Microfluidic point-of-care device for detection of early strains and B.1.1.7 variant of SARS-CoV-2 virus

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Overview

- **Introduction**

- **Methods**

- **Results**

- **Discussion**

- **What's next?**

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)
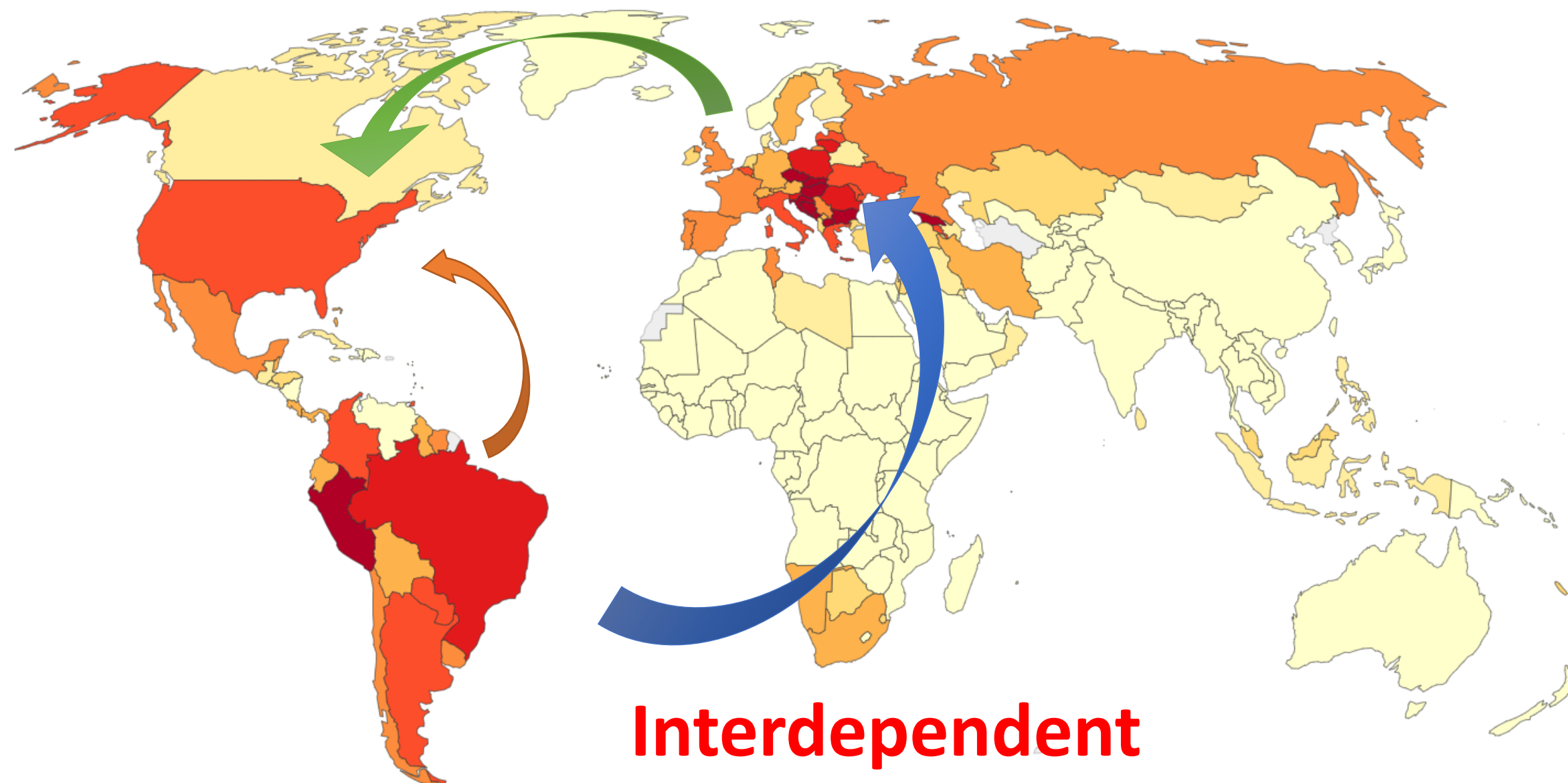
Cumulative confirmed COVID-19 deaths per millions people, Mar 19, 2022

Interdependent
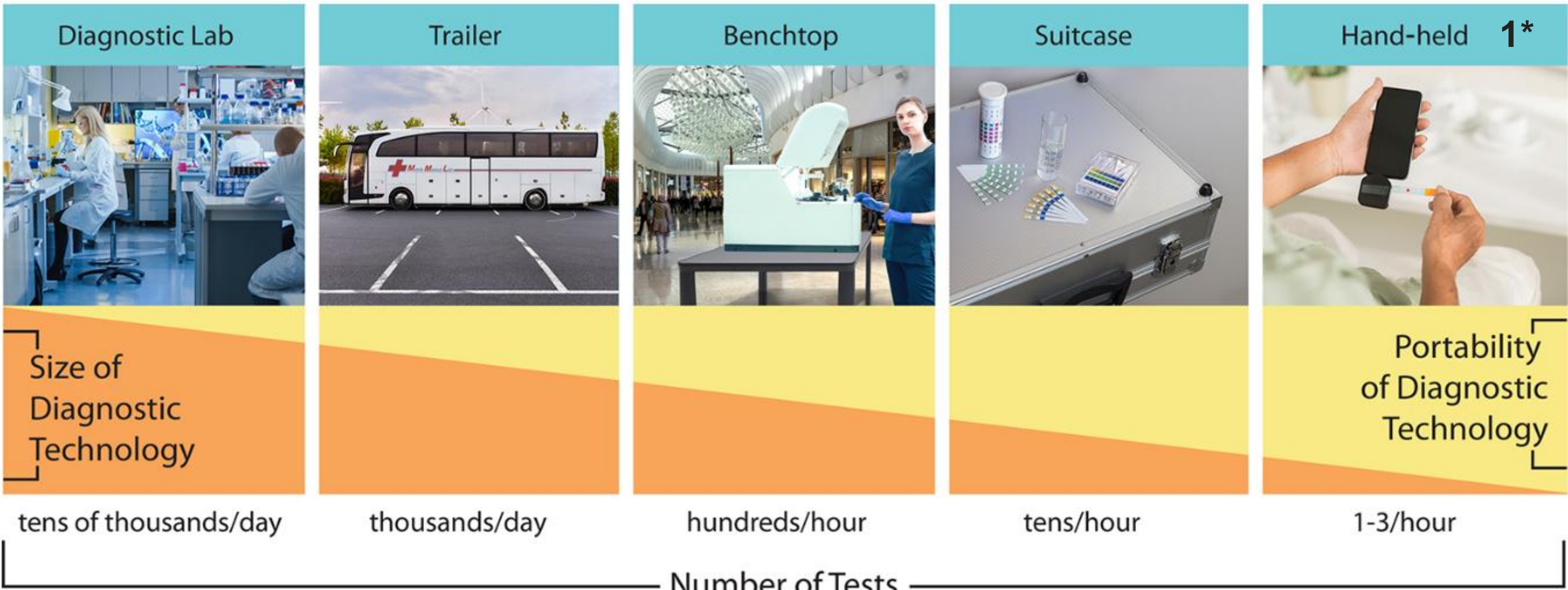
| No data | 0 | 500 | 1,000 | 1,500 | 2,000 | 2,500 | 3,000 | 3,500 |

