Understanding COVID-19: A Global Pandemic

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Microbiologist, ATCC

Credible Leads to Incredible™
About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World’s largest, most diverse biological materials and information resource for microbes – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository

- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Agenda

- What is COVID-19 and SARS-CoV-2?
- What are coronaviruses?
- Diagnostics
- Vaccines
- Therapeutics
- ATCC solutions
Major areas in scientific research to combat the pandemic

Understanding the disease (COVID-19)
Understanding the infectious agent (SARS-CoV-2)
Diagnostics
Detection & Surveillance
Development of Prophylactics
Vaccines
Development of Therapeutics
Antiviral drugs
COVID-19 timeline
Epidemiology

- There are 215 countries, areas, or territories with cases
- More than 6.9 million cases of COVID-19 and 400,000 deaths have been reported to WHO

Globally, as of 4:00pm CEST, 8 June 2020, there have been 6,931,000 confirmed cases of COVID-19, including 400,857 deaths, reported to WHO.

Data Source: WHO
People of any age can be affected by COVID-19; however, older adults (65 years +) might be at higher risk for severe illness from COVID-19.
Based on the current information available and clinical expertise, **people of any age who have serious underlying medical conditions** like heart disease, lung disease, or diabetes might be at higher risk for severe illness from COVID-19.
COVID-19
Pathogenesis & clinical manifestations

Symptoms appear 2 to 14 days after exposure
- Range from mild to severe illness with either of the following accompanying symptoms:
  - Flu or fever-like symptoms
  - Muscle pain
  - Shortness of breath or difficulty breathing
  - Temporary loss of taste or smell

Based on various risk factors, symptoms can progress to:
- Developing pneumonia in both lungs
- Extrapulmonary systemic hyperinflammation syndrome
- Acute respiratory distress syndrome (ARDS)
Major areas in scientific research to combat the pandemic

- **Understanding the disease (COVID-19)**
- **Understanding the infectious agent (SARS-CoV-2)**
- **Diagnostics**
  - Detection & Surveillance
- **Development of Prophylactics**
  - Vaccines
- **Development of Therapeutics**
  - Antiviral drugs
What do we know about Coronaviruses?

- Coronaviruses (CoVs) are **large, enveloped RNA viruses of both medical and veterinary importance**.

- These viruses cause a variety of diseases, including respiratory disease, enteric disease, neurological illness, and hepatitis.

- Zoonosis:
  - These viruses are described in various wildlife species such as swine, cattle, horses, camels, cats, and dogs. Many coronavirus infections are subclinical.
  - In humans, coronaviruses are included in the spectrum of viruses that cause the common cold.  
    - Alphacoronaviruses (229E and NL63)
    - Betacoronaviruses (OC43 and HKU1)
Recent history of Coronavirus epidemics

- During the past two decades, three zoonotic coronaviruses have been identified as the cause of large-scale disease outbreaks:
  - Severe Acute Respiratory Syndrome (SARS)
  - Middle East Respiratory Syndrome (MERS)
  - Swine Acute Diarrhea Syndrome (SADS)

- SARS and MERS emerged in 2003 and 2012, respectively, and caused worldwide pandemics that claimed thousands of human lives while SADS struck the swine industry in 2017.

- Common characteristics of these viruses:
  - Highly pathogenic to humans or livestock
  - They originated in bats

- It is highly likely that future SARS- or MERS-like coronavirus outbreaks will originate in bats.

Coronaviruses are enveloped, positive-sense RNA viruses that are characterized by three main features:

- Club-like spikes that project from their surface
- An unusually large RNA genome
- Unique replication strategy
Phylogeny

SARS-CoV-2 is more closely related to bat-related betacoronaviruses of the sub-genus Sarbecovirus.
Host range

Proposed host reservoir: bats
Possibilities of intermediate hosts: Bamboo rats, pangolins, snakes, and others?
This map reflects the average temperature data from March-April 2019 and was developed to predict the areas that are at risk for community transmission of COVID-19. Zones at the highest risk are within the green bands.

