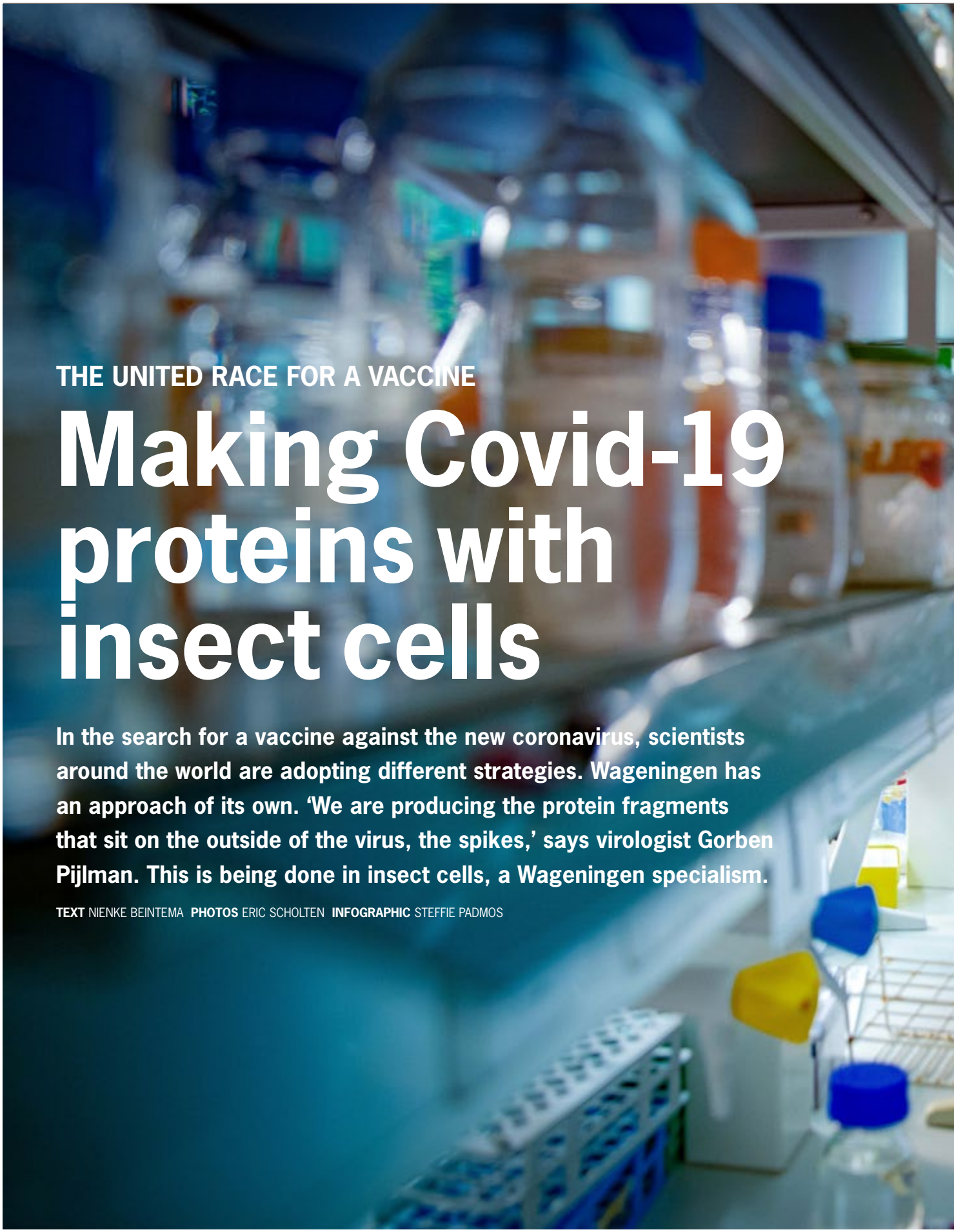


THE UNITED RACE FOR A VACCINE

Making Covid-19 proteins with insect cells

In the search for a vaccine against the new coronavirus, scientists around the world are adopting different strategies. Wageningen has an approach of its own. 'We are producing the protein fragments that sit on the outside of the virus, the spikes,' says virologist Gorben Pijlman. This is being done in insect cells, a Wageningen specialism.