- **>500 millions total cases**
- **>6 millions total deaths**
- **2/3 US people are fully vaccinated**
- **Cases are still increasing**

Johns Hopkins University CSSE COVID-19 Data
https://coronavirus.jhu.edu/map.html

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SARS-CoV-2 diagnostics

From collection to result (30min)

Bottleneck

Fast, Cost-effective, Easy to collect, Accuracy

ShieldT3 Health, https://www.shieldt3.com/
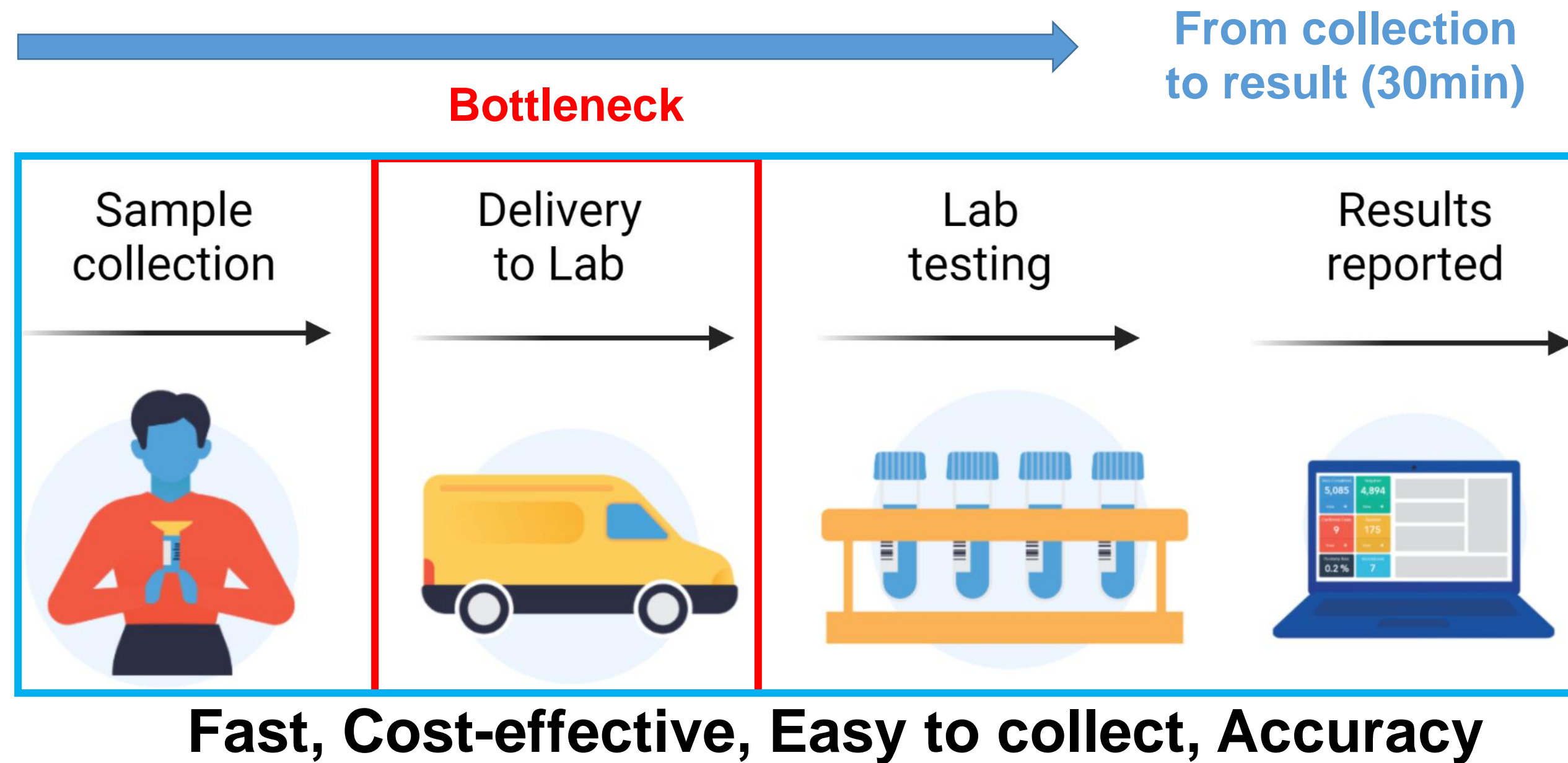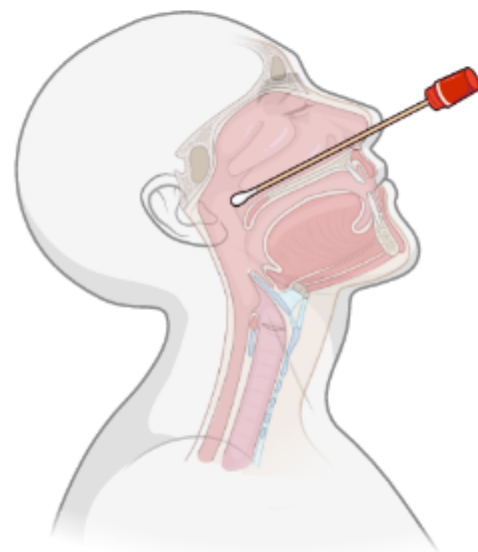
Point-of-care

Time and cost saving!

1) Valera, Enrique, et al. *ACS nano* 15.5 (2021): 7899-7906.
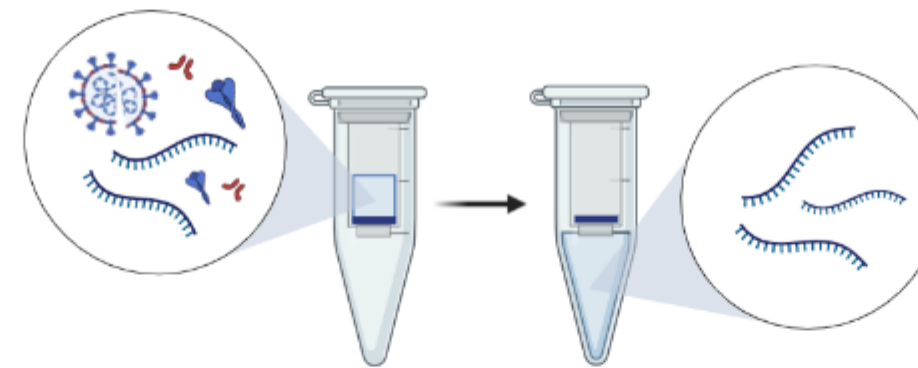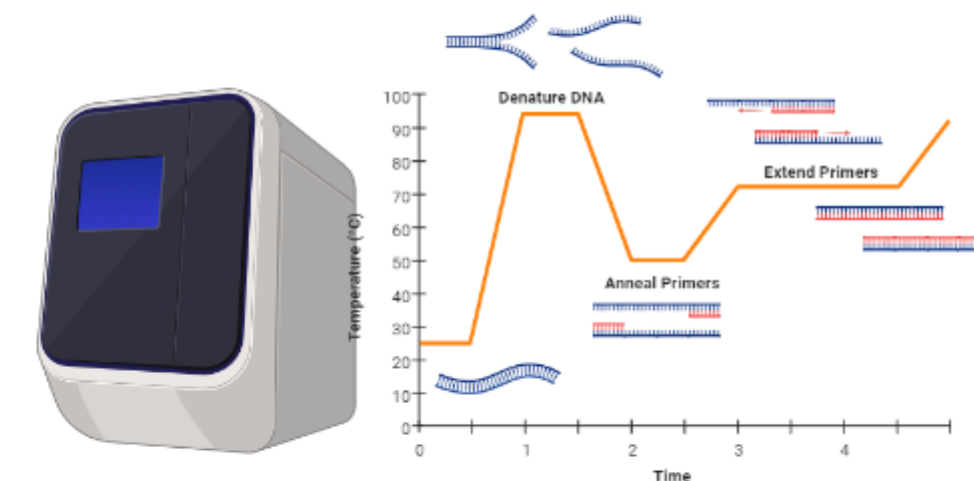
Current gold standard: RT-PCR