Given the consistent seasonal variation of the four endemic coronaviruses (229E, HKU1, NL63, and OC43), SARS-CoV-2 may be affected by the following factors:

- Climate
- Humidity
- Presence of UV light
Discovering the molecular mechanisms of pathogenesis

Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses

Michael Letko, Andrea Marzi, and Vincent Munster

Over the past 20 years, several coronaviruses have crossed the species barrier into humans, causing outbreaks of severe, and often fatal, respiratory illness. Since SARS-CoV was first identified in animal markets, global viromics projects have discovered thousands of coronavirus sequences in diverse animals and geographic regions. Unfortunately, there are few tools available to functionally test these viruses for their ability to infect humans, which has severely hampered efforts to predict the next zoonotic viral outbreak. Here, we developed an approach to rapidly screen lineage B betacoronaviruses, such as SARS-CoV and the recent SARS-CoV-2, for receptor usage and their ability to infect cell types from different species. We show that host protease processing during viral entry is a significant barrier for several lineage B viruses and that bypassing this barrier allows several lineage B viruses to enter human cells through an unknown receptor. We also demonstrate how different lineage B viruses can recombine to gain entry into human cells, and confirm that human ACE2 is the receptor for the recently emerging SARS-CoV-2.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

1. A549 - human lung epithelial - ATCC CCL-185
2. A549 - human lung epithelial - ATCC CCL-185
3. A549 - human lung epithelial - ATCC CCL-185
4. BRK - human kidney - ATCC CCL-10
5. Caso-2 - human colon epithelial cells - ATCC HMB-37
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  - Vaccines
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  - Antiviral drugs
Current methods for detection & surveillance

Molecular and serological/immunological assays

**Molecular-based assays**

- Reverse transcription-polymerase chain reaction (RT-PCR)
- Isothermal nucleic acid amplification
- Reverse transcription loop-mediated isothermal amplification (RT-LAMP)
- Transcription-mediated amplification (TMA)
- CRISPR/cas9-based assays
- Rolling circle amplification
- Nucleic acid hybridization using microarray
- Amplicon-based metagenomic sequencing

**Serological & immunological assays**

- Enzyme-linked immunosorbent assay (ELISA)
- Lateral flow immunoassays
- Rapid antigen test
- Neutralization assay
- Luminescent immunoassay
- Biosensor test

ACS Cent. Sci. 6: 591–605, 2020
Variation in diagnostic tests relative to symptom onset

![Chart showing the variation in diagnostic tests relative to symptom onset.](chart.png)

JAMA. Published online May 6, 2020. doi:10.1001/jama.2020.8259
A majority of PCR-based assays target the ORF1ab, RNA-dependent RNA polymerase (RdRp), E, N, and spike regions. Currently authorized serological tests for SARS-CoV-2 measure IgM and/or IgG antibodies, total antibodies, or the spike protein.

- **FDA:**

- **CDC:**

- **WHO:**

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<table>
<thead>
<tr>
<th>Institute</th>
<th>Gene targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>China CDC, China</td>
<td>ORF1ab and N</td>
</tr>
<tr>
<td>Institut Pasteur, Paris, France</td>
<td>Two targets in RdRP</td>
</tr>
<tr>
<td>US CDC, USA</td>
<td>Three targets in N gene</td>
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<tr>
<td>National Institute of Infectious Diseases, Japan</td>
<td>Pancorona and multiple targets, Spike protein</td>
</tr>
<tr>
<td>Charité, Germany</td>
<td>RdRP, E, N</td>
</tr>
<tr>
<td>HKU, Hong Kong SAR</td>
<td>ORF1b-nsp14, N</td>
</tr>
<tr>
<td>National Institute of Health, Thailand</td>
<td>N</td>
</tr>
</tbody>
</table>
Diagnostics
Validation methods & ATCC solutions

- Limit of detection/Analytical sensitivity
- Cross-reactivity/Analytical specificity
- Clinical evaluation/Agreement