TEXT NIENKE BEINTEMA **PHOTOS** ERIC SCHOLTEN **INFOGRAPHIC** STEFFIE PADMOS


VIRO 3

> ‘The point is to get a vaccine fast, not to be the first to make one’

A bizarre summer is ahead of us: without full pavement cafes, without crowded beaches and swimming pools, without festivals, sports camps or foreign holidays. How quickly daily life and the global economy can get back to normal depens on a single crucial factor: the development of a vaccine. Only when there is an effective vaccine can we slowly let go of social distancing. Wageningen scientists are collaborating in the worldwide quest.
‘We are working with several partners on a vaccine against Covid-19,’ says Gorben Pijlman of the Laboratory of Virology. ‘The initiative came from the Danish company ExpreS2ion Biotechnologies, who we’ve been working with for quite a while.’ The collaboration primarily has focused on diagnostic tests and vaccines against chikungunya and Zika, two tropical viral diseases. Pijlman: ‘We now want to apply the same strategies to Covid-19-19.’ The universities of Copenhagen and Tübingen are collaborating on this too, as is Leiden University Medical Centre, which has the expertise for working with live coronavirus. The new collaboration was set up at lightning speed. ‘The European Commission earmarked funding for research on a coronavirus vaccine in February, and put out a call for proposals.’ says Pijlman. ‘In such cases, you always stand a better chance with an international collaboration. The Danes brought a consortium together in no time, based on existing partnerships. So we got a phone call too, and we said “yes” without hesitation.’

STIMULATING IMMUNE SYSTEMS

The key to vaccination is that the immune system is stimulated in a way that is specific to the target pathogen. The immune system then manufactures antibodies against the pathogen, as well as ‘memory cells’. These specialized white blood cells enable the body to go straight into action if it is infected again, and to produce large numbers of antibodies to defend itself against the attack.
Classic vaccines, such as the measles vaccine, consist of a weakened or deactivated version of the virus. People who are immunized with it do not fall ill, but they do develop immunity to the pathogen. Another approach is to give people fragments of the genetic material of the target virus. The very first coronavirus vaccine, for which testing started in the US in April, is of this kind. In this case, small, characteristic fragments of RNA from the virus are introduced into the body. RNA functions as a blueprint for manufacturing proteins. On the basis of the virus RNA, cells in the immunized person’s body start manufacturing pieces of virus protein themselves, after which the immune system produces antibodies against them.

PROTEIN FRAGMENTS

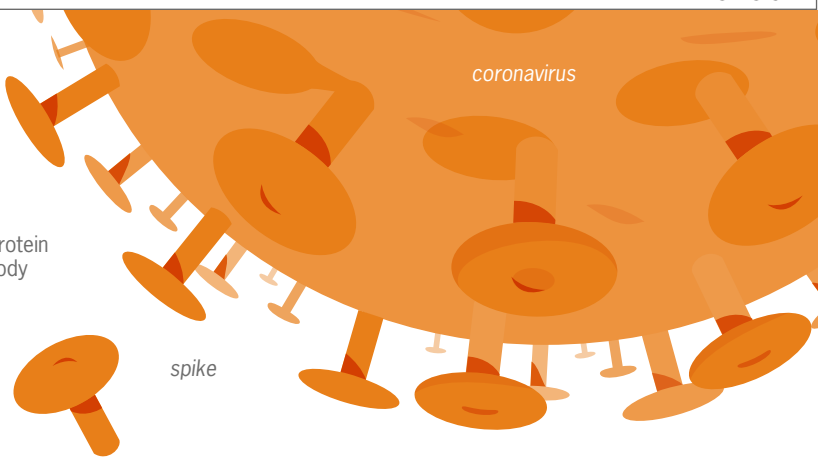
‘We are going for a different approach,’ says Pijlman. ‘In our lab we manufacture protein fragments that are found on the outside surface of the virus. These are the spikes you can see, and we produce the spikes that are specific to the coronavirus.’
The idea is that our immune system recognizes those spikes as foreign and starts producing antibodies against them. If ever the complete virus then enters the body, the spikes on the virus will immediately spark off an immune response. ‘The big advantage is that to make these spikes, we can rely on technology that is already in use in the pharmaceutical industry,’ says Pijlman. ‘RNA vaccines are much newer. Nobody knows yet whether you can easily produce them on a large scale.’
And that is one of the main success factors in the development of vaccines, independently of the question of whether they are safe and effective: are they also fast, reliable, cheap and producible on a large scale? ‘That is the case for our spikes,’ claims Pijlman. That production is now going on in insect cells, a Wageningen specialism.

