OPTICA
Advancing Optics and Photonics Worldwide
Formerly OSA

Molecular Probes and Nanobio-Optics Technical Group

miSHERLOCK: CRISPR-enabled POC diagnostic platform for SARS-CoV-2

Dr. Helena De Puig

Broad Institute of MIT and Harvard

15 November 2021 @ 10 AM PST/ 1PM EST

About Our Technical Group

This technical group focuses on using novel nano-probes such as QDs, fluorescent proteins, and plasmonic nanoparticles, to deepen our understanding of biological tissues. Applications span novel therapeutics, tissue imaging, and point-of-care diagnostics.

Our mission is to accelerate the development of the state-of-the-art technologies and connect the 1000+ members of our community through technical events, webinars, networking events, and social media.

Past Webinars:

- **Medical Hyperspectral Imaging: Artificial Intelligence and Image-Guided Surgery** by Prof. Baowei Fei in January 2021.
- **Molecular Understanding of Electromagnetic Field Biomatter Interaction** by Dr. Michal Cifra on October 29 2021.

Connect With Our Technical Group

Join our online community to stay up to date on our group's activities. You also can share your ideas for technical group events or let us know if you're interested in presenting your research.

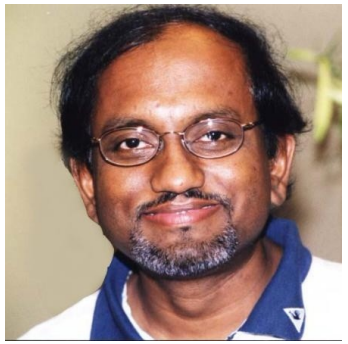
Ways to connect with us:

- Our website at www.optica.org/BP
- On LinkedIn at www.linkedin.com/groups/12561256/
- On Facebook at www.facebook.com/groups/opticamolecularprobestg
- Email us at TGactivities@optica.org

Technical Group Executive Committee

Chair
Girija Gaur, Ph.D.
University of California, Irvine

Vice Chair
Prof. Michel Meunier
Polytechnique Montreal, Canada
Co-founder Vega BioImaging

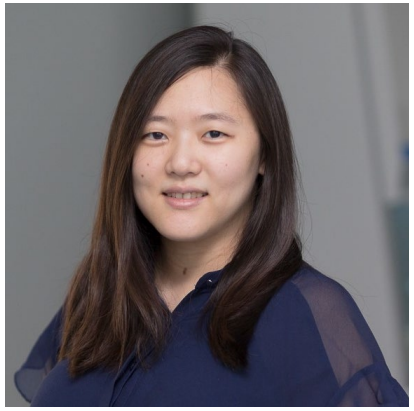


Conference Chair
Achyut Dutta, Ph.D.
CEO, Banpil Photonics

Global Events Officer
Shuren Hu, Ph.D.
Lidar System Architect, Aurora

Planning & Social Media Officer
Atrouli Chatterjee
University of California, Irvine

Technical Group Executive Committee



Events-Asia Pacific
Assistant Prof. Hyeon Jeong Lee
Zhejiang University, China

Events Officer
Assistant Prof. Lucien Weiss
Polytechnique Montreal, Canada

Events Officer
Assistant Prof. Aniruddha Ray
University of Toledo

Today's Speaker

Dr. Helena De Puig

Postdoctoral Researcher at the Broad Institute of MIT and Harvard

Dr. Puig uses synthetic biology principles to build new molecular diagnostics and smart materials. Dr. Puig has been awarded over $350,000 in prestigious fellowships in Spain and at MIT. Dr. Puig earned a S.M. in Mechanical Engineering at MIT under the guidance of Dr. Kimberly Hamad-Schifferli and then obtained a Ph.D. in Mechanical Engineering at MIT, in the laboratory of Prof. Lee Gehrke where she developed nanoparticle-assisted detection methods to diagnose tropical diseases, such as dengue, zika, chikungunya or ebola. She is a co-inventor on 11 patent applications and has published over 20 articles in journals including Science and Science Translation Medicine.

Minimally instrumented SHERLOCK (miSHERLOCK) for CRISPR-based point-of-care diagnosis of SARS-CoV-2 and emerging variants

Helena de Puig

Wyss Institute for Biologically Inspired Engineering, Harvard University

Institute for Medical Engineering and Sciences. Massachusetts Institute of Technology

Diagnosing SARS-CoV-2 and emerging variants

SARS-CoV-2

Worldwide: 253M cases – 5.1M deaths.
Economic return from increased testing and contact tracing is 30 times the cost.

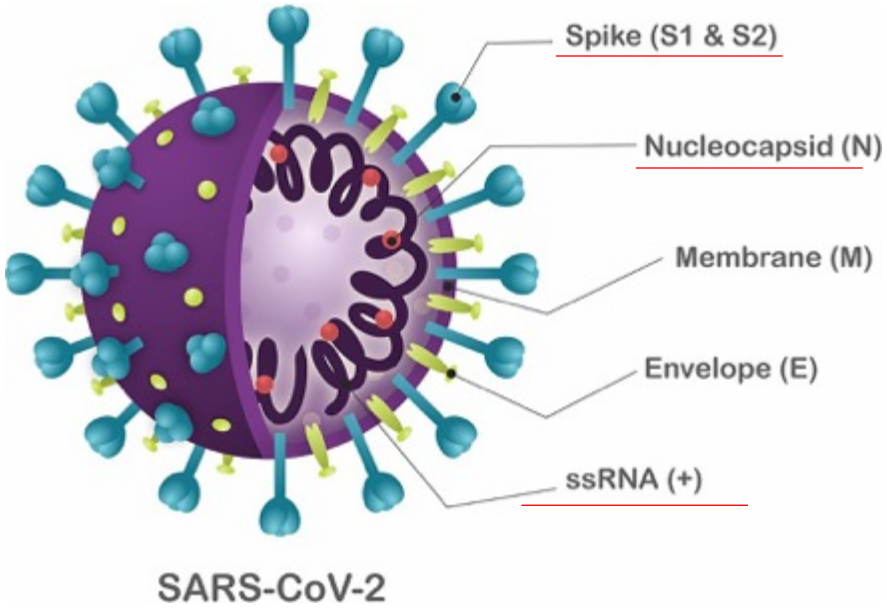
All outbreaks

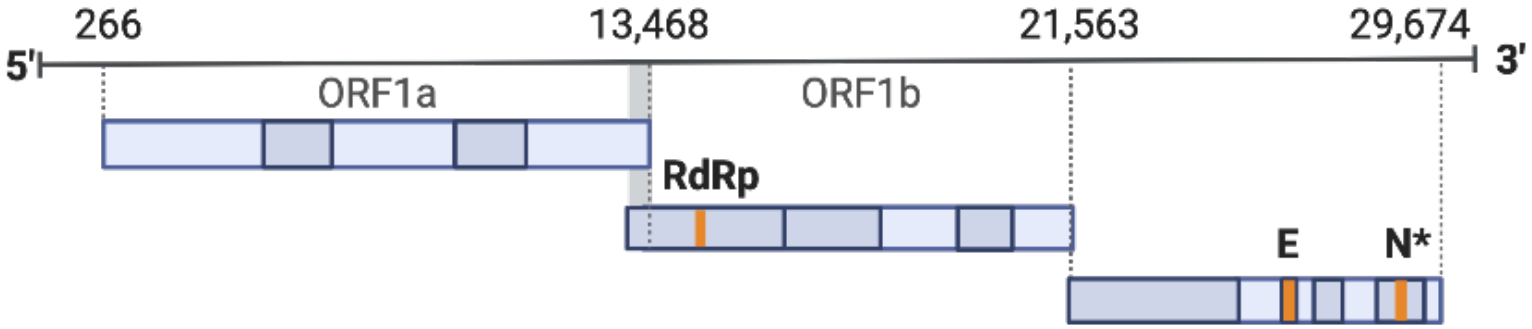
During epidemics, the development of rapid, de-centralized diagnostics is #1 priority.
Development of new assays takes months: This is too long during an outbreak.

Diagnostics are fundamental for:

- Patient management
- Surveillance and epidemiology
- Prevention (e.g. quarantine)
- Treatment
- Vaccine efficacy studies

Virus genome (RNA) contains information to make new virions





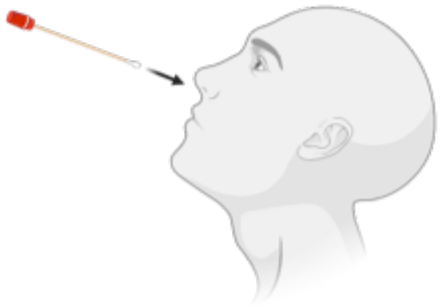
Sequencing assays (to detect virus variants) as well as nucleic acid tests (LAMP/PCR) target SARS-CoV-2 viral RNA.

RT-qPCR to detect viral RNA

Genome detection

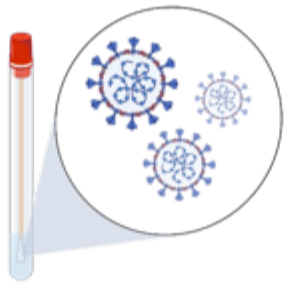
1 Nasopharyngeal swab <15 min

Cotton swab is inserted into nostril to absorb secretions.



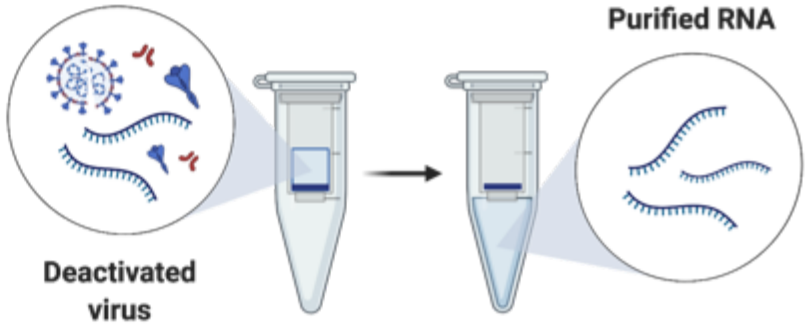
2 Collected specimen 0-72 h

Specimen is stored at 2-8°C for up to 72 hours or proceed to RNA extraction.



3 RNA extraction ~45 min

Purified RNA is extracted from deactivated virus.

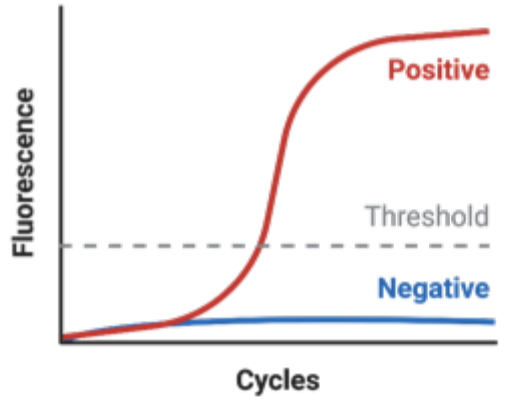


4 RT-qPCR ~1 h per primer set

Purified RNA is reverse transcribed to cDNA and amplified by qPCR.