SARS-CoV-2 Reference Materials
SARS-CoV-2 Synthetic Molecular Standards
Materials for Inclusivity Testing
Materials for Exclusivity [Specificity] Testing
Policy for COVID-2019 tests

Introduction

Contains Nonbinding Recommendations

Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised)

Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff


- Limit of Detection/Analytical Sensitivity
- Cross-reactivity/Analytical Specificity
- Microbial Interference
- Clinical Agreement Study
Policy for COVID-2019 tests

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(1) Limit of Detection

FDA recommends that developers document the limit of detection (7) of their SARS-CoV-2 assay. FDA generally does not have concerns with spiking RNA or inactivated virus into artificial or real clinical matrix (e.g., Bronchoalveolar lavage [BAL] fluid, sputum, etc.) for LoD determination.

FDA recommends that developers test a dilution series of three replicates per concentration with inactivated virus on actual patient specimen, and then confirm the final concentration with 20 replicates. For this guidance, FDA defines LoD as the lowest concentration at which 19/20 replicates are positive. If multiple clinical matrices are intended for clinical testing, FDA recommends that developers submit in their EUA requests the results from the most challenging clinical matrix to FDA. For example, if testing respiratory specimens (e.g., sputum, BAL, nasopharyngeal [NP] swabs, etc.), laboratories should include only results from sputum in their EUA request.
Policy for COVID-2019 tests
Cross-reactivity/analytical specificity & microbial interference

Policy for Coronavirus Disease-2019
Tests During the Public Health
Emergency (Revised)

Immediately in Effect Guidance for
Clinical Laboratories, Commercial
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Food and Drug Administration Staff


Contains Nonbinding Recommendations

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(3) Inclusivity

developers should document the results of an in silico analysis indicating the percent identity matches against publicly available SARS-CoV-2 sequences that can be detected by the proposed molecular assay. FDA anticipates that 100% of published SARS-CoV-2 sequences will be detectable with the selected primers and probes.

(4) Cross-reactivity

FDA recommends cross-reactivity wet testing on common respiratory flora and other viral pathogens at concentrations of $10^6$ CFU/ml or higher for bacteria and $10^5$ pfu/ml or higher for viruses, except for SARS-Coronavirus and MERS-Coronavirus, which can be accomplished by in silico analysis. As an alternative, FDA believes an in silico analysis of the assay primer and probes compared to common respiratory flora and other viral pathogens can be performed. For this guidance, FDA defines in silico cross-reactivity as greater than 80% homology between one of the primers/probes and any sequence present in the targeted microorganism. In addition, FDA recommends that developers follow recognized laboratory procedures in the context of the sample types intended for testing for any additional cross-reactivity testing.

Additional information for the validation of molecular diagnostics is included in the manufacturer and developers EUA templates available for download on our website.
Policy for COVID-2019 tests

Clinical agreement

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(2) Clinical Evaluation

The availability of positive samples has increased as the pandemic has progressed. As such, FDA now recommends that developers use positive clinical samples for clinical validation. Moreover, due to the increased availability of clinical samples, FDA recommends that developers confirm performance of their assay by testing a minimum of 30 positive specimens and 30 negative specimens as determined by an authorized assay. If you do not have access to clinical samples as determined by an authorized assay, contrived clinical specimens may be considered. Contrived reactive specimens can be created by spiking RNA or inactivated virus into leftover clinical specimens, of which the majority can be leftover upper respiratory specimens such as NP swabs, or lower respiratory tract specimens such as sputum, etc. If contrived samples are used, FDA recommends that twenty of the contrived clinical specimens be spiked at a concentration of 1x-2x LoD, with the remainder of specimens spanning the assay testing range. For this guidance, FDA defines the acceptance criteria for the performance as 95% agreement at 1x-2x LoD, and 100% agreement at all other concentrations and for negative specimens.
ATCC portfolio

