بسم الله الرحمن الرحيم

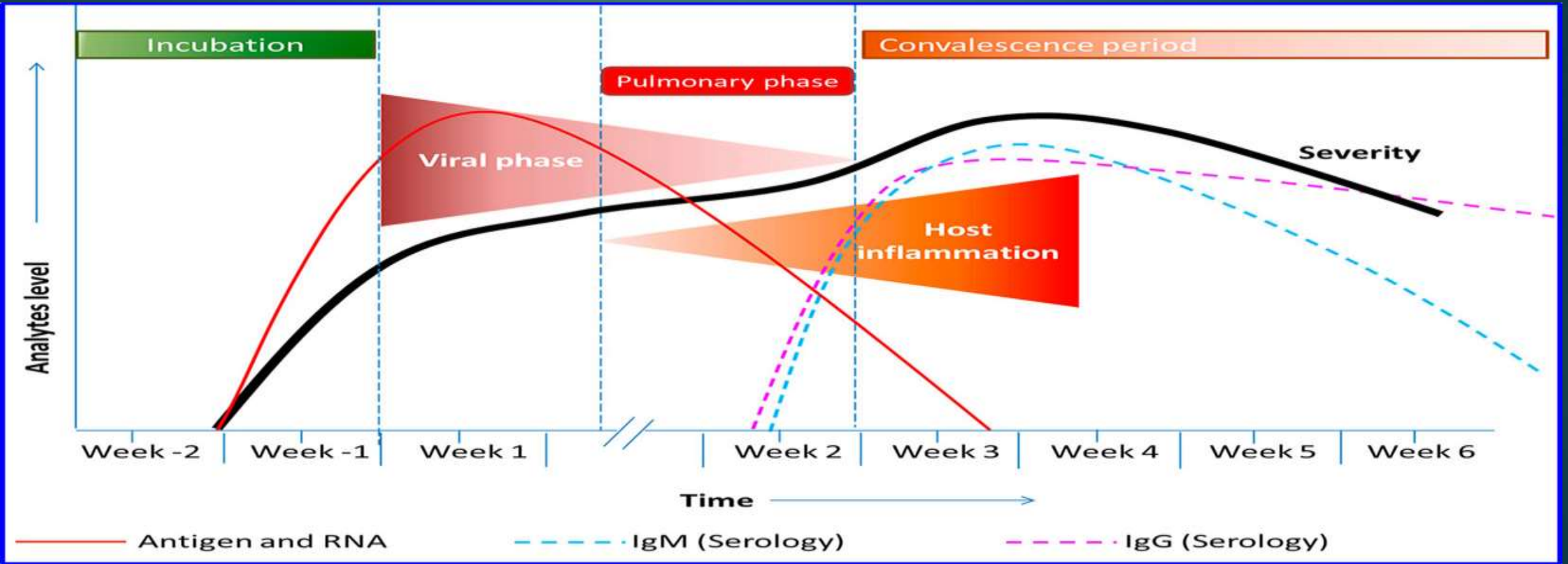
COVID 19 PERIOD

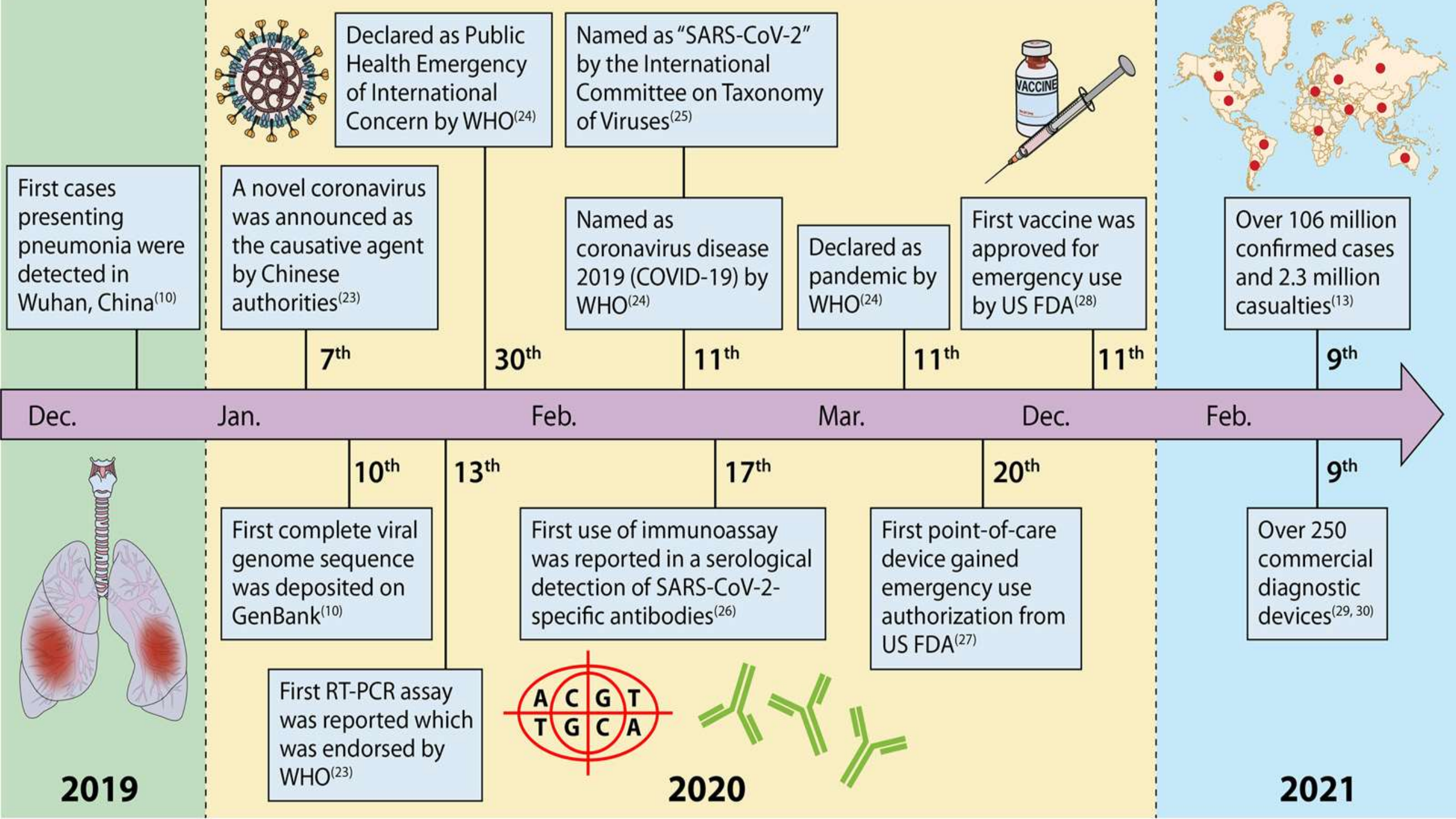
International Reviews of Immunology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iiri20