Sample collection → Nasal swab

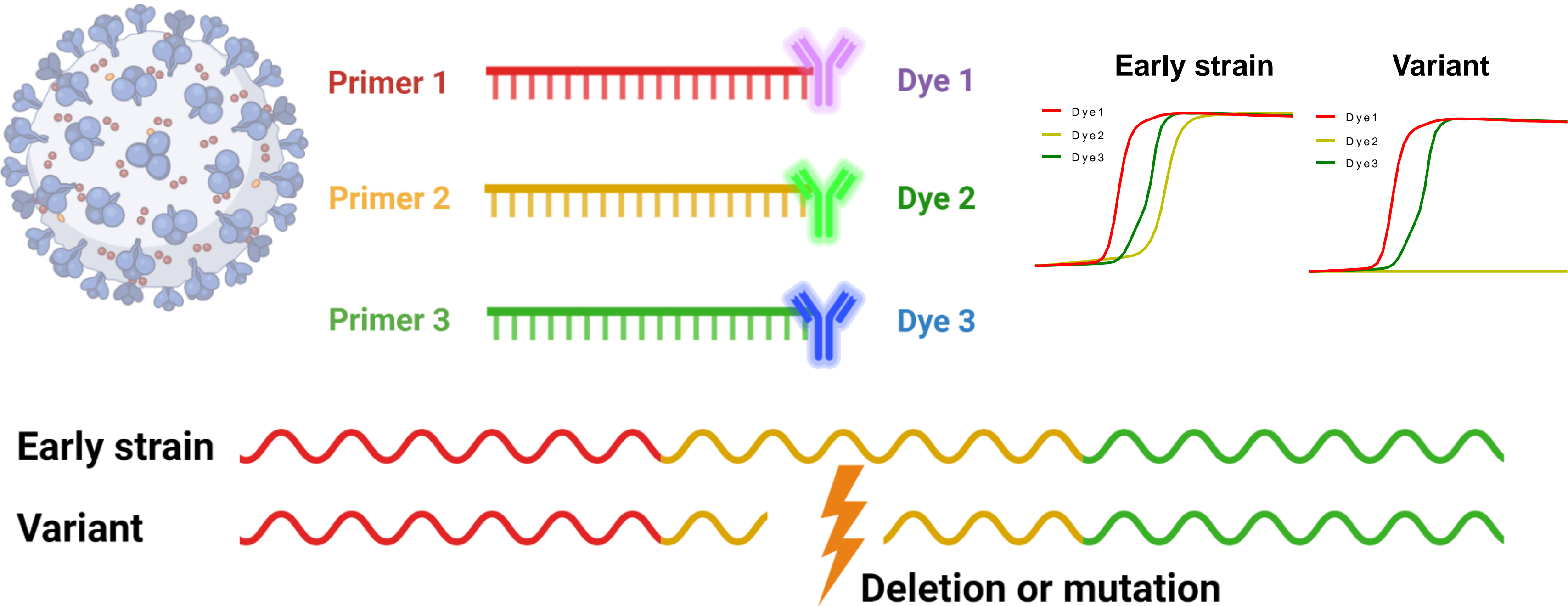Treatment → RNA Extraction and purification