5 Test results real-time

Positive SARS-CoV-2 patients cross the threshold line within 40.00 cycles (< 40.00 Ct).

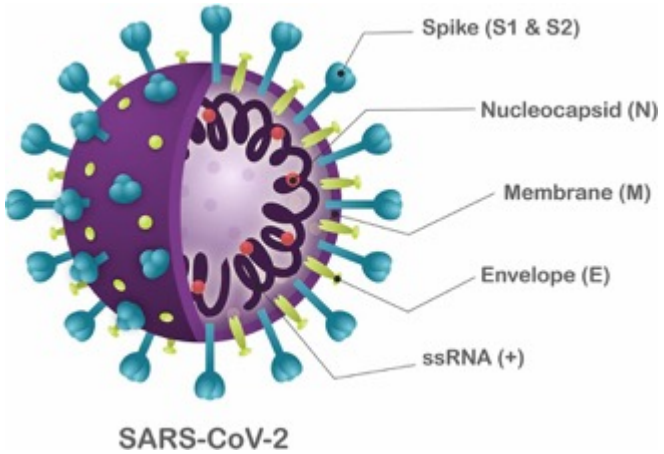


<u>Current SARS-CoV-2 diagnostic methods: PCR</u>

- **Slow** turnaround for readout
- Requires **expensive** reagents, equipment and trained personnel
- **Complex** methodology
- **Fixed** location
- Cold-chain transport of samples and reagents

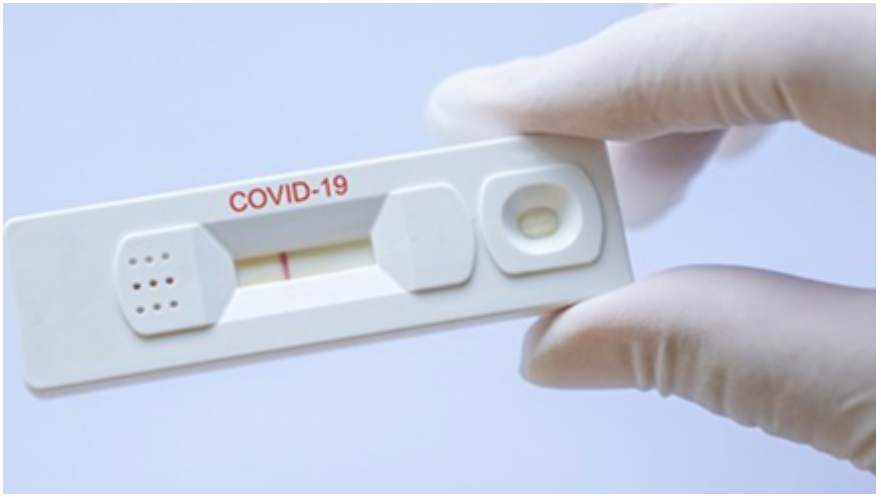
<u>Gold standard to diagnose variants</u>: Sequencing: more expensive and slower than PCR.

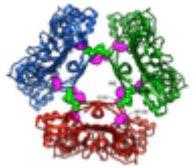
Antigen detection (ASSURED criteria)



SARS-CoV-2

Affordable
Sensitive & **S**pecific
User-friendly
Rapid
Equipment-free
Deliverable





Antigen detection

ASSURED criteria are key for diagnosis of disease at home and/or in remote areas

Rapid antigen tests are in general not sensitive enough to detect SARS-CoV-2 in all stages of disease

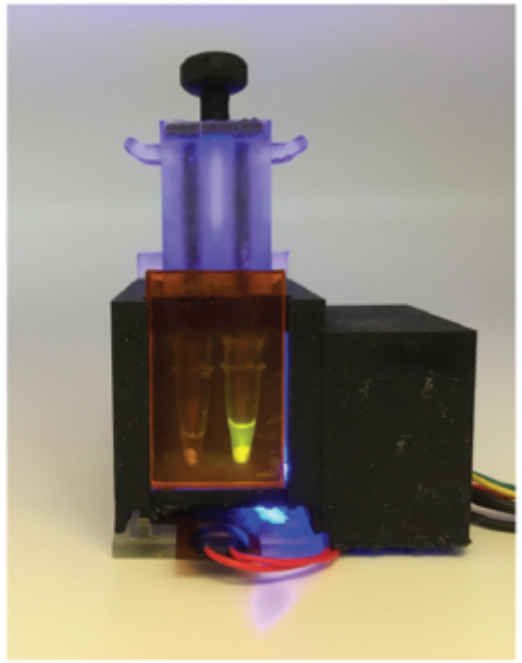
WHO guidelines, diagnostics

Peeling et al., Nature Reviews Microbiology, 2006

Alternative diagnostics (ASSURED criteria) for genome detection

Genome detection

Affordable
Sensitive & **S**pecific
User-friendly
Rapid
Equipment-free
Deliverable



miSHERLOCK is an ideal candidate for diagnosis of disease at home and/or in remote areas
Sensitivity and specificity comparable to PCR
Also complies with other ASSURED criteria
Cost $2-$15; sample-to-answer in 1h

WHO guidelines, diagnostics
Peeling et al., Nature Reviews Microbiology, 2006

Portable, rapid SARS-CoV-2 diagnostics for at-home and de-centralized testing

Sample type and pre-treatment:

- Easily accessible sample: self-collected saliva
- Integrated virus lysis and nuclease inactivation
- Viral RNA collection and concentration in solid substrates

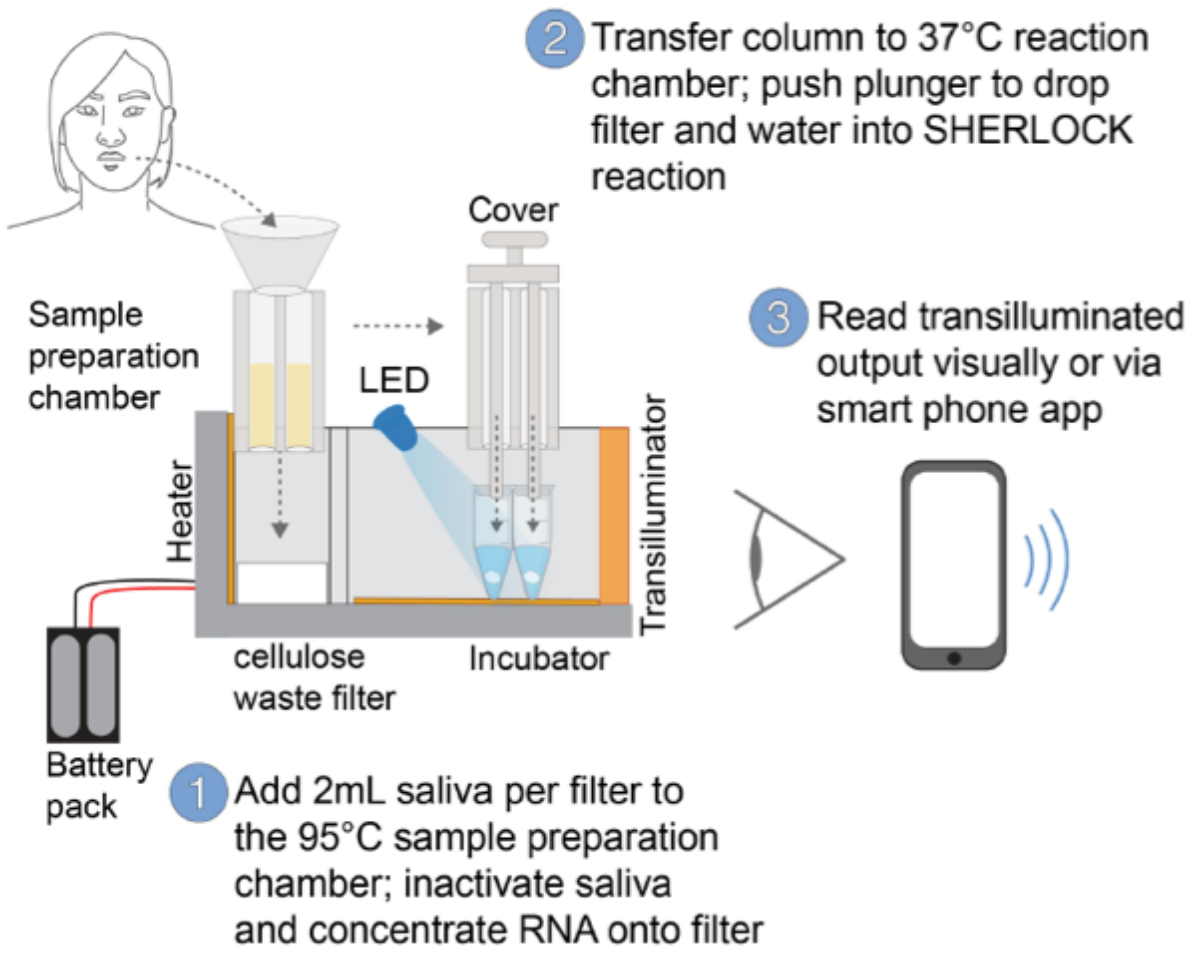
Biochemical assay:

- Isothermal amplification + signal through CRISPR-Cas enzymes (SHERLOCK with Cas12a or Cas13a).
- Limit of detection: ~1cp/μl
- Stabilized dehydrated enzymes for room temperature transport/storage

Device integration:

- Format: 3D-printed, low-cost instrument and consumable cartridges.
- Easy to use microfluidics device integrates sample preparation, assay development and diagnostic readout.