Rapid diagnosis of SARS-CoV-2 using potential point-of-care electrochemical immunosensor: Toward the future prospects

Pushpesh Ranjan, Ayushi Singhal, Shalu Yadav, Neeraj Kumar, S. Murali, Sunil K Sanghi & Raju Khan




First cases presenting pneumonia were detected in Wuhan, China[10]
A novel coronavirus was announced as the causative agent by Chinese authorities[23]
Declared as Public Health Emergency of International Concern by WHO[24]
Named as "SARS-CoV-2" by the International Committee on Taxonomy of Viruses[25]
Named as coronavirus disease 2019 (COVID-19) by WHO[24]
Declared as pandemic by WHO[24]
First vaccine was approved for emergency use by US FDA[28]
Over 106 million confirmed cases and 2.3 million casualties[13]
VACCINE
7th
30th
11th
11th
11th
9th
Dec.
Jan.
Feb.
Mar.
Dec.
Feb.
10th
13th
17th
20th
9th
First complete viral genome sequence was deposited on GenBank[10]
First RT-PCR assay was reported which was endorsed by WHO[23]
First use of immunoassay was reported in a serological detection of SARS-CoV-2-specific antibodies[26]
First point-of-care device gained emergency use authorization from US FDA[27]
Over 250 commercial diagnostic devices[29, 30]
A C G T
T G C A
2019
2020
2021

ANTI COVID DRUGS

Review

JOURNAL OF HEPATO

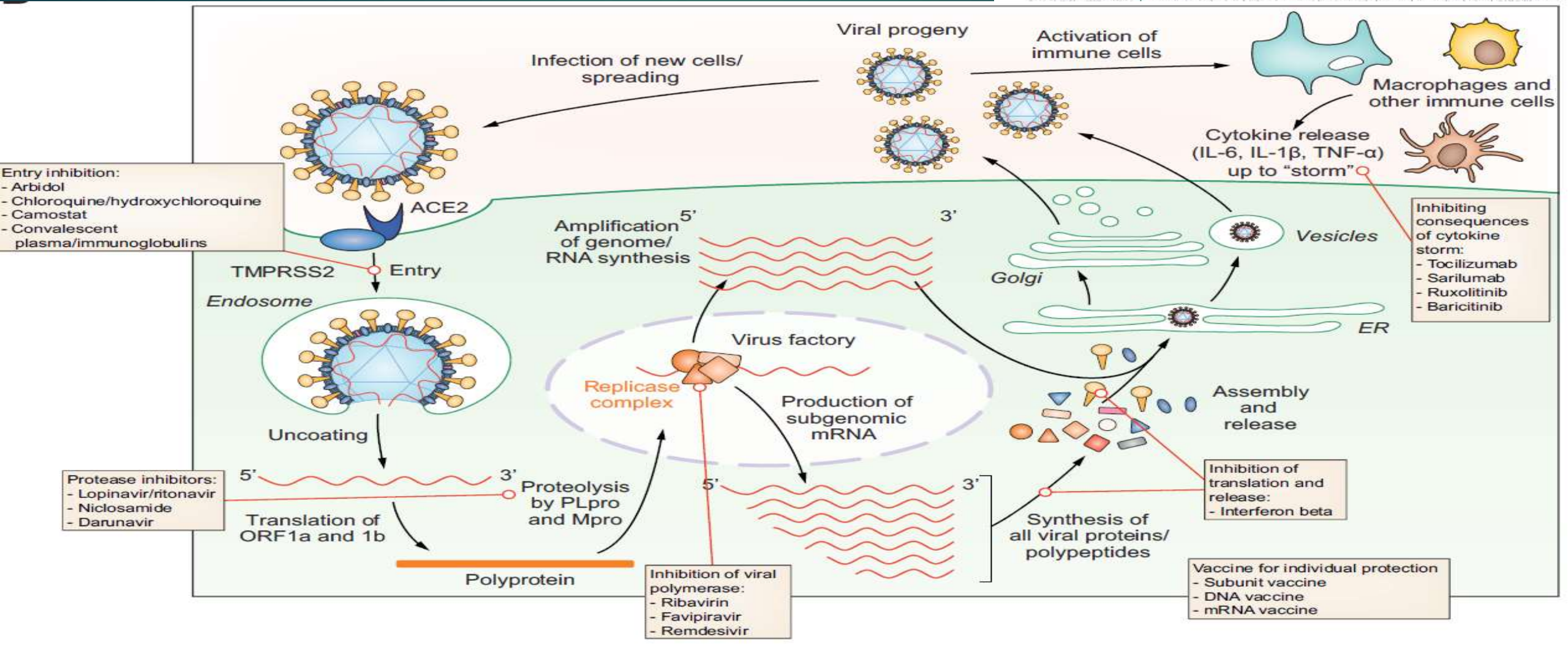
COVID-19: Discovery, diagnostics and drug development