Detection → Thermocycler

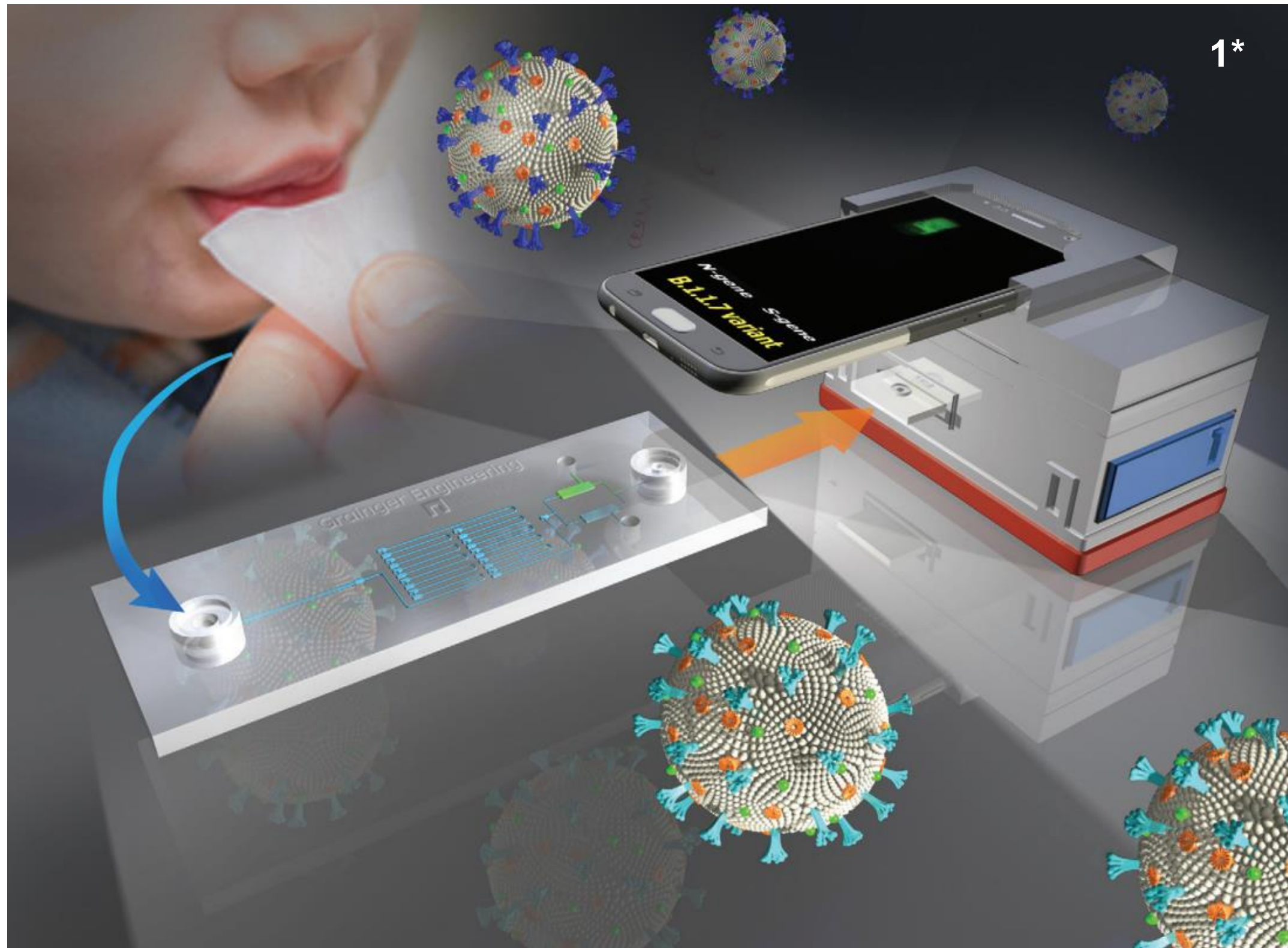**Hard to translate into point-of-care**

Multiplexed RT-PCR and Variant identification

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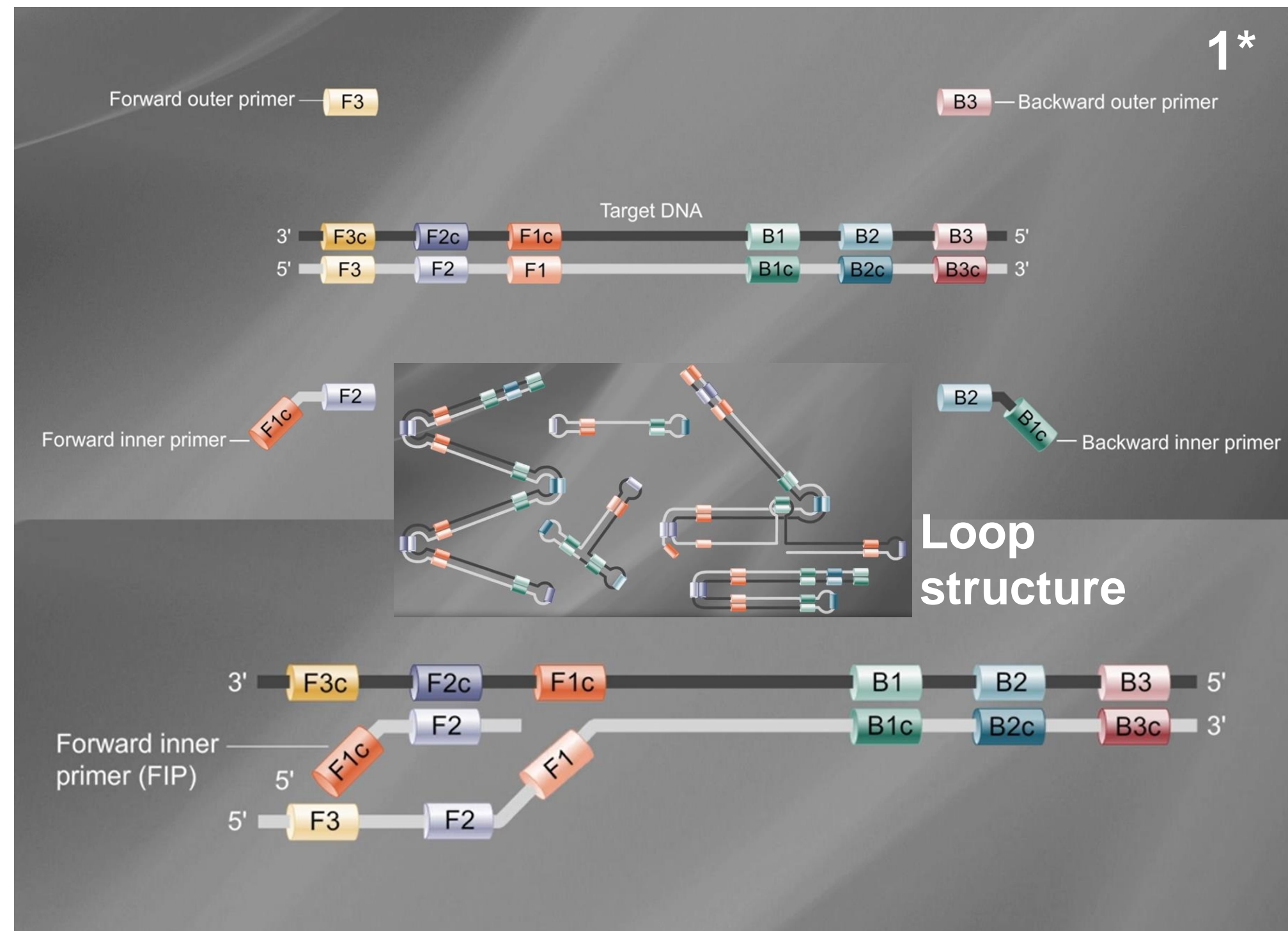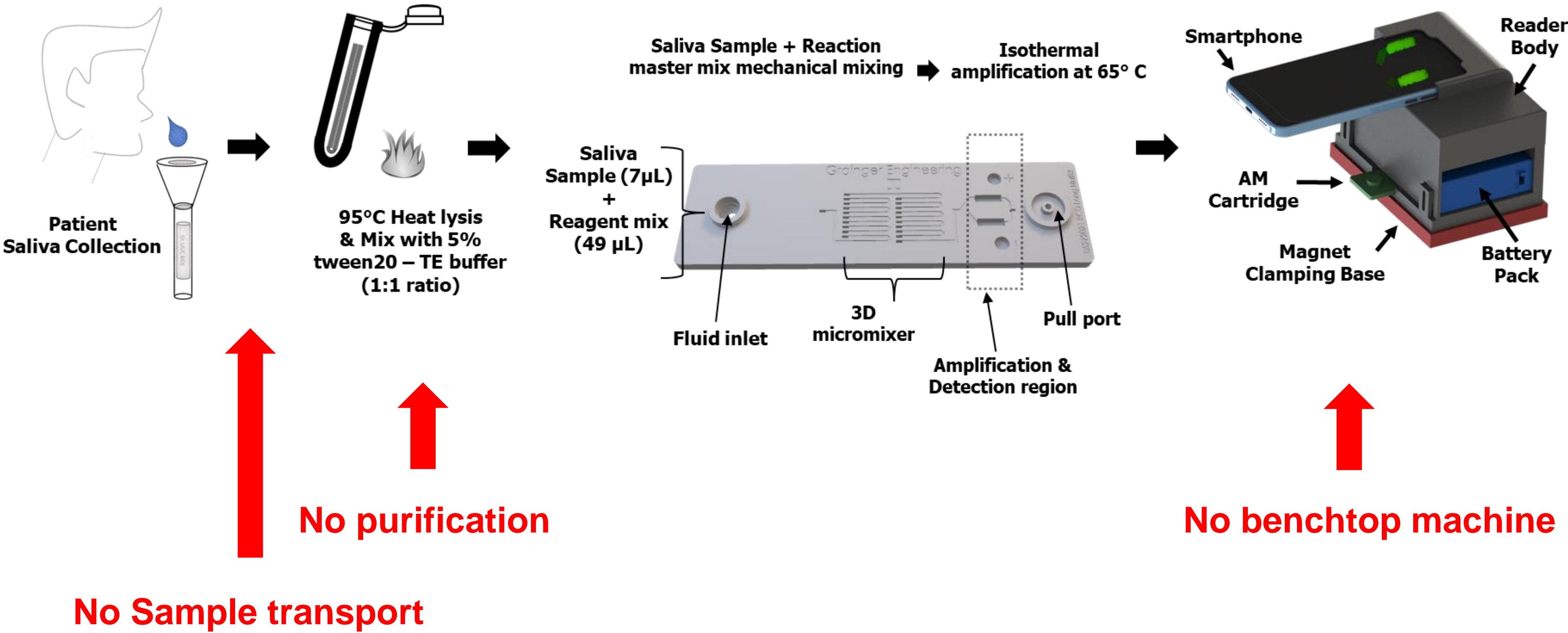Bioengineering