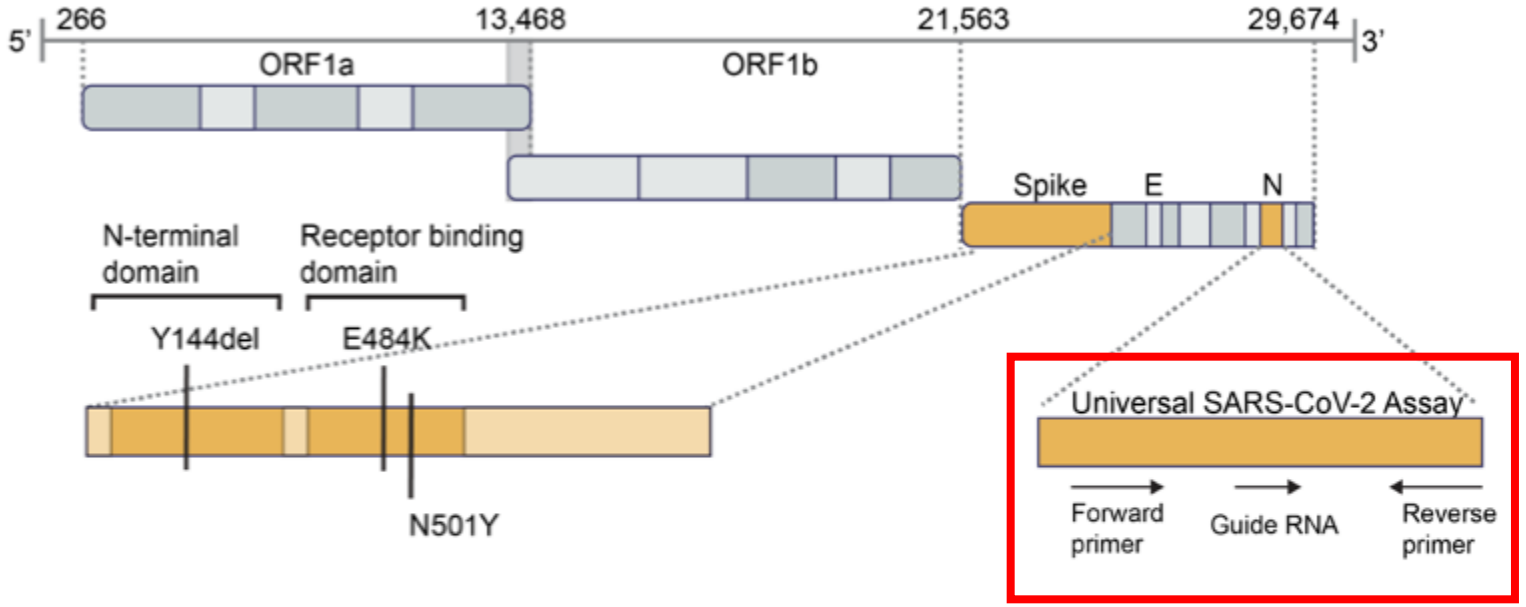
Portable, rapid SARS-CoV-2 diagnostics for at-home and de-centralized testing



miSHERLOCK is a device that integrates:

- **Biochemical reactions:**
  - Isothermal amplification + signal through CRISPR-Cas enzymes (SHERLOCK with Cas12a or Cas13a).
  - Limit of detection: ~1cp/μl
  - Stabilized dehydrated enzymes for room temperature transport/storage

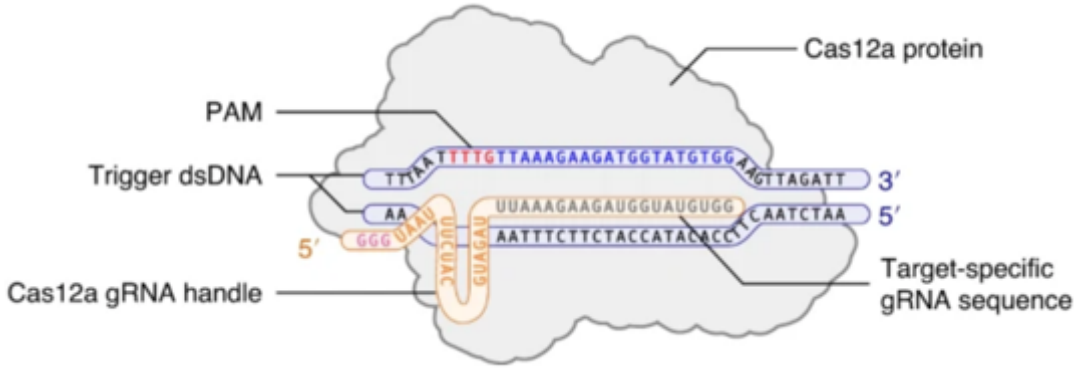
Key SARS-CoV-2 target regions



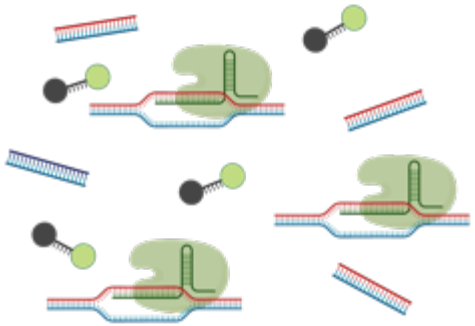
Spike Region Mutations of the Key SARS-CoV-2 Variants

N501Y: A23063T E484K: G23012A	**P.1, B.1.351** **(Brazil, S. Africa Variant)**
N501Y: A23063T Y144 del: 21991-21993 deletion	**B.1.1.7** **(UK Variant)**

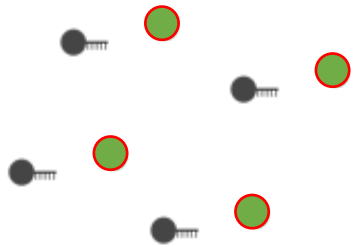
Cas12a-based diagnostics



Cas12a detection



Collateral cleavage produces fluorescence

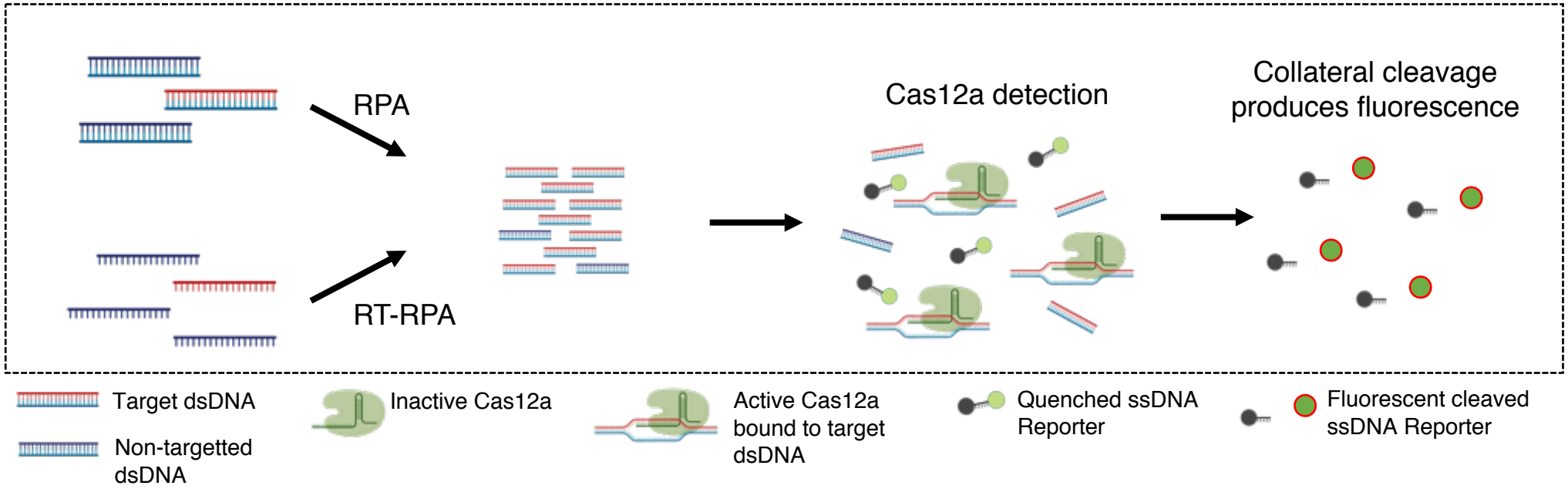


→ Cas12a activates through recognition of its dsDNA target. Once activated, it exhibits promiscuous, non-specific DNase activity and cleaves non-target ssDNAs.

→ Advantage: highly specific.

SHERLOCK diagnostics

CRISPR-based diagnostics (SHERLOCK) use CRISPR/Cas enzymes to detect viral RNA

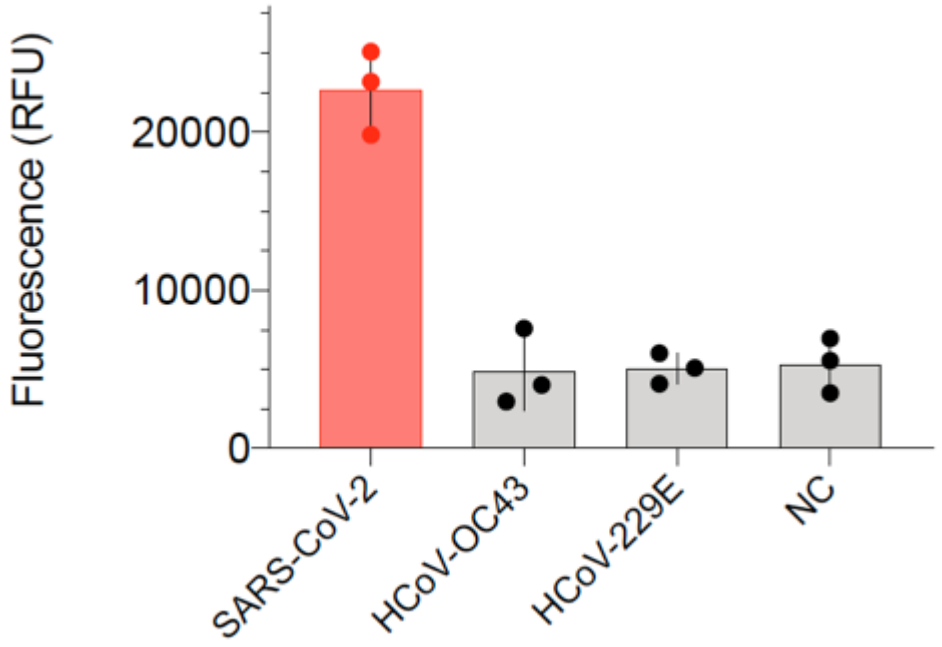


→ Cas12a activates through recognition of its dsDNA target. Once activated, it exhibits promiscuous, non-specific DNase activity and cleaves non-target ssDNAs.

→ Isothermal amplification (RPA, NASBA) is compatible with Cas12a detection.

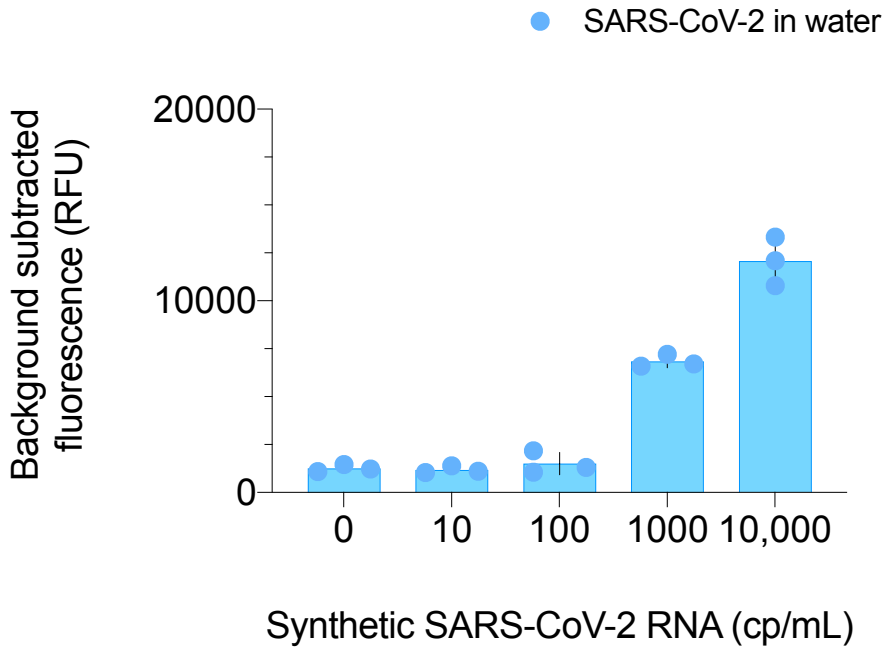
→ Advantage: sensitive + specific.

