RApid Molecular and SErological aSsays and Inhibitors for novel coronavirus

RAMSES-I

List of participants

Participant No. *	Participant organisation name	Country
1 (Coordinator)	University of Helsinki **(UH)**	Finland
2	University of Turku **(UTU)**	Finland
3	University of Tartu **(UTA)**	Estonia
4	University of Uppsala **(UU)**	Sweden
5	Technical Research Centre of Finland **(VTT)**	Finland
6	Reagena **(REAG)**	Finland
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1. Excellence

Our mission is to respond rapidly to the global public health threat caused by the novel SARS-coronavirus-2. We will build upon existing excellence and preparedness for emerging infectious diseases allowing us to develop swiftly **rapid diagnostic tools** for detection of both viral nucleic acids as well as acute antibody response or past immunity. These will be both field-deployable and usable also in low-incoming settings, which will be of key importance under a pandemic scenario. **Pathogen traits** and genetic signatures associated with these traits will be assessed in cell culture and animal models, which are also essential for identifying and evaluating both **new and repurposed antivirals**. In addition, we will produce both neutralizing and diagnostic **monoclonal antibodies** for therapeutic candidates, as well as diagnostic reagents for evaluating e.g. neutralizing responses rapidly. With our clinical PIs and collaborators in China, we will be able to evaluate the diagnostic tools using clinical samples already collected in China and those accumulating in Europe. All therapeutic and diagnostic tools, as well as better understanding of coronavirus emergence will significantly aid preparedness also for future coronavirus outbreaks.

1.1 Objectives

OBJECTIVE 1 – TO IDENTIFY AND DEVELOP ANTIVIRALS

Novel highly pathogenic viruses emerge and cause severely debilitating conditions. Most of these are vector-borne and zoonotic viruses with neither vaccines nor drugs for prevention and treatment. This low level of preparedness has been illustrated by the recent outbreaks of Ebola and Zika viruses, and again now by SARS-CoV-2. No vaccines and drugs are available for prevention, prophylaxis and treatment of any coronavirus infection in humans (Eurosurveillance Editorial, 2020). *Our consortium will test safe-in-man broad-spectrum antivirals (BSAAs) as rapidly usable drug candidates for prophylaxis and treatment of SARS-CoV-2 coronavirus infections. We will also analyze the potential of natural antiviral substances, interferons (IFN-α/β, IFN-λ and IFN-γ) alone or in combinations to inhibit the replication of SARS-CoV-2 in cell culture and animal infection models. We will also develop novel protease inhibitors, entry inhibitors, as well as therapeutic monoclonal antibodies.*

OBJECTIVE 2 – TO DEVELOP RAPID AND VERSATILE DIAGNOSTIC TOOLS

Timely identification of all infected individuals is a key component in limiting the spread of any infectious disease. Such identification tools are not available, as none of the previously developed methods for detecting coronaviruses are adequate for the detection of SARS-CoV-2. *Our consortium will develop a toolbox for detecting the novel coronavirus SARS-CoV-2 in clinical as well as animal and environmental samples rapidly and reliably in different scenarios and settings.* We will also develop tools to monitor antibodies induced against this virus for diagnostic use and surveillance to understand the epidemiology. All these tools will be useful in the search of the animal reservoir and for identifying other coronaviruses that could pose a future threat.

1.2 Relation to the action

Our consortium addresses the topic **Development of therapeutics and diagnostics combatting coronavirus infections** (H2020-JTI-IMI2-2020-21-single-stage), and the specific challenge to rapidly gain a better understanding of the newly identified virus, especially in relation to potential clinical and public health measures that can be put to immediate use to improve patients' health and/or contain the spread of SARS-CoV-2.

Specific scope of the topic	How RAMSES-I addresses the programme scope
Development of antivirals as well as other types of therapeutics to address a rapid response to the current COVID-19 outbreak	• By identifying safe-to-man BSAAs and effectiveness of interferons as a first line of therapeutics • By developing therapeutic monoclonal antibodies
Development of therapeutics to address the current and/or future coronavirus outbreaks	• By developing novel antivirals: protease inhibitors, entry inhibitors and siRNAs
Development of diagnostics, ensuring rapid evaluation of candidates based on existing technologies	• By producing a catalog of antigens and virus isolates for product development • By rapidly developing point-of-care immunochromatographic tests, TR-FRET –based tests as well SMIA-based tests • By developing a novel point-of-care RT-PCR method that is rapid and field-deployable
Development of fast and reliable tools that go beyond the state of the art for detection of COVID-19 carriers and symptomatic individuals suspected of COVID-19 infection.	•

1.3 Concept and methodology

(a) Concept

Our central concept is to address the current threat of SARS-CoV-2 by the quick translation of knowledge and experience gained on other emerging infectious diseases, such as the recent Ebola and Zika virus outbreaks. We will bring together a unique mixture of experts in latest technologies with experts in virology to quickly respond to the current epidemic by a twofold approach of advanced diagnostic solutions and antiviral treatment. We are in an excellent position to tackle this problem because of ongoing and previous collaboration of the consortium partners, which has already resulted in several breakthroughs in the field, including a number of diagnostic rapid tests already in use and identification of potential drug candidates in use against some of the most devastating viral diseases. Here, multiple technological solutions to both point of care and laboratory diagnostics and treatment of SARS-CoV-2 will be developed simultaneously and evaluated using both clinical samples and animal models. In RAMSES-I project the new antigens and antibodies will be developed by the project participants to be utilized in the development of point-of-care diagnostic methods, such as lateral flow assays. These are discussed in detail in the following paragraphs on methodology.

The consortium has a unique mixture of both scientific and commercial know how to enable rapid implementation of the results to diagnostic laboratories and medical manufacturers as well as to the wider scientific community. We bring multinational technological innovation, cutting edge virology and commercial laboratory diagnostics together to tackle the current international health crisis as

well as develop a model for quick response tactics to future epidemics wherein all available resources are efficiently used and not in competition.

Table: National and international research and innovation activities linked with the project.

Acronym	Partner	Description
VEO	UH, UU	Versatile Emerging infectious disease Observatory generates and distributes high quality actionable information for evidence-based early warning, risk assessment and monitoring of EID threats by public health ators and researchers in the One-Health domain.
EbolaMoDRAD	UH, UTU	IMI project on Ebolavirus diagnostics
Arbovirus threats and disease associations	UH	A project aiming at discovery and characterization of viruses/viromes in arthropods in Finland and Kenya, their possible disease associations, and to understand pathogenesis of emerging viruses
Preparedness for Emerging Infections in Kenya	UH	One Health project in collaboration with Univ. of Nairobi for screening wildlife, domestic animals and febrile patients for emerging infections in Kenya, including capacity building
DropPen	UH, VTT	Business Finland project for commercialization of portable droplet device for single cell analytics and digital PCR
Virus-host interactions	UTU	A platform of dentification of viral signalling inhibitors regulating innate immunity as novel targets on drug development
City mosquitoes	UU	A project investigating how the threats of mosquito-borne viral diseases (e.g. dengue and Japanese encephalitis) changes in rapidly growing mega cities (Vietnam, India)
Avian influenza –host switch	UU	A project aiming to better understand host-switches of avian influenza virus
CENDRtrap	UH	The development of new antiviral molecules to block multiple viruses
Influenza	UTA	Genetic susceptibility to severe influenza infections
Influenza	UTA	Influenza diagnostics
Influenza	UTA	Novel antivirals against influenza
HEDIMED	VTT	Human Exposomic Determinants of Immune Mediated Diseases. EU Horizon 2020 project
DengueDetect	VTT	Detection and burden of Dengue

HTRob	VTT	Development of high throughput selection and screening methods for antibody libraries
Horizon 2020 Phase 1	REAGENA	Phase 1 feasibility study during May – October 2015
Eurostars Programme	REAGENA	The coordinator and industrial partner in the project "Rapid Diagnostics of Tick-borne infections"

(b) Methodology

THERAPEUTICS – 6 DIFFERENT SOLUTIONS

The first line of therapeutic compounds against SARS-CoV-2 – repurposed drugs and interferons

Developing novel virus-specific vaccines and antiviral drugs can be time-consuming and costly (Bekerman & Einav, 2015; De Clercq & Li, 2016). To overcome these issues, academic institutions and pharmaceutical companies focused on repositioning of existing antivirals from one viral disease to another, considering that most viruses utilize the same host factors and pathways to replicate inside a cell (Yu et al, 2013; Bekerman & Einav, 2015; Debing et al, 2015; Check Hayden et al, 2018; Jaishankar et al, 2018; Schor & Einav, 2018; Yuan et al, 2019). Drug repurposing is a strategy for generating additional value from an existing drug by targeting disease other than that for which it was originally intended (De Clercq & Li, 2016). It has significant advantages over new drug discovery and development since chemical synthesis steps, manufacturing processes, reliable safety, and pharmacokinetic properties in pre-clinical (animal model) and early clinical developmental phases (phase 0, I and IIa) are already done.

Here, we will start with **broad-spectrum antivirals (BSAAs)**, small-molecules that inhibit human viruses belonging to 2 or more viral families. We have recently reviewed BSAAs and summarized the information on 120 safe-in-man agents in freely accessible database (https://drugvirus.info/). These and other BSAAs may represent drug candidates for prophylaxis and treatment of SARS-CoV-2 infections. For example, teicoplanin, oritavancin, dalbavancin, monensin and emetine could be repurposed for treatment of SARS-CoV-2 infections. Oritavancin, dalbavancin and monensin are approved antibiotics, whereas emetine is an anti-protozoal drug. These drugs have been shown to inhibit several coronaviruses (Figure 1) (Shen et al., 2019; Zhou et al., 2016). Moreover, chloroquine and remdesivir were shown to effectively inhibit SARS-CoV-2 virus infection in vitro (Wang et al., 2020). In addition, clinical investigations into the effectiveness of lopinavir, ritonavir, and remdesivir against SARS-CoV-2 infections have started recently (NCT04252664; NCT0425487; NCT04255017). Other safe-in-man BSAAs (such as hydroxychloroquine and arbidol) may also represent drug candidates for prophylaxis and treatment of SARS-CoV-2 coronavirus infections (NCT04261517, NCT04260594). A panel of 900 compounds is approached to be searched for suitable therapeutic approaches. Natural antiviral substances, **interferons**, are also an option for treating severely ill SARS-CoV-2 patients. IFNs are established biological antiviral substances/drugs that can readily be given to humans. IFN-α/β, IFN-λ and IFN-γ may have antiviral effects given alone or in combinations, and together with other pharmacological antiviral drugs. The antiviral activities of IFNs are systematically tested in *in vitro* cell culture models and animal infection experiments.

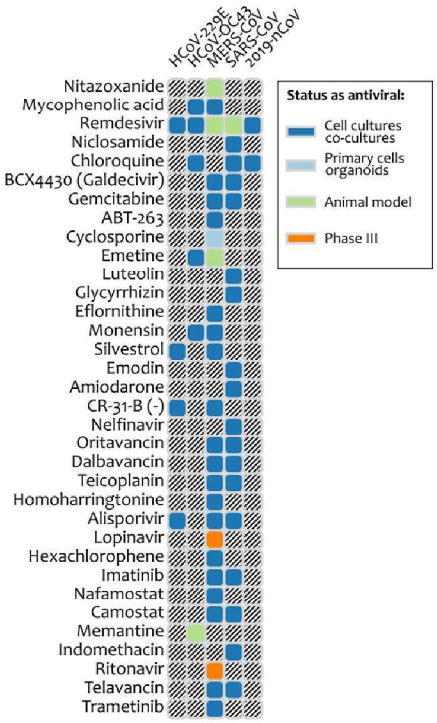


Figure 1. Safe-in-man broad-spectrum antiviral agents and coronaviruses they inhibit. A snapshot is taken from https://drugvirus.info/ website. Different shadings indicate different development status of BSAAs. Grey shading indicates that the antiviral activity has not been either studied or reported.

Protease inhibitors and siRNAs

Viral proteases have been successfully used as targets for the development of efficient Hepatitis C and HIV antiviral treatments. The SARS-CoV-2 presents a 3C-like cysteine protease (3CL protease) essential for the virus life cycle and thus representing a promising target for the development of antiviral prophylaxis/treatment. We have already begun to identify protease inhibitors against 2019nCoV 3CL protease for the development of therapeutics. For this purpose, we will employ a combination of *in silico*, enzymatic, biophysical, and cell/virus-based methods. Selected compounds will finally be evaluated in a SARS-CoV-2-Ferret infection model.

Compounds with inhibitory activity against SARS-CoV-2 3CL protease will be identified by screening SARS 3CL protease inhibitors, already approved drugs and libraries of small lead-like compounds. Each category of compounds presents different advantages: SARS 3CL and 2019nCoV 3CL protease share 96% amino acid sequence identity. Therefore, it is most probable that known SARS 3CL protease inhibitors will show inhibitory activity also against 2019nCoV 3CL protease. Approved drugs have the advantage of a well established safety profile. Thus, the use of already

approved drugs for the treatment SARS-CoV-2 3CL may represent the fastest route toward an antiviral treatment of 2019nCoV infections. Lastly, non-peptidic small lead-like compounds provide high flexibility for further design of novel protease inhibitors.

Known SARS protease inhibitors will be synthetized and screened using a FRET-based fluorogenic protease enzymatic assay. The assay will use recombinant SARS-CoV-2 3CL protease similar to assays already established in our laboratory (partner 4) for analysis of protease inhibitors against Zika and Tick-borne encephalitis viruses. Large libraries of approved drugs and lead-like compounds will be screened *in silico* using a combination of molecular docking and molecular dynamics simulations. The binding affinity of the most promising compounds will be estimated using The Linear interaction energy method (LIE). The most promising compounds will then be tested using the FRET-based fluorogenic protease enzymatic assay introduced above.

The structural basis of inhibition of compounds with high inhibitory activity against 2019nCoV 3CL protease will be studied by x-ray crystallography. Crystal structures of selected compounds in complex with the 3CL protease will help guiding the compounds further optimization. Several compounds variants will thus be synthetized and tested establishing a structure activity relationship profile. The antiviral activity of optimized compounds will be assessed using cell-based assays. Cell-based experiment will be carried out in adequate BSL3 lab facility.

All compounds will be synthetized by Anja Sandström group, Dept. of Medical Chemistry, Uppsala University (UU). The Sandström group has vast experience on the design and synthesis of protease inhibitors for viral diseases i.e. HIV and Hepatitis C, also in collaboration with Pharmaceutical companies. We have our own BSL3 facility at the Zoonosis Science Center (ZSC), UU. Advance computational resources will be provided by the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX), UU. Protein Science Facility (PSF) Karolinska Institute, Stockholm, is a core facility which rapidly provides recombinant proteases, as e.g. during 2019 Zika and TBE recombinant proteases. They delivered (?) the SARS-CoV-2 3CL protease in Feb 2020.

Recent technology developed in Julkunen group laboratories (UTU) (Jiang et al., 2019a) enables the production of virus-specific RNA molecules in high quantities that enable further production of Dicer cleaved **small interfering RNA** (DsiRNA) swarms that can target multiple regions in viral genomes. Proof-of-principle analysis of DsiRNA antiviral activity against highly pathogenic H5N1 and other influenza viruses in primary human macrophages and DCs has just recently been described by partner 2 (Jiang et al. 2019b). The method is based on a synthetic large viral RNA molecule representing conserved viral sequences followed by *in vitro* synthesis of ssRRNA and dsRNA molecules by bacteriophage T7 and Phi6 RNA-dependent RNA polymerase (RdRp) enzymes, respectively (Jiang et al., 2019a). SARS-like coronaviruses, like SARS-CoV and SARS-CoV-2 have highly conserved sequences within their genome, which enables the construction of a synthetic 2-3 kb SARS-CoV-2 and SARS-CoV-specific gene fragment. This chimeric gene functions as the template for the production of SARS-CoV-2-specific DsiRNA swarm enabling the analysis of its antiviral potential against the novel coronavirus. DsiRNA swarm delivery into target human primary cells and stable lines have been previously optimized (Jiang et al. 2019a). With this approach we are able to provide information on the applicability of a DsiRNA approach as an antiviral substance against SARS-CoV-2. In case SARS-CoV-2 is inhibited by DsiRNA in *in vitro* cell models, in primary human macrophages and DCs and in stable cell lines this approach can also be tested experimental animal models.

New antivirals – entry inhibitors

The rationale of this approach is that as obligate parasites, viruses rely on cellular functions to complete their life cycle and to spread. Hence, identifying and blocking these functions will provide new avenues for therapeutic intervention against viral diseases. Each step of the viral infection cycle depends on hundreds of cellular factors, organized in functional clusters, and encoded in the genome of the host cell. Each cluster regulates fundamental cellular processes, such as membrane trafficking, protein synthesis, and immune responses that viruses have to hijack and manipulate to their advantage. To efficiently interfere with viral spreading and its pathogenic outcome, we aim to identify and characterize the host factors essential for the establishment of SARS-CoV-2 infection, both as individual molecules and as part of regulated networks.

Monoclonal antibodies

Monoclonal antibodies are a well-known therapy for virus infections. The problem is the characterization and efficient production of these antibodies. Using convalescent plasma is limited in large scale epidemics and production of immunopotential serum in animals requires either an animal host resistant but infection competent to the virus or a molecule comparable to a vaccine.

Our intention is to use new techniques where we analyse convalescent blood for antibody-producing cells, sequence and clone the genes generating neutralizing antibodies. We thereafter produce the antibodies as well as, truncated, bioactive forms which can then be used to study neutralization efficiency, produce better diagnostics and provide a direct therapeutic measure. The production processes are optimized to enable even large scale production sufficient for drug production.

