Diagnostics of SARS-CoV-2

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Introduction - What *is* SARS-CoV-2?

- Outbreak originated in China in late 2019
- Disease caused by this virus is called: coronavirus disease 2019 (a.k.a. COVID-19)
- Global pandemic declared March 11, 2020
- Symptoms include:
  - Fever
  - Dry cough
  - Shortness of breath
  - Fatigue
  - Loss of smell and/or taste

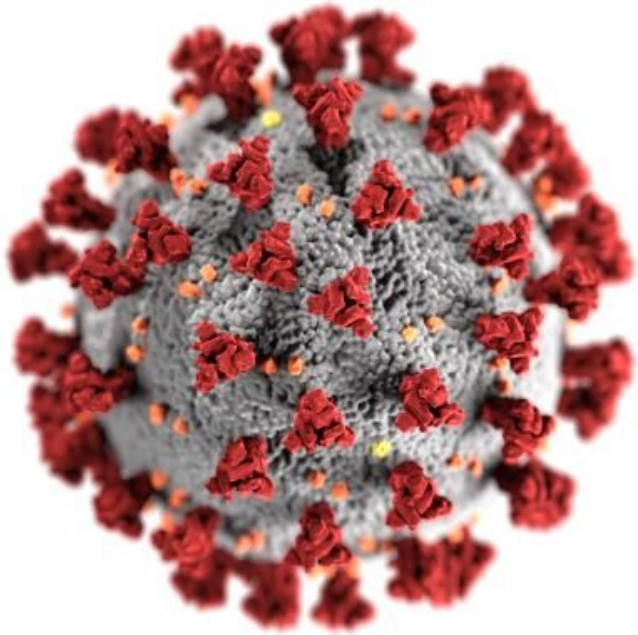
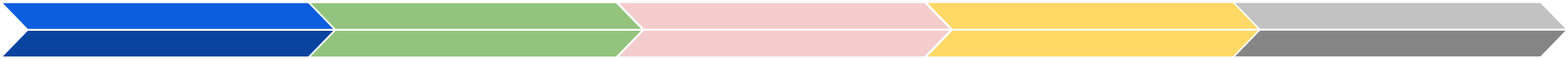


Illustration of SARS-CoV-2. Adapted from Eckert & Higgins, 2020.

(Sheposh, 2021)(WHO, 2021)

Diagnostic Overview



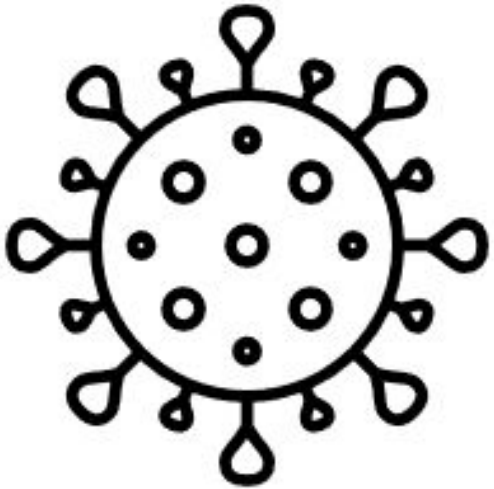
Clinical Performance

- Analytical sensitivity: reliably detect minimum amount of target substance within sample (limit of detection)
- Analytical specificity: ability of test to detect only the analyte being measured
- Clinical sensitivity: True positive rate
- Clinical specificity: True negative rate

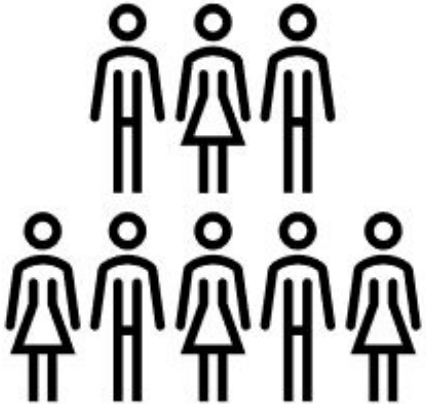
ACRONYMS TO KNOW
RT-PCR: Reverse transcription polymerase chain reaction
RT-LAMP: Reverse transcription loop-mediated isothermal amplification
ELISA: Enzyme-linked immunoassay
LFA: Lateral flow assay
CRISPR: Clustered Regularly Interspaced Short Palindromic Repeats
AI: Artificial Intelligence