SARS-CoV-2 universal assay (N gene) is specific



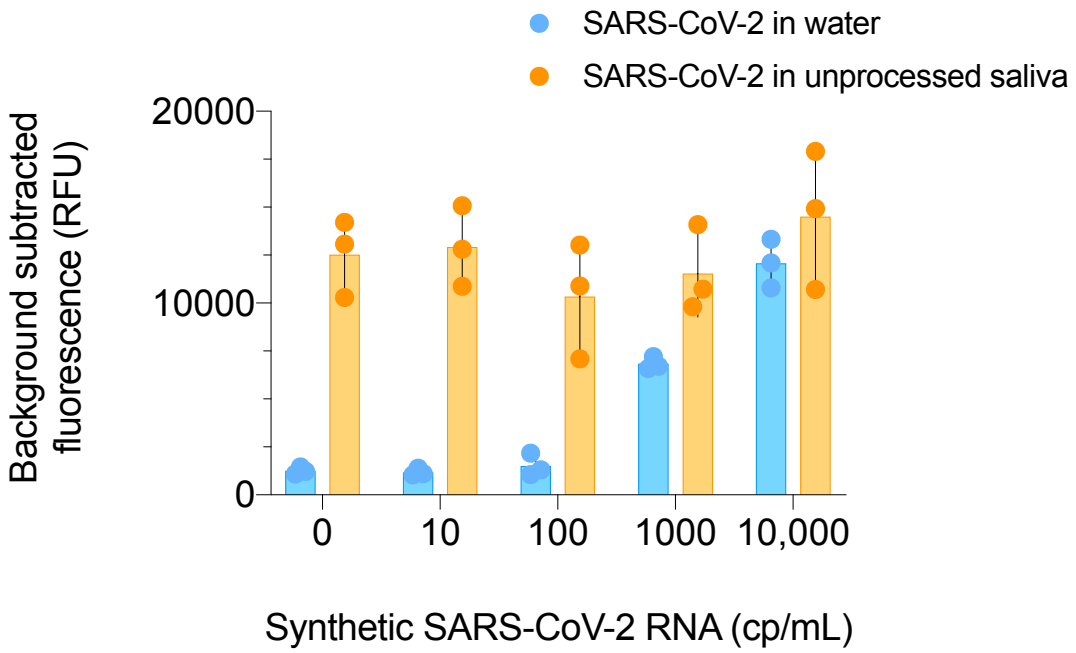
SARS-CoV-2 universal assay detects SARS-CoV-2 and other endemic coronaviruses are not detected

SARS-CoV-2 universal assay (N gene) is sensitive



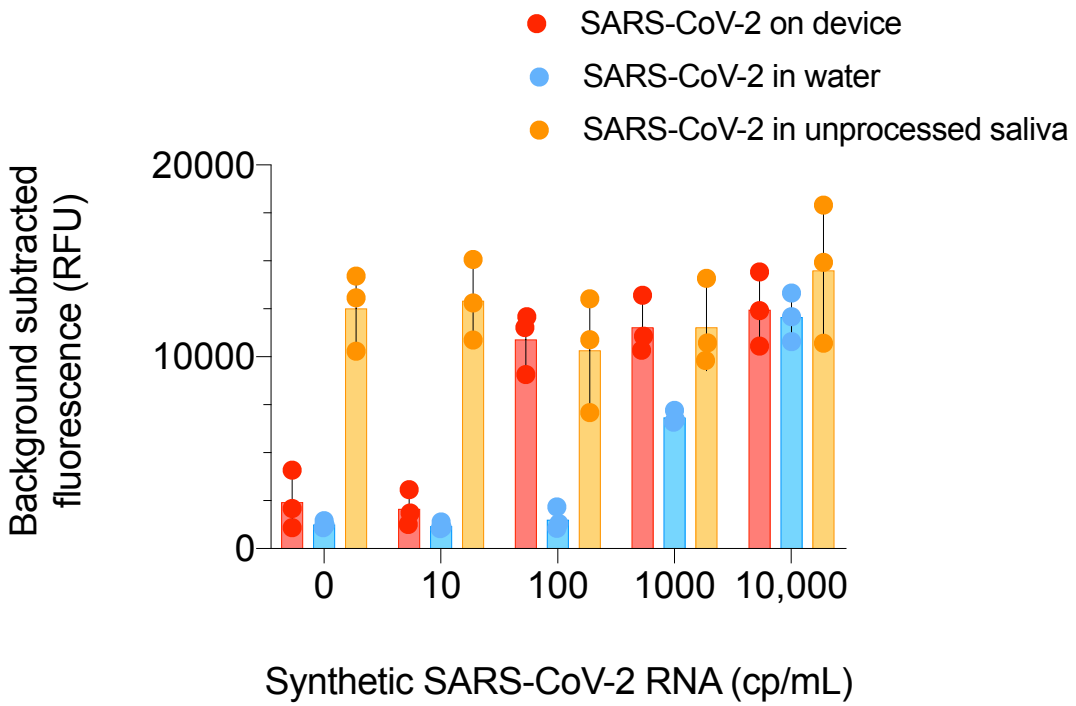
LOD 1cp/μl in water

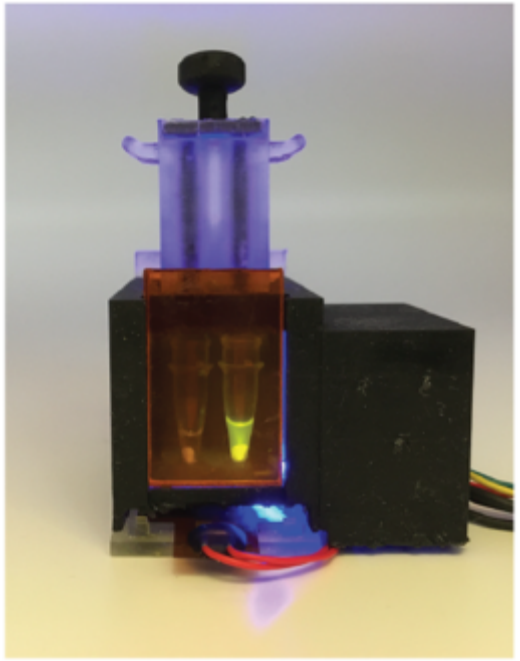
Saliva contains nucleases that must be deactivated



LOD 1cp/μl. However, unprocessed saliva leads to false positive signals

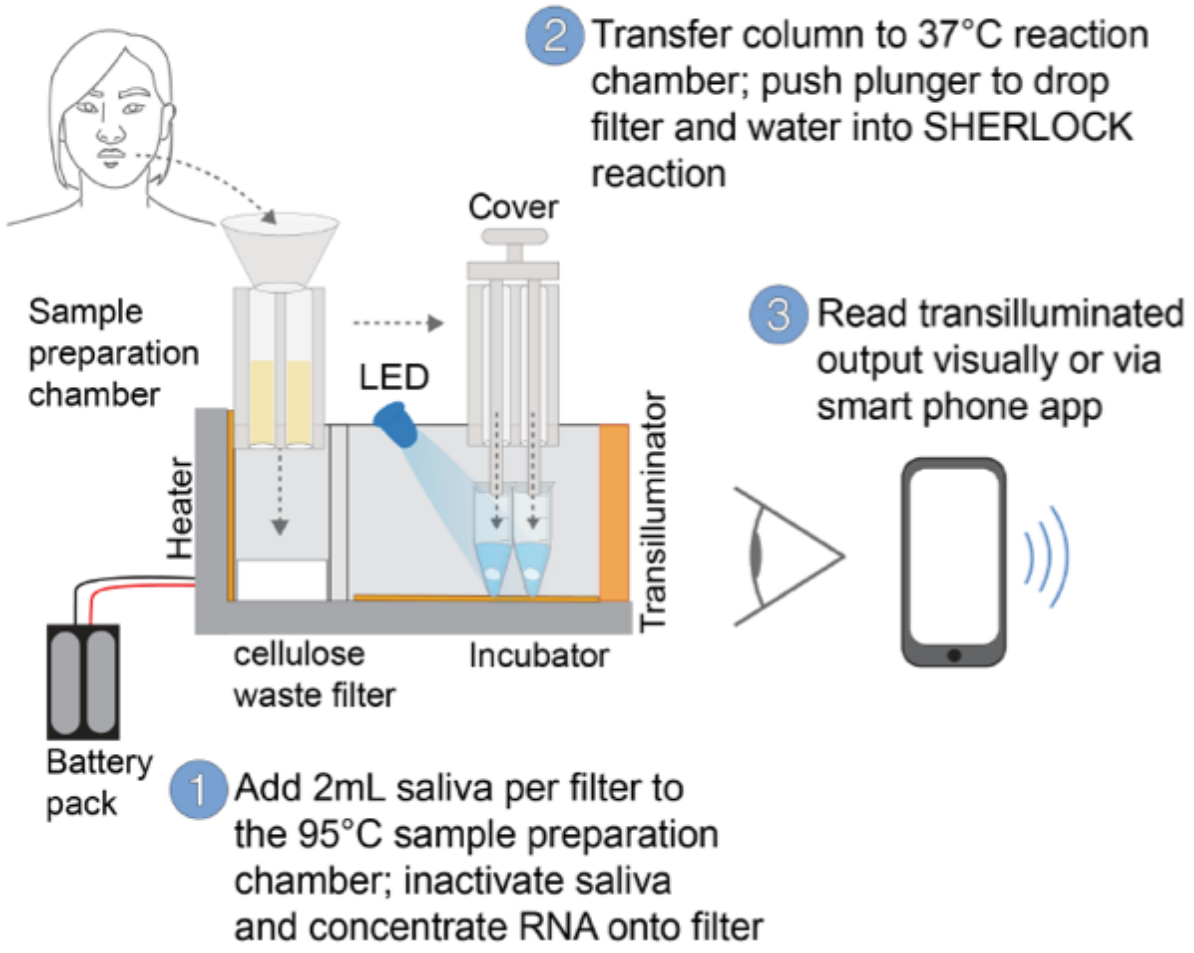
SARS-CoV-2 universal assay (N gene) on miSHERLOCK