Solution

1*

- **Point-of-care diagnostics**

- **Hand-held device and smartphone**

- **Disposable microfluidic cartridge**

- **Multiplex detection**

- **Variant identification**

1) Lim, Jongwon, et al. *Lab on a Chip, 22.7* (2022): 1297-1309.

Method

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- **High strand displacement activity**

- **Single temperature (65 ºC)**

- **Rapid amplification (< 30 mins)**

- **4-6 primers**

- **Can be extended to RT-LAMP**

1) Loop Mediated Isothermal Amplification (LAMP) Tutorial New England BioLabs,
https://www.neb.com/tools-and-resources/video-library/loop-mediated-isothermal-amplification-lamp-tutorial?autoplay=1

Protocol workflow

Patient Saliva Collection

95°C Heat lysis & Mix with 5% tween20 – TE buffer (1:1 ratio)

Saliva Sample + Reaction master mix mechanical mixing ➡ Isothermal amplification at 65° C

Saliva Sample (7µL) + Reagent mix (49 µL)

Fluid inlet

3D micromixer

Amplification & Detection region

Pull port

Smartphone

Reader Body

AM Cartridge

Magnet Clamping Base

Battery Pack

No purification

No Sample transport

No benchtop machine

Wyllie, Anne L., et al. *New England Journal of Medicine* 383.13 (2020): 1283-1286.
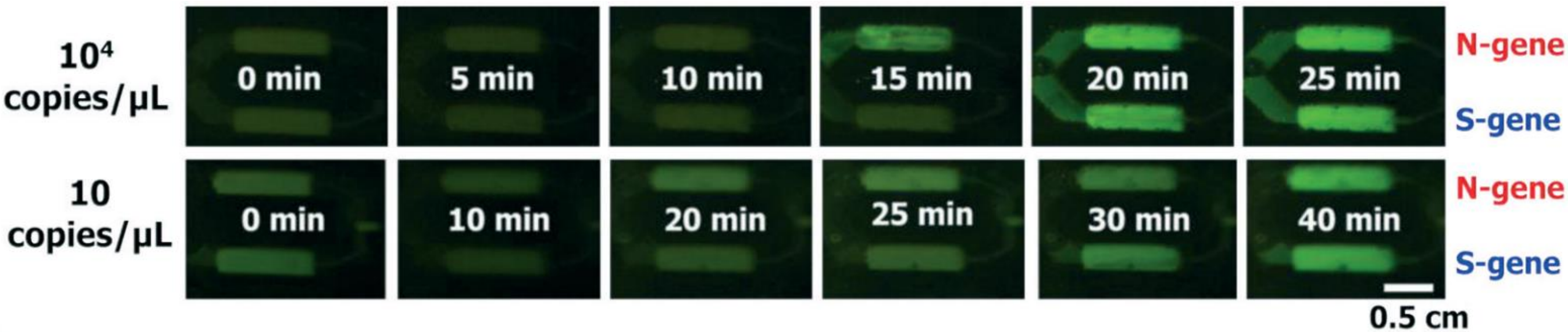
Mock sample – Early Strain

Figure. On-cartridge detection of inactivated SARS-CoV-2 virus spiked in saliva using an additively manufactured cartridge and a handheld POC device. Fluorescence images of the real-time RT-LAMP reaction (65 °C, 50 min.) for multiplexed detection (N- and S-genes). The pre-dehydrated primers allowed the simultaneous detection of the two target genes in a single cartridge (upper detection region for N-gene and lower detection region for S-gene). Scale bar = 0.5 cm
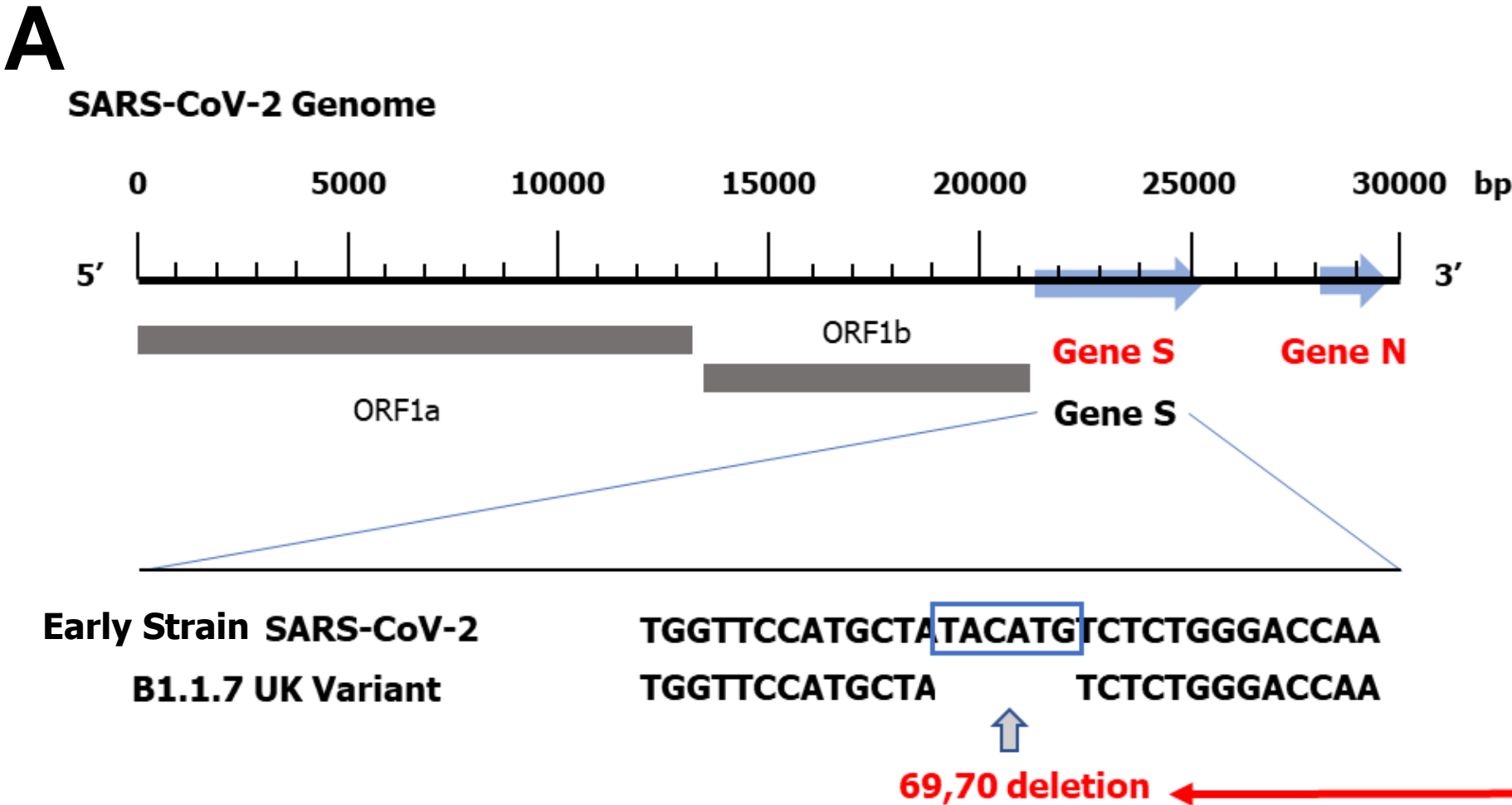
1) Larremore, Daniel B., et al. *Science advances* 7.1 (2021): eabd5393.