(Chau et al., 2020)

Importance of Diagnostic Testing



☑ COVID-19 **+ve**
❑ COVID-19 **-ve**

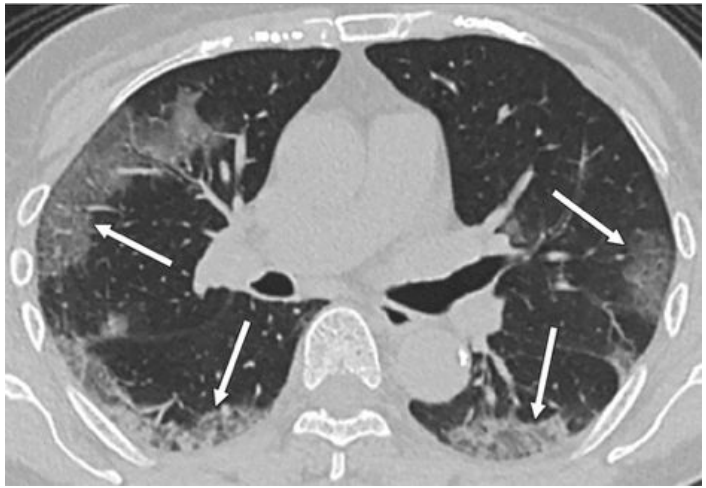


Epidemiological information for Public Health

Prevent spread of COVID-19

CT-Scans

- Series of X-rays of lungs
- Earliest Diagnostic test
- Lower specificity and sensitivity (even lower)
- Expensive and specialist reliant
- Quick results



Presence of bilateral ground-glass opacities in upper lobe

(Bernheim et al, 2020)(Bai et al, 2020)

rRT-PCR

- Initially tested for the presence of SARS-CoV-2 RdRp, N or E genes

- Later narrowed down testing to the RdRp Helicase gene alone due to lower cross-reactivity with other viruses

The New York Times

You're Infected With the Coronavirus. But How Infected?

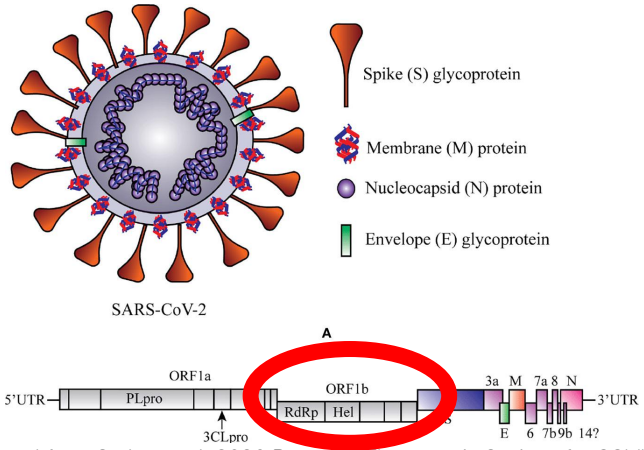


Figure adapted from Sarkar et al. 2020 Potential Therapeutic Options for COVID-19: Current Status, Challenges, and Future Perspectives

(Sarkar et al. 2020)

rRT-PCR

- Samples are retrieved and treated
- Marker DNA sequences complementary to the viral gene are added
- Standard PCR testing begins with updates occurring in real time