Building a toolbox for rapid diagnostics

Production of recombinant coronavirus proteins for antibody production and serodiagnostic tools

A bottle-neck for diagnostic applications is often the availability of viruses and viral proteins. To overcome this problem SARS-CoV-2 as well as SARS-CoV and MERS-CoV structural proteins are expressed by baculovirus and other transient expression systems. Synthetic coronavirus genes encoding all of the four viral structural proteins S, E, M and N are inserted into baculovirus and mammalian expression vectors and proteins are produced as GST- and His-tagged fusion constructs to enable their fast and easy purification. Protein are also produced in their native form without any tags. Previously, partner UTU has successfully expressed and purified SARS-CoV and Ebola virus proteins (Ziegler et al., 2005; Melen et al., 2017). High level expression of SARS-CoV-2, SARS-CoV and MERS-CoV structural proteins enables the production of polyclonal and monoclonal antibodies in various animal species. Coexpression of CoV surface glycoprotein (S) and membrane (M) protein with or without viral envelope (E) and nucleoprotein (N) in the baculovirus likely enables the production of virus-like particles (VLPs) that are excellent immunogens for antibody production and targets for serological methods (EIA, microarray, lateral flow rapid tests) to analyse human IgG and IgM response against SARS, MERS and 2019 coronaviruses. Baculovirus-produced viral structural proteins enable the identification of virus specific immune target proteins that are specific or common to different coronavirus infections. Likely, the S protein enables the type-specific serological diagnostics of SARS-CoV-2 infections from other dangerous and mild coronavirus infections. If necessary, structural proteins from coronaviruses causing mild infections can be produced for the purpose of differential diagnostics. Large scale antibody analyses for population seroprevalence studies or diagnostics for acute viral infections are carried out by

classical EIA or novel dual-mode microarray immunoassay which allows simultaneous detection of viral IgG and IgM antibodies against up to 20 different antigens in a single microtiter well format (Kazakova et al., 2019 and 2020). The assay was developed to efficiently measure antibody levels in a quantitative and specific manner against multiple respiratory viruses or influenza vaccine antigens. Efficient expression of SARS-CoV-2 and other coronavirus proteins by baculovirus ensures basically an unlimited source of viral proteins for multiple immunoassays including rapid point-of-care antibody assays. An additional advantage of recombinant protein production as individual proteins and VLPs is that these proteins do not provide any biohazard compared to work with infective viruses. We also use coronavirus S protein encoding genes to create recombinant Vesicular stomatitis viruses (VSV) expressing coronavirus surface glycoproteins. This enables the analysis of neutralizing antibodies in lower, BSL-2 containment conditions.

Multiple approaches to quick antigen and antibody tests

Antibodies are important markers of disease and can reveal both past and recent infections. There is now an urgent need for rapid development of rational ways of detecting and quantifying antibodies to SARS-CoV-2, both from humans and from animals (potential natural reservoirs). It is evident that the spike protein (S) is the main antigen, and we will express the N-terminal regions of this protein to detect sarbecovirus-like specific IgM and IgG responses that will be tested in the formats below for acute immunity testing. In particular the C-terminal part of the S contains the receptor binding domain, which is expressed for use in specific tests as rapid proxy neutralization tests (see below). A separate test utilizing green fluorescent VS-particles is being constructed as a neutralization test, and our consortium can already use the virus isolated in cell culture (from Chinese tourist in Finland) for a classical reference neutralization test.

We have recently reported that various recombinant proteins as well as peptides of 100 amino acids or longer (megapeptides), designed and synthesized for optimal serological performance, can successfully be used as detection antigens in a Luminex-based suspension multiplex immunoassay (SMIA). Megapeptides can be created quickly from pathogen sequences, which means that we will have SARS-CoV-2 antigens ready for evaluation within a few days. SARS-CoV-2 antigens (peptides, megapeptides and recombinant proteins produced within our consortia) will be evaluated by patient samples provided by our collaborators and by SARS-CoV-2-specific sera produced in mice and rabbits. Established highly sensitive and specific SARS-CoV-2 SMIAs will be used both for patient diagnostics and for future rapid and efficient seroepidemiological studies.

Point-of-care (POC) or point-of-impact diagnostics is a megatrend in today's medicine. To that end, we have created an innovative diagnostic concept based on time-resolved Förster Resonance Energy Transfer (TR-FRET) for on-site rapid measurement of pathogen-specific antibodies. We have provided the proof-of-concept for our patented assay platform, dubbed LFRET, in serodiagnosis of infectious diseases such as acute hantavirus and Zika virus infection. The assay relies on simultaneous binding of fluorophore-labeled antigen and protein L to an immunoglobulin molecule, thus forming a FRET pair. The assay is homogenous (i.e. wash-free) and after combining the reaction components (labeled antigen, labeled protein L, and patient sample) the results are ready in about 15 min. Our studies show LFRET to have a good performance against the gold standard tests performed in central laboratories. Most recently, we demonstrated that LFRET detects antibodies from urine sample, which enables non-invasive testing.

During RAMSES-I, we will test the LFRET's ability to measure anti-nCoV-2019/anti-CoV antibodies from serum/plasma, urine, and saliva samples. We will also set up a TR-FRET based test for the detection of CoV neutralizing antibodies. Our preliminary data shows that a competitive assay, in which the antibodies of patient serum outcompete the binding of a labelled neutralizing

monoclonal antibody to the labelled antigen. We will employ virus-like particles, VSVs pseudotyped with CoV S protein, and recombinant CoV S protein as the antigens for setting up the TR-FRET assays.

Lateral flow point-of-care assay technology offers several advantages over the traditional laboratory methods. Lateral flow rapid immunochromatographic assays do not require central laboratory facilities or equipment and thus can be performed with minimal training in field conditions, hospital wards and doctors' offices. Results are available near the patients in minutes rather than hours and there is no need to send the samples to central laboratories thus shortening the start of appropriate therapeutic and biosafety measures.

For manufacturing the antibody tests, we include a company, Reagena LTD with long experience in validating and manufacturing in-vitro diagnostic CE-marked lateral flow assay, e.g. launched the world's first rapid tests for 1) the detection of acute hantavirus infection (Hujakka H et al. 2001) 2) acute tick-borne encephalitis (TBE) (Korhonen V-P et al. 2014) and 3) chemokine CXCL13 (Pietikäinen et al. 2018) in human samples as a marker of Lyme neuroborreliosis (LNB) patients. The result of Reagena rapid tests is read by the ReaScan rapid test reader which reports the result in a numerical form allowing no room for subjective interpretations with similar performance as the central laboratory enzyme immuno assays. In RAMSES-I project Reagena will develop and commercialize lateral flow assay(s) to detect coronavirus antibodies in human samples within 15 minutes after application of the sample on the test.

RAPID AND SENSITIVE RT-PCR TESTS ARE THE KEY FOR THE DETECTION OF SARS-COV-2 RNA IN PATIENT

Rapid and sensitive RT-PCR tests are the key for the detection of SARS-CoV-2 RNA in patient specimens. To limit the spread of the epidemics and evaluate the need and length of quarantine period for increasing number of asymptomatic risk groups of potentially infected individuals, there is an urgent need for portable point-of-care tests that are sensitive enough for even minute amounts of virus. Higher sensitivity to RT-PCR infection tests could be achieved by digital PCR (dPCR) where the bulk reaction volume of tens of microliters is divided to tens of thousands of pico/nanoliter sized reaction chambers such as water-in-oil droplets or microwell arrays. dPCR allows very accurate and absolute counting of target molecules or copy-number variations in the sample, without the need of controls or standard curves, and with significantly enhanced sensitivity and specificity of the assays. Infection monitoring and diagnostics with dPCR is already utilized in clinical use for many pathogens (Li et al, 2018). However, wider use of dPCR technology in clinical settings is limited by the large, complex, expensive, relatively slow and low throughput equipment (e.g. Bio-Rad QX200 and QX ONE ddPCR systems, Stilla Technologies Naica Chrystal Digital PCR, Thermo Fisher Scientific QuantStudio 3D, Qiagen dPCR). Furthermore, none of the systems are portable and quick enough to enable point-of-care usage of dPCR in remote locations and for infection diagnostics, where the rapid readout would be often crucial.

Saavalainen group (UH) has invented DropPen - a microfluidic chip design actuated by portable manual pressure device that allows rapid generation of monodisperse droplets on chip. Within these all-in-one DropPen chips, tens of thousands of droplets accumulate into single layer of droplets in a collection chamber, where the target DNA/RNA amplification and SybrGreen (or TaqMan) signal enhancement takes place in thermocycler. The chip is then imaged with fluorescent microscope and positive and negative droplets counted with image analysis software (Aiforia Technologies). Commercial scale mass fabrication process is underway with a novel thermal imprinting process for polydimethyl siloxane (PDMS) developed recently by the VTT partner. UH and VTT groups have a joint commercialization funding from Business Finland, to develop DropPen for single cell analytics and dPCR applications. In this EU-consortium project the chip and device will be rapidly

applied and validated to point-of-care diagnostic tool for ultrasensitive and rapid detection of SARS-CoV-2 from human samples. We begin from Technology Readiness Level TRL3 (experimental proof-of-concept) and aim to rapidly mature during project to at least TRL6-7 or higher in the 3 year project. Rapid commercialization via spin-off company, co-development or licencing to existing diagnostic companies already during the project would speed up this process. DropPen aims to be the first ever portable point-of-care tool utilizing dPCR accuracy.

INFORMATION RELEVANT TO DEVELOPMENT OF THERAPEUTIC AND DIAGNOSTIC MODALITIES

Several partners (UH, UTU) have previously analysed the replication of emerging viruses including SARS and MERS-CoVs, avian influenza and Zika viruses in primary human leukocytes, neuronal cell models and other established stable cell lines (Tynell et al., 2016; Westenius et al. 2014, 2018; Kuivanen 2016; Österlund 2019). Immune cells as resident cells also in the respiratory track of humans are often the primary targets for viral infections. In addition, they form the first line defence against viral pathogens, since they play an important role in innate immunity (macrophages, DCs) and regulating the activation of adaptive immunity (DCs). Human primary macrophages and DCs have receptors for SARS-CoV and MERS-CoV and they can be readily infected by these viruses (Ziegler et al., 2005; Tynell et al., 2016). However, SARS and MERS-CoVs do not efficiently replicate in primary human leukocytes. Viral RNA is expressed, but there is no efficient translation of viral RNAs and the production of progeny viruses is impaired. However, SARS and MERS-CoVs readily replicated in lung-originated Caco-3 epithelial cell line (Tynell et al., 2016). One of the first things to be analysed is to study the replication of SARS-CoV-2 in primary human macrophages and DCs and in other human cell lines. This work is done by several partners (UH, UTU and UU), which all have well-functioning BSL-3 facilities for analysing the replication of SARS-CoV-2. It is expected, but not known for sure before it is analysed, that the novel SARS-CoV-2 is able to replicate in primary human cells and cell lines, since the virus has been efficiently spreading in the human population.

Innate immunity contributes both to protection and immunopathology of severe respiratory viral infections. Infections with avian influenza, SARS-CoV, MERS-CoV and the novel SARS-CoV-2 can in some individuals lead to a severe lung infection and inflammation characterized as an acute respiratory distress syndrome (ARDS). Highly pathogenic viruses trigger ARDS more often. *In vitro* analysis of macrophage and DC cultures with highly pathogenic avian H5N1 influenza virus, but not those of seasonal influenza or low pathogenic avian H7N9 viruses, indicated that the H5N1 virus efficiently spreads in primary immune cell cultures and induces a strong inflammatory and antiviral IFN response (Westenius et al., 2018). This response may contribute to the development of ARDS. Analysis of SARS-CoV-2 infection in primary human immune cell models provides important information whether the new virus is able to efficiently replicate in macrophages and/or DCs and whether the infection induces strong inflammatory responses. The replication of SARS-CoV-2 is analysed by RT-PCR to detect the expression of viral RNA and Western blotting and flow cytometry to analyse viral protein expression in different cell types. Viral production is analysed by classical plaque or TCID50 assays or the secreted virus is quantitated by RT-PCR. At the same time host innate cytokine gene expression and activation of host cell signalling pathways are analysed by established methods (Tynell et al., 2016, Westenius et al., 2014, 2018, Österlund et al., 2019). These analyses provide information of the pathogenesis of SARS-CoV-2 infection in the human system and may lead to identification of new mechanism in virus – host cell interactions and provide novel targets for antiviral therapy that are targeted to either stimulate antiviral response and/or reduce excessive inflammatory response.

Infectious diseases affect both men and women, therefore they are relevant to both sexes. At present, little is known regarding possible sex and/or gender differences in the risk of exposure to SARS-CoV-2 and if sex differences contribute to the varying severity of these infections. Interestingly, there is sex-specificity in recognized cases of some zoonotic infections, and this has also been suggested now for SARS-CoV-2. As there is a wide gap of knowledge regarding possible gender differences in the susceptibility, disease presentation, severity, and outcome in SARS-CoV-2 infections, additional epidemiological studies are sorely needed.

1.4 Ambition

Our ambition is to enable versatile and point-of-care deployable detection of SARS-CoV-2 and to provide therapeutics for curtailing the impact of this pandemic, while preparing simultaneously for future coronavirus threats.

The first line of therapeutic compounds to be tested for use against SARS-CoV-2 are those that have already been tested and accepted for use in humans. Depending on the results and the stocks available for the drugs, we can rapidly translate these or their combinations to trials in patients when safe levels are known and available. Depending on the development of the epidemic/pandemic, we will do this either in China or Europe – a separate ethical review will take place for evaluating the process after preclinical studies are completed. Accessing materials to create a library of monoclonals protecting from virus attachment will commence immediately and will result in cloning protective antibodies from humans, as well as animals. As soon as the candidate MAbs show protection in animal models, we will rapidly translate the findings to human trials

While the real-time RT-PCR assays have been already developed and distributed with unforeseen speed, a key hurdle is the availability of the assays in resource-poor areas, their dependence on central hospital -level laboratory facilities with RT-PCR machinery, and delays in sample logistics, laboratory queues and workflows. All of these are hampering the timely decision-making for individual patients at the point of care, or e.g. individual travellers. In particular, as some individuals with SARS-CoV-2 may be subclinically infected, rapid screening e.g. at ports of entry or from environmental samples may be needed in some scenarios. The technical solution, DropPen device and chips, that we develop and commercialize in this project will bring the outperforming sensitivity and accuracy of digital PCR concept first time ever to point-of-care testing of SARS-CoV-2.

As already known, most individuals contracting the SARS-CoV-2 will be mildly and some subclinically infected. These individuals will become immune to SARS-CoV-2 often without being diagnosed during the period of excreting virus (and being positive for viral RNA in nucleic acid tests). A complicating issue is also the fact that during the SARS-CoV-2 outbreak many more individuals will be infected by the 150-200 other human respiratory viruses causing similar symptoms. Furthermore, as the clinical course of SARS-CoV-2 infection may be prolonged with severe symptoms beginning only in the second week after the onset of illness, serology will play an important role both in the acute diagnostics as well as helping large segments of the population in an epidemic situation to know whether they have been already infected and are immune, or if they are susceptible. Serological tests are not available yet, and the newly developed tests need to be very specific and sensitive. We have an excellent track-record in developing rapid immunochromatography tests to zoonotic infections and we will transfer this knowledge to rapidly provide and upscale production for SARS-CoV-2 IgM and IgG tests for diagnostics and immunity

detection. In addition we will use a technology for measuring antibodies competing the neutralizing antibodies binding to nCoV receptor binding site, to be used as a proxy test of having protective immunity to SARS-CoV-2. This will be further useful in a scenario of epidemic returning repeatedly to an area (as known for influenza pandemics) or large use of vaccinations, and possibly if level of neutralizing antibodies associates with outcome or course of disease, also in patient care.

Finally, during the 3 years we will gather essential basic information on cellular pathways used by the SARS-CoV-2, as well as innate immunity responses and structural insights to human antibody response, and monitor the viral variants evolving. A possibility is that in severe infections, the virus may have mutated in its properties. The will help to adjust the diagnostic tests, but also to show "weak points" on the viral replication that could be targeted by antivirals, and to e.g. modify the antibodies used for this purpose. Furthermore the studies on innate immunity will inform on possible human use of interferons in therapy (already taking place in China). Our collaborator at KAVI institute in Kenya are specifically competent and experienced in running trials on infectious disease patients.

2. Impact

In the current situation of SARS-CoV-2 emergence, the main way to control the spread is through identifying rapidly those that are infected, efficient contact-tracing and quarantines, as well as travel restrictions. In this case, as well as in the case of pandemic spread or larger epidemics in Europe or elsewhere, a key component in either control or efficient patient care is rapid, reliable and accessible diagnosis. With increasing case numbers, burden for the economy and society is lesser if adequate knowledge on the infection status is acquired instantly for informed decision making. As mild and subclinical cases are known to be common, much of the population may not know if they have been infected with the SARS-CoV-2 - or some other of the more than 150 respiratory viral pathogens circulating. Being able to either acutely in convalescence or later in retrospect to give reliable information on immunity will be essential also for normal function of the society – immune people will not need to be further protected. When a safe vaccine will be implemented, rapid and reliable immunity tests will be further helpful in the evaluations. The rapid test for molecular detection of the SARS-CoV-2 will also assist in assessing possible viral contaminations in the environment. The method developed in this project proposal will be applicable in low-income countries, which will be the weakest point in controlling possible pandemic threat.