Tarik Asselah[1,*], David Durantel[2], Eric Pasmant[3], George Lau[4], Raymond F. Schinazi[5]

Keywords: SARS-CoV-2; Coronavirus; Pathogenesis; Drug repurposing; Remdesivir.

Summary

Coronavirus disease 2019 (COVID-19) started as an epidemic in Wuhan in 2019, and has since b
pandemic. Groups from China identified and sequenced the virus responsible for COVID-19, n
vere acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and determined that it was



The AAPS Journal (2021) 23: 14
DOI: 10.1208/s12248-020-00532-2

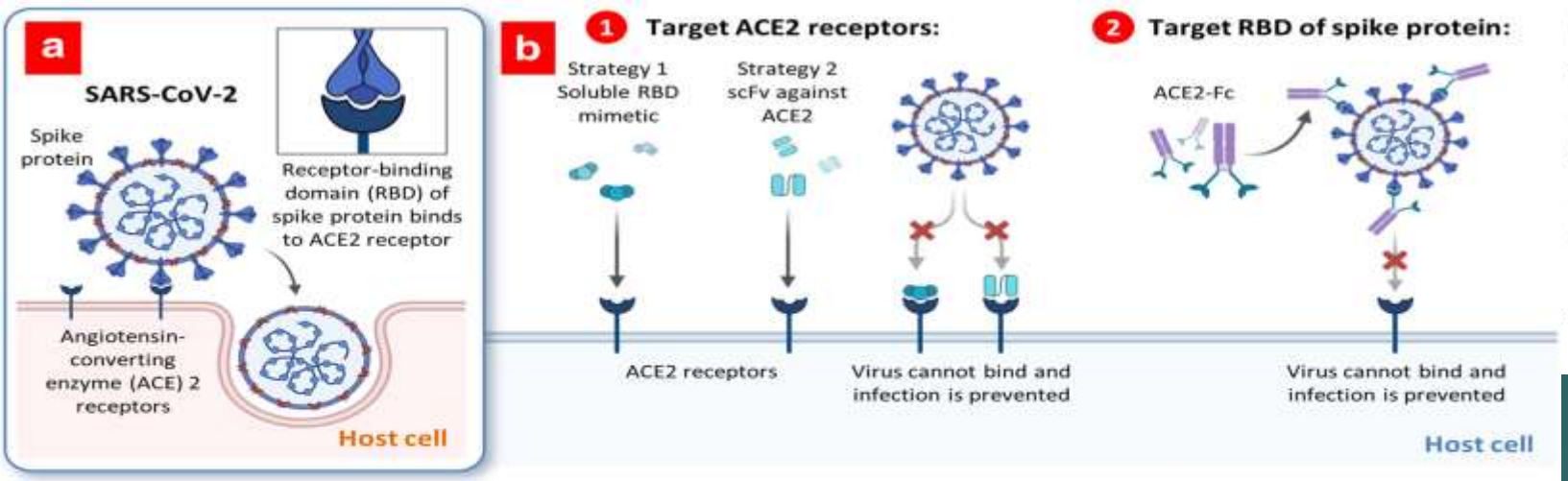
Review Article

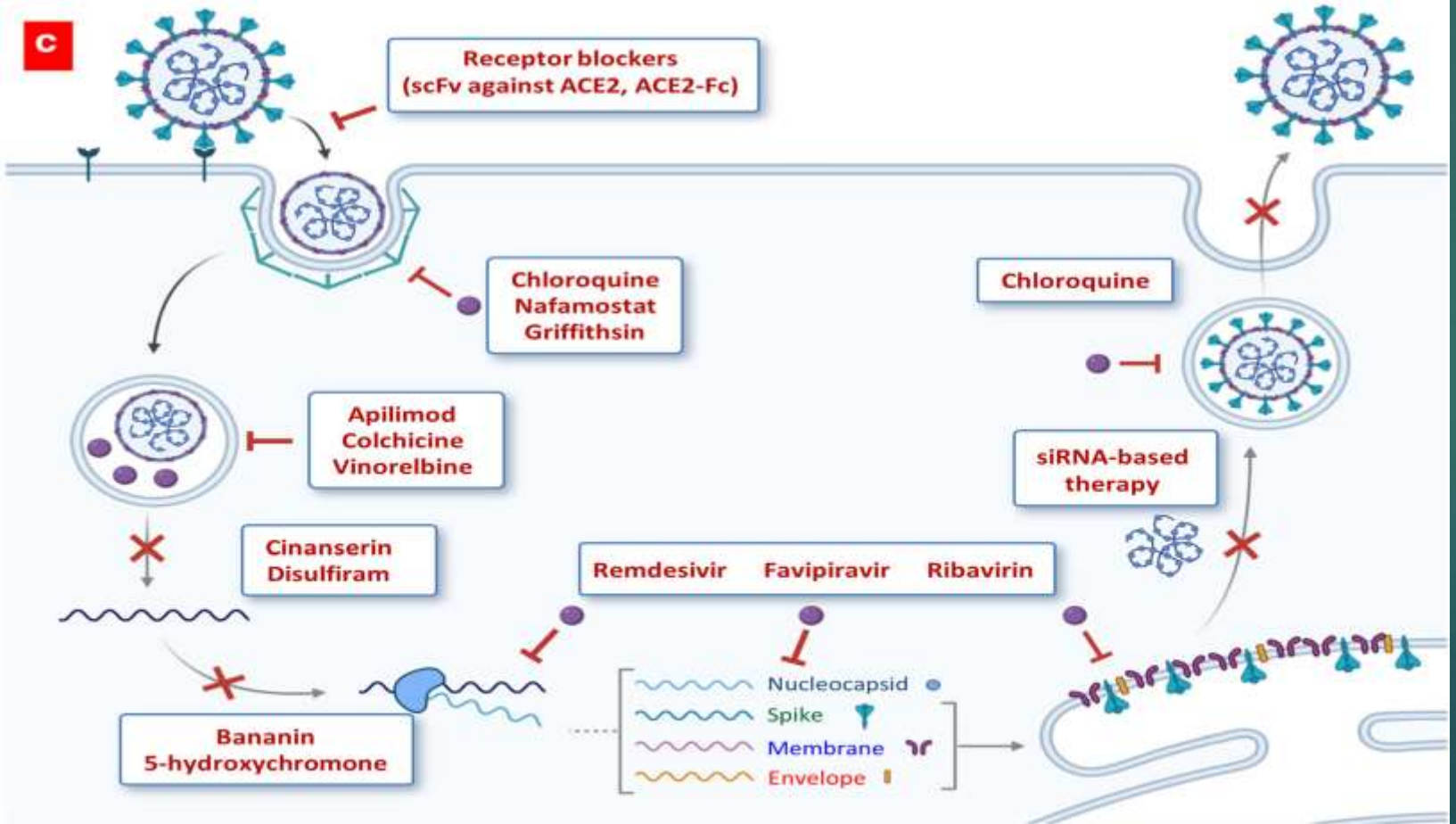
Theme: Celebrating Women in the Pharmaceutical Sciences
Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

Recent Developments on Therapeutic and Diagnostic Approaches for COVID-19

Joydeb Majumder[1,2,3] and Tamara Minko[1,2,3,4]