SARS-CoV-2 reference materials for inclusivity testing

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Product Description</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-1986HK™</td>
<td>Heat-inactivated SARS-CoV-2, Washington</td>
<td>Available</td>
</tr>
<tr>
<td>VR-1991D™</td>
<td>Genomic SARS-CoV-2 RNA, Hong Kong</td>
<td>Available</td>
</tr>
<tr>
<td>VR-1992D™</td>
<td>Genomic SARS-CoV-2 RNA, Italy</td>
<td>Available</td>
</tr>
<tr>
<td>VR-1994D™</td>
<td>Genomic SARS-CoV-2 RNA, Germany</td>
<td>June 2020</td>
</tr>
</tbody>
</table>

- **Limit of Detection/Analytical Sensitivity**
- **Cross-reactivity/Analytical Specificity**
- **Microbial Interference**
- **Clinical Agreement Study**

- **Downgraded from BSL-3 to BSL-2 (gRNA) and BSL-1 (heat-inactivated)**

- **Applications:**
  - Positive controls for RT-PCR or other RNA-based assays
  - Monitoring run-to-run variation within each step of the procedure, such as:
    - Nucleic acid extraction
    - Process verification
    - Amplification

www.atcc.org/coronavirus
The genome of each strain is sequenced and assembled using our standardized workflow.

- Genes are annotated and the species identity is confirmed.

- Annotated genome sequences are provided on the ATCC Genome Portal.
## ATCC portfolio

### Synthetic molecular standards

<table>
<thead>
<tr>
<th>ATCC® No.</th>
<th>Product Description</th>
<th>Availability</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR-3276SD™</td>
<td>Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N</td>
<td>Available</td>
</tr>
<tr>
<td>VR-3277SD™</td>
<td>Quantitative Synthetic SARS-CoV-2 RNA: Spike 5'</td>
<td>Available</td>
</tr>
<tr>
<td>VR-3278SD™</td>
<td>Quantitative Synthetic SARS-CoV-2 RNA: Spike 3'</td>
<td>Available</td>
</tr>
<tr>
<td>VR-3279SD™</td>
<td>Quantitative Synthetic SARS-CoV-2 RNA: nsp9, nsp12 (RdRp)</td>
<td>June 2020</td>
</tr>
</tbody>
</table>

- **Limit of Detection/Analytical Sensitivity**
- **Cross-reactivity/Analytical Specificity**
- **Microbial Interference**
- **Clinical Agreement Study**

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www.atcc.org/coronavirus
ATCC portfolio

Synthetic molecular standards

<table>
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<th>ATCC® No.</th>
<th>Product Description</th>
<th>Compatible Assays</th>
</tr>
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</table>
| VR-3276SD™ | Quantitative Synthetic SARS-CoV-2 RNA: ORF, E, N | - China CDC Primers and probes for detection 2019-nCoV (24 January 2020)  
- Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany (17 January 2020)  
- Detection of 2019 novel coronavirus (2019-nCoV) in suspected human cases by RT-PCR – Hong Kong University (23 January 2020)  
- PCR and sequencing protocol for 2019-nCoV - Department of Medical Sciences, Ministry of Public Health, Thailand (Updated 28 January 2020)  
- US CDC panel primer and probes – U.S. CDC, USA (28 January 2020) |
| VR-3277SD™ | Quantitative Synthetic SARS-CoV-2 RNA: Spike 5' | - Detection of WN-Human1 sequence from clinical specimen. – National Institute of Infectious Diseases Japan (17 January 2020) |
| VR-3279SD™ | Quantitative Synthetic SARS-CoV-2 RNA: nsp9, nsp12 (RdRp) | - Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany (17 January 2020)  
- Real-time RT-PCR assays for the detection of SARS-CoV-2 - Institut Pasteur, Paris (02 March 2020) |

Applications:
- Positive controls for RT-PCR or other RNA-based assays
- Generation of a standard curve for quantitative RT-PCR to determine viral load
- Monitoring run-to-run, assay-to-assay, and lot-to-lot variation within each step of the procedure, such as:
  - Process verification
  - Amplification
- Assay development, verification, and validation
- To assign a genome copy number to secondary calibrators – for example, to establish a ratio of plaque or colony forming units to genome copies
- Can be used in BSL-1
ATCC Portfolio – Synthetic Molecular Standards

FAQs on Testing for SARS-CoV-2

What If I Do Not Have...?