MOTH CELLS

Many laboratories use mammalian cells for the production of complex proteins, but Pijlman sees a number of clear advantages to using insect cells. They are easier to grow in a bioreactor than mammalian cells. The researchers infect the cells of a moth with a genetically modified baculovirus into which they have introuced the gene for the target protein, in this case the spike protein. After being infected with the baculovirus, the insect cell’s machinery goes into action to produce the target protein. ‘This is done in large concentrations,’ says Pijlman. ‘One cell can produce up to 30 per cent of its own dry weight in protein.’
Established vaccines are already produced by this method, for example the vaccine against the human papillomavirus (HPV), which causes cervical cancer. ‘In this case, the insect cell produces small protein particles that strongly resemble HPV: these are known as virus-like particles,’ explains Pijlman. ‘We use this approach to make more complex vaccines as well, such as those against Zika and chikungunya. And now against the novel coronavirus. Our Danish partners are going to glue our spikes onto virus-like particles, which will create something resembling a real coronavirus.’
Combined approach
The production of the spikes is under way, says Pijlman, but there are still challenges. ‘The spikes themselves are so small >

THE WAGENINGEN ROUTE TO A CORONAVIRUS VACCINE

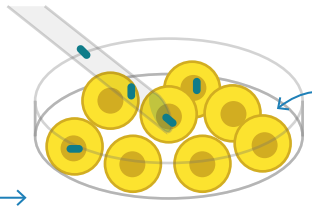
Insect cells are used to replicate the spikes – the protein fragments on the outside of the coronavirus. The body identifies this protein as foreign and manufactures antibodies against it.

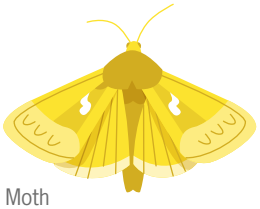


The gene that codes for the spike proteins is put into a baculovirus

Baculoviris

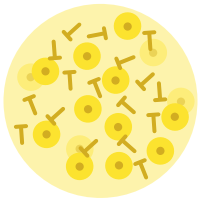
Cells from a moth are infected with the virus

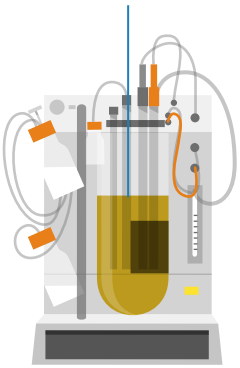




Moth cell

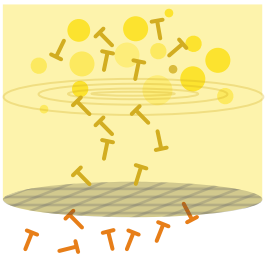
The insect cells produce the spike proteins in large quantities in a bioreactor



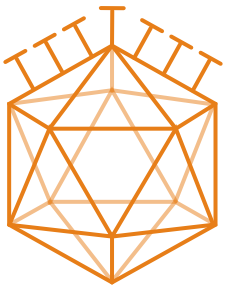


Bioreactor

The spikes are purified



The spike proteins are linked to virus-like particles



Virus-like particle

TESTING FOR COVID-19

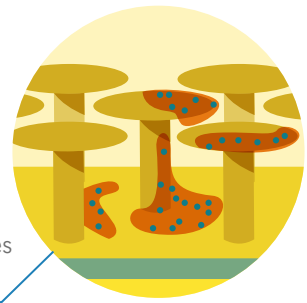
The spike proteins produced by insect cells are used in serological tests which identify antibodies to the coronavirus in the blood. This kind of test can also serve to reveal how well a vaccine works.