2.1 Expected impacts

Expected impact	Stakeholders beneficiaries	Project outcomes
To contribute to the clinical management of patients infected by SARS-CoV-2.	Patients, hospitals and health care providers, pharmaceutical sector	Efficacious therapeutics identified and made available (Partners 1,2,3,4,5)
To contribute to the public health preparedness and response in the context of the ongoing epidemic of SARS-CoV-2.	Public health sector, decision-makers, health-care providers, travel sector	Tools for detecting infection and immunity for rapidly acquiring knowledge for informed monitoring and controlling the spread of infection, knowledge of mutations affecting clinical outcomes (Partners 1,2,4,5,6)
To contribute to knowledge on SARS-CoV-2 infection and immune responses from cellular to population level	Scientific community, public health sector, decision-makers, health-care providers, pharmaceutical and diagnostic sector	Virus-cell interactions and their inhibitors, host responses and kinetics of infection markers revealed, guiding further translational treatment and control efforts (Partners 1,2,4)
To contribute to the diagnosis of patients infected by SARS-CoV-2, including detection of immunity.	Patients, public, Health care providers, hospitals, public health institutes, travel sector	Novel serological and molecular diagnostic tests developed/provided, produced, evaluated, validated and distributed. Enhanced (point-of-care) tools for use in patient diagnosis and follow-up and in infection control (Partners 1,2,4,5,6)

One of the impacts will be use and distribution of novel cutting edge technologies to fight the pandemic threat. While immunochromatography test for rapid serodiagnosis for SARS-CoV-2 will be made with already established production lines to ensure immediate access to and rapid distribution of the most sensitive and specific tests technologies that are currently available, also new cutting edge methodologies are used to develop tests using e.g. new Time-resolved Förster Resonance Fluorescent Transfer for detection of antibodies (acute infection or immunity) developed by us, including a proxy test for neutralizing antibodies. We have recently also reported that peptides of 100 amino acids or longer ("megapeptides") can successfully be used as detection antigens in a Luminex-based suspension multiplex immunoassay (SMIA; Albinsson 2018, 2019, Rönnberg 2017, Rizwan 2016). Megapeptides can quickly be created just from pathogen genome sequences, which means that we will have 2019nCoV antigens ready for evaluation within a few days.

The methodology also for the molecular diagnosis is unforeseen and will provide for the first time the advantages of a platform on the impact of the DROPPEN test.sensitive and accurate digital PCR

in a portable point-of-care DropPen platform developed for COVID-19 diagnostics in this project. This paves the way of the same technology to be rapidly applied later also to other emerging diagnostic needs, e.g. for point-of-care testing of antibiotic resistance in bacteria.

As this is a new disease and the virus causing it was only recently identified, a lot is yet unknown. In order to guide the immediate work ahead, it will be essential to fill some of these knowledge gaps, such as the length of excretion of the virus, kinetics of antibody responses, and correlates of virus and viral antibody determinants with clinical outcomes. Through the emerging literature and collaboration with Chinese colleagues at China CDC and Wuhan, we will rapidly learn more details. We will exploit and utilize the knowledge on other coronaviruses, in particular SARS-CoV sharing the same receptors, origins and pathways of human emergence. However, our work will contribute to the basic knowledge on innate immunity, viral attachment and cellular pathways employed by the virus, and this research done by us and by the international scientific community will guide the translational work. For example, the group in Turku, leaders in innate immunity studies of MERS and SARS will lay grounds on how interferons play a role also in therapeutic use. The virus-interactions studied in detail by Giuseppe Balistreri's group will help understand candidate selection of agents for inhibiting viral attachment or proteases. While the research in Tartu by Denis Kainov's group of repurposing existing human-use-accepted compounds will provide possible treatment modalities, this work will increase our understanding of viral pathways and identify weak points in the viral replication.

A key factor is to follow the viral mutations emerging within the epidemic and also within the host: how do these affect transmission, the clinical picture - how do they affect treatment or resistance. We will closely monitor this development in real time and adapt the strategies accordingly. This will enable us to adjust biosecurity measures, patient management and treatment in tandem with these changes.

The front most technologies and therapies for combatting emerging infections include use of monoclonal antibodies preventing the virus from entering cells. We will employ both single cell cloning as well as phage display libraries to select highly binding antibodies for inhibition. Rapid access to the sources of such antibodies is through the Chinese collaborators in this consortium. Characterization of these antibodies will further provide new knowledge on the human immune responses and how they combat the disease. This will reveal therapies and vaccine targets for future development. These high-binding human antibodies can also be generated de novo with in vitro mutations driving enhanced binding enabling increased speed and accuracy in analysis.

The societal impact consists of shortened diagnostic time at the place where the patient is first seen, better accessibility of serology both in the society and health care, possibility to estimate individual and population level immunity, and an increased spectrum of treatment options for the diseased. As we speak, we are already doing these experiments on the SARS-CoV-2 virus isolated in Finland.

2.2 Measures to maximise impact

a) Dissemination and exploitation of results

The situation for this call is exceptional: an imminent threat to the wellbeing, health and economy of the whole world. While writing this proposal we cannot know how the situation will evolve: whether the epidemic will resolve with current or heightened control measures, or become a pandemic with direct spread to different regions of the globe, or invade the third world, e.g. Africa.. Despite this, in all scenarios the rapid dissemination of the results is essential, with issues like publications or patenting only secondary. Therefore, all methods developed will be immediately distributed and reported to public health officials and made public for other researchers, clinicians

and other stakeholders, whenever applicable and relevant. Said that, and while using e.g. BioRxiv and project websites, as well as social media (twitter)) to disseminate the results, it is also important and evident to report all findings in peer-reviewed journals to maintain quality control of research data and reports, even if this comes with a time lag. With the actual test formats, it is also important that those are made in the standards and formats of best current quality, without typical "high gain high risk" -type of agendas. This will ensure rapid dissemination of the outcomes to become in use without delays or significant risks of technical problems in production or eventual use (pls. see exploitation). Therefore the management WP9 will have specific tasks to maintain frequently updated website for reporting results and a person dedicated to science communication using different routes of media. Our clinical collaborations abroad and within the project will further be updated on a weekly or when needed, daily basis, on the outcomes by a weekly telecon between the partners and collaborators. Particularly, we will communicate also with our Kenyan collaborators on developments of laboratory testing and controls in Kenya and beyond in Africa. Naturally we will be fully open with all our results when their quality and significance is ensured, with other consortia and research groups working on the novel coronavirus and its control.

In this situation it is critical that there is a prompt start for the project - actually we have started it already - to ensure that the results will be available when needed in the rapidly emerging situation.

Exploitation. As for the diagnostic tests, there is already a distributor and key opinion leader network in place to disseminate the rapid immunochromatography tests by an SME (Reagena) that has a solid track-record in development and production of high-performance rapid tests. Detailed commersialization and distribution plans will be prepared for each diagnostic tool during the RAMSES-I project. Similar plans will be developed for other tools. An officially approved registry for clinical patient data and test results will be generated and used according to the current data protection rules. This registry will be used to collect the information for case occurrence and public health purposes. Transfer of patient data between research centers will carried out using appropriate protected data transfer systems. Ethics approvals will be applied for in each participating research centre according to the local rulings.

The neutralization tests based on VLPs are typically for use in standard laboratories, and the VSV reagents can be multiplied by these laboratories or provided by a central laboratory. The TR-FRET format tests will require a fluorescence reader, which is available in laboratories, and which will further be available from a company to provide a field deployable reader. As currently SARS-CoV-2 qRT-PCR-test are already being distributed by several commercial providers in different laboratory formats, there is no need to duplicate what has already been done there, the rapid RT-PCR tests format suggested in this proposal will make the diagnosis more rapid and accessible to low-income setting, why among other things we will be testing it in Kenya with our collaborators at University of Nairobi KAVI institute.

The candidate compounds that will arise from the cell-virus interaction and innate immunity studies, followed by studies in animals will be subjected to analysis of further value in clinical use.

Evaluation of the selected antivirals

As a first line approach, we will select safe-in-man BSAAs, which have shown excellent therapeutic and minimal side effects *in vitro* and *in vivo*. We will evaluate the clinical relevance of BSAAs considering whether drug concentration used for the primary disease indication reaches the pharmacological concentration needed for the repurposed disease indication. We will also estimate effectiveness of treatment using our in vitro and in vivo results. In addition, we will perform economic evaluation based on the costs of treatment from the perspective of the health care payer and society. These analyses will use relevant subgroup characteristics to estimate subgroup-specific transition probabilities and outcomes (i.e.

hospitalization rates, case-fatality ratios, quality-adjusted life expectancy) in the selection of countries involved in the project, for which relevant data is accessible. All evaluations will be subjected to statistical analyses (probabilistic sensitivity and variable importance analysis, as well as bi-variate and threshold analyses on BSA price and relative efficacy). Together with clinicians from China, we will prioritize BSAAs for the treatment of infected patients and draft case study protocols. As for the novel therapeutics, wewe will apply for funding and for relevant ethical permits to initiate as many as possible clinical case studies in accordance with the national and EU regulations (Directive 95/46/EC).

A consortium agreement with a full data management plan will be formulated as soon as possible. A special focus will be on the managing the ownership and access to key knowledge. Consortium members will make available their research data generated in this project in accordance with the relevant option of Article 29.3 of the H2020 model grant agreement. (In a situation of public health emergency, all measures will be taken to provide open access to data.)

b) Communication activities

As noted above, the circumstances of this call are exceptional with high pressure to gain rapidly relevant knowledge on SARS-CoV-2 infection and communicate it rapidly. The release of new information still needs to be performed in a controlled way when the reliability of the data is secured. We will have a person dedicated to public information through project website and twitter accounts and University of Helsinki has specific staff for preparing **press releases**. All **relevant publications will be made accessible when submitted** to journals, also to public servers (BioRxiv) and relevant knowledge is immediately reported to public health officials directly. Naturally all research eventually will lead to **peer-reviewed open-access publications**. OV and ÅL are board members of Emerging Viral Disease Laboratory Network (**EVD-LabNet**) under ECDC, a network specifically dedicated to rapid laboratory specialist information and communication, which will ensure rapid flow of relevant information from the project and to the project within EU researchers and ECDC. Development of all methods will also be communicated and interactions with the pharmaceutical and diagnostic industry will be done in early phase for enabling efficient production of tests developed. Depending on the evolving scenario of the epidemic, the interactions to the clinical collaborations will be communicated through our infectious disease specialist collaborators in Wuhan, Nairobi, Helsinki and Uppsala.

The management WP will ensure communications within the consortium occur through **weekly telecons** between PI:s, and **montly larger consortium meetings** either electronically or physically (the groups are reachable with <1h flight distance). The clinical collaborators abroad, when relevant are invited to weekly PI meeting or in minimum monthly project general meeting. Particularly, we will communicate also with our Kenyan collaborators on developments of laboratory testing and controls in Kenya and beyond in Africa. Communication with other researchers in other consortia and research groups working on the novel coronavirus and its control will be active beyond regular scientific meetings.

The **project website** is weekly updated, and boosted with active use of other media such as through project **twitter** account. The RAMSES-I website will be the main site where regularly updated project information, results and achievements will be made publicly available in English. The website will include and describe the project's objectives, activities, results, and outputs, such as publications and presentations. A blog will be included as a forum where researchers can convey their findings and their significance and implications to end-users more informally. The blog will also allow the posting of moderated comments and questions from the public. The RAMSES-I website will be amended by up-to-date posts on relevant research findings, upcoming meetings, events and other activities in Facebook and Twitter. These social media platforms will be the most

accessible sources of information for the general public in all participating countries and beyond, and also the most accessible media for the public to approach researchers with questions and comments regarding our work.

The main researchers have also actively been in general media for informing public about the SARS-CoV-2 virus and its spread, and project members included in this proposal are involved in capacity building, coordination and communication regarding monitoring, control and handling of outbreaks of zoonotic diseases, so there are also various channels for communication.

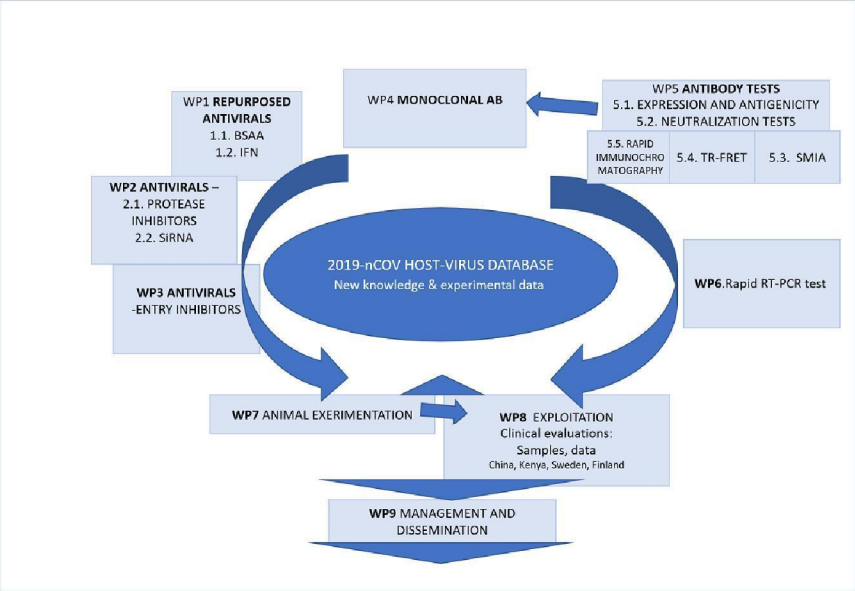
Experience with other projects on Public Health have amply demonstrated that significant effort needs to be directed at translating the scientific and research outputs into material that is actually useful to end-users. Experience has also shown that the required translation process needs to be two-way; both the research scientists and the target users need to be 'educated' in each other's capabilities and language.

3. Implementation

3.1 Work plan — Work packages, deliverables

Table 3.1a: List of work packages

WP No	Work Package Title	Lead No	Lead	Person-Months	Start Month	End month
1	Repurposed antivirals				1	24
2	Antivirals 2				1	24
3	Antivirals 3				1	24
4	Monoclonal AB				1	24
5	Antibody tests				1	24
6	Rapid RT-PCR tests				1	24
7	Animal experimentation				6	24
8	Exploitation				1	24
9	Management and dissemination				1	24


WP1 REPURPOSED ANTIVIRALS
1.1. BSAA
1.2. IFN
WP4 MONOCLONAL AB
WP5 ANTIBODY TESTS
5.1. EXPRESSION AND ANTIGENICITY
5.2. NEUTRALIZATION TESTS
5.5. RAPID IMMUNOCHROMATOGRAPHY
5.4. TR-FRET
5.3. SMIA
WP2 ANTIVIRALS –
2.1. PROTEASE INHIBITORS
2.2. SIRNA
WP3 ANTIVIRALS
-ENTRY INHIBITORS
2019-nCOV HOST-VIRUS DATABASE
New knowledge & experimental data
WP6.Rapid RT-PCR test
WP7 ANIMAL EXERIMENTATION
WP8 EXPLOITATION
Clinical evaluations:
Samples, data
China, Kenya, Sweden, Finland
WP9 MANAGEMENT AND DISSEMINATION

Work package number	1	Lead beneficiary			UTA	
Work package title	Antivirals – repurposed drugs - interferons					
Participant number	1	2	3	4	5	6
Short name of participant	UH	UTU	UTA	UU	VTT	REAG
Person months per participant:	10	9	30			
Start month	1		End month	36		

OBJECTIVE 1 – TO IDENTIFY AND DEVELOP ANTIVIRALS

WP1.1. BSAAs

- To catalog and evaluate toxicity and antiviral efficacy of available BSAAs against SARS-CoV-2 in cell cultures.
- To identify BSAA combinations with synergistic and additive effects.
- To evaluate most promising BSAAs and their combinations against SARS-CoV-2 in primary human cells and organoids.
- To identify drug targets, to elucidate the mode of action of drug substances, and to investigate their on/off-target and adverse effects.
- To evaluate most promising BSAAs and their combinations in vivo.

WP1.2. Interferons

- To analyse the antiviral actions of different doses of type I (IFN-* * *), type II (IFN-*) and type III (INFs-*) against SARS-CoV-2 in human macrophages, DCs and stable cell lines
-To study possible synergistic or additive antiviral activity of different IFN types against SARS-CoV-2
-To study the antiviral actions of IFNs combined with BSAAs and other potentially effective antiviral substances against SARS-CoV-2

Description of work, lead partner and role of participants

Task 1.1. Systematic discovery of novel activities of BSAAs against SARS-CoV-2. Partners: UTA, UH

Our literature review as well as our proof/of concept experiments allowed us to identify 120 safe-in-man BSAAs. We made a database to bring the underlying information on known as well as emerging BSAAs into public domain (https://drugvirus.info/). Here, we will test available BSAAs against SARS-CoV-2 in cell culture. We will test viability and death of compound/drug-treated mock/virus-infected cells and titer the viral progeny or monitor reporter protein expression as described in our previous studies (Bulanova *et al*., 2017; Ianevski *et al*., 2018, Bosl *et al*., 2019).

The half-maximal cytotoxic concentration (CC50) and the half-maximal effective concentration (EC50) for each compound will be analysed. The relative effectiveness of drugs will be quantified

as the selectivity indexes (SI = CC50/EC50). We will select BSAAs with SI>3. Thus, we will identify novel activities for BSAs in cell cultures.

Task 1.2. BSAA combinations. Partners: UTA, UH

Several BSAAs could be combined to inhibit SARS-CoV-2. Combination therapies could result in better efficacy and decreased toxicity against particular virus, and such combinations have become a standard for the treatment of HIV and HCV. To test BSAA combinations, we will treat cells with increasing concentrations of one drug, second drug and combinations of the drugs. We will infect the cells with virus and monitor cell viability/death. The observed responses will be compared with expected combination responses calculated by means of zero interaction potency (ZIP) model (Ianevski *et al*., 2017).

We will determine if combinations have synergistic or additive effects on a viral infection using Bliss Independence and Loewe Additivity models. We will also define a spectrum of viral infections which could be targeted by such combinations ('magic bullets'). Thus, we will identify promising BSAA combinations.

