelen*)
CCD:	Central Authority for Scientific Procedures on Animals (*Centrale Commissie Dierproeven*)
CCMO:	Central Committee on Research Involving Human Subjects (*Centrale Commissie Mensgebonden Onderzoek*)
Challenge studies:	Studies in which the protective effect of a vaccine or medicine is assessed by administering the virulent microorganism
CHMP:	Committee for Medicinal Products for Human Use
CRO:	Contract research organisation
DEC:	Animal Tests Committee (*Dierexperimentencommissie*)
EDQM:	European Directorate for the Quality of Medicines |
FDA:	US Food and Drug Administration
IvD:	Animal Welfare Body (*Instantie voor Dierenwelzijn*)
Pathogenesis:	The usually gradual onset, development and progression of a condition or disease.
WHO:	World Health Organization

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