Received 4 September 2020; accepted 2 November 2020; published online 5 January 2021





Drug	Mode of Action	Mode of Administration
Antiviral		
Remdesivir	Nucleotide analogue	intravenous
Chloroquine/hydroxychloroquine (Aralen/Plaquenil)	Heme polymerase inhibitor	Oral
Lopinavir + ritonavir (Kaletra)	Protease inhibitor	Oral
Favipiravir (Avigan)	RNA polymerase inhibitor	Oral
Umifenovir (Arbidol)	Inhibits membrane fusion (entry)	Oral
Camostat	Protease inhibitor	Oral
Ribavirin	Lower respiratory tract infection due to RSV	Inhalation
Anti-inflammatory		
Interferon alfa-2b	Immune modulator	Sub-cutaneous
Tocilizumab (Actemra)	IL-6R Ab	intravenous
Sarilumab (Kevzara)	IL-6R Ab	intravenous
Baricitinib (Olumiant)	Inhibition of JAK	Oral

COVID Vaccines

International Immunopharmacology 96 (2021) 107763

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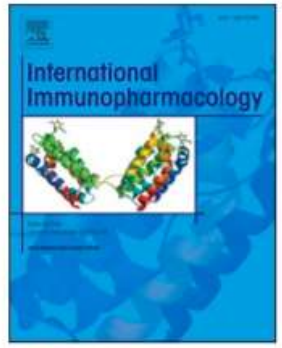
journal homepage: www.elsevier.com/locate/intimp