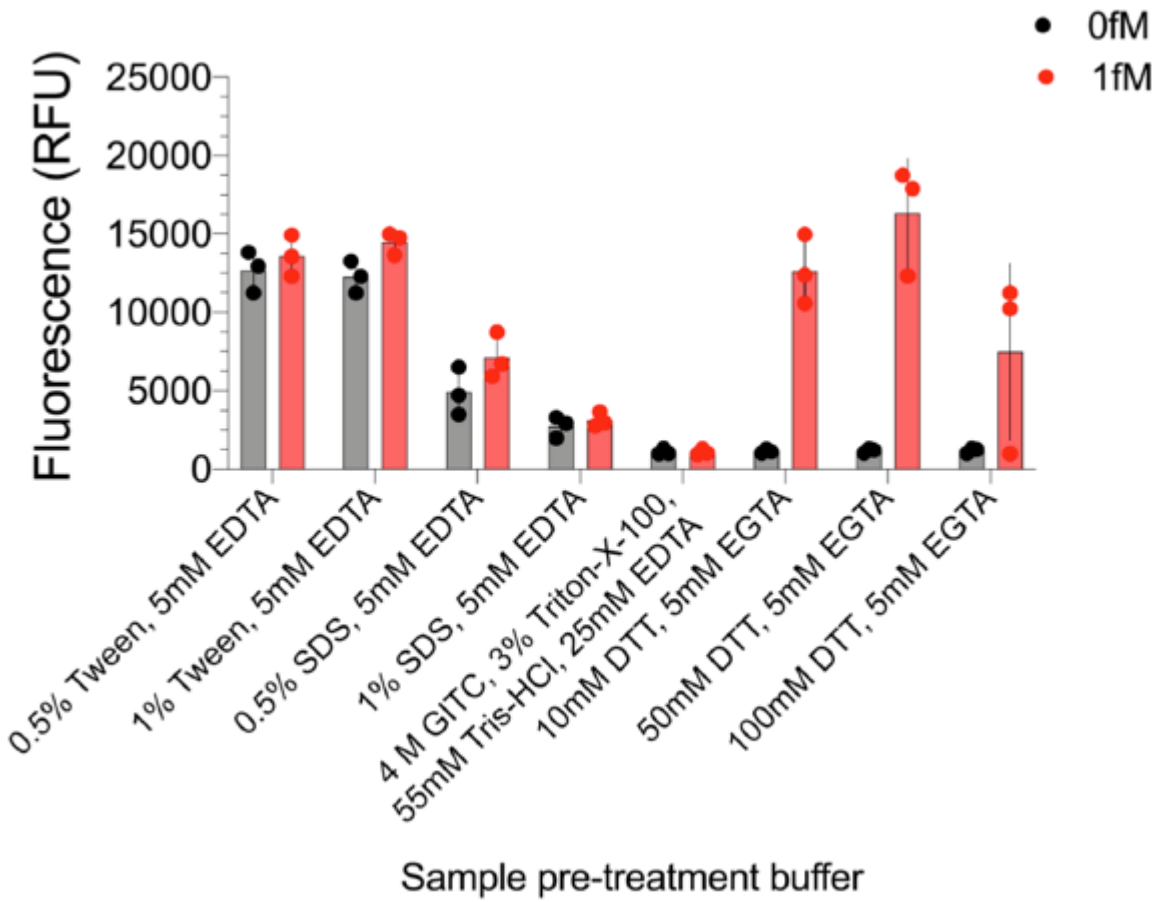
LOD 0.1cp/μl in processed saliva due to: (1) nuclease inactivation and (2) RNA concentration

Portable, rapid SARS-CoV-2 diagnostics for at-home and de-centralized testing



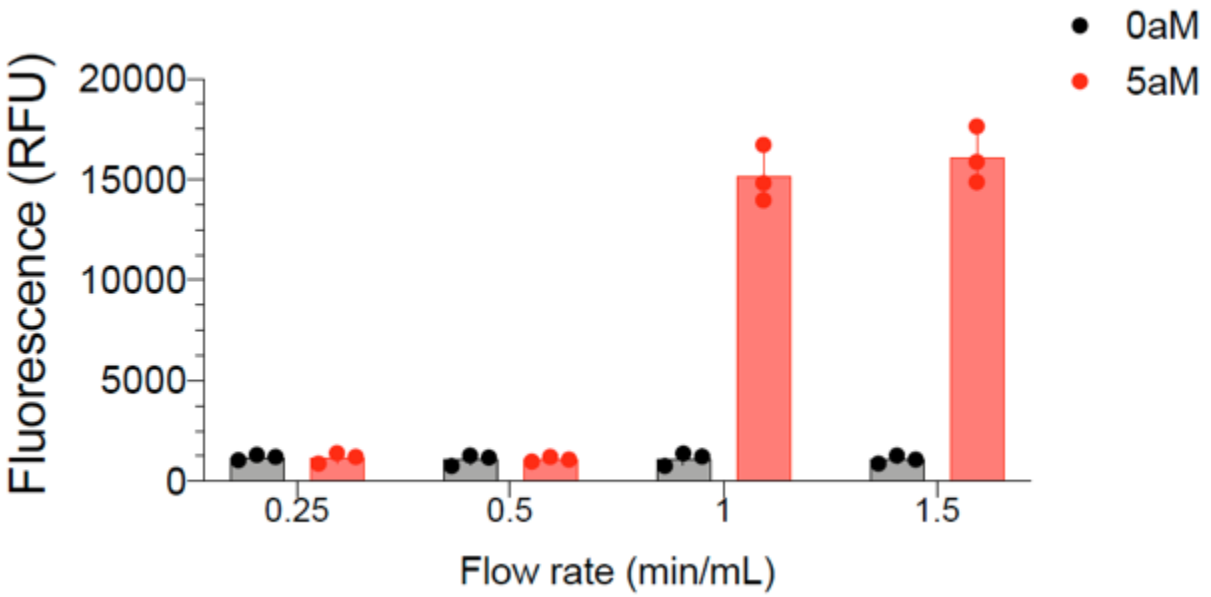
- Sample pre-treatment:
  - Easily accessible sample: self-collected saliva
  - Integrated virus lysis and nuclease inactivation
  - Viral RNA collection and concentration in solid substrates

Using saliva as a sample requires inactivating nucleases



EGTA/DTT combinations lead to highest signal/noise

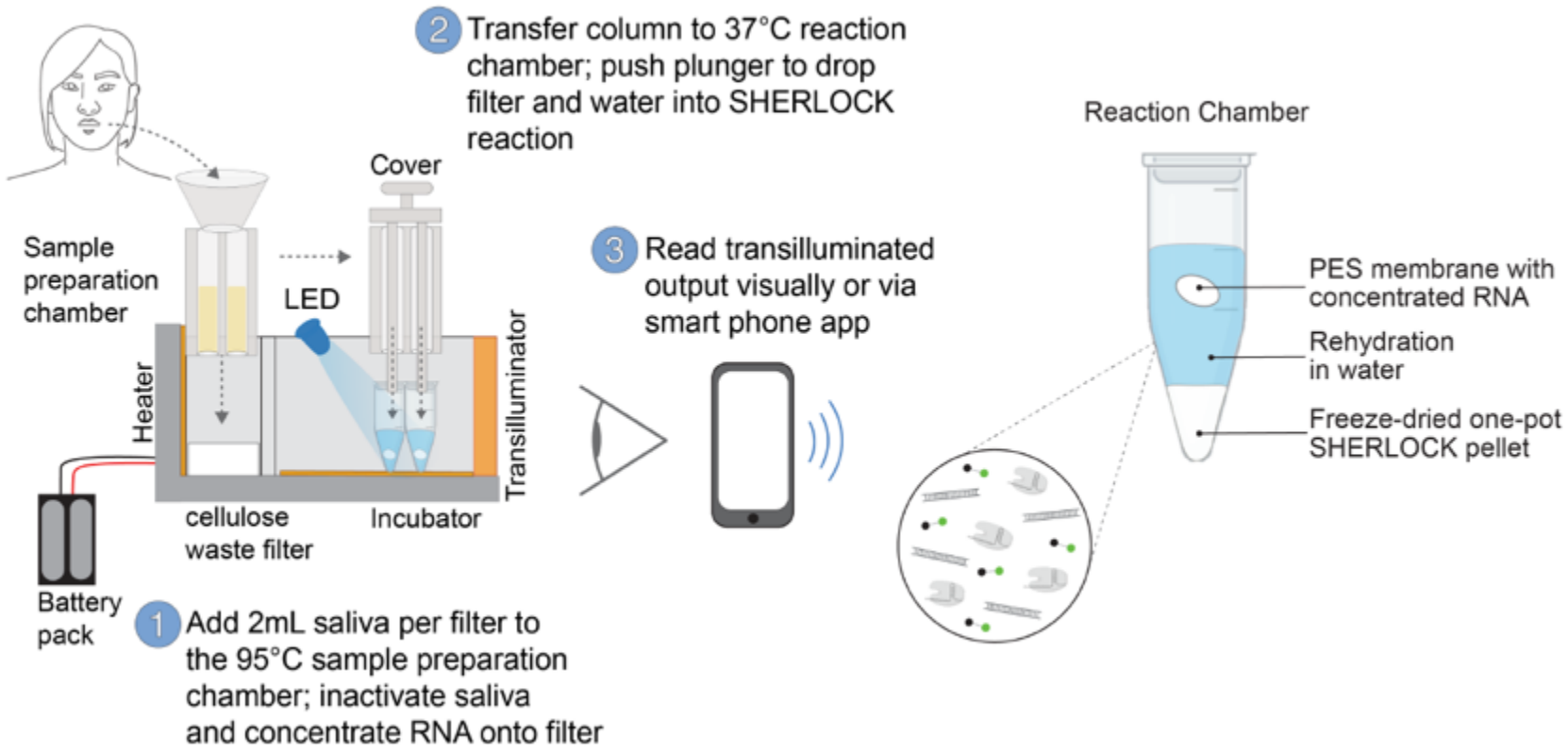
Viral RNA can be captured and concentrated in PES filters