Task 1.3. Ex vivo studies of BSAAs.

We will validate novel activities of selected BSAAs and their combinations in primary human cells isolated from healthy donors (such as human peripheral blood mononuclear cells-derived macrophages and dendritic cells). Cell toxicity, viability, and viral titers will be monitored to validate BSAAs. Next, we will test the BSAAs in human organoids. Organoids virtually recapitulate viral diseases, and serve as an excellent platform for BSAA development, including efficacy evaluation, toxicity testing and pharmacokinetics analysis (R. Vries https://hub4organoids.eu - human epithelial organoids).

Task 1.4. Mechanisms of action of BSAAs.

It is important to understand which stage of SARS-CoV-2 virus infection BSAAs inhibit. For this, we will perform time-of-compound-addition experiments. To retrieve information on BSAA targets, we will individually knock out (KO) corresponding host genes in cell lines using the CRISPR/Cas9 approach 23. KO clones will be selected, amplified, and validated by DNA sequencing, RT-qPCRs and WB analyses. KO cells will be infected with appropriate viral strains and cell death/viability and production of infectious virus particles will be monitored. Wild type (WT) cells will be used as control to determine individual contribution of drug target to viral replication/cell response. Next, we will analyse immuno- and neuro-modulating properties of validated BSAAs using RNA sequencing, cytokine profiling and targeted mass-spectrometry as described in our previous studies (Denisova *et al*., 2014; Fu *et al*., 2016; Soderholm *et al*., 2016; Gaelings *et al*., 2017; Zusinaite *et al*., 2018).

Deliverables

D1.1. novel activities of selected BSAAs in cell culture
D1.2. information on MOA of BSAAs

D1.3. at least 1 efficient BSA combinations D1.4. validated activities of BSAs or their combinations in vivo

For each work package:

Work package number	**2**	**Lead beneficiary**			UU and UTU		
Work package title	Antivirals – protease inhibitors and siRNAs						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:		12		15			
Start month	1			**End month**	36		

OBJECTIVE 1 – TO IDENTIFY AND DEVELOP ANTIVIRALS

Description of work , lead partner and role of participants

Task 2.1. Protease inhibitors. Viral proteases have been successfully used as target for the development of efficient Hepatitis C and HIV antiviral treatments. The 2019nCoV presents a 3C-like cysteine protease (3CL protease) essential for the virus life cycle and thus representing a promising target for the development of antiviral prophylaxis/treatment.

We have already in Jan. 2020 begun to identify protease inhibitors against 2019nCoV 3CL protease for the development of therapeutics. For this purpose, we will employ a combination of *in silico*, enzymatic, biophysical, and cell/virus-based methods. Selected compounds will finally be evaluated in a 2019nCoV-Ferret infection model.

Compounds with inhibitory activity against 2019nCoV 3CL protease will be identified by screening SARS 3CL protease inhibitors, already approved drugs and libraries of small lead-like compounds. Each category of compounds presents different advantages: SARS 3CL and 2019nCoV 3CL protease share 96% amino acid sequence identity. Therefore, it is most probable that known SARS 3CL protease inhibitors will show inhibitory activity also against 2019nCoV 3CL protease. Approved drugs have the advantage of having a well establish safety profile. Thus, the use of already approved drugs for the treatment 2019nCoV 3CL may represent the fastest route toward an antiviral treatment of 2019nCoV infections. Lastly, non-peptidic small lead-like compounds provide high flexibility for further design of novel protease inhibitors.

Known SARS protease inhibitors will be synthetized and screened using a FRET-based fluorogenic protease enzymatic assay. The assay will use recombinant 2019nCoV 3CL protease similarly to assays already establish in our laboratory for analyzes of protease inhibitors against Zika and Tick-borne encephalitis viruses. Large libraries of approved drugs and lead-like compounds will be screened *in silico* using a combination of molecular docking and molecular dynamics simulations. The binding affinity of the most promising compounds will be estimated using The Linear interaction energy method (LIE). The most promising compounds will then be tested using the FRET-based fluorogenic protease enzymatic assay introduce above.

The structural basis of inhibition of compounds with high inhibitory activity against 2019nCoV 3CL protease will be studied by x-ray crystallography. Crystal structures of selected compounds in complex with the 3CL protease will help guiding the compounds further optimization. Several compounds variants will thus be synthetized and tested establishing a structure activity relationship profile. The antiviral activity of optimized compounds will be assessed using cell-based assays. Cell-based experiment will be carried out in adequate BSL3 lab facility.

All compounds will be synthetized by our collaborator Anja Sandström, Dept. of Medical Chemistry, Uppsala University (UU). Anja's group has vast experiences on the design and synthesis of protease inhibitors for viral diseases i.e. HIV and Hepatitis C, also in collaboration with Pharmaceutical companies. We have our own BSL3 facility at the Zoonosis Science Center, UU. Advance computational resources will be provided by the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX), UU. Protein Science Facility (PSF) Karolinska Institute, Stockholm, is a core facility which rapidly provides recombinant proteases, as e.g. during 2019 Zika and TBE recombinant proteases. They will deliver the 2019nCoV 3CL protease by the end of Feb 2020.

Task 2.2. Recent technology developed by partner 2 and coworkers allows the production of basically any viral sequences for in vitro production of large quantities of virus-specific siRNA swarms (Jiang *et al*., 2019a). The technology is based on chimeric SARS-CoV-2 sequences in plasmids that allow in vitro ssRNA synthesis by T7 RNA polymerase followed dsRNA synthesis by bacteriophage Phi6 RNA-dependent RNA polymerase (RdRp). Up to 3 kb dsRNAs are cleaved by Giardia intestinalis Dicer enzyme into 21-23 nt long DsiRNAs creating a swarm of siRNA molecules with more than 100 target sequences in the coronavirus RNAs. DsiRNA swarm is transfected into target cells and their ability to inhibit virus replication is monitored by analysing virus replication by RT-PCR, Western blotting, flow cytometry and viral plaque assay. Proof-of-principle of the functionality of the assay was demonstrated against highly pathogenic avian and other influenza strains (Jiang *et al*., 2019b). In case SARS-CoV-2-specific DsiRNA effectively inhibits nCoV replication their in vivo efficacy in animal experiments are warranted.

Deliverables

D.2.1. Production of recombinant 2019nCoV 3CL protease and establishment of an in vitro FRET-based enzymatic assay (month 1)
D.2.2. Determination of 2019nCoV 3CL protease in complex with a potential inhibitor by x-ray crystallography (month 2)
D.2.3. *In vitro* screening (by enzymatic assay) of known SARS 3CL protease inhibitors against 2019nCoV 3CL protease (month 6).
D.2.4. *In silico* screening of approved drugs (against other diseases) against 2019nCoV 3CL protease. (month 5).
D.2.5. *In vitro* screening (by enzymatic assay) of approved drugs (against other diseases) with predicted activity against 2019nCoV 3CL protease (month 7)
D.2.6 *In silico* screening of non-peptidic lead-like compounds against 2019nCoV 3CL protease (month 5).
D.2.7. *In vitro* screening (by enzymatic assay) of non-peptidic lead-like compounds with predicted activity against 2019nCoV 3CL protease (month 7)

D.2.8.Crystallization of 2019nCoV 3CL protease in complex with best-identified compounds (month 8)
D.2.9. Optimization of the best compounds (month 11)
D.2.10. Cell-based testing of optimized compounds (month 14)
D.2.11. Selection of drug resistance mutations by cell-culture assays (month 18)
D.2.12. Structural study of 2019nCoV 3CL protease drug resistance mutations with x-ray crystallography (month 22)
D.2.13. Efficient production of SARS-CoV-2-specific siRNAs and demonstration their efficacy as antiviral substances (month 18)
D.2.14. Inhibitory activity of SARS-CoV-2-specific srRNAs in vivo in animal infection experiments (months36)

Work package number	3	Lead beneficiary			UH		
Work package title	Antivirals – Entry inhibitors						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:	30						
Start month	1			End month	36		

1. Objectives (GB; UH):

We aim to rapidly identify and characterize the host factors essential for the establishment of 2019NewCV infection, both as individual molecules and as part of regulated networks.

The rationale of this approach is that as obligate parasites**, viruses rely on cellular functions to complete their life cycle and to spread**. Hence, **identifying and blocking these functions will provide new avenues for therapeutic intervention** against viral diseases. Each step of the viral infection cycle depends on hundreds of cellular factors, organized in functional clusters, and encoded in the genome of the host cell. Each cluster regulates fundamental cellular processes, such as membrane trafficking, protein synthesis, and immune responses that viruses have to hijack and manipulate to their advantage. To efficiently interfere with viral spreading and its pathogenic outcome, **we aim to identify and characterize the host factors essential for the establishment of 2019NewCV infection, both as individual molecules and as part of regulated networks.**

Main objectives:

I) Identify and characterize all cellular genes required for establishing infection of selected model viruses by integration of state-of-the-art genome wide CRISPR screens.

II) Assemble gene networks describing host factors functionally interconnected and essential for infection by a specific virus or multiple viruses.

III) Determine the molecular mechanism by which our top-ranked host factors regulate viral replication and identify compounds that alter the function of the identified cellular factor(s) - i.e. combinatorial therapy- to inhibit viral infection.

How the project is linked to previous international and/or national research (state of the art). Large-scale genetic screens based on state-of-the-art gene-editing approaches have been recently performed to successfully identify key cellular regulators of virus infection[1]. During the last five years, these studies have led to important discoveries including (i) the receptors for Ebola virus[2] , (ii) pan-cellular regulators of virus maturation for mosquito-borne epidemic viruses, such as Chikungunya and West Nile virus[3], (iii) antiviral proteins that are able to restrict infection of multiple respiratory viruses[4], and (iv) cellular regulators of viral protein synthesis shared by a broad range of negative-sense RNA viruses, such as rabies and measles viruses, among others [5]. The list of genes that are either required, or that restrict the infection of medically important viruses keeps growing. However, the specific host requirements for numerous viruses are mostly unknown, and little is known of the cellular repertoire of genes and pathways that play a pro- or antiviral role in infection. By integrating two genome-wide loss- and gain-of-function screening approaches, **we will obtain a comprehensive characterization of how the cell genome as a whole is exploited by the SARS-CoV-2 to favour replication and spreading**. A schematic workflow of the consortium project is shown in Figure 1.

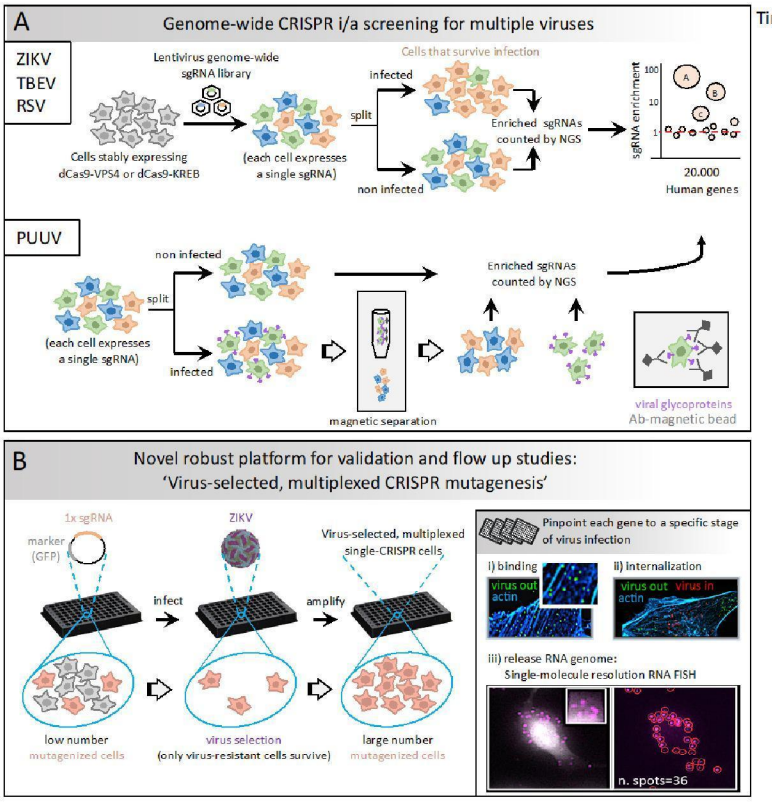


Figure 1. *Workflow of the screening methods currently used by the consortium members GB, OV, JH. A) Genome-wide CRISPRi/a screens for indicated viruses. B) Validation pipeline of the screen top hits, and high-content imaging approach to pinpoint each identified gene to a specific stage of the virus life cycle.*

The results obtained from the screen (Figure 1 A) will provide a list of genes organized into functional clusters. Using a set of automated, image-based assays (Figure 1 B), each of these clusters will be assigned to a specific stage of the virus life cycle, including cell binding and penetration, intracellular delivery of viral genomes, viral protein synthesis and genome replication, as well as virion assembly and release (Figure 1 B, inset). The technical details of each approach are outlined in sections 2 of this proposal.

The top-ranked hits obtained from the screen will be systematically characterized by a combination of cell biology and biochemical approaches, including single-molecule resolution live-cell imaging[6] and structural studies using Cryo-electron microscopy (CryoEM)[7]. These techniques are already established in the laboratories of the consortium members and collaborators. As model systems we will use a SARS-CoV-2 isolated in Finland from a patient on February 10th 2020.

Research questions and/or hypotheses: The strength of the consortium lies on the availability of quantitative assays to monitor each step of the virus life cycle, providing a strong competitive advantage for the post-screening phase of the project.

Our main hypothesis is that by inhibiting or activating cellular factors that play crucial roles in virus infection, we will be able to find new targets for therapeutic intervention. In addition to deciphering the interplay between the virus and the infected cell, we have the possibility to assign new physiological functions to proteins that were previously not characterized.

The specific research questions:

Q1. Which cellular genes are required, or restrict, 2019NCV infection? *Utilizing state of the art screening techniques, such as CRISPRi and CRISPRa, we will systematically identify cellular factors required for viral infection.*

Q2. Can we block virus infection by chemical inhibition of the identified cellular genes required for virus replication? *To develop resistance to a cellular inhibitor, a virus needs to evolve a different infection strategy – a process that in nature occurs very slowly. Therefore, we aim to inhibit infection utilizing readily available chemical libraries to probe the hits identified above. Biophysical and molecular structure determination of cellular targets in complex with hit compounds to further antiviral development.*

Expected research results and their anticipated scientific impact, potential for scientific breakthroughs and for promoting scientific renewal: The consortium brings together a unique collection of top-notch experts and state-of-the-art methods that for the first time will unravel the complex regulatory networks that constitute viral infection. By pinpointing top-ranked identified genes to specific stages of the infection cycle, we will expand the current knowledge of virus-cell interactions, assign new functions to poorly characterized genes, and reveal new targets for antiviral intervention. The specific outcomes are:

R1) From screens to single molecules – mapping gene networks: In the CRISPRi approach, decreasing the levels of a gene product that is required for a given step of the virus life cycle (e.g. decreasing the levels of the virus receptor) will result in strong inhibition of virus infection, allowing the respective cells to survive. Similarly, in the CRISPRa approach, the upregulation of a gene product that interferes with virus infection (e.g. a gene related to antiviral innate immunity) will render the cell resistant to infection. The combined results obtained from CRISPRi and CRISPRa will permit us to identify these critical host factors. The outcome of the screens will be grouped into functional clusters. Each identified gene and gene-cluster will be correlated to a specific stage of the virus life cycle. This approach will assign new functions to poorly characterized cellular factors and unveil key aspects of virus-host interactions. *Rather than focusing on a single gene, the outcome of the consortium is to create a map of gene networks that play key regulatory roles in the life cycle of target virus.*

R2) Defining the "Achilles' heel" of virus infections: The strength of the consortium lies on the availability of quantitative assays to monitor each step of the virus life cycle, providing a strong competitive advantage for the post-screening phase of the project. A key aspect of translation regulation that we aim to clarify is how viruses are able to produce their proteins while the bulk of cellular protein synthesis shuts down. We believe this could be the "Achilles' heel" of virus infection. Protein synthesis and stress responses are also at the core of neurodegenerative diseases and metabolic dysfunction. Thus, our discoveries can provide new paradigms of protein synthesis regulation that can be invaluable to the broad scientific community. Finally, from the follow-up phase of the CRISPRa we expect to identify host factors with intrinsic antiviral activity.

2. Description of work (where appropriate, broken down into tasks), lead partner and role of participants

2.2. Research data and material, methods, and research environment

2.2.1 Research data to be used, justifications and information on data collection/acquisition and use, considering issues such as intellectual property rights (IPR). Gene databases, structural information, and results from previous screens are all publicly available and will be used according to copyright and intellectual property regulations of respective institutions/journals. All data produced during the project will be available to each of the consortium members under confidentiality agreement according to University of Helsinki regulations. The raw sequencing data will be stored securely at CSC (IT Center for Science, https://www.csc.fi/home), to which the researchers at University of Helsinki have access. All sequencing data will be made available upon request and after publishing the major findings the data will also be made available via public repositories such as the Sequence Read Archive, https://www.ncbi.nlm.nih.gov/sra). Structural data will be deposited in the public worldwide Protein Data Base after publication (see the DMP for more details).

2.2.2 Research methods and how they will contribute to answering the research questions or confirming the hypotheses, or how they will support the chosen approach. The consortium utilizes carefully selected virus model systems and complementary experimental approaches to elucidate the genomic networks of viral infection. To this end, we will utilize the following key methods:

Robust model systems: The consortium will rapidly identify the most suitable human cell line to perform the genome-wide screens using fully-automated, high-content image-based assays. The screening platforms are already optimized in in several cell lines, including lung A549 cells, which will be tested first.