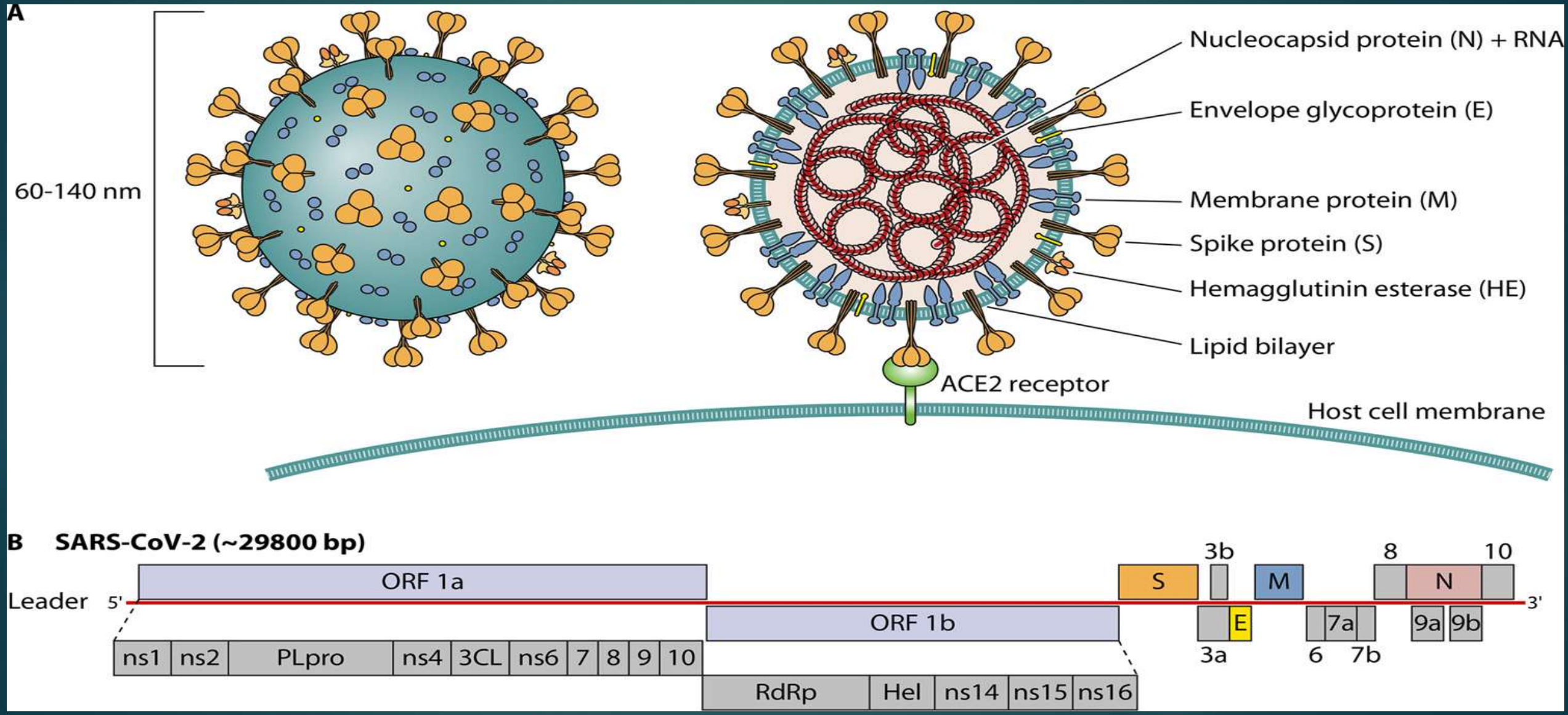


Review

An update review of globally reported SARS-CoV-2 vaccines in preclinical and clinical stages

Hamid Motamedi [a], Marzie Mahdizade Ari [b], Shirin Dashtbin [b], Matin Fathollahi [a], Hadi Hossainpour [a], Amirhoushang Alvandi [a,c], Jale Moradi [a], Ramin Abiri [a,d,*]

SARS-CoV-2 GENOME AND STRUCTURE



LAB DIAGNOSIS TESTS

LABORATORY METHODS FOR THE DETECTION OF SARS-CoV-2

Testing methods	Time to results	Commercially available	Advantages	Disadvantages	LOD* (copies/μl)
RT-PCR	Hours	Yes	High sensitivity, high specificity, reliable, ideal for high-throughput analysis	Requiring sample transportation, substantial equipment, regents and trained personnel	0.009–150
Isothermal PCR	15–60 mins	Yes	High sensitivity, high specificity, point-of-care testing, user friendly	Complex primer design, prone to non-specific amplification and false-positive results	0.13–7.0
ddPCR	Hours	Yes	Ultra-high sensitivity, high specificity, ideal for pooling analysis	More expensive than common RT-PCR	0.01–0.63
Antibody test	15 mins–several hours	Yes	Convenient and safe, high specificity	Moderate sensitivity, cross-reactivity, retrospective nature (**not suitable for diagnosis**)	NA
Rapid antigen test	15–30 mins	Yes	Rapid, visual readout	Requiring strict design of synthetic antibody and expert knowledge on viral etiology, prone to false-negative results	10^5 times higher than RT-PCR
CRISPR	15–60 mins	No	High sensitivity, high specificity, reliable, visual readout, multiplexing	Have not been extensively tested for SARS-Cov-2	6.8
Toehold switch	15–60 mins	No	High sensitivity, high specificity, safe and inexpensive, easy to store and distribute, ideal for wearable diagnostics	Have not been tested for SARS-Cov-2	NA

SPECIMEN TYPES

Specimen Types

Specimens collected from the upper respiratory tract:

Flocked nasopharyngeal (NP) coated with multilength fibers swab that is placed in universal or viral transport medium (UTM or VTM, respectively)

Sampling of the anterior nares (Na)

Oropharyngeal (OP) swabs

Washes/aspirates from the nasopharynx, nose, or throat OP swab along with sampling of the anterior nares