Q: I am having trouble obtaining viral transport media/universal transport media (VTM/UTM) and a flocked nasopharyngeal swab to collect and transport patient samples. Are there alternatives that I can use? (Updated 5/6)

Q: What happens if I do not have the extraction platform referenced in the authorization of CDC’s EUA-authorized test? (Updated 5/26)

Q: What happens if I do not have the instruments referenced in the authorization of the CDC’s EUA-authorized test?

Q: If I do not have assay positive control material, how can I obtain it? (Updated 4/22)

A: Below is information regarding positive control material. Links provided are for information purposes only and not a recommendation by FDA to use that product. FDA encourages other suppliers of test materials to email COVID19DX@fda.hhs.gov to discuss whether materials they have available may also be appropriate for use.

Control material specific for the CDC EUA is available from the following resources:

- ATCC: Order through their website [https://www.atcc.org/Landing_Pages/Coronavirus_Resources]
  - Product # VR-3276SD: Quantitative Synthetic SARS-CoV-2: ORF, E, N
  - Product # VR-3278SD: Quantitative Synthetic SARS-CoV-2 RNA: Spike 3'
Having access to a variety of coronavirus strains is essential for establishing the inclusivity and exclusivity of a novel assay.

To support this need, ATCC provides microbial strains that have a wide spectrum of temporal and geographical diversity.

www.atcc.org/coronavirus
Major areas in scientific research to combat the pandemic

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Understanding the infectious agent (SARS-CoV-2)

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Detection & Surveillance

Development of Prophylactics
Vaccines

Development of Therapeutics
Antiviral drugs
Vaccine development

Platforms

Vaccine Candidates

Development and Implementation Phases

GMP process development

Clinical trials

Phase I → Phase II → Phase III

Licensure

FDA, EMA etc.

Large scale production and distribution

Administration

Immunity

Vaccine development
ATCC biological materials – Meeting the need

Cell Lines for SARS-CoV-2 Propagation
- Vero E6 cells (ATCC® CRL-1586™)
- Vero CCL-81 (ATCC® CCL-81™)
- MRC-5 (ATCC® CCL-171™)
- HCT-8 (ATCC® CCL-244™)
- Media and reagents to support cellular growth

Cell Lines for Enhanced Virus Production
- STAT1 knockout cell lines capable of producing high-titer viral stocks:
  - Vero.STAT1 KO (ATCC® CCL-81-VHG™)
  - MDCK.STAT1KO (ATCC® CCL-34-VHG™)
- Additional cell lines can be found on ATCC’s website

www.atcc.org/coronavirus
Vaccine development

Published research & news

Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine

Ede Qiu,1,4 Huiling Shi,1, Lin Tang,1, Cuie Wang,1, Guohui Chang,1, Zhihen Ding,1, Kai Zhao,1, Jian Wang,1, Ze Chen,1, Man Yu,1, Bingyin Shi,1, Jianyuan Liu,1, Dongli Wu,1, Xiaojie Cheng,1, Baosen Yang,1, Wenming Peng,1, Qingwen Meng,1, Bohua Liu,1, Weiguo Han,1, Xumin Yin,1, Hongyuan Duan,1, Dawei Zhao,1, Long Tian,1, Shangli Li,1, Jingong Wu,1, Gang Tan,1, Yi Li,1, Yuchuan Li,1, Yonggang Liu,1, Hong Liu,1, Fushuang Lv,1, Yu Zhang,1, Xiangdong Kong,1, Baochang Fan,1, Tao Jiang,1, Shuli Xu,1, Xiaomei Yang,1, Xiaohong Wu,1, Yongjia Dong,1, Min Zhao1,4, Qingshuang Sui2