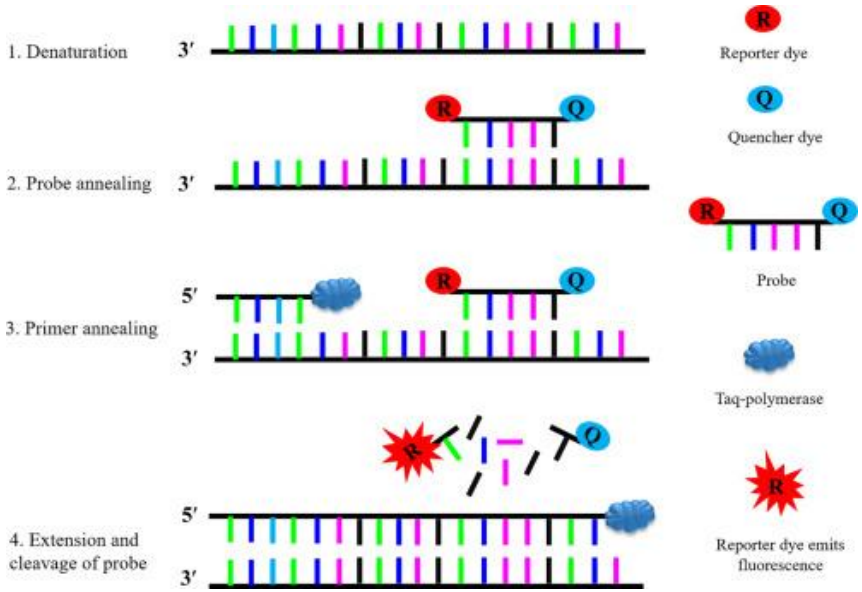


Figure adapted from Roy et al. 2019. Chapter 5 - Small RNA proteome as disease biomarker: An incognito treasure of clinical utility

(Roy et al 2019)

rRT-PCR

Advantages:

- Little time between sample collection and test results
- No cross-reactivity with other respiratory viruses or other coronavirus strains

Disadvantages:

- Fairly expensive
- Unable to retroactively diagnose COVID-19 infections

Virus[a]	Viral titer ($TCID_{50}$/ml)[b]	Cross-reactivity[c]		
		COVID-19-RdRp/Hel	COVID-19-N	RdRp-P2
SARS-CoV	1.0×10^3	−	−	+
MERS-CoV	5.6×10^3	−	−	−
HCoV-OC43	3.2×10^3	−	−	−
HCoV-229E	5.0×10^2	−	−	−
HCoV-NL63	3.2×10^1	−	−	−
Adenovirus	1.0×10^2	−	−	−
hMPV	3.2×10^2	−	−	−
IAV (H1N1)	4.2×10^3	−	−	−
IAV (H3N2)	5.6×10^3	−	−	−
IBV	3.2×10^3	−	−	−
ICV	5.6×10^2	−	−	−
PIV1	1.0×10^2	−	−	−
PIV2	1.0×10^3	−	−	−
PIV3	1.0×10^3	−	−	−
PIV4	1.0×10^3	−	−	−
Rhinovirus	7.9×10^3	−	−	−
RSV	1.0×10^3	−	−	−

Figure Adapted from Chan et al. 2020. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay

(Chan et al. 2020) (ThermoFisher, 2020)

RT-LAMP

One-step nucleic acid amplification method

Advantages:

- Faster and less expensive alternative to RT-PCR
- High sensitivity and specificity
- Special training or equipment not required

Disadvantages:

- Designing compatible LAMP primers to target sequence

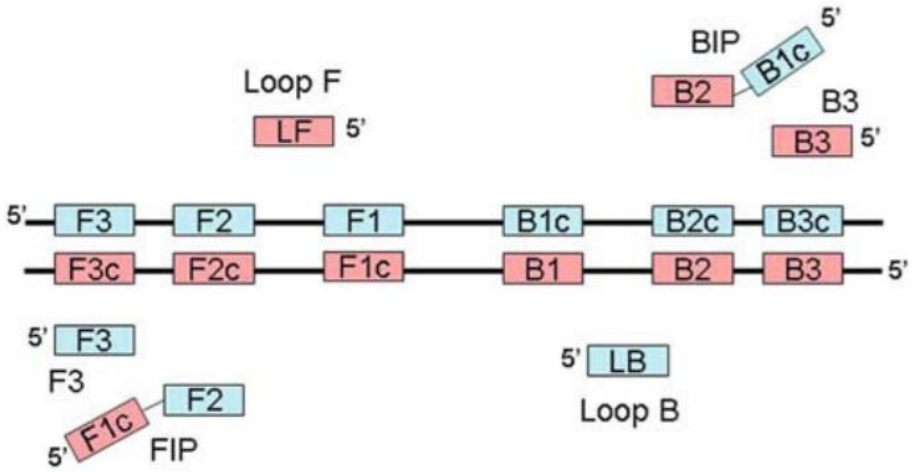


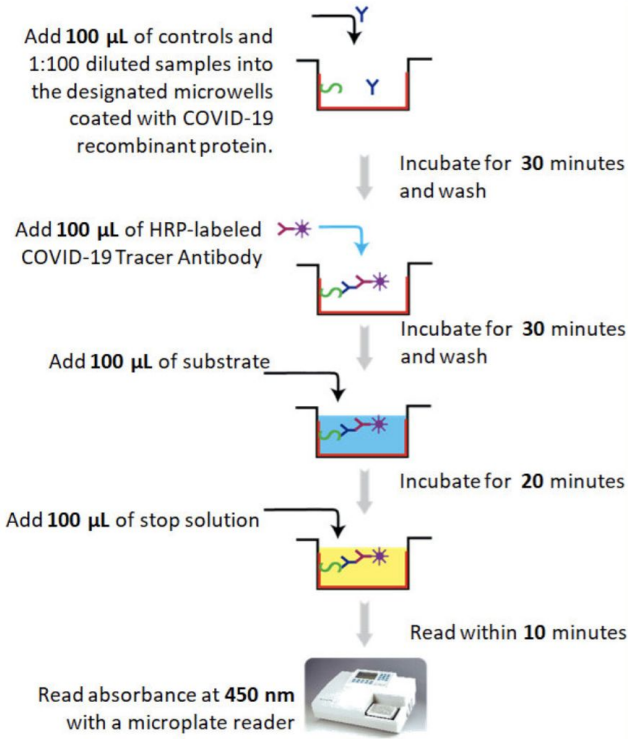
Figure Adapted from Mori & Notomi 2009. Schematic illustrating the loop-mediated isothermal amplification (LAMP) primers

(Lamb et al., 2020) (Mori & Notomi, 2009)(Schilling, 2015)

Serological Testing: ELISA

ELISA:

- High specificity and sensitivity after seroconversion
- Contained purified SARS-COV-2 protein to bind human antibody
- Indirect: Presence of secondary antibody
- Added Substrate and fluorescence indicator
- Retrospective Testing



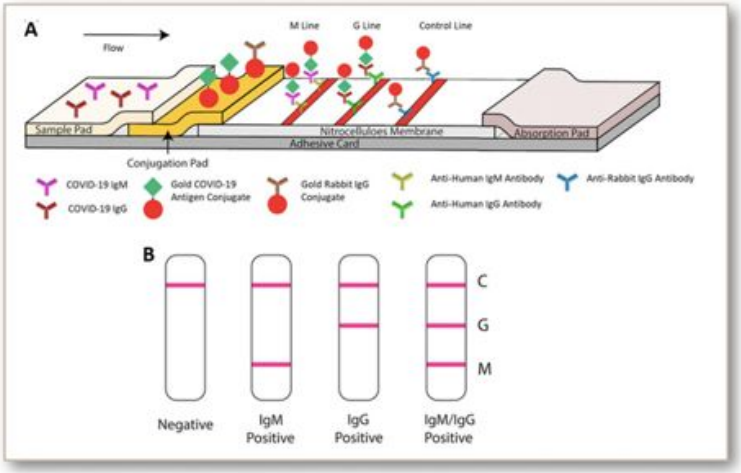
COVID-19 ELISA test Protocol

(Epitope Diagnostics Inc., 2020) (Serrano et al, 2020) (Gibbs et al, 2020) (Michigan Diagnostics, 2020) (Choe et al 2021)

Serological Testing: Rapid Lateral Flow Assay

Rapid Lateral Flow Assay:

- Use of Anti-Human IgG and IgM to capture potential SARS-COV-2 specific antibodies
- Easy to read results based on coloured bands
- Quick Processing Time
- Point of Care Testing
- Inexpensive
- Lowered Specificity and Sensitivity