Genome-wide CRISPRi/a screens: we will perform two complementary genome-scale screens, a loss-of-function CRISPR interference (CRISPRi) and a gain-of-function CRISPR activator (CRISPRa) screens. Both methods target the promoter regions of each human protein coding gene. CRISPRi causes strong gene repression, CRISPRa gene activation. The feasibility of these approaches has been recently demonstrated. CRISPRa has been successfully employed for influenza virus[4] and for a laboratory strain of ZIKV. We will follow a four-step screening process as described by Sanson *et al.* 2018 (Figure 1A): 1) generation of stable Cas9 expressing cell lines (already completed in A549 and HeLa), 2) transduction of the cell line with the sgRNA library and selection of successfully transduced cells, 3) viral infection, and 4) identification of host genes modulating infection. To confirm the robustness of our methodologies and to improve the likelihood of our success, we already have generated the A549, HeLa and SK-N-SH cells expressing Cas9 variants, which are the cell culture models for other human pathogens such as RSV, ZIKV and TBEV. We have performed a CRISPRa screen for RSV, and a KO CRISPR screen has been set up and sequencing is in progress to identify potential host effectors for TBEV infection. If these cells are not susceptible to infection, a CRISPRa screen will identify activators allowing SARS-CoV-2 to replicate effectively in human cells. This approach is currently being used in our lab in collaboration with consortium members OV and JH for Puumala virus (Figure 1 A, PUUV). Otherwise Vero and MDCK cell lines can be used.

Robust platform for follow-up studies: from gene networks to single molecules: In published studies where CRISPR screens have been used to study virus-host interactions, only one or two cellular proteins were chosen for in-depth, follow-up studies. *We will set up a novel and effective method to overcome this limitation and validate multiple genes at the same time, therefore gaining the full potential of the genome-wide approach: 'Virus-selected, multiplexed CRISPR mutagenesis'.* The sgRNAs of each identified gene will be purchased and introduced by high-throughput reverse transfection into recombinant dCas9-stable cell lines that we have produced in our laboratories (Figure 1B)[13]. To assign each identified gene to a specific stage of virus infection GB has previously implemented an automated, quantitative image-based assay platform for several viruses

including RSV (Figure 1B, inset). The same methods have been used for other viruses (Flu and RSV)[14,15] and will be adapted to the viruses included in this project. Selected hits will be characterized by single-molecule resolution live cell imaging approaches. Proof-of-concept for the successful implementation of these methodologies is shown in Figure 2.

Figure 2. Identification of host regulators of RSV infection using single molecule imaging. *A) Schematic presentation of the single-molecule imaging approaches to study virus mobility and nanoclustering on the PM (uPAINT) and upon internalization (sdTIM). B-C) Validation of top hits identified by genome-wide siRNA screen for RSV. Depletion or inhibition of Na^+K^+ATPase subunit components (B) inhibits a post-entry step of RSV infection (arrowheads), whereas depletion of ribosomal component rpL40 (C) inhibits RSV translation. D) RSV (red spots) induces dramatic, time-dependent changes in the cellular actin cytoskeleton. E) Single molecule tracking (LifeAct-mEos) of actin dynamins during virus infection. Scale bar is 5 µm and the color-coding refers to the time-frame of image acquisition. F) Quantification of single molecule mobility (mean square displacement, MSD; $\mu m^2 s^{-1}$) of LifeAct-mEos in RSV-infected (red line) or non-infected (blue line) cells. The area under the MSD curve (AUC; in arbitrary units [a.u.]) is shown in the inset. (G) Significant decrease in the mobility of LifeAct-mEos upon virus infection indicates a higher binding rate of the construct to F-actin, which most likely is the result of induced actin polymerization. (H-I) Changes in actin dynamics upon RSV infection are transient, showing a trend of initial decrease and subsequent return of the actin mobility to control levels based on (H) MSD, AUC, (I) $Log_{10}D$ and M/IMM quantifications. Statistical analyses of independent experiments were performed using the Student's t test and the statistical significance is indicated as $*<0.05$, $***<0.001$. Error bars are ±SEM.*

A novel antiviral molecule to block SARS-CoV-2 entry

All viruses whose proteins are cleaved by furin will expose peptides that end with the C-terminal consensus R/KxxR/KOH. The 2019nCOV surface protein has a specific furin-like cleavage site that is absent in lineage b CoVs, including SARS-CoV (Coutard et al., Antiviral Research, 2020). Taking advantage of this conserved feature, as a collaborative effort with Estonian consortium partner TT we have developed an innovative method to inhibit virus entry using molecules that selectively bind the R/KxxR/K sequence present at the surface of multiple viruses. Our preliminary experiments demonstrate that the new antiviral molecule, which we called CENDRtrap, is effective in preventing cell entry of Chikungunya virus and Semliki Forest virus, both having surface proteins activated by furin, but not vesicular stomatitis virus, which is not activated by furin cleavage. We will test our novel approach for the SARS-CoV-2 in cell lines, primary airway epithelial cells cultivated in 2D and 3D-air/liquid interface. These cultures are already available in the lab of GB and currently used for RSV entry studies (Coutard et al. Antiviral Res. 2020).

Deliverables

3.1. List of cellular factors/pathways required for SARS-CoV-2. Month 6-12
3.2. Identification/characterization of available small molecule inhibitors against selected hits. Month 12-24
3.3 Molecular details of interactions between selected hits and viral components. Month 24-36

Work package number	4	**Lead beneficiary**			VTT		
Work package title	Monoclonal AB						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:		12			34		
Start month	1			**End month**	36		

Objectives Generation and characterization of neutralizing antibodies from coronavirus patients's peripheral blood mononuclear cells

Description of work (where appropriate, broken down into tasks), lead partner and role of participants

Task 4.1. Expression of SARS-CoV-2 structural proteins spike (S), membrane (M), envelope (E) and nucleoprotein (N) by baculovirus expression. Protein are expressed in native form (no tag) and as GST and His-tagged forms to enable efficient purification of viral fusion proteins. Native protein-expressing baculoviruses are used in Sf9 cell coinfection experiments to produce virus-like particles (VLPs). VLP production may occur in the present S and M proteins, but E and/or N proteins may also be required. Efficient SARS-CoV-2 recombinant protein and VLP production allows immunization of various animal species for the production of monoclonal and polyclonal antibodies. Recombinant SARS-CoV-2 proteins are used for the development of nCoV-specific IgG and IgM antibody assays in EIA, microarray immunoassay and point-of-care rapid assays.

Task 4.2 Generation of recombinant anti-coronavirus antibodies (VTT)

We have two alternative strategies to isolate new neutralizing antibodies. The first strategy is based on cloning antibody genes from human peripheral blood mononuclear cells (PBMC), while the second strategy relies on a naïve human antibody library.

In the first alternative strategy, we clone antibody genes from PBMCs collected from voluntary donors who have survived the SARS-CoV-2 infection. After total RNA isolation and cDNA synthesis, antibody genes will be amplified by PCR using primer sets targeting the variable regions of the heavy and light chain coding regions. A library of the antibody genes will be cloned to a phage display vector, consisting of different heavy-light chain combinations of the original amplified genes, displayed as a single-chain Fv fragments fused to at the N terminus of the phage pIII protein.

In the second strategy, we use a naïve human antibody library that has been constructed at VTT by combining diverse antibody-coding genes from 50 healthy donors (Niemi *et al.* 2011). Thus antibodies are displayed on phage as scFv fragments, and the library is ready to use. In comparison to the first strategy, the coronavirus-specific antibodies in the naïve library may have lower affinity initially, but they can be affinity-matured *in vitro* using targeted random mutagenesis on selected CDR loop residues, followed by screening of the high affinity variants.

Coronavirus-specific antibodies will be searched from the phage display libraries by panning against the coronavirus envelope protein S, i.e. the spike protein. We will use recombinant fragments of the S protein (produced in UH laboratory) containing the external subunits S1 and S2, or specifically just truncated fragments containing the receptor binding domain (RBD), which is a prominent target of neutralizing antibodies for coronaviruses. After 2-4 rounds of panning, we will screen positive clones from miniculture supernatants by ELISA, using antigen-coated microtiter plates and/or protein arrays at the bottom of the wells (Pérez-Gamarra *et al.*, 2017, Lamminmäki *et al.*, 2018). Selected clones will be converted to recombinant Fab fragments and produced in E coli at 1-5 mg amounts for further characterization (see Task 2.3). The purified, endotoxin-free Fab fragments will also be used for neutralization studies in cell culture models of the virus infection (WP1)

Task 4.3 Whole IgG production in CHO cells (VTT)

We will produce selected antibodies also as whole IgG molecules in CHO hosts. For this, we use a mammalian expression vector designed for rapid cloning and production of whole human antibodies, which has been constructed by modifying the pVITRO1-hygro-mcs vector (InvivoGen). In this vector heavy and light chain genes are cloned under different promoters, and cloning sites for VH- and VL-gene fragments have been designed into the junction regions of the variable and constant coding regions. This expression vector has been used for efficient production of human antibodies of different isotypes (Dodev *et al.*. Scientific reports 2014).

Task 4.4. Antibody characterization (VTT)

Selected antibodies will be characterized by Biacore T200 for affinity and kinetic properties (on-rate, off-rate). Specificity and cross-reactivity of antibodies to the spike protein of SARS-CoV-2 versus those of other coronaviruses will be determined by an array-in-well approach, i.e. protein arrays printed in the bottom of microtiter plate wells (Talha *et al.*, 2016). Also, the antibody specificity to variants (mutants) of the spike protein of SARS-CoV-2 will be studied, pending the availability of recombinant proteins and/or sequence data for synthetic peptide libraries. As a result, we will produce reactivity profiles for the new antibodies consisting of K_d, K_{on}, K_{off}, and specificity for different coronavirus S protein variants.

Deliverables (brief description and month of delivery)

D4.1. Production and purification of recombinant SARS-CoV-2 structural proteins and VLPs (M12)

D4.2. Production of monoclonal and polyclonal antibodies against SARS-CoV-2 (M18)

D4.3. Recombinant anti-coronavirus antibodies (M10)

D4.4. Whole IgG molecules for neutralization studies (M12)

D4.5. Antibody reactivity profiles (M18)

Work package number	**5**	**Lead beneficiary**			UTU		
Work package title	Antibody tests						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:	30	4		10		55	
Start month	1			**End month**	36		

Objectives: Development of antibody tests

Description of work (where appropriate, broken down into tasks), lead partner and role of participants

Antibodies are important markers of disease and can reveal both past and recent infections. There is now an urgent need for rapid development of rational ways of detecting and quantifying antibodies to 2019nCoV, both from humans and from animals (potential natural reservoirs).

WP5.1 (UT) For antigen production, the S, M, N and E genes of SARS-CoV-2 will be synthesized and cloned into EBB vector with HA tag for use in mammalian and to a GST vector for use in insect cells (as described for ebola- and zika viruses (Melen et al. 2017; Lundberg et al., 2019)). The respective genes of other human-infecting coronaviruses will serve as controls. These and additional S protein constructs will serve in setting up the TR-FRET assays (see below). We will study the antigenicity of these recombinant proteins, kinetics of IgM and IgG responses as well as cells infected with SARS-CoV-2 virus (Finnish strain) or viral particles purfied from the cell culture media in different formats, including IFA and WB, using Finnish case and Chinese collaborators serum panels.

CoV infected cells produce the S glycoprotein in abundance, and the protein served multiple functions in viral entry and pathogenesis (1, 2). Furin or a related protease cleaves the S protein into S1 and S2 subunits (1, 2). The S1 subunit mediates virus binding to cells through its receptor-binding domain (RBD), while the S2 subunit mediates virus-cell membrane fusion. Several studies have shown that potent neutralizing antibodies (NAbs) against alpha- or beta-CoVs target the RBD region of the S1 subunit (2, 3). We will develop various assays for the detection of human antibodies to SARS-CoV-2 S protein, using S1 and S2 subunits as well as on RBD region of SARS-CoV-2 S protein virus (318-541 aa) as the antigens. The S1, S2 and RBD domains with his-tag at C-terminus are synthesized (alternatively, if delays in gene synthesis, we will amplify them from the SARS-CoV-2 Finnish isolate), cloned into pCAGGS vector (4, 5) under control of CAG promoter, and expressed in HEK293T cells. In addition, we will study the use of alternate expression systems and fusion tags. The corresponding regions of other human-infecting coronaviruses will serve as controls. After the initial evaluations, we will employ the generated antigens in different assay formats, as detailed below.

WP5.2. (UH) Assays for NAb detection. The detection of NAbs will identify if a person is immune to a given disease. While neutralization tests requires cell cultures maintained at proper biosafety level conditions (BSL-3 for SARS-CoV-2), the VSV-based neutralization tests are more rapid and simple, and require the more accessible BSL2. To produce a specific assay for distinguishing infections by different coronaviruses, we offer the VSVΔG-eGFP system in which VSV's surface glycoprotein G is replaced by the enhanced green fluorescent protein gene (eGFP) as adapted in our laboratory (4, 5). The system provides a safe viral entry model because of its inability to produce infectious progeny virus, while cell tropism identical to the native virus. The eGFP marker gene provides identification of infected cells at 12-16 h post-infection. Pseudotyping of VSVΔG-eGFP with SARS-CoV-2 S protein will also serve in studying the functions of

SARS-CoV-2 S protein. Earlier reports show that a pseudotyped VSV bearing a SARS-CoV S protein truncated (Δ18 aa) from the cytoplasmic domain incorporates into VSV particles more efficiently than the full-length S protein (6). To distinguish between different CoVs, we will compare the neutralizing titers of VSV's pseudotyped with the S proteins of various coronaviruses to those pseudotyped with SARS-CoV-2 S protein.

WP 5.3. (UU)

We have recently reported that various recombinant proteins as well as peptides of 100 amino acids or longer ("megapeptides"), designed and synthesized for optimal serological performance, can successfully be used as detection antigens in a Luminex-based suspension multiplex immunoassay (SMIA; Albinsson 2018, 2019, Rönnberg 2017, Rizwan 2016). We can quickly create megapeptides from pathogen sequences, and we will thus have SARS-CoV-2 antigens ready for evaluation within a few days. We will evaluate the SARS-CoV-2 antigens (peptides, megapeptides and recombinant proteins produced within our consortia) using patient samples provided through collaborators and by SARS-CoV-2 specific sera produced in mice and rabbits during RAMSES-I. We will employ the established highly sensitive and specific SARS-CoV-2 SMIAs for patient diagnostics and seroepidemiological studies.

WP 5.4. (UH/Hepojoki) To enable rapid, point-of-care type neutralization proxy testing we will modify our previous approach on TR-FRET (Time-Resolved Förster Reseonance Energy Tranfer), in which patient antibodies compete in binding with mAb against an epitope of the test antigen, for which we provided the proof-of-concept using hantavirus infection as a model (Hepojoki S, et al J Clin MIcrobiol 2015). We will employ libraries generated from PBMCs of those that have recovered of SARS-CoV-2 infection in WP4 as candidate NAbs and test their applicability in TR-FRET based assay for NAb detection. In addition, we will employ the antibodies generated during experimental infections on mice using S1-receptor-binding domain for setting up the assays. The test development begins by identification of the best antigen (recombinant SARS-CoV-2 S protein or its fragments, particularly the receptor-binding domain, SARS-CoV-2 VLPs, or VSV pseudotypes with nCoV-2019 S protein) and system for antigen production, all which are addressed in WPs 5.1-5.3. We will then set up an LFRET assay (based on bacterial superantigen L protein and antigen binding to the same immunoglobulin molecule to produce a FRET pair) for the detection of IgM antibodies to account for acute disease diagnostics (timeline months 0-4). In parallel, we will fine-tune the assay for the detection of anti-nCoV-2019 IgG (and IgA) antibodies. Once we have the NAbs available (from WP4) against nCoV-2019, we will set up the assay for the detection of nCoV-2019 NAbs in patient serum (timeline months 5-12). By the end of RAMSES-I, we will have TR-FRET based assays for identifying an acute nCoV-2019 infection, and determining immunity against nCoV-2019. We estimate to complete the development of TR-FRET assays during 12 months.

WP 5.5. (REAG) Research, development and commercialization of rapid test for the detection of coronavirus antibodies in human samples. REAG will develop a new in-vitro diagnostic lateral flow rapid test for detection of coronavirus specific antibodies in human blood, serum and plasma. Similar approach between the members of this consortium has previously led to sensitive and specific, commercialized rapid tests for hantaviruses and tick-borne encephalitis.
During RAMSES-I, we will develop a unique test, which fulfils the general safety and performance requirements of a diagnostic test set forth in the EU Regulation 2017/746 on in vitro diagnostic (IVD) medical devices. We will perform risk assessments according to the ISO 14971 standard. We will employ the antigens described in WPs 5.1.-5.3. in the test development, and test and optimize the final combination of materials for the assay. In addition, we will evaluate the pilot batches of the developed tests with human sample panels obtained during WP4. We will further provide verification and validation of the developed test and its manufacturing process to warrant that the produced test fulfills its intended purpose, which we will perform in concert with project partners of WP4. The final steps in the R&D project are the registration process and preparation of the EU declaration of conformity. We will make the new product available through Reagenas's distributor(s) and the key opinion leader network. We will advertise the product in relevant scientific exhibitions and conferences, and publish the evaluation results in recognized peer-reviewed scientific journals.
The objective is to ensure that the project meets its targets and milestones within budget and time schedules.