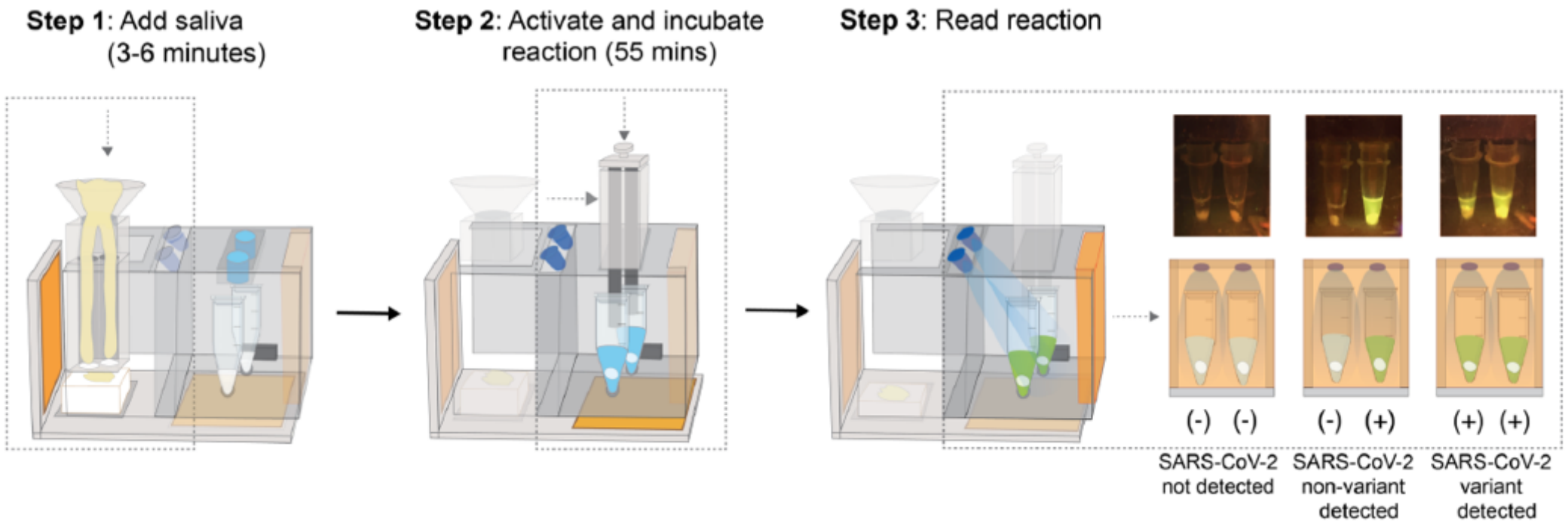
Slower low rates lead to more viral RNA captured

miSHERLOCK is a device that integrates:

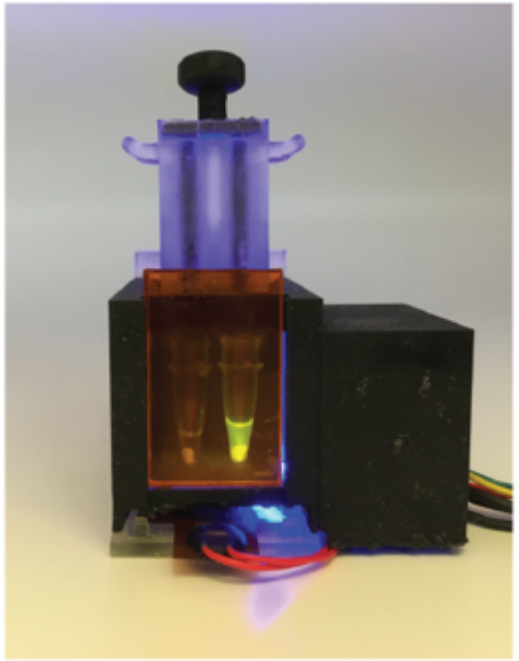
- Easy to use format and readout:
- Format: Low-cost instrument and consumable cartridges.
- Easy to use microfluidics device integrates sample preparation, assay development and diagnostic readout
- Phone-app for results visualization



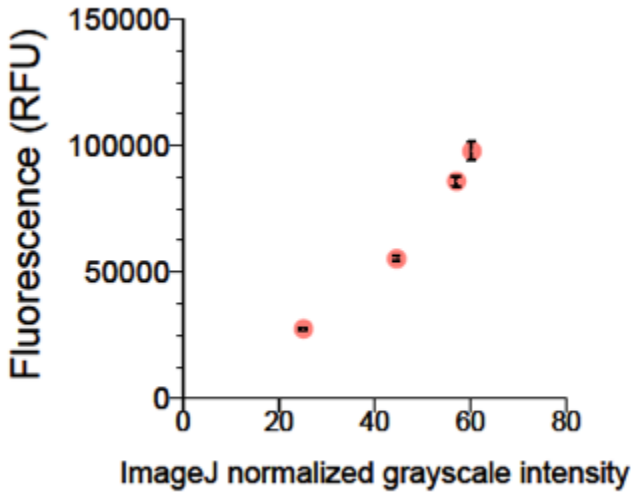
miSHERLOCK step by step:

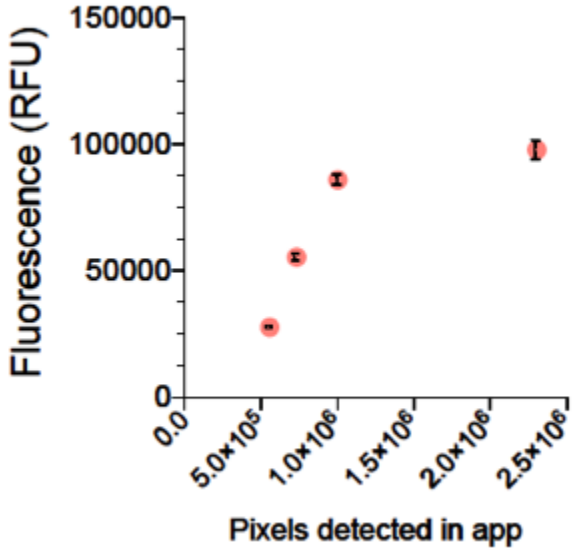


miSHERLOCK: a 3D printed device for SARS-CoV-2 diagnostics in saliva

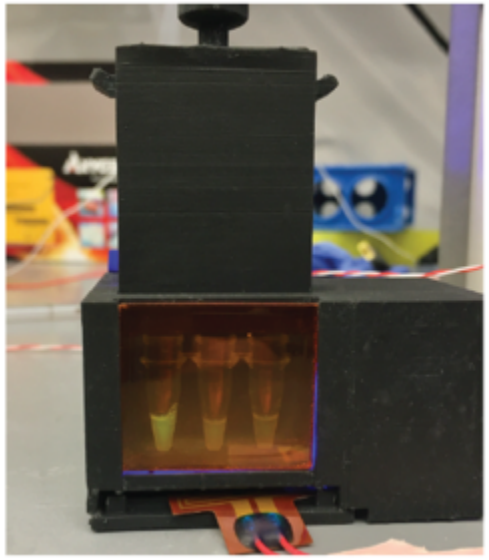


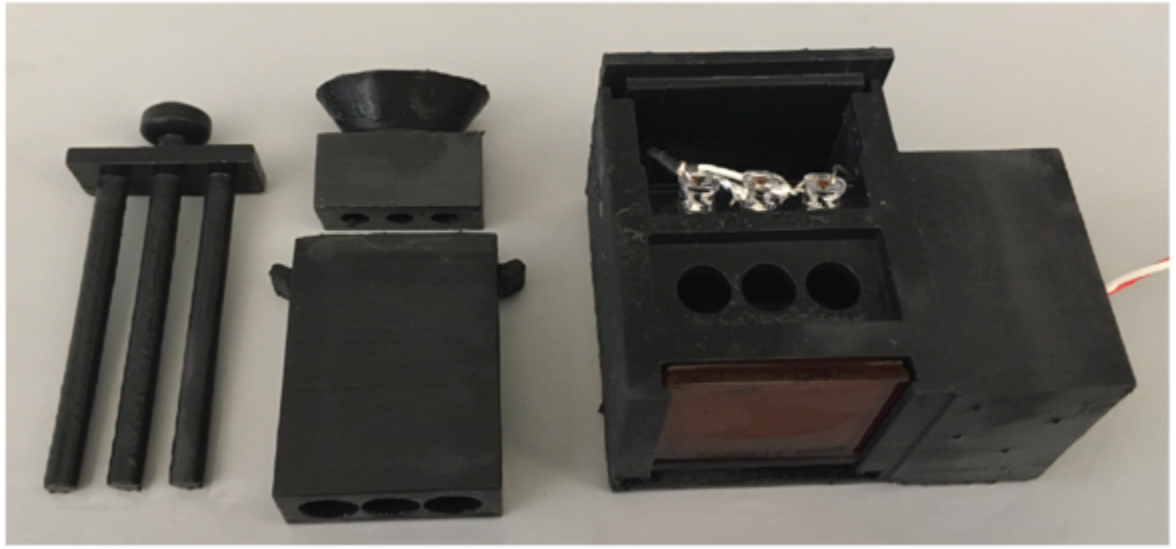
Fluorescence readouts in phone app/fluorimeter and ImageJ quantification are comparable