1 Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, No. 29 Dongruo Street, Pang, Beijing 100071, China
2 Beijing Genomics Institute (BGI), Chinese Academy of Sciences, I Zone, Shenzhen, Beijing 518057, China
3 Harbin Institute of Veterinary Medicine, The Chinese Academy of Agricultural Sciences, Huizhou, Guangdong Province, 516000, PR China

Received 2 September 2004; accepted 16 June 2005
Available online 12 September 2005

Vero cells (ATCC® CCL-81™)


Molecular and Biological Characterization of Human Monoclonal Antibodies Binding to the Spike and Nucleocapsid Proteins of Severe Acute Respiratory Syndrome Coronavirus

Edward N. van den Brink,1 Jan ter Meulen,1 Freerk Cox,1 Manday A. C. Jongeneelen,1 Alexandra Thissée,1 Mark Thorsby,1 Wilfred E. Marissen,1 Pauline M. L. Rood,1 Alexander B. H. Bakker,2 Hans R. Gelderblom,2 Byron F. Martina3 Albert D. M. E. Osterhaus,3 Wolfgang Preiser,4 Hans Wilhelm Doerr,4 John de Kruif,1 and Jaap Goudsmit1,4

ARTICLE INFO

Article history:
Received 1 March 2003
Revised 16 March 2003
Accepted 16 March 2003
Available online

ABSTRACT

Background: Coronaviruses pose a serious threat (SARS), Middle East Respiratory Coronavirus (MERS-CoV), and the so-called SARS-CoV-2, are the causative SaS vaccines that saddle flu-like onset

An Efficient Method to Make Human Monoclonal Antibodies From Memory B Cells: Potent Neutralization of SARS Coronavirus

Elisabetta Traggia1,3, Stephan Becker, Kanta Subbarao, Larissa Kolesnikova, Yasushi Uematsu, Maria Rita Gismondo, Brian R Murphy, Rino Rappuoli, Antonio Lanzavecchia

Affiliations + expand

PMID: 15247913
PMCID: PMC7095806
DOI: 10.1038/nm1080


Published online 2007 Jul 9. doi: 10.1073/rnas.0701001014
Medical Sciences

Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies

Jo,1 Arianne Roberts,2 Tim Sheahan,3 Xiaodong Xiao4,5,6
E. A. Sidky,2 Davide Catt2,7,8,9 Beatrice Vignali4,8 Yu-Feng10,11,12 Lanzavecchia,13,14,15,16,17,18,19

$limite$8

PMCID: PMC1924550
PMID: 17620608
Major areas in scientific research to combat the pandemic

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A SARS-CoV-2 protein interaction map reveals targets for drug repurposing

Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro

Vero E6 cells (ATCC® CCL-1586™)

Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro

Vero E6 cells (ATCC® CCL-1586™)
Two groups of drugs that affect the virus and the two different ways that it happens were identified from screening old drugs:

- **Disrupting translation of the virus**
  - Ternatin-4
  - Zotatifin
  - Plitidepsin

- **Sigma receptors**
  - Two antipsychotics: haloperidol and melperone
  - Two potent antihistamines:
    - Clemastine
    - Cloperastine
  - Compound PB28
  - Female hormone progesterone
Ongoing COVID-19 clinical trials

341,642 studies for COVID-19

Summary

- COVID-19 is a high global and public health threat.
- The development of safe and effective diagnostic methods, prophylactics, and therapeutics will depend on solid scientific research.
- ATCC has expedited scientific research by quickly providing a variety of research materials for the development of diagnostic assays, vaccines, and therapeutics.
- We must proactively protect ourselves and our community from COVID-19 infection.
- Everyone is a part of the solution.

www.atcc.org/coronavirus
Viral Metagenomics and the Use of Standards: From Biology to Clinical Applications

Presented by Tasha M. Santiago-Rodriguez, Ph.D.

12 ET, June 25, 2020