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Carle

Patient sample – Early strain
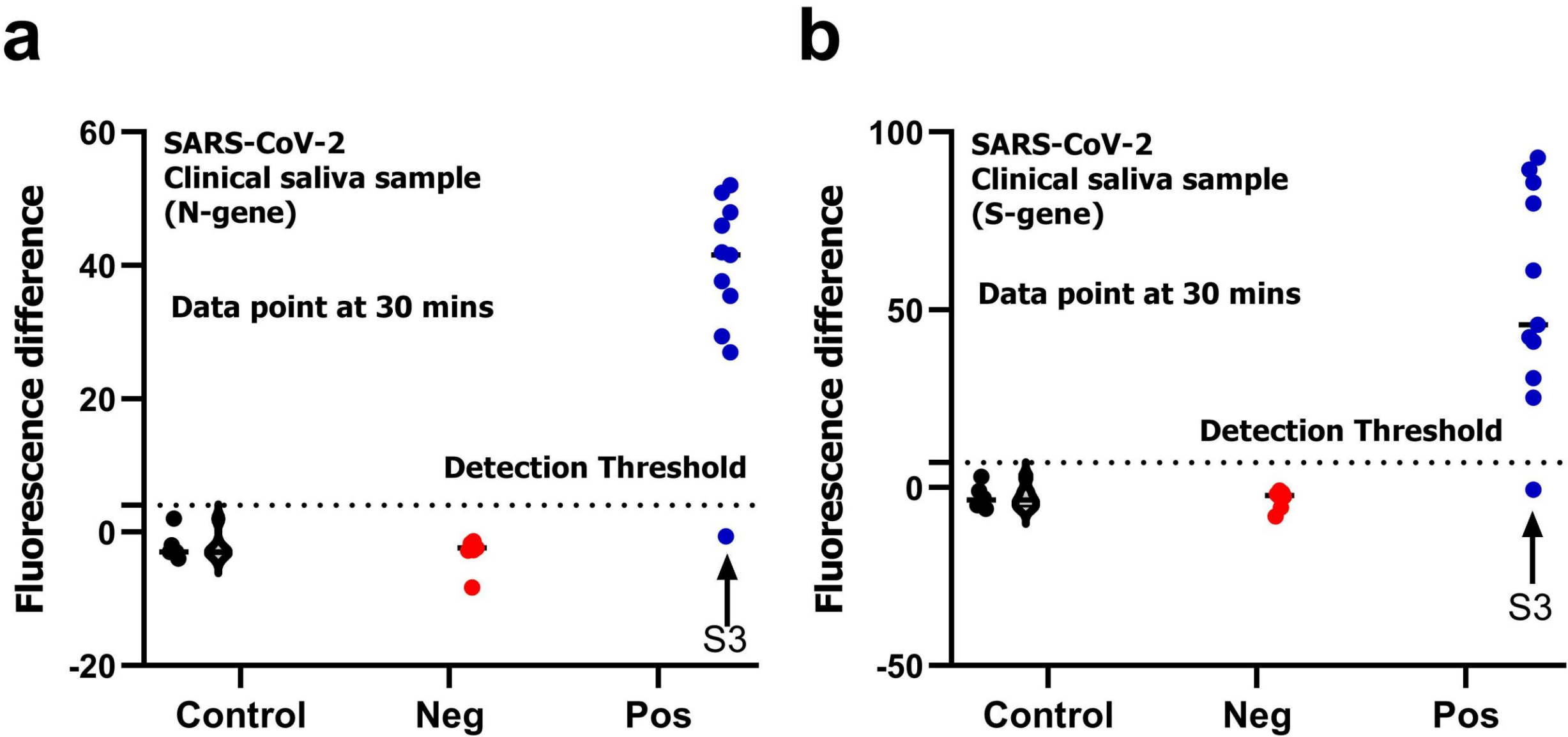
- 11 positive 7 negative

- Confirmed by RT-PCR

- 91% sensitivity (10/11)

- 100% specificity (0/7)

- S3 showed low viral load

Larremore, Daniel B., et al. *Science advances* 7.1 (2021): eabd5393.

A SARS-CoV-2 Genome

B N-gene primer

Primer	Sequence
F3	GAC TTC TCA GTG GAA GCA A
B3	ACG TGG TGT TTA TTA CCC T
FIP	TAC ATG TCT CTG GGA CCA ATG GTA CTA TAA ACA CCA TCA TTA AAT GGT AGG
BIP	TAG CAT GGA ACC AAG TAA CAT TGG AGA CAA AGT TTT CAG ATC CTC A
Loop B	GAA AGG TAA GAA CAA GTC CTG AGT T

C S-gene primer specific to detect SGTF

Primer	Sequence
F3	GAC TTC TCA GTG GAA GCA A
B3	ACG TGG TGT TTA TTA CCC T
FIP	TAC ATG TCT CTG GGA CCA ATG GTA CTA TAA ACA CCA TCA TTA AAT GGT AGG
BIP	TAG CAT GGA ACC AAG TAA CAT TGG AGA CAA AGT TTT CAG ATC CTC A
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(A) SARS-CoV-2 genome map highlighting detection regions. Multiplexed RT-LAMP assay was developed: (B) N- and (C) S-gene in the early strains and the other assay use two primer sets to detect the N- and S-gene in the Alpha variant using the SGTF.