BAL fluid

Endotracheal secretions

Sputum

Saliva

Transport Media

UTM or VTM

Amies transport medium, sterile normal saline

Phosphate-buffered saline PBS

M4 medium

Minimal Essential Medium (MEM)

Timing of Specimen Collection

SARS-CoV-2 RNA can be detected early in the pre symptomatic stage of the disease and later on, even after recovery

Real-Time RT-PCR false-negative rates could be minimized by testing 2 to 3 days after symptom onset, with an average time of symptom onset of 5 days post exposure

Repeat testing can be considered for individuals with an initial negative test result but for whom there is a high level of clinical suspicion

Detecting SARS-CoV-2 RNA from stool is possible in the presence or absence of gastrointestinal symptoms

The magnitude of the viral load and duration of shedding depend on:

- The Specimen type
- The anatomical site of illness
- The severity of illness
- The host immune response to infection

In patients with mild disease

In Upper respiratory tract:

From 7.9 to 20 days after symptom onset

From 6 to 30.8 days in cases with moderate to severe illness

In the lower respiratory tract:

From 8 to 38.4 days for mild cases

Between 6 and 26.9 days for moderate to severe illness

The mean duration of SARS-CoV-2 RNA detection from symptom onset in mild adult cases

12.1 days (95% CI, 10.1 to 14.1 days) in the upper respiratory tract and 24.1 days (95% CI, 10.0 to 38.2 days) in the lower respiratory tract

For moderate to severe cases

The pooled estimates for the duration of SARS-CoV-2 RNA positivity:

In the upper respiratory tract were 15.8 days (95% CI, 11.1 to 20.6 days)

23.2 days (95% CI, 21.5 to 25.0 days) in the lower respiratory tract

In cases of mild adult disease

SARS-CoV-2 RNA:

In the upper respiratory tract was maximal on day 4, at approximately 6.6108 copies/ ml

In lower tract viral loads peaked at approximately 2.7108 copies/ml on day 6 after symptom onset

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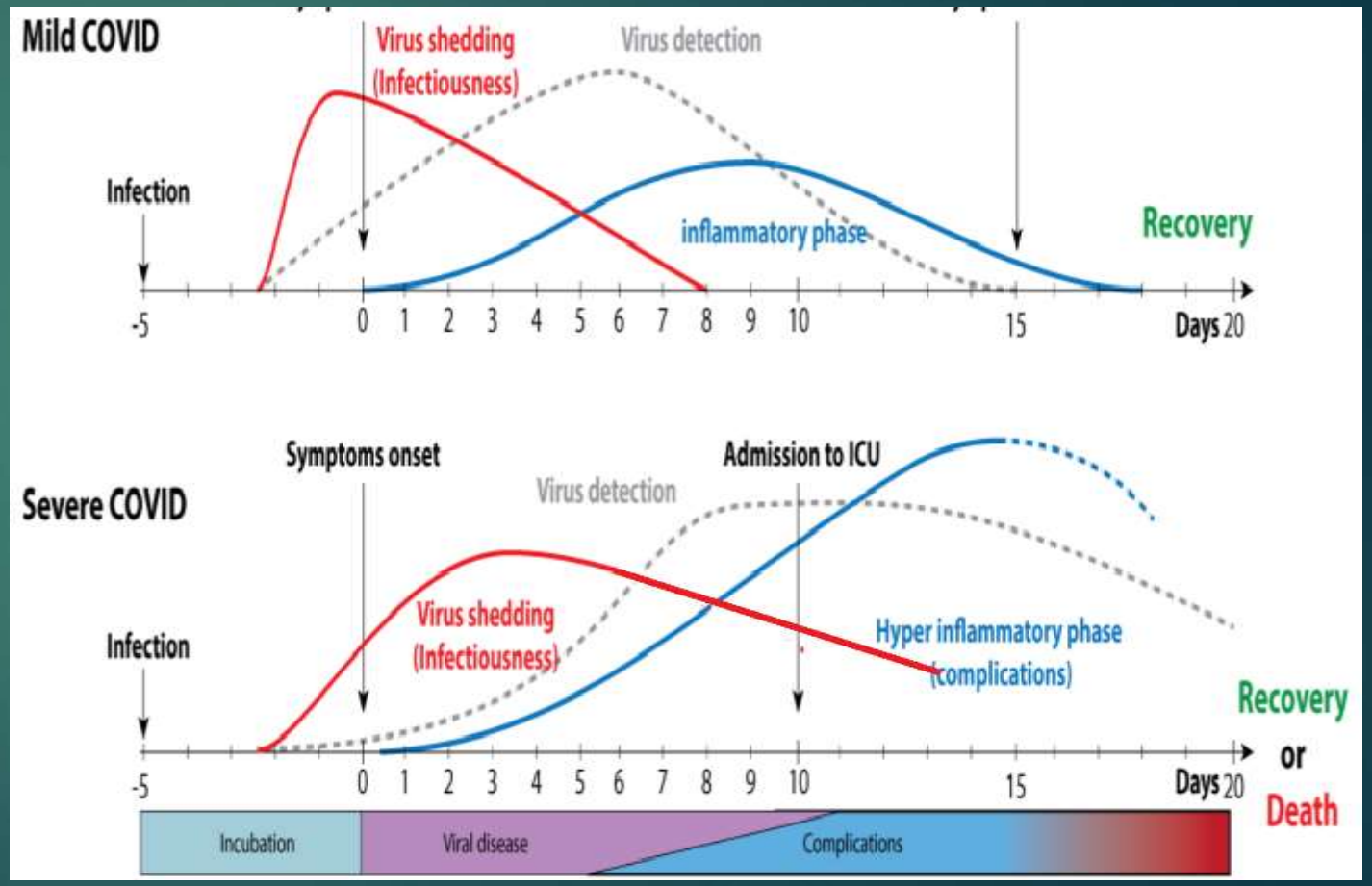
Viral Shedding