REAG – Specific tasks

T 1 Product development process

T 1.1 Feasibility study
- search for critical components such as antigens, antibodies, carrier molecules, detection particles and assay membranes
- preparation and testing of pilot batches

T 1.2 Preparation of design and development plan
T 1.3 Product development
- the final combination of the selected materials is optimized

T 1.4 Preparation of design and development report, verification & validation plan and performance evaluation plan
T 1.5 Validation and verification of the product and the manufacturing process
- production and testing of validation batches
- verification of the test
- manufacturing process validation
- sending the assay kits to be evaluated in WP7

T 1.6 Risk assessment
T 1.7 Post market surveillance plan
T 1.8 Preparation of technical file
T 1.9 Registration process and preparation of EU declaration of conformity
T2 Commercialization plan
T3 Management of regulatory issues
- Reagena follows the mandatory guidelines set forth in the EU directive for in vitro diagnostic devices (98/79/EY) and the EU Regulation 2017/746 on in vitro diagnostic (IVD) medical devices. In addition, risk assessment will be done according to the ISO 14971 standard. Reagena's certified EN ISO 9001:2015 and EN ISO 13485:2016 Quality Management Systems cover all the processes.

T4 Management of IPR issues
- Reagena will use its own patented proprietary technology when applicable. If needed, Reagena will perform the Freedom-to-Operate (FTO) studies through patent attorneys and makes licensing agreements to have the necessary proprietary rights.

Deliverables

Development of clinically applicable SARS-CoV-2-specific IgG and IgM antibody assays

A validated new in-vitro diagnostic lateral flow assay for detection of coronavirus specific antibodies in human samples ready for market entry

A validated Luminex-based suspension multiplex immunoassay (SMIA) for detection of coronavirus specific antibodies (IgM and IgG) in human samples.

A validated Luminex-based suspension multiplex immunoassay (SMIA) for detection of coronavirus specific antbodies (IgM and IgG) in animal (rodent, bat, bird) samples.

Work package number	6	Lead beneficiary			UH		
Work package title	Diagnostic tools – RT-PCR						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:	26				3		
Start month	1			End month	36		

Objectives

To develop a novel point-of-care droplet digital PCR diagnostic platform for sensitive virus RNA detection from human specimens

Description of work (where appropriate, broken down into tasks), lead partner and role of participants

Task 6.1 DropPen digital RT-PCR assay development and proof-of-concept (UH)

Feasibility of the previously developed DropPen system with portable manual pressure device and disposable all-in-one microfluidic droplet chips in SARS-CoV-2 RT-PCR diagnostics will be tested first with recently published and commercially available corona virus specific RT-PCR assays (Corman et al. 2019). Oligos, fluorescent labelled probes and in vitro transcribed positive control RNA of the tests will be ordered from European Virus Archive (https://www.european-virus-archive.com/evag-news/wuhan-coronavirus). The sensitivity of the DropPen droplet digital assay will be characterized with dilution series with the positive control RNA and compared with the standard RT-PCR results of the same assay. As soon as clinical samples are available and ethical permissions granted, the sensitivity and specificity of the digital DropPen assay will be studied further, and rapid sample preparation methods evaluated and optimized for DropPen platform. Clinical and epidemiological data of the tested patients and potentially infected carriers will be analysed retrospectively in order to determine how low virus counts the detection levels can reach and how well that performs in estimation of quarantine needs of asymptomatic individuals.

Along with PCR amplification based assays, the SARS-CoV-2 RNA targets will be tested also for isothermal amplification in ambient 40-50 C temperature and rapid detection in less than 30 min. Various available protocols (e.g. RT-LAMP, RT-SIBA) and their feasibility, sensitivity and accuracy in DropPen droplet digital format will be evaluated. Isothermal assays would allow even faster read-out of rapid tests that are not dependent on thermocycler availability, enabling the development of a truly portable point-of-care test platform for SARS-CoV-2.

Task 6.2 Product design and mass production process development (UH, VTT)

The proof-of-concept prototype of the DropPen device and chips will be preliminary piloted within the consortium, with members representing also the future end users such as researchers, nurses and laboratory technicians of the diagnostic laboratories and point-of-care units. Based on their user

experience and feedback, the final changes to the design will be made, fabricated and tested in prototype level. After this the most optimal materials (PDMS; polydimethyl siloxane, or thermoplastics) and mass production methods (roll-to-roll thermal imprinting, hot embossing or injection molding) will be selected. The pilot runs will take place in VTT premises in Oulu, Finland. VTT partners have already experience with PDMS thermal imprinting of DropPen chips in our ongoing joint project. Further processes of chip functionalization and lidding will be set up.

Task 6.3 Preparation of the regulative process, partnerships and commercialization

Regulatory processes needed for the eventual use of DropPen as diagnostic platform will be defined and considered throughout the project. Validation and verification of the platform and it's imaging and data analysis components, as well as further piloting and performance evaluation both with consortium partners and in independent customer and end user groups in the health care chain will be done. These include e.g. medical doctors, nurses, diagnostic laboratories, hospitals and health care centers, crematory staff, airport and other public transportation staff. Once the safety and performance requirements are met (EU Regulation 2017/746) and risk assessments done (ISO 14971 standard), the registration process and preparation of the EU declaration of conformity will be finalized. Possible fast-track approval processes may, however, apply to diagnostics due to the emergency of SARS-CoV-2 epidemic, and these will be applied to DropPen platform if available.

Potential partners from diagnostics industry will be actively searched throughout the project, in order to speed up the joint development and/or licencing process of the DropPen technology from academic R&D project to diagnostic tool of potentially urgent global need. Preliminary discussions are under way with the project partner Reagena ((10)(2e)) and Aidian (previous Orion Diagnostica; Mirko Brummer). Partners and suppliers with supporting technology such as fluorescent microscopy and image analysis software, are also searched for. The current chip prototype is imaged with standard fluorescent microscopes detecting SybrGreen/FAM/FITC labels, which are commonly available in research labs and institutes. However, low cost and mobile microscope solutions would be optimal for a rapid point of care test environment in future. In the prototype level we currently use Aiforia image analysis software to count the positive vs negative droplets. Analysis solutions for the future point-of-care platform will be characterized and developed during the project, with potential partner companies or providers.

Along with the regulation and partnerships search, market and freedom-to-operate analyses will be performed, novel IPR created during the project will be secured, and finally the productization and commercialization plans done, with a go-to-market strategy.

Deliverables

D6.1 Proof-of-concept data and publication of DropPen digital platform feasibility to SARS-CoV-2 diagnostics (month 12-18)

D6.2 Tested mass fabrication method for the DropPen chips and device (month 24)

D6.3 Strategic partners and go-to-market strategy for DropPen platform (month 24)

Work package number	7	**Lead beneficiary**	UU

Work package title	Animal experimentation						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:			5	40			
Start month	1			**End month**	36		

Objectives

To test newly developed 2019-CoV antivirals in animal models assessing i) effect; ii) toxicity; and iii) PK/PD.

Description of work

An effective antiviral for treatment and/or prophylaxis of SARS-CoV-2 represents a crucially important step in mitigating the disease. This WP will take antiviral candidates identified in WP1-3 from *in vitro* through two required *in vivo* steps in preparation for human trials.

Rapid testing in relevant animal models is an essential step to get SARS-CoV-2 antivirals on the market as soon as possible. To do the testing in an efficient manner, and to satisfy the need for testing in two distinctly different animal species, we will use a step-wise approach of mouse and ferret animal models.

Mice constitute a natural first step in animal models because of availability and cost- and space-efficiency. It is not yet established if SARS-CoV-2 infects mice, but SARS-CoV gives rise to viremia and clinical signs in aged BALB/c mice and thus this will be our first step to establish a mouse animal model to screen multiple antivirals. Of note is that young BALB/c mice do not get the same clinical picture, so inclusion of animals at different ages will be an important variable.

Ferrets are the gold standard for influenza studies in mammals, can produce ferret-to-ferret transmissible SARS-CoV infection and is therefore also likely a suitable model for SARS-CoV-2. Josef Järhult is currently a visiting scientist at the Australian Animal Health Laboratory (AAHL) in Geelong, Australia. At AAHL, BSL-4 compatible animal facilities and labs are available and pilot ferret trials to establish an animal model for SARS-CoV-2 are being conducted Feb-April 2020. Thus, we have a ready template on how to set up this relevant animal SARS-CoV-2 model. The ferret model will then be set-up at the BSL-3 animal facility at Karolinska Institute (KI), Stockholm, Sweden.

Newly developed antivirals will be tested with regard to i) effect; ii) toxicity; and iii) PK/PD. Effect evaluation will be based on mortality, clinical signs, virus detection by PCR and histopathology, as compared to untreated controls. Toxicity will be assessed by clinical signs, clinical chemistry tests regarding liver, heart, kidney and muscular damage, and histopathology in treated, uninfected animals, as well as in the regular treatment groups. Different doses will be used to determine an appropriate balance of toxicity and effect. Antiviral concentrations will be measured in serum and lung tissue to construct relevant PK/PD models.

Deliverables (brief description and month of delivery)

D7.1. SARS-CoV-2 animal model in mice established at KI (month 6)
D7.2. 12 months – Mouse model up-and-running, clinical, biochemical, virological and histopathological natural course of SARS-CoV-2 determined, infectious dose and experimental protocol established. (month 12)
D7.3. Ferret model established at KI (learning from method presently being established at AAHL) (month 15)
D7.4. Pilot testing of first antiviral candidate in mice finished (month 18)
D7.5. Ferret model up-and-running, clinical, biochemical, virological and histopathological natural course of SARS-CoV-2 determined, infectious dose and experimental protocol established (21 month)
D7.6. Testing of at least 2 antiviral candidates in the mouse model finished using final, optimized protocol (month 24)
D7.7. Pilot testing of first antiviral candidate in ferrets finished (month 27)
D7.8. Testing of at least 4 antiviral candidates in final mouse model finished (month 30)
D7.9. Testing of at least 2 antiviral candidates in the ferret model finished using final, optimized protocol (month 36)
D7.10. One antiviral candidate most suitable for testing in primates/first human trials (month 36)

For each work package:

Work package number	8	**Lead beneficiary**				UH	
Work package title	Evaluation						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:	10		4	4		4	
Start month	1			**End month**	36		

Objectives

This WP will focus on coordinating and arranging data and clinical materials for evaluations of the molecular and serological tests, as well as to arrange for clinical testing of the lead antiviral candidates and analysing the results in the context of patient data. Depending on the scenarios – whether the epidemic will be contained with majority of cases from China; full-blown global pandemics; or sustained circulation in resource-poor countries: we have arrangements in place to secure access to samples and clinical collaboration for the project.

Description of work

We have already secured collaborative arrangements to have samples from Wuhan, China, collaborating with Dr Xiong Yong, Director and chief physician, Department of infectious disease, Zhongnan hospital Wuhan, China as well as Professor Yong-Zhen Zhang PhD, Head of Department of Zoonoses, National Institute for Communicable Disease Control and Prevention, Chinese CDC, Beijing, China, the research consortium which was first to report and release the sequence of the SARS-CoV-2 genome.

In Kenya we are collaborating with University of Nairobi, KAVI institute for clinical research which is particularly experienced and authoritative in clinical trials on infectious diseases, and with whom the UH group has also collaborated on preparedness on emerging infections.

For Finland and Sweden, Professors of Infectious Diseases at universities (and university hospitals) of Helsinki and Uppsala are part of the partner teams (Prof (10)(2e) and (10)(2e), respectively) and will coordinate additional patient samples and data of relevance to the project, in addition Professor Vapalahti is a chief physician at UH hospital laboratory with access, through ongoing research projects, to patient samples.

A separate ethical review will take place for all patient materials and data where relevant; due to the rapid call, this was not possible beforehand.

The patient samples needed for evaluation include respiratory extracts (nasopharungeal swabs, nasal aspirates, throat swabs, conjunctival swabs etc.) from suspect and diagnosed patients, serum blood, urine and fecal samples. We will extract 40 ml of blood from 2-5 recovered patients for preparing peripheral blood mononuclear cells for subsequent antibody library to screen for

neutralizing or diagnostic human monoclonal antibodies (see WP 4).

Our clinical collaborators will test BSAAs or their combinations against drug-resistant infections or severe viral infections. For this, they will select subjects among infected patients from the wards or the intensive care units (ICU). The assessment for hospitalization will be carried out according to local practices: if the clinician evaluates the patient as not being able to manage at home, the patient will be hospitalized. As for those attending ICU, the principles of evaluation will be consistent with the Infectious Diseases Society of America guidelines on community-acquired pneumonia 25. The patients will give their written informed consent. For ICU patients, consent for sample collection will be received from relatives or from the patients after treatment. For children, consent for sample collection will be received from their parents. Clinicians will treat patients with BSAAs in accordance with good clinical practice, study protocols, ethics, etc. Our clinical partners will also collect medical data and samples from patients during and after infection. Sample collection time will depend on the origin of viral disease and other factors. The samples will contain valuable information to assess the therapeutic and adverse effects of the treatments. We will detect viruses in the samples, test host immune factors using standard assays and report the results to the clinicians. Clinicians will invite patients who recovered from viral diseases after treatment with BSAAs to participate in our annual meetings/teleconferences and share their experience on a voluntarily basis. All patients' data and samples will be treated anonymously. The anonymized patient information will include age (0-10, 11-20, 21-30 and so on), sex (f/m), hospitalization (yes/no), date of sample collection (week, year), and diagnosis. The anonymized patient data will be stored in secure local databases under restricted access for only the named scientists of the present project. All patient data and samples will be destroyed at the end of the project.

Deliverables (brief description and month of delivery)

8.1. Patient respiratory sample panels for evaluation of RT-PCR-tests

8.2. Patient serum/plasma sample panels for evaluation of serological test

8.3. PBMCs for establishing a human antibody library

8.4. Ethical review timelines and conditions on potential use of antivirals in clinical trials

8.5. Establishment of permits and guildelines for possible clinical trials in Nordic Countries/Kenya/China depending on pandemic/epidemic scenarios

For each work package:

Work package number	9	**Lead beneficiary**				UH	
Work package title	Management and dissemination						
Participant number	1	2	3	4	5	6	
Short name of participant	UH	UTU	UTA	UU	VTT	REAG	
Person months per participant:	20	1	1	1	1	1	
Start month	1			**End month**	36		

Objectives:

To implement the project management structure, and oversee the project

To promote awareness and coordinate communication strategy targeted at the key end users

Description of work:

WP9 is responsible for overall consortium management of RAMSES-I, which consists of the tasks coordinated by UH (Tarja Sironen) with the assistance of the other lead investigators, and the management and advisory boards. Furthermore, this WP will manage communication and dissemination efforts with the assistance of UH communications services. Timely sharing of accurate and evidence-based information is of key importance in managing the public response to the emergence of SARS-CoV-2.

Task 9.1. Implementation of management bodies and decision-making processes (UH)

Task 9.2. Organization of regular meetings, managing internal progress reports (UH)

Task 9.3. Technical and financial reporting to the Commission (All partners)

Task 9.4. Development of data management plan (All partners)

Task 9.5. Online visibility and presence (Lead UH, all partners): development of a public website, set up of social media account (Twitter) and regular press releases and appearances in media

Task 9.5. Coordination of publications (UH) – ensuring proper acknowledgment of the RAMSES-I project

Deliverables (brief

D9.1. Consortium agreement (Month 0)

D9.2. Project management plan (Month 1)

D9.3. Dissemination and communication plan (Month 1)

D9.4. Data Management Plan (Month 6)

D9.5. Exploitation Plan (Month 12)

D9.6. RAMSES-I website launched (Month 1) D9.7 Communications reports (Month 6, 12, 18, 24, 30, 36)

For each work package:

Work package number		**Lead beneficiary**					
Work package title							
Participant number							
Short name of participant							
Person months per participant:							
Start month	1			**End month**	24		

Objectives:

Description of work:

Deliverables (brief

Timing of the work packages

	3	6	9	12	15	18	21	24	27	30	33	36
WP1												
WP2												
WP3												
WP4												
WP5												
WP6												
WP7												
WP8												

WP9 [redacted]

Table 3.1c: List of Deliverables

Nr.	Deliverable name	WP	Lead partner	Type	Dissemination level	Del. month
1.1.	Novel activities of selected BSAAs in cell culture	1	UTA	R	PU	6
1.2.	Information on MOA of BSAAs	1	UTA	R	PU	6
1.3.	At least 1 efficient BSAA combination	1	UTA	R	PU	6
1.4.	Validated activities of BSAAs or their combinations in vivo	1	UTA	R	PU	36
2.1	Production of recombinant SARS-CoV-2 3CL protease and establishment of an in vitro FRET-based enzymatic assay	2	UU	R, DEM	PU	1
2.2	Determination of SARS-CoV-2 3CL protease in complex with a potential inhibitor by x-ray crystallography	2	UU	R	PU	2
2.3	In vitro screening (by enzymatic assay) of known SARS 3CL protease inhibitors against SARS-CoV-2 3CL protease	2	UU	R	PU	6
2.4	In silico screening of approved drugs (against other diseases) against SARS-CoV-2 3CL protease	2	UU	R	PU	5
2.5	In vitro screening (by enzymatic assay) of approved drugs (against other diseases) with predicted activity against SARS-CoV-2 3CL protease	2	UU	R	PU	7
2.6	In silico screening of non-peptidic lead-like compounds against SARS-CoV-2 3CL protease	2	UU	R	PU	5
2.7	In vitro screening (by enzymatic assay) of non-peptidic lead-like compounds with predicted activity against SARS-CoV-2 3CL protease	2	UU	R	PU	7
2.8	Crystallization of SARS-CoV-2 3CL protease in complex with best-identified compounds	2	UU	R	PU	8
2.9	Optimization of the best compounds	2	UU	R	PU	11
2.10	Cell-based testing of optimized compounds	2	UU	R	PU	14
2.11	Selection of drug resistance mutations by cell-culture assays	2	UU	R	PU	18
2.12	Structural study of SARS-CoV-2 3CL protease drug resistance mutations with x-ray crystallography	2	UU	R	PU	22
2.13.	Efficient production of SARS-CoV-2-specific siRNAs and demonstration their efficacy as antiviral substances	2	UTU	R	PU	18