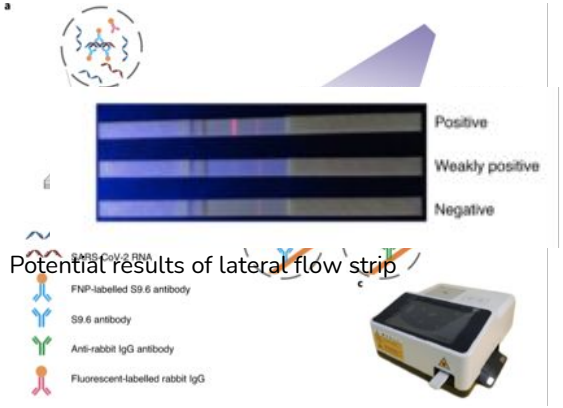
Steps in COVID-19 specific Lateral Flow Assay

(Augustine et al, 2020) (Hackner et al, 2020) (Serrano et al, 2020)

Antigen Based Testing

Lateral Flow Assay:

- Adapted technique
- Use of DNA probes
- Easy to read results based on fluorescence cut off value



Potential results of lateral flow strip

Steps in COVID-19 specific antigen Lateral Flow fluorescence immunoassay

Sandwich ELISA:

- Use of lab made Capture, primary and secondary antibody

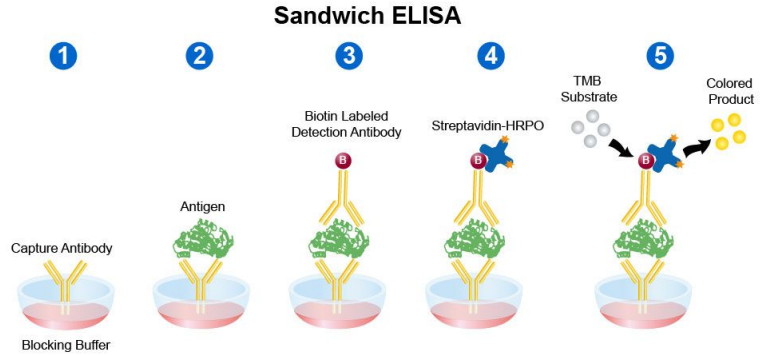
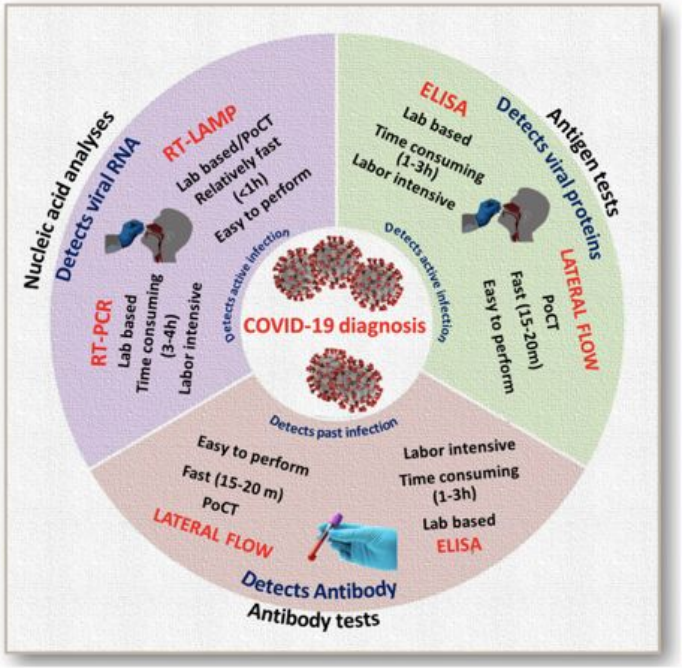


Figure adapted from Leinco Technologies inc. Sandwich ELISA protocol

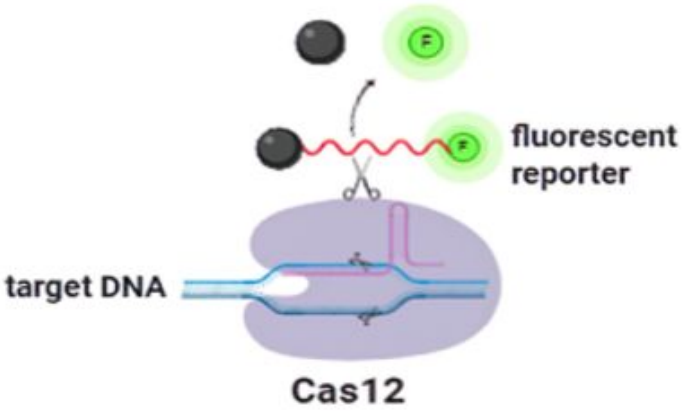
(Wang et al, 2020) (FIND, 2020) (Augustine et al, 2020)