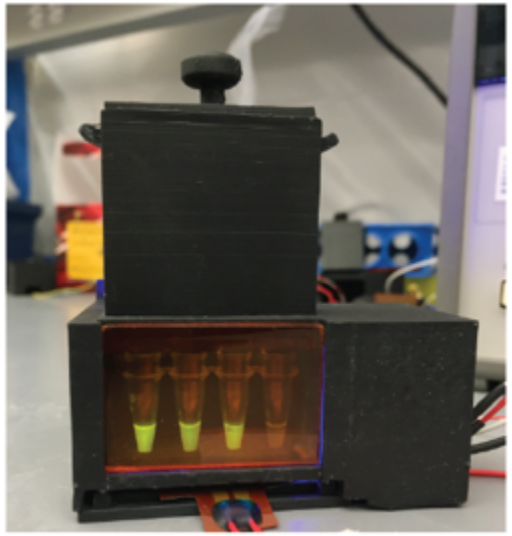




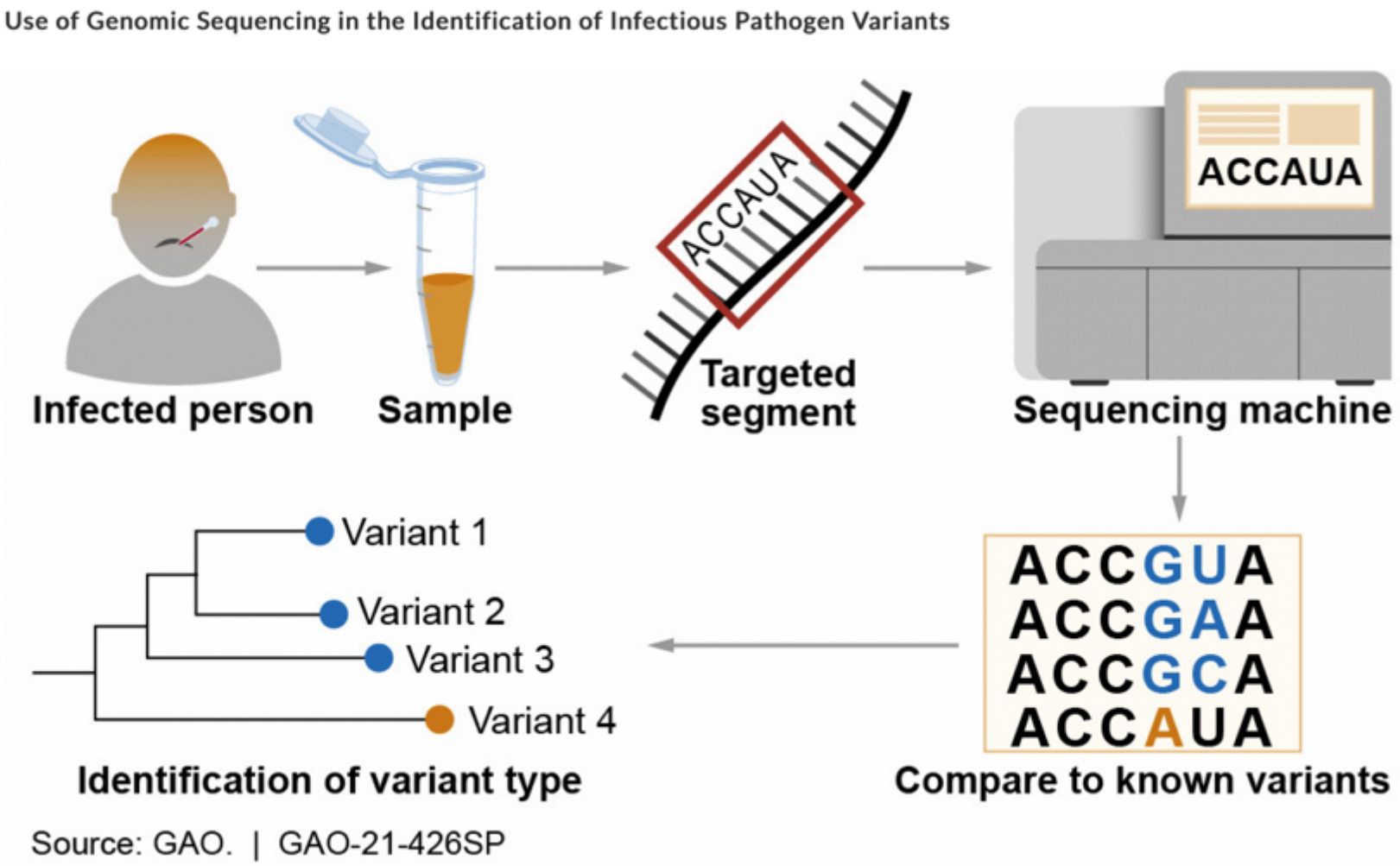
Device is easy to modify for higher order multiplexing



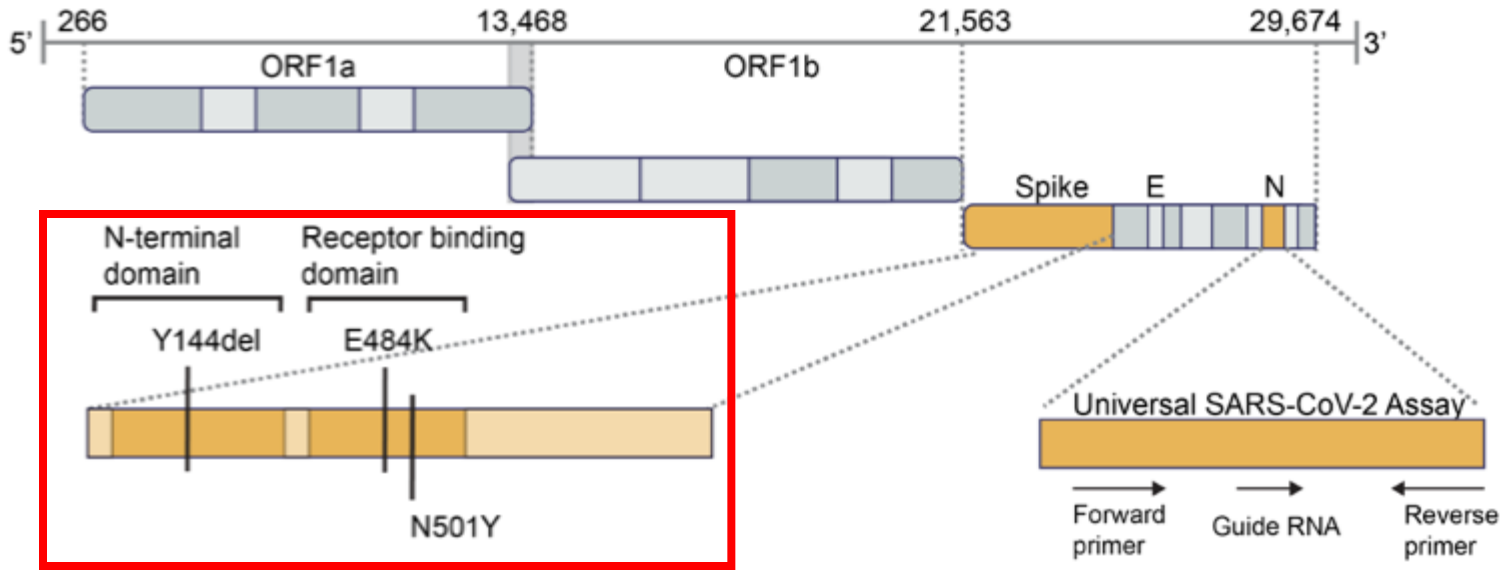




miSHERLOCK can be used to monitor SARS-CoV-2 variants



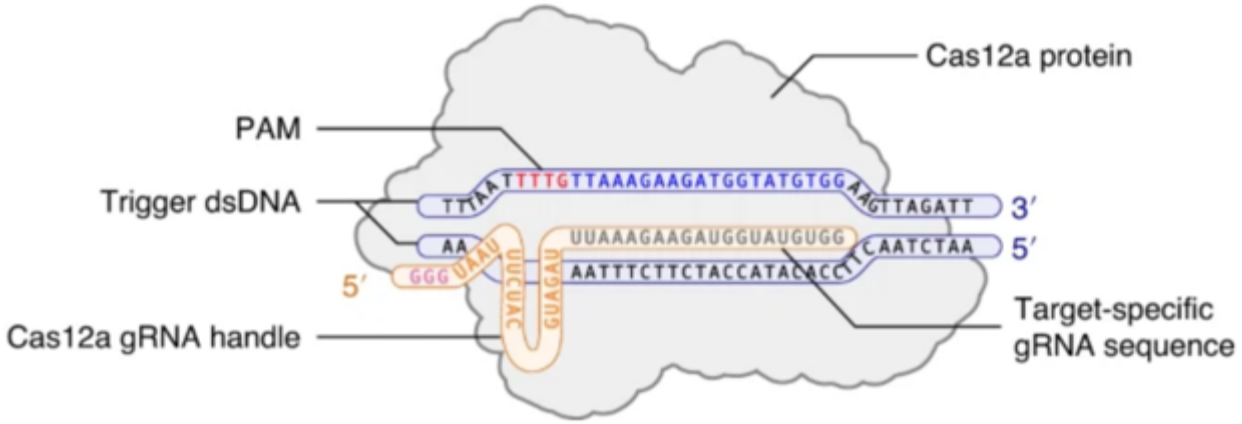
Key mutations to identify SARS-CoV-2 variants are located in the S gene

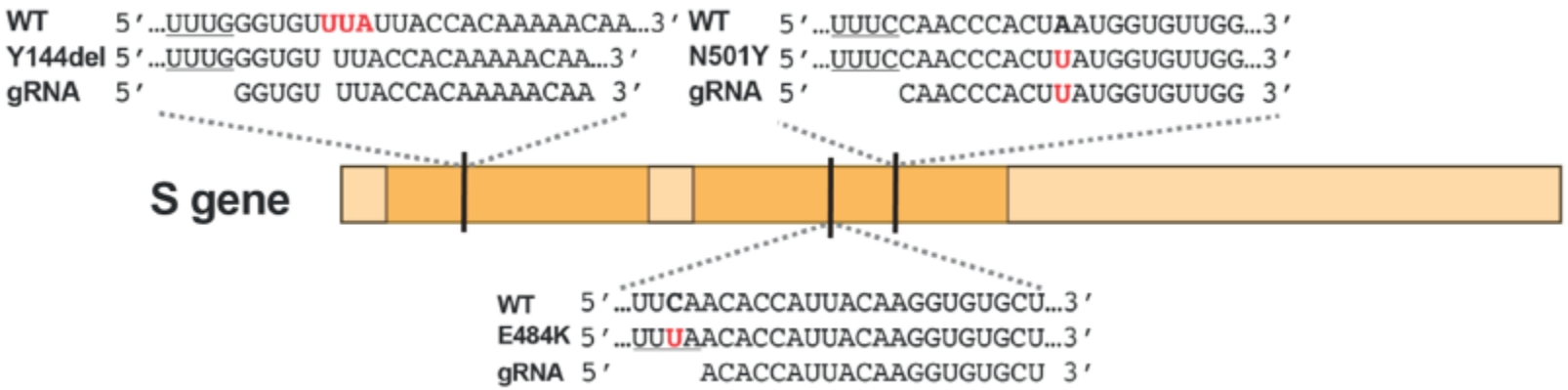


Spike Region Mutations of the Key SARS-CoV-2 Variants

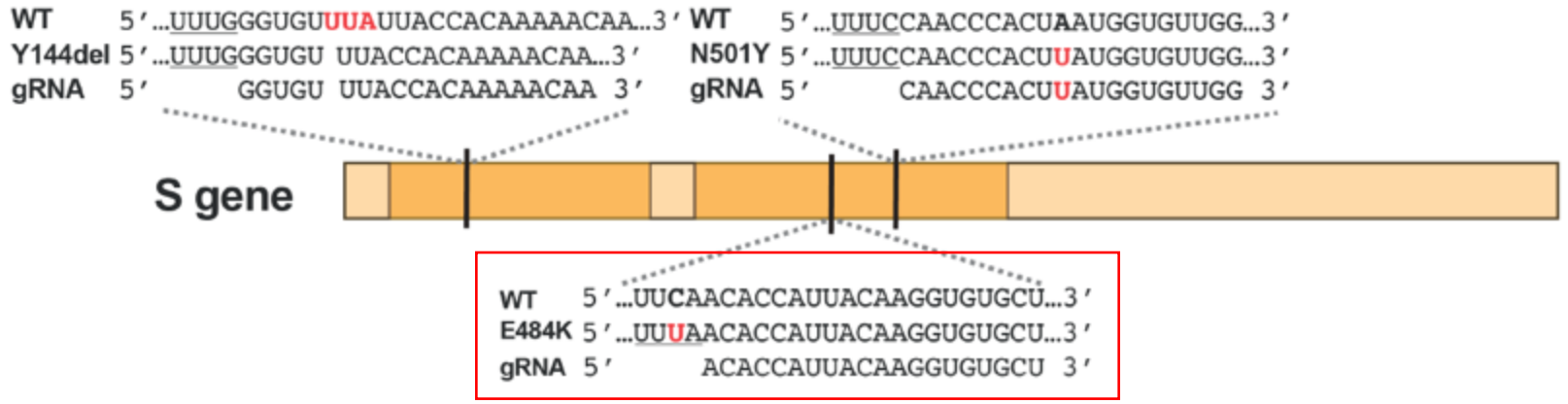
N501Y: A23063T E484K: G23012A	**P.1, B.1.351** **(Brazil, S. Africa Variant)**
N501Y: A23063T Y144 del: 21991-21993 deletion	**B.1.1.7** **(UK Variant)**