While most individuals with mild disease clear the virus within 10 to 20 days, in some cases with severe COVID-19, the duration of shedding can be prolonged 83 and 111 days after



Gastrointestinal Specimens

Detecting SARS-CoV-2 RNA from stool is possible in the presence or absence of gastrointestinal (GI) symptoms , however, only 1% of patients had detectable RNA in their stool in the absence of positive respiratory Specimens

For some patients, viral shedding in stool can occur for a longer period than in the respiratory samples and could help diagnose infection if upper and lower respiratory tract specimens are negative but there is a high suspicion of disease

Of individuals who test positive with GI specimens, the median duration of RNA shedding in the GI tract is 12.5 days following negative respiratory tract specimens

Less frequently, shedding in stool can be prolonged and has been documented up to 70 days after symptom onset or 33 days following clearance from the respiratory tract

Serum Specimens

Immunological appear 6 days after symptom onset, as viral RNA levels begin to decline

The first detectible antibody in human blood is IgM, followed by IgG

Both IgA and IgM decline rapidly over the course of infection

The median seroconversion times for total antibody, IgM, and IgG were 9, 10, and 12 days after symptom onset (or 15, 18, and 20 days after exposure), respectively.

Specimen Preprocessing Requirements

Preprocessing step like heat lysis or inactivation using guanidinium salts before nucleic acid extraction and amplification

Specimen types such as sputum may require mucolytic agents such as dithiothreitol (DTT), N-acetyl-L-cysteine (NALC), or proteinase K (PK)

Centrifugation for specimens like stool and PK digests for tissues (e.g., lung biopsy specimens), Specimen aliquoting

MOLECULAR METHODS FOR VIRAL RNA DETECTION

Molecular Methods for Viral RNA Detection

- Real-time RT-PCR
- Isothermal amplification technologies
  - Reverse transcription-recombinase polymerase amplification
  - Transcription-mediated amplification
  - Nicking enzyme-assisted reaction
  - Reverse transcription–loop-mediated isothermal amplification
- CRISPR-CAS technology

REAL-TIME RT-PCR

Real-time RT-PCR

Device/assay (manufacturer)	Method	Target gene(s)	Specimen type(s)	Authorized setting(s)	Time/throughput	LoD[b]
cobas 6800/cobas SARS-CoV-2 (Roche Molecular Systems, USA)	RT-PCR	ORF1ab + E	NS, NPS, OPS	H, M, H-pooling	3 h for the first-run results but 90 min per run in continuous mode/864 samples per 8 h	46 copies/ml
Abbott m2000/RealTime SARS-CoV-2 (Abbott Diagnostics, USA)	RT-PCR	RdRp + N	NS, NPS, OPS, BAL fluid	H	7 h per run/470 samples per 24 h	100 copies/ml
NeuMoDx 288/NeuMoDx SARS-CoV-2 (NeuMoDx Molecular, USA)	RT-PCR	Nsp2 + N	NS, NPS, OPS, BAL fluid, saliva	H, M	1.3 h per run/288 samples per 8 h	150 copies/ml
Panther Fusion/Aptima SARS-CoV-2 (Hologic, USA)	TMA	ORF1ab	NS, NPS, OPS, MTS, NPW, NPA, NA	H, pooling	2.4 h per run/500 samples per 8 h	0.026 $TCID_{50}$/ml
Liaison MDX/Simplexa COVID-19 Direct (DiaSorin Molecular, Italy)	RT-PCR	ORF1ab + S	NS, NPS, NW, NA, BAL fluid	H, M	1 h per run/8 samples per run	500 copies/ml
FilmArray/BioFire Respiratory Panel 2.1 (BioFire Diagnostics, USA)	RT-PCR	S + M	NPS	H, M	2-min hands-on time/1 h per run	160 copies/ml
ePlex/ePlex SARS-CoV-2 (GeneMark Diagnostics, USA)	RT-PCR	N	NPS	H, M	2-min hands-on time/1.5 h per run	750 copies/ml
GeneXpert Xpress/Xpert Xpress SARS-CoV-2 (Cepheid, USA)	RT-PCR	E + N2	NS, NPS, OPS, MTS, NW, NA	H, M, W	1-min hands-on time/45 min per run	0.02 PFU/ml
Accula Dock/Accula SARS-CoV-2 (Mesa Biotech, USA)	RT-PCR	N	NS, MTS	H, M, W	5-min hands-on time/30 min per run	150 copies/reaction

Contents lists available at ScienceDirect

Journal of Clinical Virology

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Short communication

Detection of SARS-COV N2 Gene: Very low amounts of viral RNA or false positive?