1. The spike proteins are fixed to a surface

2. A drop of blood is added

3. Antibodies against the coronavirus attach themselves to the spike proteins

Assay



4. A dye is used to make the antibodies visible

> ‘There is no way around very careful testing’

WEAKENING THE VIRUS FOR LIFELONG IMMUNITY

Jelke Fros of the Laboratory of Virology in Wageningen is working on another vaccine strategy: using weakened, or attenuated viruses. If you vaccinate people with an attenuated virus, they don’t fall ill but their immune systems do produce antibodies.

‘That weakening can be done in a variety of ways,’ says Fros. ‘For example, you can switch off some of the virus’s genes. But if you want to do that effectively, you need a lot of biological knowledge about that virus. Are you sure you are making it inocuous, for example? And might it reverse-mutate? This makes safety a bigger issue.’

Fros and his colleagues are therefore using another special method. In all living organisms the genetic code consists of just four bases, or ‘letters’. ‘Bacteria and some viruses have the bases C and G next to each other more often than do humans and other vertebrates,’ says Fros. A recently discovered component of our immune system makes use of this, he explains. ‘That component is geared to recognizing genetic codes that have C and G next to each other more often. We try to offset this adaptation by introducing extra CG combinations in certain places. The immune system reacts more strongly to this, and therefore cleans up the virus more efficiently.’

Meanwhile, the immune system learns to recognize the virus, so that it can react even faster next time. ‘Because the virus is still functional, and can reproduce a little bit, it activates other parts of our immune system too. That gives you a bigger chance of lifelong immunity.’

The method offers advantages over conventional methods of weakening viruses, says Fros. ‘It means we can play with the number of added CG combinations to optimize the degree of attenuation, the effectiveness and the safety of the vaccine.’

This approach is still in its infancy, says Fros, but it could form an important piece of the overalll puzzle. ‘One good thing might be that in the long term, our approach could reinforce other strategies.’

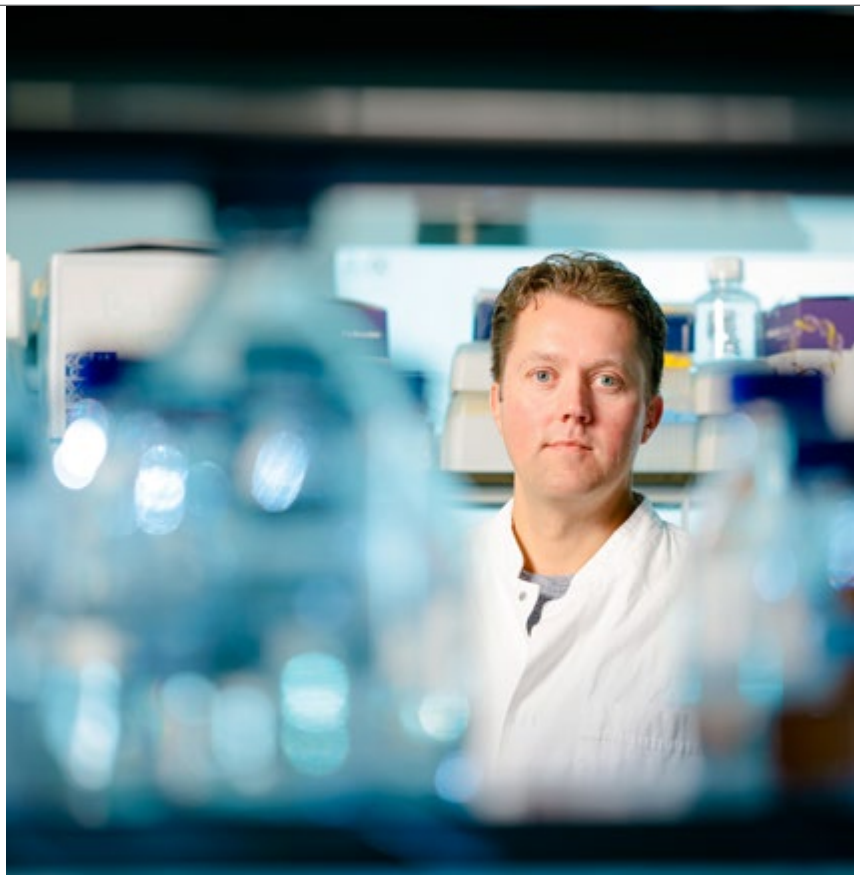
that the immune system does not efficiently recognize them individually,’ he explains. ‘So we are now working on ways of attaching them to those virus-like particles in repeating sequences.’