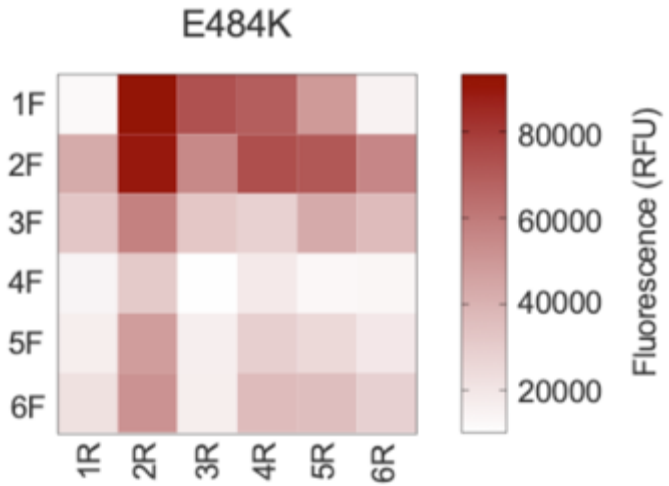
Cas enzymes have evolved to be very specific in nucleic acid detection





Example: E484K assay for the Brazil variant

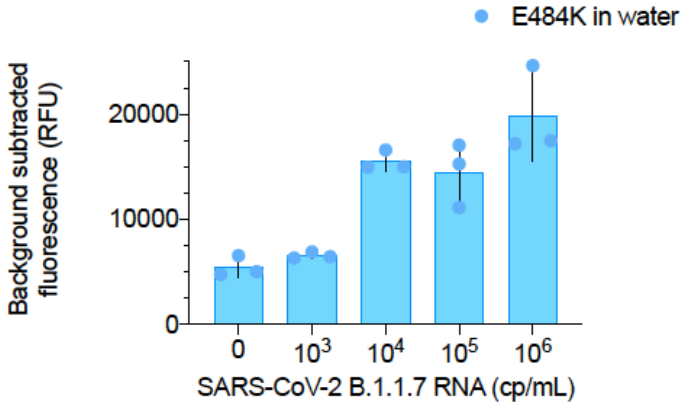




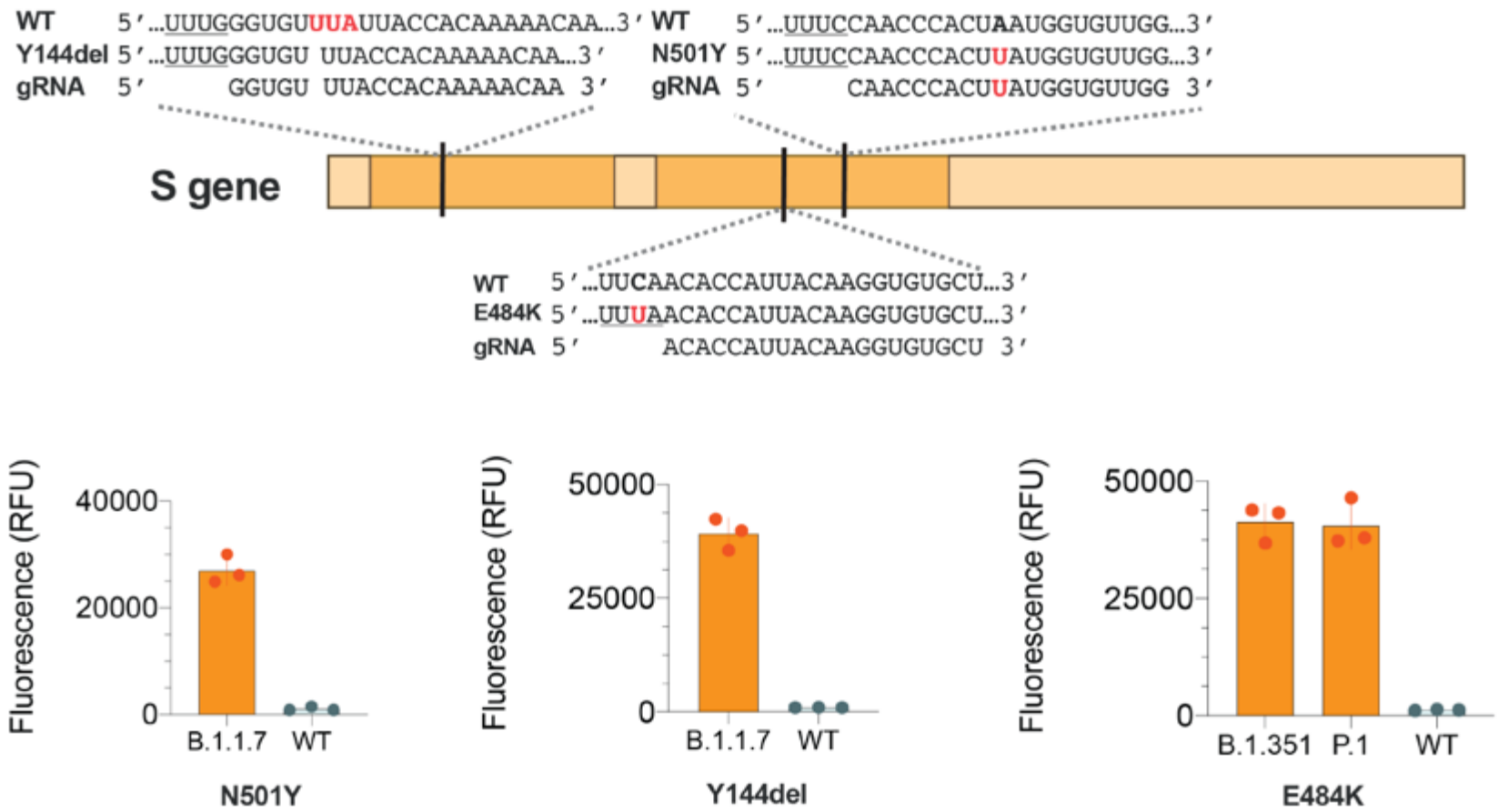
WT 5′...UUCAACACCAUUACAAGGUGUGCU...3′

E484K 5′...UUUAACACCAUUACAAGGUGUGCU...3′

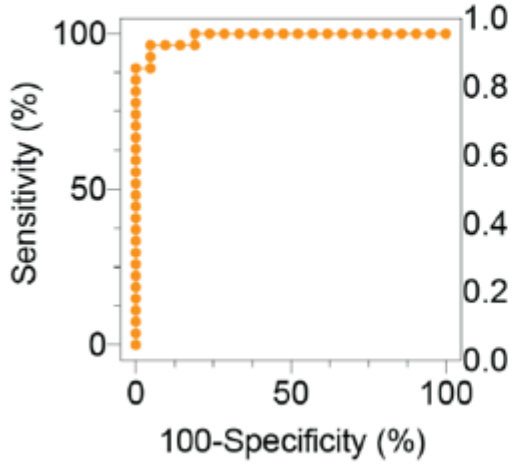
gRNA 5′... ACACCAUUACAAGGUGUGCU 3′

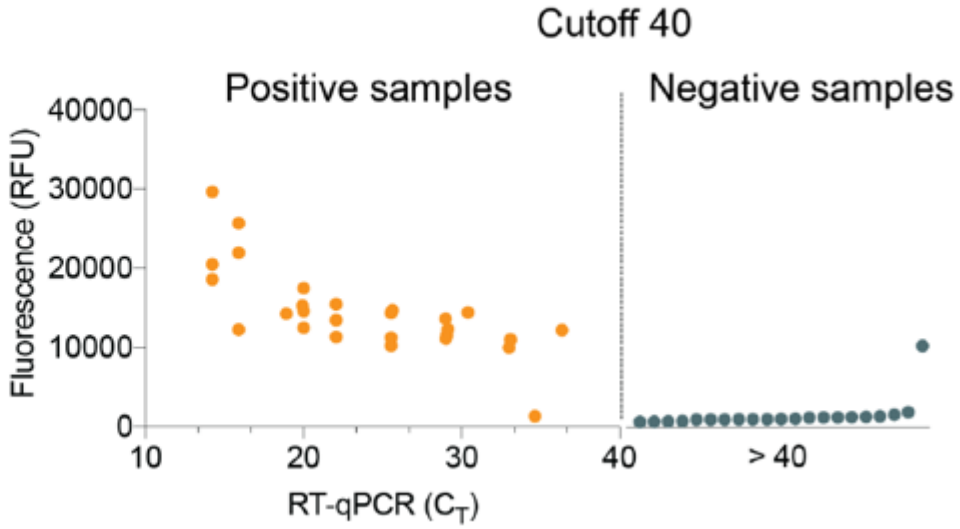


Assays on miSHERLOCK distinguish WT from variant RNA



miSHERLOCK performance with clinical samples



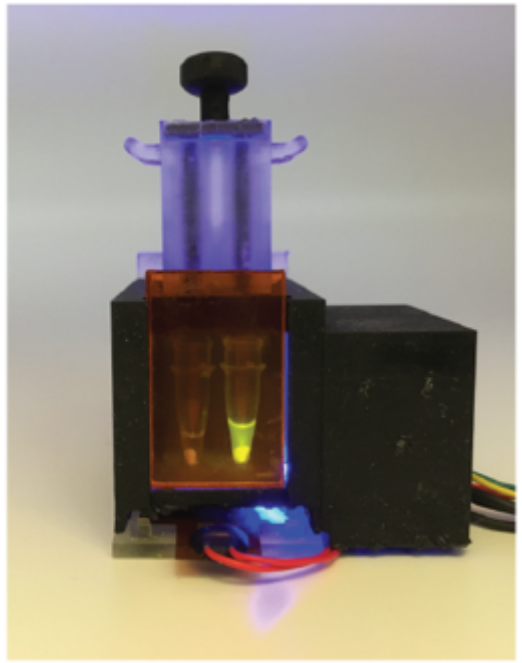


Results with clinical samples are comparable to RT-qPCR.

SUMMARY: miSHERLOCK device for remote/at home diagnostics

Genome detection

Affordable
Sensitive & **S**pecific
User-friendly
Rapid
Equipment-free
Deliverable



miSHERLOCK is an ideal candidate for diagnosis of disease at home and/or in remote areas
Sensitivity and specificity comparable to PCR
Also complies with other ASSURED criteria
Cost $2-$15; sample-to-answer in 1h

WHO guidelines, diagnostics
Peeling et al., Nature Reviews Microbiology, 2006

Thanks!

R. A. Lee, D. Najjar, X. Tan, L. R. Soekensen, N. M. Angenent-Mari, N. M. Donghia, N. E. Weckman, A. Ory, C. F. Ng, P. Q. Nguyen, A. S. Mao, T. C. Ferrante, G. Lansberry, H. Sallum, J. Niemi, **J. J. Collins.**

Funding: Paul G. Allen Frontiers Group, the Wyss Institute for Biologically Inspired Engineering, Harvard University and the Harvard University Center for AIDS Research (CFAR)