2.14.	Inhibitory activity of SARS-CoV-2-specific srRNAs in vivo in animal infection experiments	2	UTU	R	PU	36
3.1	List of cellular factors/pathways for SARS-CoV-2	3	UH	R	PU	6
3.2	Identification/characterization of available small molecule inhibitors against selected hits	3	UH	R	PU	18
3.3.	Molecular details of interactions between selected hits and viral components	3	UH	R	PU	36
4.1	Production and purification of recombinant SARS-CoV-2 structural proteins and VLPs	4	UH	R	PU	12
4.2	Production of monoclonal and polyclonal antibodies against SARS-CoV-2	4	VTT, UTU	R	PU	18
4.3	Patient-derived anti-coronavirus antibodies	4	VTT	R	PU	10
4.4	Whole IgG molecules for neutralization studies	4	VTT	R	PU	12
4.5	Antibody reactivity profiles	4	VTT, UH	R	PU	18
5.1	Development of clinically applicable SARS-CoV-2-specific IgG and IgM antibody assays	5	UH, UTU, REAG	R	PU	24
5.2	A validated new in-vitro diagnostic lateral flow assay for detection of coronavirus specific antibodies in human samples ready for market entry	5	REAG	R	PU	36
5.3	A validated Luminex-based suspension multiplex immunoassay (SMIA) for detection of coronavirus specific antibodies (IgM and IgG) in human samples.	5	UU	R	PU	36
5.3	A validated Luminex-based suspension multiplex immunoassay (SMIA) for detection of coronavirus specific antbodies (IgM and IgG) in animal (rodent, bat, bird) samples.	5	UU	R	PU	36
6.1	Proof-of-concept data and publication of DropPen digital platform feasibility to SARS-CoV-2 diagnostics	6	UH	R	PU	18
6.2	Tested mass fabrication method for the DropPen chips and device	6	UH	R	PU	24
6.3	Strategic partners and go-to-market strategy for DropPen platform	6	UH	R	PU	24
7.1	SARS-CoV-2 animal model in mice established at KI	7	UU	DEM, R	PU	6
7.2	Mouse model up-and-running, clinical, biochemical, virological and histopathological natural course of SARS-CoV-2 determined, infectious dose and experimental protocol established	7	UU	DEM, R	PU	12
7.3	Ferret model established at KI (learning from method presently being established at AAHL	7	UU	DEM ,R	PU	15
7.4	Pilot testing of first antiviral candidate in mice finished	7	UU	DEM, R	PU	18
7.5	Ferret model up-and-running, clinical, biochemical,	7	UU	DEM	PU	21

	virological and histopathological natural course of SARS-CoV-2 determined, infectious dose and experimental protocol established			, R		
7.6	Testing of at least 2 antiviral candidates in the mouse model finished using final, optimized protocol	7	UU	R	PU	24
7.7	Pilot testing of first antiviral candidate in ferrets finished	7	UU	R	PU	27
7.8	Testing of at least 4 antiviral candidates teste in final mouse model finished	7	UU	R	PU	30
7.9	Testing of at least 2 antiviral candidates in the ferret model finished using final, optimized protocol	7	UU	R	PU	36
7.10.	Selection of one antiviral candidate most suitable for testing in primates/first human trials	7	UU	R	PU	36
8.1	Patient respiratory sample panels for evaluation of RT-PCR test	8	UH	R	PU	3
8.2	Patient serum/plasma sample panels for evaluation of serological tests	8	UH	R	PU	3
8.3	PBMCs for establishing a human antibody library	8	UH	R	PU	3
8.4	Ethical review timelines and conditions on potential use of antivirals in clinical trials	8	UH	R	PU	12
8.5.	Establishment of permits and guidelines for possible clinical trials in Nordic countries/Kenya/China depending on pandemic/epidemic scenarios	8	UH	R	PU	24
9.1	Consortium agreement	9	UH	R	PU	0
9.2	Project management plan	9	UH	R	PU	1
9.3	Dissemination and communication plan	9	UH	R	PU	1
9.4	Data Management Plan	9	UH	R	PU	6
9.5	Exploitation Plan	9	UH	R	PU	12
9.6	RAMSES-I website launched	9	UH	DEC	PU	1
9.7	Communications reports	9	UH	R	PU	6, 12, 18, 24, 30, 36

3.2 Management structure, milestones and procedures

Due to the special nature of this call, the partners have agreed that the organisational structure and the decision-making protocols will be established during grant agreement negotiations. This will occur at the first workshop of RAMSES-1 to be organized as early as possible. UH will coordinate RAMSES-I as a whole, and oversee the establishment of effective innovation management team, a scientific advisory board as well as identify a user stakeholder panel.

Table 3.2a: List of milestones (to be updated upon start of the project)

Milestone number	Milestone name	Related WPs	Due month	Means of verification
M1	*Potential therapeutics identified*	*1-4*	*18*	*WP reports*
M2	*Rapid diagnostic tests developed*	*4-6*	*18*	*WP reports*
M3	*Clinically relevant and efficacious therapeutics evaluated*	*1-8*	*36*	*WP reports*
M4	*Management and communication plans established*	*9*	*6*	*Initial workshops organized*

Table 3.2b: Critical risks for implementation

Description of risk (indicate level of likelihood: Low/Medium/High)	WP(s)	Proposed risk-mitigation measures
Limited number of antivirals with novel anti-SARS-CoV-2 activities.	1	The discovery of novel antiviral activities depends on the choice of cell lines, viruses and viral strains, concentration range and purity of compounds, as well as on the endpoint measurements. To overcome these issues, we will implement harmonized antiviral bioactivity data annotation, standardization, curation, and intra-resource integration. To increase the discovery rate of novel anti-SARS-CoV-2 antivirals, we will also test emerging drug candidates. We have at least 31 drug candidates to test in vitro. However, we can combine these agents with each other virus-specific antivirals to identify synergistic/additive effects, as we did in our previous studies [21,28].
Incomplete MOA information.	1	Task will provide only limited knowledge on MOAs of antivirals due to technical bottlenecks, but we will integrate the

		omics data to reconstitute the global portrait of antivirals' MOAs as we have done previously [27].
No response to treatment with BSA combinations.	1	We have enough BSAAs to test their combinations in vitro. However, we can combine BSAAs with virus-specific drugs to identify synergistic/additive effects, as we did in our previous studies [21,28].
Failure to obtain ethical permits.	1	In order to obtain human primary cells amendments to existing ethical permission will be submitted to the national ethical committees immediately after the funding decision will become available. The same applies to genetically modified viral strains and human cells. All mouse experiments will be conducted in collaborators laboratories under existing ethical permissions following 3R policy.
Competing IPR	1 (others?)	There is a risk of competing IPR concerning novel antiviral activities of BSAAs. We will systematically monitor and manage the risks at an early stage together with our TTO by reviewing the scientific literature and patents.
Lack of communication.	1 (others?)	The data is expected to be novel and at the cutting edge of biomedical research and thus will be published in the best biomedical and general publication series.

3.3 Consortium as a whole

The objectives of this call request accessing diagnostics of SARS-CoV-2 with rapid tests and to provide possible antivirals, which led to collecting the gallery of partners now in this RAMSES-I proposal having relevant experience and excellence and with previous collaborations on development of tools and test to combat emerging viral infections. The partners include an SME with long traditions in collaborating with academic partners and for producing rapid tests which have been evaluated to be highly sensitive and specific in the case of other viral diseases (REAG), We have groups working on specifically emerging viral zoonoses and doing translational development research with hospital diagnostic laboratoriees (UH, UTU, UU), groups specialized in antiviral development (UTa, UU) and virus-host interactions (UH, UTU) and a group with a novel concept for nucleic acid testing, and infectious disease research on animal models (UU). This is supplemented with key collaborators on clinical research from China and Nairobi. Altogether the consortium members share previous successful collaborative projects on (including EU grant funding) e.g. concerning hantaviruses (UU and UH), ebola preparedness (UU, UTU) zika (UH, UTU, UTa and VTT). The UTU group has worked previously on innate immunity of highly pathogenic coronaviruses. Päivi Saavalainen's group has already developed a new approach to performing RT-PCR and the Kainov, Vapalahti and Julkunen groups have previously collaborated on new antivirals, and UH and VTT on developing human monoclonal antibodies. Each partner complements the consortium from diagnostic laboratories to technological innovations and basic and translation research on cellular and molecular interactions. The work is already ongoing with the recently isolated SARS-CoV-2 strain from Finland and the partners are in frequent contact all the time.

The commercialization status is described in more detail in the workpackages, and the exploitation particularly of the rapid serology tests will be immediate.

For clinical collaborations, the part of the globe most involved depends on which epidemic or pandemic scenarios will take place, thereby we have collaborators for clinical samples and studies in Europe, Kenya and China.

Our main clinical collaborators In Nordic countries will include within UU, MD, Professor of Infectious Diseases, Dr (10)(2e), with long track record on studying e.g influenza, and part of the Zoonoses Research Center in UU; and within UH, MD, PhD, Professor of Infectious Diseases, Dr. (10)(2e), Senior Medical Officer at Helsinki University Hospital (HUH, head of Aava Travel Clinic in Helsinki, and the founder and head of Meilahti Vaccination Research Center, MeVac at HUH.

For Kenya we collaborate (through longstanding collaborations with UH Viral Zoonsoes group and University of Nairobi KAVI institute of Clinical Research, College of Health Sciences University of Nairobi, an institute conducting clinical research projects in Kenya. Our main collaborators are Dr. **Omu Anzala**, professor of virology and immunology at the University of Nairobi with vast experience in infectious disease research and central figure establishment of KAVI-Institute of Clinical Research. He is Principal Investigator and co-investigator in several clinical trials and basic research projects on HIV, Ebola and influenza, among others. He has a rich network of collaborators and research partners across the country and the globe. He is also a member and consultant for various local and international bodies, including a taskforce on the 2019 novel coronavirus, Ministry of Health, Kenya. Within KAVI, another important team member and collaborator is Dr. Moses Masika, a physician, lecturer and research scientist specialized in tropical and infectious diseases and global health leadership and management, who is also leading efforts to improve laboratory infrastructure and skills for diagnosis of emerging and reemerging pathogens through next-generation sequencing. Author in article: Ommeh et al: Genetic evidence of MERS-CoV .. in camels in Kenya, Virol Sin 2018.

In China we collaborate with **Dr. Xiong Yong**, Director and chief physician, in the department of infectious disease, Zhongnan hospital Wuhan, China, who has currently involvement in two papers about nCoV outbreak: Wang et al, Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China, JAMA 2020 and Jin et al: A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (SARS-CoV-2) infected pneumonia (standard version), Mil. Med Res 2020.

In China we collaborate also with **Professor Yong-Zhen Zhang**, Professor and head of the Dept of Zoonoses at National Institute for Communicable Disease Control and Prevantion, Chinese CDC, Beijing, China. An intertnationally known scholar on zoonotic viruses, virus discovery and the leader of the consortium who first identified the causative agent: Wu, Zhao, … Holmes, Zhang YZl: A new coronavirus associated with human respiratory disease in China.Nature 2020

3.4 Resources to be committed

Tables for section 3.4

Table 3.4a: Summary of staff effort

Please indicate the number of person/months over the whole duration of the planned work, for each work package, for each participant. Identify the work-package leader for each WP by showing the relevant person-month figure in bold.

	WP1	WP2	WP3	WP4	WP5	WP6	WP7	WP8	WP9	Total Person-Months per Participant
UH										
UTU										
UTA										
UU										
VTT										
REAG										
Total										

Table 3.4b: 'Other direct cost' items (travel, equipment, other goods and services, costs of internally invoiced goods and services, large research infrastructure)

1 - UH	**Cost (€)**	**Justification**
Travel		**project workshops and scientific meetings**
Equipment		
Other goods and services		**Reagents and laboratory consumables, outsourcing to Kenya and China**
Costs of internally invoiced goods and services (MGA Art. 6.2.D.5)1		
Total		
2 - UTU	**Cost (€)**	**Justification**

Travel		Travel expenses to international congresses and project network meetings.
Equipment		
Other goods and services		Sequencing, proteomics and antibody production
Costs of internally invoiced goods and services		All laboratory reagents, kits, cell culture media, sera, chemical, publication costs
Total		
3 - UTA	**Cost (€)**	**Justification**
Travel		Travel expenses to international congresses and project network meetings.
Equipment		**small equipment updates**
Other goods and services		**laboratory consumables**
Costs of internally invoiced goods and services		
Total		
4 - UU	**Cost (€)**	**Justification**
Travel		Travel expenses to international congresses and project network meetings.
Equipment		
Other goods and services		**laboratory consumables, animal experimentation**
Costs of internally invoiced goods and		
Total		
5 - VTT	**Cost (€)**	**Justification**
Travel		Travel expenses to international congresses and project network meetings.
Equipment		
Other goods and services		laboratory consumables
Costs of internally invoiced goods and services		
Total		

6 – REAG	**Cost (€)**	**Justification**
Travel		**Travel expenses to international congresses and project network meetings.**
Equipment		**Small laboratory equipment**
Other goods and		**Reagents and materials for assay development.**

services		
Costs of internally invoiced		
Total		

7 –	**Cost (€)**	**Justification**
Travel		**Travel expenses to international congresses and project network meetings.**
Equipment		**Small laboratory equipment**
Other goods and services		**Reagents and materials for assay development.**
Costs of internally invoiced		
Total		

8 –	**Cost (€)**	**Justification**
Travel		**Travel expenses to international congresses and project network meetings.**
Equipment		**Small laboratory equipment**
Other goods and services		**Reagents and materials for assay development.**
Costs of internally invoiced		
Total		

9 –	**Cost (€)**	**Justification**
Travel		**Travel expenses to international congresses and project network meetings.**
Equipment		**Small laboratory equipment**
Other goods and services		**Reagents and materials for assay development.**
Costs of internally invoiced		
Total		

10 –	**Cost (€)**	**Justification**
Travel		**Travel expenses to international congresses and project network meetings.**
Equipment		**Small laboratory equipment**
Other goods and services		**Reagents and materials for assay development.**
Costs of internally invoiced		
Total		

Abdelnabi, R. et al. A novel druggable interprotomer pocket in the capsid of rhino- and enteroviruses. PLoS Biol 17, e3000281, doi:10.1371/journal.pbio.3000281 (2019).

Akaberi D, Chinthakindi PK, Båhlström A, Palanisamy N, Sandström A, Lundkvist Å, Lennerstrand J. Identification of a C2-Symmetric diol based Human immunodeficiency virus protease inhibitor targeting Zika virus NS2B-NS3 protease. J Biomol Struct Dyn. 2019 Dec 27:1-11. doi: 10.1080/07391102.2019.1704882.

Akaberi D, Bergfors A, Kjellin M, Lidemalm L, Kameli N, Kolli B, Shafer RW, Palanisamy N and Lennerstrand J. Baseline dasabuvir resistance in Hepatitis C virus from the genotypes 1, 2 and 3 and modeling of the NS5B-dasabuvir complex by the in silico approach. Infect Ecol Epidemiol. 2018 Oct 5;8(1):1528117.

Akaji, K., Konno, H., Mitsui, H., Teruya, K., Shimamoto, Y., Hattori, Y., Ozaki, T., Kusunoki, M., & Sanjoh, A. (2011). Structure-based design, synthesis, and evaluation of peptide-mimetic SARS 3CL protease inhibitors. Journal of Medicinal Chemistry, 54(23), 7962–7973. https://doi.org/10.1021/jm200870n

Albinsson B, Rönnberg B, Vene S, Lundkvist Å. Antibody responses to tick-borne encephalitis virus non-structural protein 1 and whole virus antigen-a new tool in the assessment of suspected vaccine failure patients. Infect Ecol Epidemiol. 2019 Dec 3;9(1):1696132. doi: 10.1080/20008686.2019.1696132. eCollection 2019.

Albinsson B, Vene S, Rombo L, Blomberg J, Lundkvist Å, Rönnberg B.
Distinction between serological responses following tick-borne encephalitis virus (TBEV) infection vs vaccination, Sweden 2017. Euro Surveill. 2018 Jan;23(3). doi: 10.2807/1560-7917.ES.2018.23.3.17-00838.

Anand, K., Ziebuhr, J., Wadhwani, P., Mesters, J. R., & Hilgenfeld, R. (2003). Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. Science (New York, N.Y.), 300(5626), 1763–1767. https://doi.org/10.1126/science.1085658

Andersen, P. I. et al. Novel Antiviral Activities of Obatoclax, Emetine, Niclosamide, Brequinar, and Homoharringtonine. Preprints 2019090128, doi:10.20944/preprints201909.0128.v1 (2019).

Balistreri, G. et al. The host nonsense-mediated mRNA decay pathway restricts Mammalian RNA virus replication. Cell Host Microbe 16, 403-411, doi:10.1016/j.chom.2014.08.007 (2014).

Balistreri, G. et al. Oncogenic Herpesvirus Utilizes Stress-Induced Cell Cycle Checkpoints for Efficient Lytic Replication. PLoS Pathog 12, e1005424, doi:10.1371/journal.ppat.1005424 (2016).

Banerjee, I., Yamauchi, Y., Helenius, A. & Horvath, P. High-content analysis of sequential events during the early phase of influenza A virus infection. PLoS One 8, e68450, doi:10.1371/journal.pone.0068450 (2013).

Bekerman, E. & Einav, S. Infectious disease. Combating emerging viral threats. Science 348, 282-283, doi:10.1126/science.aaa3778 (2015).