Gold standard: Overview of Current Methods

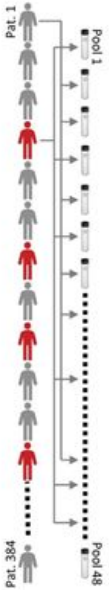


(Augustine et al, 2020)

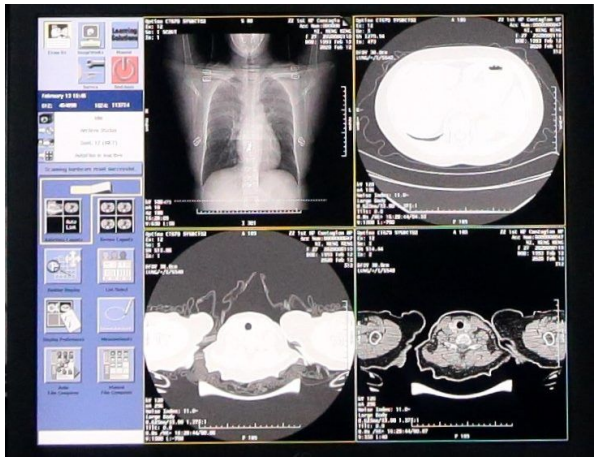
Novel methods summary



CRISPR-Cas12



P-BEST



Artificial Intelligence

(Jolany Vangah et al., 2020; Shental et al., 2020)