Variant identification
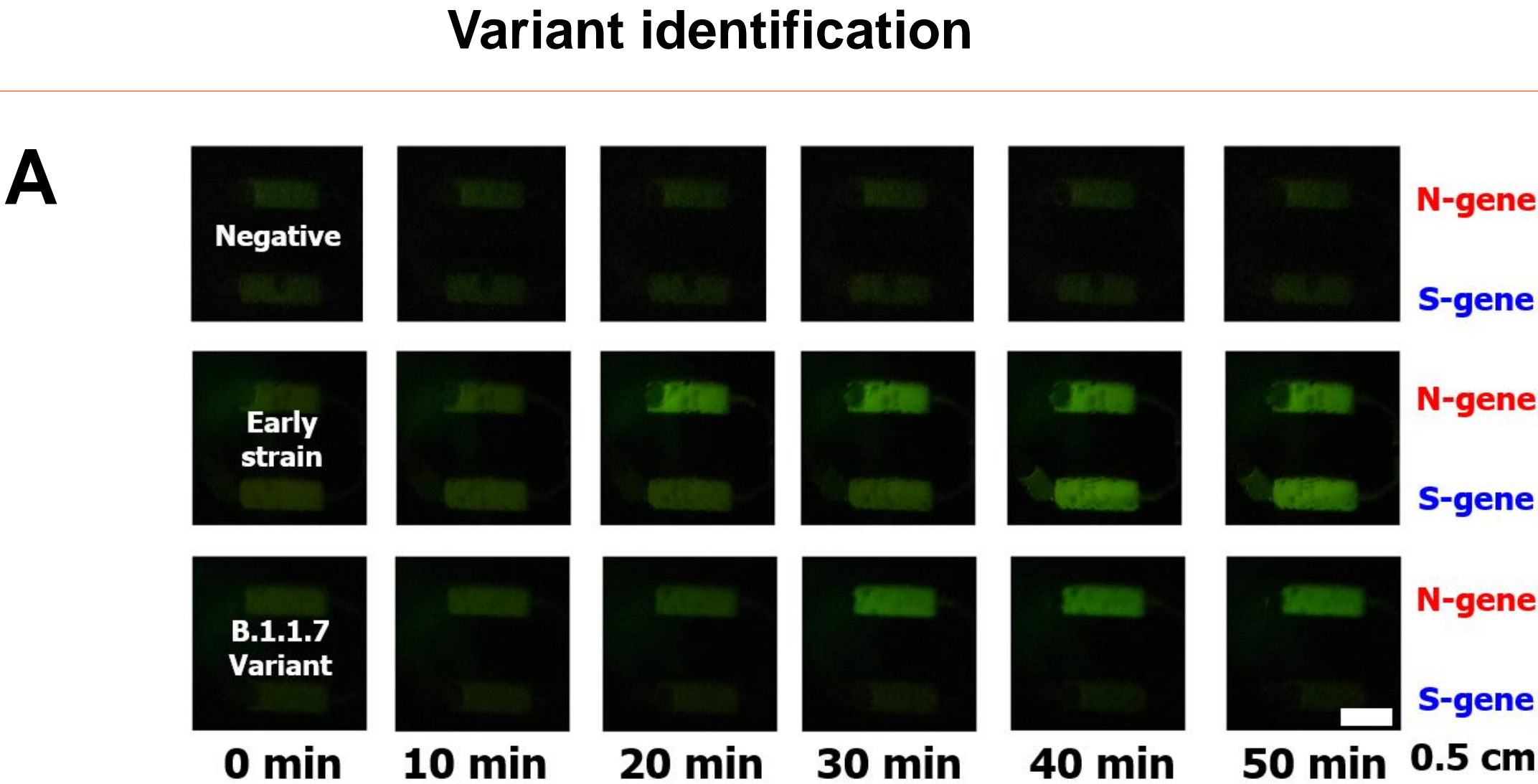
Differentiation assay		Strain decision
N-gene	S-gene specific to detect SGTF	
1	1	Early Strains
1	0	B.1.1.7 variant
0	0	Negative

(A) On-cartridge images to distinguish the SARS-CoV-2 early strains from the B.1.1.7 variant. N-gene primer set, and S-gene primer set (specific to SGTF) are pre-dehydrated in the upper and lower detection region, respectively. Scale bar = 0.5 cm. **(B)** Binary strain decision table based on the amplification fluorescence results

Patient sample – B.1.1.7 Variants
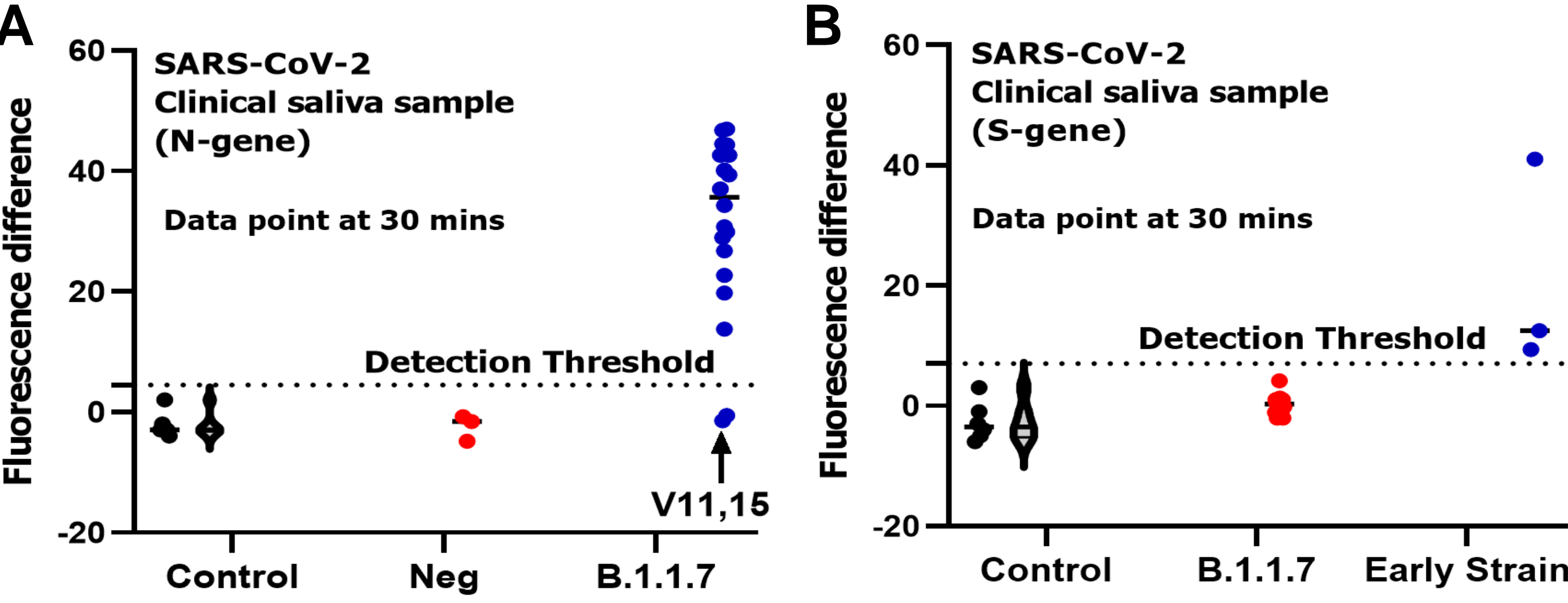
- **20 positive for B.1.1.7**
- **3 positive for Early strain**
- **3 negative**
- **Confirmed by RT-PCR**