Bosl, K. et al. Common Nodes of Virus–Host Interaction Revealed Through an Integrated Network Analysis. Front. Immunology Accepted, doi:doi: 10.3389/fimmu.2019.02186 (2019).
Ianevski, A. et al. Novel activities of safe-in-human broad-spectrum antiviral agents. Antiviral Res 154, 174-182, doi:10.1016/j.antiviral.2018.04.016 (2018).

Bulanova, D. et al. Antiviral Properties of Chemical Inhibitors of Cellular Anti-Apoptotic Bcl-2 Proteins. Viruses 9, doi:10.3390/v9100271 (2017).

Carette, J. E. et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477, 340-343, doi:10.1038/nature10348 (2011).

Check Hayden, E. Experimental drugs poised for use in Ebola outbreak. Nature 557, 475-476, doi:10.1038/d41586-018-05205-x (2018).

DALYs, G. B. D. & Collaborators, H. Global, regional, and national disability-adjusted life-years (DALYs) for 359 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1859-1922, doi.10.1016/S0140-6736(18)32335-3 (2018).

Denisova, O. V. et al. Akt inhibitor MK2206 prevents influenza pH1N1 virus infection in vitro. Antimicrob Agents Chemother 58, 3689-3696, doi:10.1128/AAC.02798-13 (2014).

Debing, Y., Neyts, J. & Delang, L. The future of antivirals: broad-spectrum inhibitors. Curr Opin Infect Dis 28, 596-602, doi:10.1097/QCO.0000000000000212 (2015).

De Clercq, E. & Li, G. Approved Antiviral Drugs over the Past 50 Years. Clin Microbiol Rev 29, 695-747, doi:10.1128/CMR.00102-15 (2016).

de Groot, R., Luthi, J., Lindsay, H., Holtackers, R. & Pelkmans, L. Large-scale image-based profiling of single-cell phenotypes in arrayed CRISPR-Cas9 gene perturbation screens. Mol Syst Biol 14, e8064, doi:10.15252/msb.20178064 (2018).

Disease, G. B. D., Injury, I. & Prevalence, C. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1789-1858, doi:10.1016/S0140-6736(18)32279-7 (2018).

Dodev TS, Karagiannis P, Gilbert AE, Josephs DH, Bowen H, James LK, Bax HJ, Beavil R, Pang MO, Gould HJ, Karagiannis SN & Beavil AJ. A tool kit for rapid cloning and expression of recombinant antibodies. Sci. Rep. 4, 5885; DOI:10.1038/srep05885 (2014).

Dukhovny, A. et al. A CRISPR Activation Screen Identifies Genes That Protect against Zika Virus Infection. J Virol 93, doi:10.1128/JVI.00211-19 (2019).

Fu, Y. et al. JNJ872 inhibits influenza A virus replication without altering cellular antiviral responses. Antiviral Res 133, 23-31, doi:10.1016/j.antiviral.2016.07.008 (2016).

Gaelings, L. et al. Regulation of kynurenine biosynthesis during influenza virus infection. FEBS J 284, 222-236, doi:10.1111/febs.13966 (2017).

Gilbert, L. A. et al. Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation. Cell 159, 647-661, doi:10.1016/j.cell.2014.09.029 (2014).

Graham, D. B. & Root, D. E. Resources for the design of CRISPR gene editing experiments. Genome Biol 16, 260, doi:10.1186/s13059-015-0823-x (2015).

Heaton, B. E. et al. A CRISPR Activation Screen Identifies a Pan-avian Influenza Virus Inhibitory Host Factor. Cell Rep 20, 1503-1512, doi:10.1016/j.celrep.2017.07.060 (2017).

Hoffmann, H. H. et al. Diverse Viruses Require the Calcium Transporter SPCA1 for Maturation and Spread. Cell Host Microbe 22, 460-470 e465, doi:10.1016/j.chom.2017.09.002 (2017).

Howard, C. R. & Fletcher, N. F. Emerging virus diseases: can we ever expect the unexpected? Emerg Microbes Infect 1, e46, doi:10.1038/emi.2012.47 (2012).

Hujakka H et al. 2001. New immunochromatographic rapid test for diagnosis of acute Puumala virus infection. J. Clin. Microbiol. 39(6):2146-2150.

Ianevski, A., Andersen, P. I., Merits, A., Bjoras, M. & Kainov, D. Expanding the activity spectrum of antiviral agents. Drug Discov Today 24, 1224-1228, doi:10.1016/j.drudis.2019.04.006 (2019).

Ianevski, A., He, L., Aittokallio, T. & Tang, J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. Bioinformatics 33, 2413-2415, doi:10.1093/bioinformatics/btx162 (2017).

Ianevski, A. et al. Low Temperature and Low UV Indexes Correlated with Peaks of Influenza Virus Activity in Northern Europe during 2010(-)2018. Viruses 11, doi:10.3390/v11030207 (2019).

Jaishankar, D. et al. An off-target effect of BX795 blocks herpes simplex virus type 1 infection of the eye. Sci Transl Med 10, doi:10.1126/scitranslmed.aan5861 (2018).

Jin YH, Cai L, Cheng ZS, Cheng H, Deng T, Fan YP, Fang C, Huang D, Huang LQ, Huang Q, Han Y, Hu B, Hu F, Li BH, Li YR, Liang K, Lin LK, Luo LS, Ma J, Ma LL, Peng ZY, Pan YB, Pan ZY, Ren XQ, Sun HM, Wang Y, Wang YY, Weng H, Wei CJ, Wu DF, Xia J, Xiong Y, Xu HB, Yao XM, Yuan YF, Ye TS, Zhang XC, Zhang YW, Zhang YG, Zhang HM, Zhao Y, Zhao MJ, Zi H, Zeng XT, Wang YY, Wang XH; , for the Zhongnan Hospital of Wuhan University Novel Coronavirus Management and Research Team, Evidence-Based Medicine Chapter of China International Exchange and Promotive Association for Medical and Health Care (CPAM). A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (SARS-CoV-2) infected pneumonia (standard version). Mil Med Res. 2020 Feb 6;7(1):4. doi: 10.1186/s40779-020-0233-6. PubMed PMID: 32029004; PubMed Central PMCID: PMC7003341.

Jiang M, Österlund P, Poranen MM, Julkunen I. In vitro production of synthetic viral RNAs and their delivery into mammalian cells and the application of viral RNAs in the study of innate interferon responses. Methods. 2019a Nov 1. pii: S1046-2023(19)30238-5. doi: 10.1016/j.ymeth.2019.10.013. [Epub ahead of print] Review.

Jiang M, Österlund P, Westenius V, Guo D, Poranen MM, Bamford DH, Julkunen I.

Efficient Inhibition of Avian and Seasonal Influenza A Viruses by a Virus-Specific Dicer-Substrate Small Interfering RNA Swarm in Human Monocyte-Derived Macrophages and Dendritic Cells. J Virol. 2019b Feb 5;93(4). pii: e01916-18. doi: 10.1128/JVI.01916-18. Print 2019 Feb 15.

Joensuu, M. et al. Visualizing endocytic recycling and trafficking in live neurons by subdiffractional tracking of internalized molecules. Nat Protoc 12, 2590-2622, doi:10.1038/nprot.2017.116 (2017).

Kazakova A, Kakkola L, Ziegler T, Syrjänen R, Päkkilä H, Waris M, Soukka T, Julkunen I. Pandemic influenza A(H1N1pdm09) vaccine induced high levels of influenza-specific IgG and IgM antibodies as analyzed by enzyme immunoassay and dual-mode multiplex microarray immunoassay methods. Vaccine. 2020 Jan 24. pii: S0264-410X(20)30037-2. doi: 10.1016/j.vaccine.2020.01.022.

Kazakova A, Kakkola L, Päkkilä H, Teros-Jaakkola T, Soukka T, Peltola V, Waris M, Julkunen I. Serological Array-in-Well Multiplex Assay Reveals a High Rate of Respiratory Virus Infections and Reinfections in Young Children. mSphere. 2019 Sep 11;4(5). pii: e00447-19. doi: 10.1128/mSphere.00447-19.

Korhonen V-P, Kelo E, Koli E, Parviainen M, Mononen I, Vapalahti O, Vaheri A, Hakala A, Avsic-Zupanc T, Jelovsek M. Fast diagnosis of acute tick-borne encephalitis with rapid lateral flow test. 7th European Meeting on Viral Zoonoses, St. Raphaël, France, 24-27 May 2014, P39: 96.

Krzyzaniak, M. A., Zumstein, M. T., Gerez, J. A., Picotti, P. & Helenius, A. Host cell entry of respiratory syncytial virus involves macropinocytosis followed by proteolytic activation of the F protein. PLoS Pathog 9, e1003309, doi:10.1371/journal.ppat.1003309 (2013).

Kuivanen, S. et al. Obatoclax, saliphenylhalamide and gemcitabine inhibit Zika virus infection in vitro and differentially affect cellular signaling, transcription and metabolism. Antiviral Res 139, 117-128, doi:10.1016/j.antiviral.2016.12.022 (2017).

Kuo, C.-J., Chi, Y.-H., Hsu, J. T.-A., & Liang, P.-H. (2004). Characterization of SARS main protease and inhibitor assay using a fluorogenic substrate. Biochemical and Biophysical Research Communications, 318(4), 862–867. https://doi.org/10.1016/j.bbrc.2004.04.098

Lamminmäki U, Batra G, Saviranta P. Array-In-Well Epitope Mapping of Phage-Displayed Antibodies. Methods Mol Biol. 2018;1785:129-140. doi: 10.1007/978-1-4939-7841-0_9. PubMed PMID: 29714016.

Lee, A. S., Burdeinick-Kerr, R. & Whelan, S. P. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. Proc Natl Acad Sci U S A 110, 324-329, doi:10.1073/pnas.1216454109 (2013).

Lundberg R, Melén K, Westenius V, Jiang M, Österlund P, Khan H, Vapalahti O, Julkunen I, Kakkola L. Zika Virus Non-Structural Protein NS5 Inhibits the RIG-I Pathway and Interferon Lambda 1 Promoter Activation by Targeting IKK Epsilon. Viruses. 2019 Nov 4;11(11). pii: E1024. doi: 10.3390/v11111024.

Li H, Bai R, Zhao Z, Tao L, Ma M, Ji Z, Jian M, Ding Z, Dai X, Bao F, Liu A. Application of droplet digital PCR to detect the pathogens of infectious diseases. Biosci Rep. 2018 Nov 15;38(6).

pii: BSR20181170. doi: 10.1042/BSR20181170. Print 2018 Dec 21. Review. PubMed PMID: 30341241; PubMed Central PMCID: PMC6240714.

Mandell, L. A. et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44 Suppl 2, S27-72, doi:10.1086/511159 (2007).

Melén K, Kakkola L, He F, Airenne K, Vapalahti O, Karlberg H, Mirazimi A, Julkunen I. Production, purification and immunogenicity of recombinant Ebola virus proteins - A comparison of Freund's adjuvant and adjuvant system 03. J Virol Methods. 2017 Apr;242:35-45. doi: 10.1016/j.jviromet.2016.12.014.

Palanisamy N, Kalaghatgi P, Akaberi D, Lundkvist Å, Chen ZW, Hu P, Lennerstrand J. Worldwide prevalence of baseline mutations/polymorphisms in hepatitis C virus against present and future direct-acting antivirals. Antiviral Therapy. 2018 May 10. doi: 10.3851/IMP3237.

Palanisamy N, Akaberi D, Lennerstrand J, Lundkvist Å. Comparative genome analysis of Alkhumra hemorrhagic fever virus with Kyasanur forest disease and tick-borne encephalitis viruses by the in silico approach. Pathogens and Global Health. 2018 May 10:1-17.

Palanisamy N, Akaberi D, Lennerstrand J; Protein backbone flexibility pattern is evolutionarily conserved in the Flaviviridae family: a case of NS3 protease in Flavivirus and Hepacivirus. Molecular Phylogenetics and Evolution 2018 Jan;118:58-63.

Pietikäinen A et al. 2018. Point-of-care testing for CXCL13 in Lyme neuroborreliosis. Diagn Microbiol Infect Dis. Jul;91(3):226 - 228.

Pérez-Gamarra S, Hattara L, Batra G, Saviranta P, Lamminmäki U. Array-in-well binding assay for multiparameter screening of phage displayed antibodies. Methods. 2017 Mar 1;116:43-50. doi: 10.1016/j.ymeth.2016.12.004. Epub 2016 Dec 9. PubMed PMID: 27956240.

Puschnik, A. S., Majzoub, K., Ooi, Y. S. & Carette, J. E. A CRISPR toolbox to study virus-host interactions. Nat Rev Microbiol 15, 351-364, doi:10.1038/nrmicro.2017.29 (2017).

Rizwan M, Rönnberg B, Cistjakovs M, Lundkvist Å, Pipkorn R, Blomberg J. Serology in the Digital Age: Using Long Synthetic Peptides Created from Nucleic Acid Sequences as Antigens in Microarrays. Microarrays (Basel). 2016 Aug 10;5(3). pii: E22. doi: 10.3390/microarrays5030022.

Rönnberg B, Gustafsson Å, Vapalahti O, Emmerich P, Lundkvist Å, Schmidt-Chanasit J, Blomberg J. Compensating for cross-reactions using avidity and computation in a suspension multiplex immunoassay for serotyping of Zika versus other flavivirus infections.Med Microbiol Immunol. 2017 Oct;206(5):383-401. doi: 10.1007/s00430-017-0517-y. Epub 2017 Aug 29.

Rönnberg B, Vapalahti O, Goeijenbier M, Reusken C, Gustafsson Å, Blomberg J, Lundkvist Å. Serogrouping and seroepidemiology of North European hantaviruses using a novel broadly targeted synthetic nucleoprotein antigen array. Infect Ecol Epidemiol. 2017 Jul 26;7(1):1350086. doi: 10.1080/20008686.2017.1350086. eCollection 2017.

Sanson, K. R. et al. Optimized libraries for CRISPR-Cas9 genetic screens with multiple modalities. Nat Commun 9, 5416, doi:10.1038/s41467-018-07901-8 (2018).

Schor, S. & Einav, S. Repurposing of Kinase Inhibitors as Broad-Spectrum Antiviral Drugs. DNA Cell Biol 37, 63-69, doi:10.1089/dna.2017.4033 (2018).

Soderholm, S. et al. Immuno-modulating properties of saliphenylhalamide, SNS-032, obatoclax, and gemcitabine. Antiviral Res 126, 69-80, doi:10.1016/j.antiviral.2015.12.011 (2016).

Soderholm, S. et al. Multi-Omics Studies towards Novel Modulators of Influenza A Virus-Host Interaction. Viruses 8, doi:10.3390/v8100269 (2016).

Talha SM, Saviranta P, Hattara L, Vuorinen T, Hytönen J, Khanna N, Pettersson K. Array-in-well platform-based multiplex assay for the simultaneous detection of anti-HIV- and treponemal-antibodies, and Hepatitis B surface antigen. J Immunol Methods. 2016 Feb;429:21-7. doi: 10.1016/j.jim.2015.12.007. Epub 2015 Dec 19. PubMed PMID: 26711310.

Tynell J, Westenius V, Rönkkö E, Munster VJ, Melén K, Österlund P, Julkunen I. Middle East respiratory syndrome coronavirus shows poor replication but significant induction of antiviral responses in human monocyte-derived macrophages and dendritic cells. J Gen Virol. 2016 Feb;97(2):344-355. doi: 10.1099/jgv.0.000351. Epub 2015 Nov 24.

Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA. 2020 Feb 7. doi: 10.1001/jama.2020.1585. [Epub ahead of print] PubMed PMID: 32031570.

Westenius V, Mäkelä SM, Julkunen I, Österlund P. Highly Pathogenic H5N1 Influenza A Virus Spreads Efficiently in Human Primary Monocyte-Derived Macrophages and Dendritic Cells. Front Immunol. 2018 Jul 17;9:1664. doi: 10.3389/fimmu.2018.01664. eCollection 2018.

Westenius V, Mäkelä SM, Ziegler T, Julkunen I, Österlund P. Efficient replication and strong induction of innate immune responses by H9N2 avian influenza virus in human dendritic cells. Virology. 2014 Dec;471-473:38-48. doi: 10.1016/j.virol.2014.10.002. Epub 2014 Oct 25

WHO. WHO publishes list of top emerging diseases likely to cause major epidemics. www.who.int/medicines/ebola-treatment/WHO-list-of-top-emerging-diseases/en/ (2015).

Yu, F. et al. Approaches for identification of HIV-1 entry inhibitors targeting gp41 pocket. Viruses 5, 127-149, doi:10.3390/v5010127 (2013).

Yuan, S. et al. SREBP-dependent lipidomic reprogramming as a broad-spectrum antiviral target. Nat Commun 10, 120, doi:10.1038/s41467-018-08015-x (2019).

Ziegler T, Matikainen S, Rönkkö E, Osterlund P, Sillanpää M, Sirén J, Fagerlund R, Immonen M, Melén K, Julkunen I. Severe acute respiratory syndrome coronavirus fails to activate cytokine-mediated innate immune responses in cultured human monocyte-derived dendritic cells. J Virol. 2005 Nov;79(21):13800-5.

Zusinaite, E. et al. A Systems Approach to Study Immuno- and Neuro-Modulatory Properties of Antiviral Agents. Viruses 10, doi:10.3390/v10080423 (2018).

Österlund P, Jiang M, Westenius V, Kuivanen S, Järvi R, Kakkola L, Lundberg R, Melén K, Korva M, Avšič-Županc T, Vapalahti O, Julkunen I. Asian and African lineage Zika viruses show differential replication and innate immune responses in human dendritic cells and macrophages. Sci Rep. 2019 Oct 31;9(1):15710. doi: 10.1038/s41598-019-52307-1.