Crispr-Based Detection

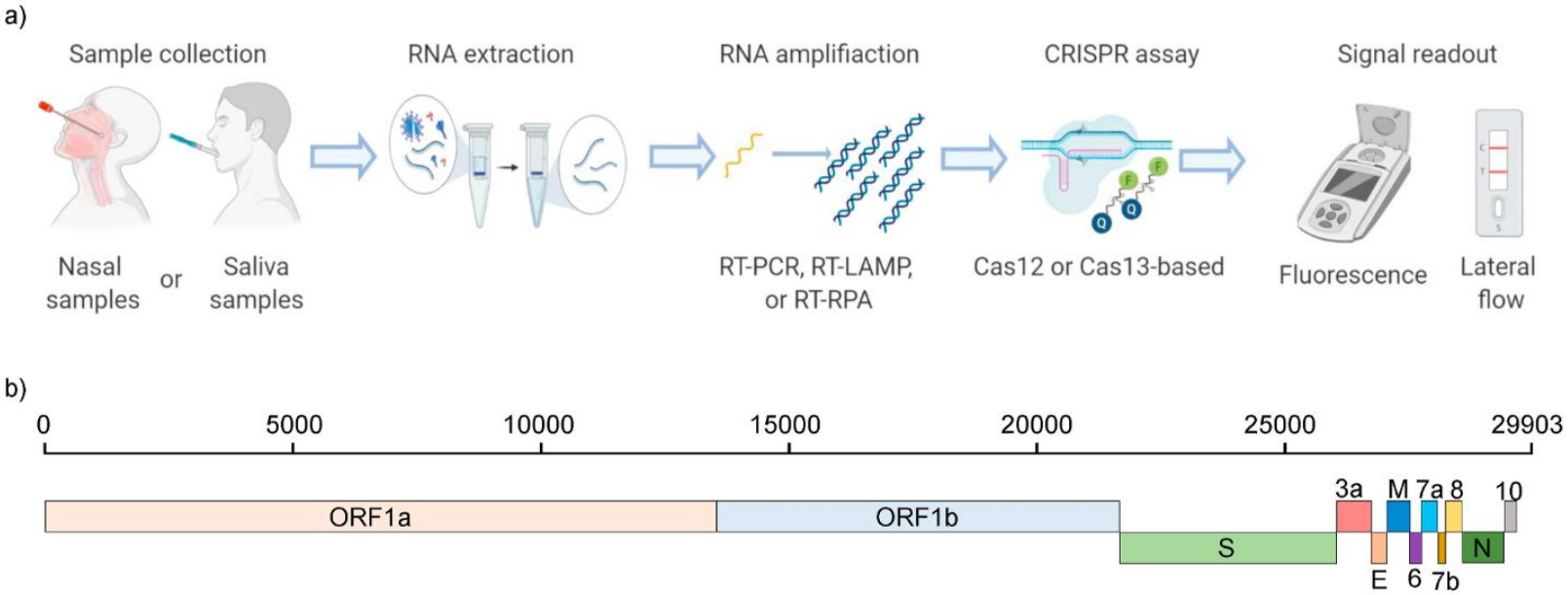


Fig. 1. a) Workflow of CRISPR-based SARS-CoV-2 diagnostic schemes from sample to answer. b) Schematic presentation of the SARS-CoV-2 genome organization (Kim et al., 2020a).

(Broughton et al., 2020)

Overview of Paper
CRISPR–Cas12-based detection of SARS-CoV-2
Broughton et al.

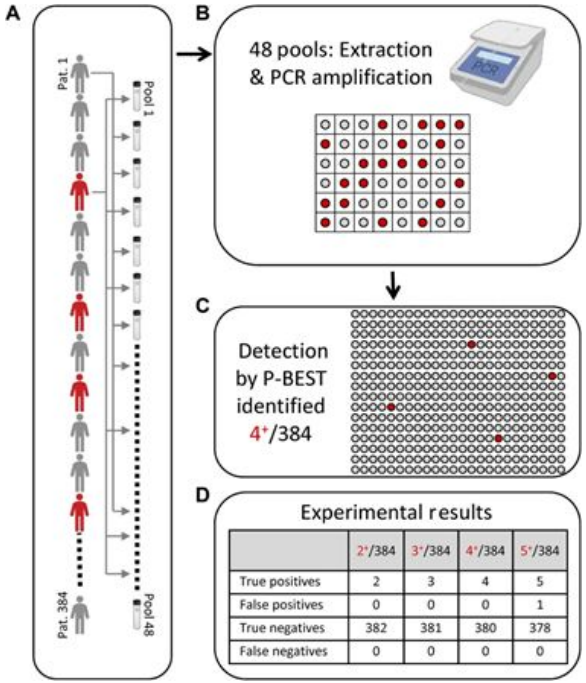
	SARS-CoV-2 DETECTR, RT–LAMP/Cas12	CDC SARS-CoV-2 qRT–PCR
Target	E gene and N gene[a]	N gene (three amplicons, N1, N2 and N3)
Sample control	RNase P	RNase P
LoD	10 copies per µl input	1 copy per µl input[b] and 3.2 copies per µl input[c]
Assay reaction time (approximate)	30–40 min	120 min
Assay sample-to-result time (approximate)	45 min (with manual RNA extraction)	4 h (including RNA extraction)
Assay results	Qualitative	Quantitative
Assay components	RT–LAMP (62 °C, 20–30 min) Cas12 (37 °C, 10 min) Lateral flow strip (RT, 2 min; no additional time if using fluorescence readout)	UDG digestion (25 °C, 2 min), reverse transcription (50 °C, 15 min), denature (95 °C, 2 min) amplification, (95 °C, 3 s; 55 °C 30 s; 45 cycles)
Bulky instrumentation required	No	Yes
US FDA EUA approval	Pending clinical validation	Yes

(Broughton et al., 2020)

Future Direction

Point of Care Testing

Mass Screening: P-BEST



	2+/384	3+/384	4+/384	5+/384
True positives	2	3	4	5
False positives	0	0	0	1
True negatives	382	381	380	378
False negatives	0	0	0	0

- Single step group testing method to test multiple people for SARS-CoV-2
- Much faster rate than testing individually
- Each individual's sample is a part of multiple groups in a combinatorial grouping method
  - Helps determine the specific positive individual from a positive group test result without the need for additional testing

(Shental et al., 2020)

AI-Based Testing

- Machine learning by previous RT-PCR verified CT scans
- Quick diagnosis
- Enhances physician diagnosis
- Improves sensitivity
- Predicts progression of illness
- Still in infancy

(Bernheim et al, 2020) (Chen and See 2020) (Zhang et al 2020)

QUESTIONS?

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