This is a unique approach worldwide, Pijlman emphasizes. But on more general issues such as practical questions about tests and how best to administer the vaccination, there is plenty of consultation between labs. ‘That is something very special about the current period,’ says Pijlman. ‘There is great community spirit and tremendous openness. The point is to get a vaccine fast, not to be the first to make one.’

So is it sensible for all those labs around the world to work with different strategies? ‘It certainly is,’ responds Pijlman. ‘The road towards an effective approved vaccine is long. During this race, a lot of candidate vaccines will fall by the wayside, either because they don’t work well enough or because of undesirable side effects.’ The point is to end up with at least one vaccine, says Pijlman. And it is possible that all those strategies could produce a combined approach. A combination of two vaccines, perhaps, which work in different ways and complement each other.

DEVELOPMENT STOPPED

One of the challenges is that there is not a single vaccine against other human coronaviruses on the market yet. ‘A prototype was developed for a vaccine against the SARS virus, and that is a coronavirus too,’ says Pijlman. ‘But the development of that vaccine stopped when the virus died out. Such a pity. If that vaccine had been fully developed, it would now only have been a question of “plug and play”: put a new gene in the cells that produce the virus protein, and you’re done.’



Virologist Gorben Pijlman

In spite of the great urgency of finding a vaccine, the researchers cannot cut many corners. They have to follow the fixed procedure, with animal studies followed by human studies. That process normally takes about three to four years. Pijlman: 'Evaluation processes are being fast-tracked in this emergency situation, but there is no way around very careful testing. All in all, we hope that our consortium will have a vaccine within a year and a half. That really would be superfast.'

Wageningen will not be the first to produce a working vaccine, guesses Pijlman. 'I think we shall start testing it on people relatively late in the day, maybe only in November,' he says, 'because we want to do really good animal studies first.' American researchers started testing a new RNA vaccine on people in April. They skipped the animal testing stage. But that won't speed up the process, according to Pijlman. 'In the race for a vaccine, you never know how it will go,' he says. 'It might well be that one after the other, they have to go back to the lab because there is something that is not optimal, either to do with effectiveness or with safety.'

The animal tests that are needed to test potential vaccines are coordinated by the working group on animal models set up by the World Health Organization (WHO). Wim van der Poel, professor of Emerging and Zoonotic Viruses at Wageningen Bioveterinary Research (WBVR), is in this working group. The group discusses questions such as which animals are best suited for testing a Covid-19 vaccine, how best to expose these animals to infection in a research setting, and how you can objectively establish how sick an animal gets or how much virus an animal spreads.

The animal research must be as efficient as possible, stresses Van der Poel. 'Not only because we want to get good results as quickly as possible,' he says, 'but also because we want to minimize the number of animals we use. As far as we can, we always research the effectiveness of a vaccine in artificial systems, such as cell cultures. But there comes a point when you really need to shift to living animals, to be able to research the effectiveness and the safety exhaustively. But you have to do that as efficiently as possible, too. That is what makes WHO coordination so important.' Cross-pollination between human and veterinary vaccine research is very important, says Van der Poel. 'There are some things, such as effective ways of administering vaccines, that you can test quicker and more easily on animals than on humans.'

TESTING FOR IMMUNITY

Meanwhile, the Wageningen research could be applied in another area, says virologist Pijlman. The spikes could be of use in blood tests that indicate whether people have had the virus and therefore have antibodies in their blood. You can for example fix the spikes to a plastic surface, and then add some of the person's blood serum. If this contains antibodies to the coronavirus, they will attach themselves to the spikes. You can then use a dye to make them visible. 'That way you can get an idea of what percentage of the population might already be immune,' says Pijlman. 'A test like that can also help to determine the effectiveness of a vaccine by finding antibodies that can neutralize the virus.'

Pijlman and his colleagues started their vaccination project on 1 April. 'Bioprocess engineers are helping us to develop the right process conditions,' he says. 'A team of colleagues in Biochemistry are on hand to purify the spikes. And the company Applikon is putting large bioreactors at our disposal free of charge. Help is coming from all sides. That is the wonderful thing about this period.' ■

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