- **90% sensitivity (18/20)**
- **100% specificity (0/20)**

- **V11,15 showed low viral load**

Detection of B.1.1.7 variant samples and differentiation from the negative and early strains virus samples. Based on the data points at 30 min, discerning criterion **(A)** to differentiate B.1.1.7 variant from negative samples using the amplification of the N-gene and **(B)** to differentiate B.1.1.7 variant from the SARS-CoV-2 early strains using the amplification of the S-gene specific to SGTF.
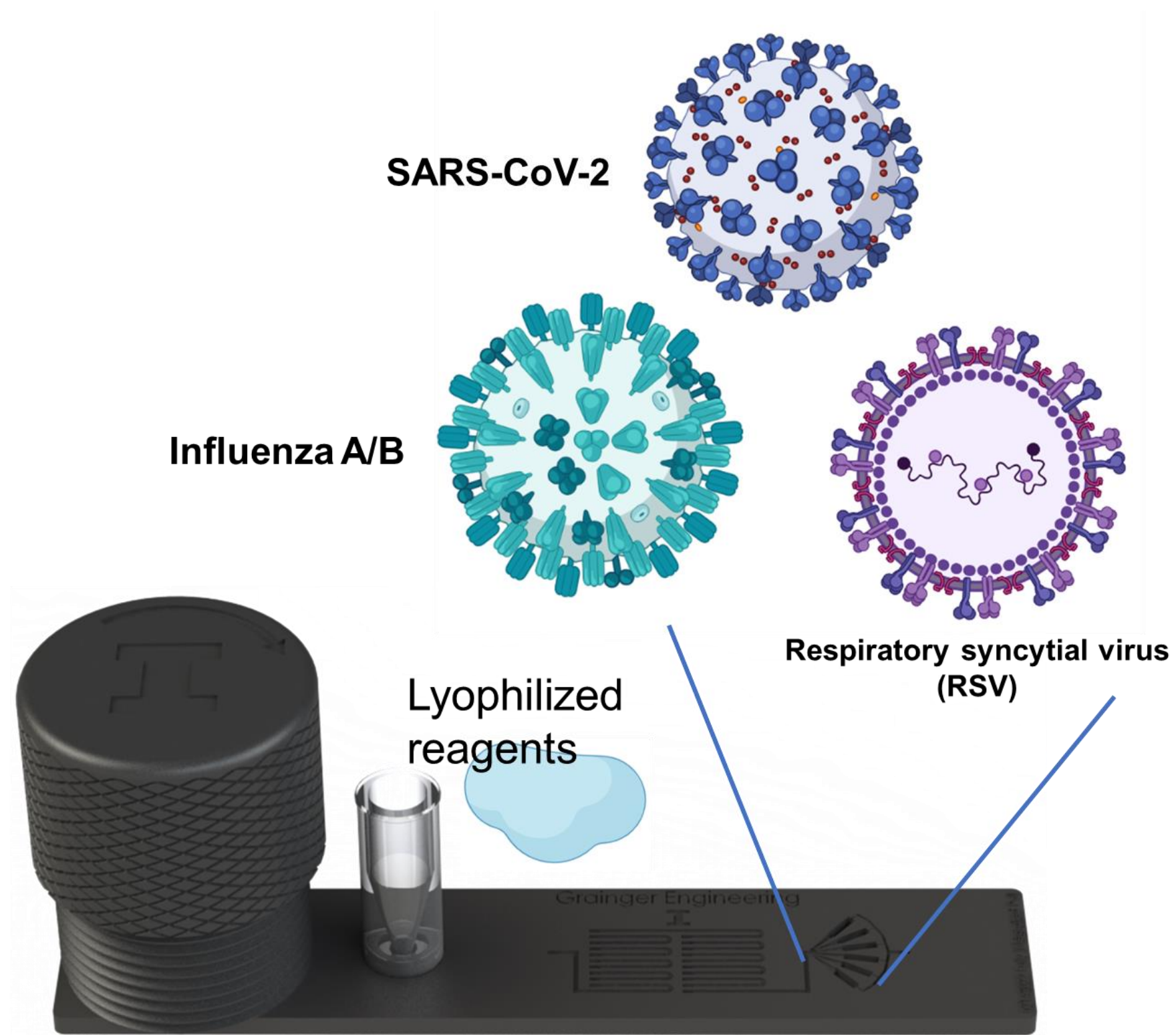
Discussion

Multiplex PCR

Point-of-care
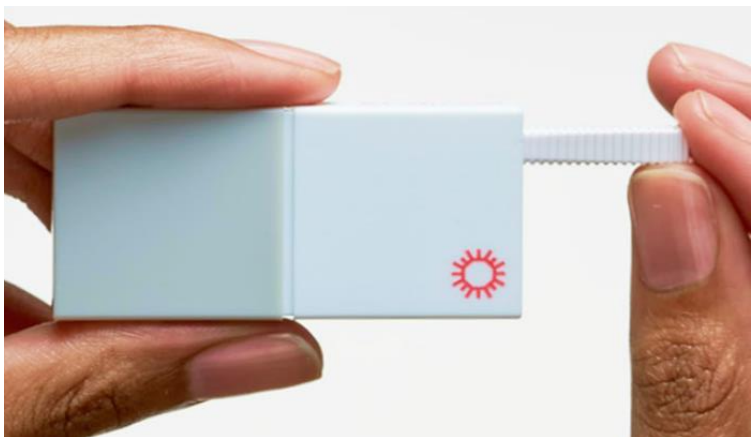
No.	Technology	LOD (copies/µL)	Sensitivity (%)	Specificity (%)	Assay time (min)	POC	Variant detection
1	RT-PCR	0.1 - 100	90-100	100	120 - 140	Low	Yes
2	RT-LAMP	0.75 - 75	90	100	30 - 60	High	No
3	CRISPR	5 - 20	86	100	45 - 70	High	No
4	Our approach	10	91	100	30	High	Yes

What's next?

SARS-CoV-2

Influenza A/B

Respiratory syncytial virus (RSV)

Lyophilized reagents

ASSURED	Previous work	Future work
Affordable	O	O
Sensitive	O	O
Specific	O	O
User-friendly	Δ	O
Rapid & Robust	O	O
Equipment-free	X	O
Deliverable to user	X	O

- **4 virus tests (SARS-CoV-2, Influenza A/B, and RSV) with controls**
- **Deliverable to user (lyophilized reagents, no cold-chain required)**
- **User-friendly (simple operation using turning valve, metering)**
- **Equipment-free (no pipet used)**

Red – confirmed
Blue – working on
Black – in the future

Acknowledgments

Image Sources

- BioRender

Lim et al, Lab on a Chip (2022) *Cover

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