Francesca Falasca [b,1], Ilaria Sciandra [b,1], Daniele Di Carlo [a], Massimo Gentile [a,b], Alberto Deales [b], Guido Antonelli [a,b], Ombretta Turriziani [a,b,*]

[a] Department of Molecular Medicine, Sapienza University, Rome, Italy
[b] Sapienza University Hospital "Policlinico Umberto I", Rome, Italy

Xpert® Xpress SARS-CoV-2 (Cepheid, Sunnyvale, CA) detect the nucleocapsid gene (N2 region of the N-gene) and envelope gene (E) in respiratory specimens

The limit of detection (LoD) was reported at 100 copies/ml

We suggest suspecting false positive results when only the N gene is detected with Ct values >39. Ct (>39)

Received: 27 October 2020 | Revised: 13 November 2020 | Accepted: 20 November 2020
DOI: 10.1002/jmv.26691

RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

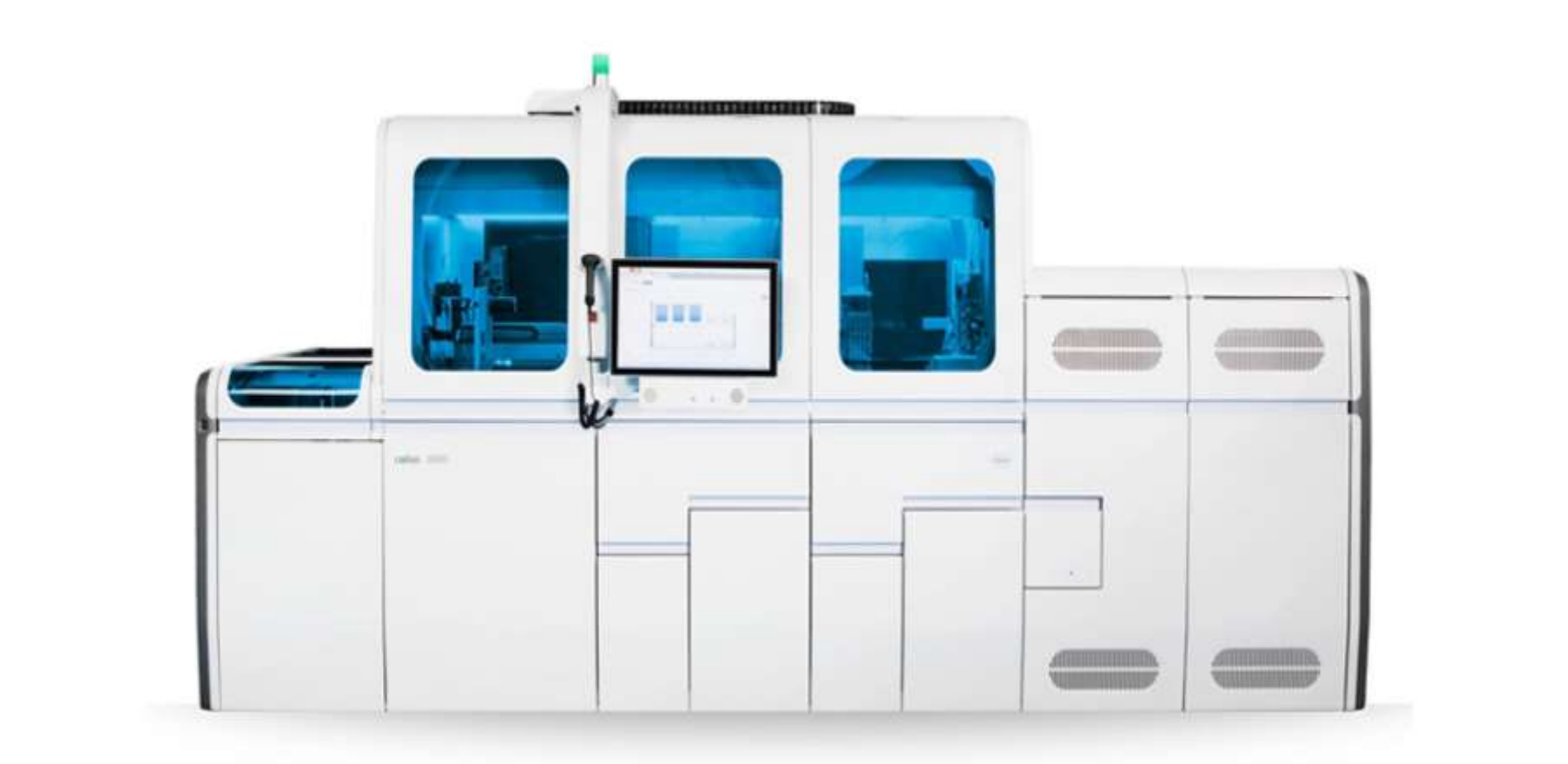
Evaluation of seven commercial RT-PCR kits for COVID-19 testing in pooled clinical specimens

Atul Garg | Ujjala Ghoshal | Sangram S. Patel | D. V. Singh | Akshay K. Arya | Shruthi Vasanth | Ankita Pandey | Nikki Srivastava

S No	Name of Kit	Manufacturer	Regulatory clearance	Target genes	Limit of detection	Kit interpretation
1	Allplex 2019-nCoV assay	See gene	US-FDA	E, N, RdRP	4167 copy/ml	$C_t < 40$ positive
2	Patho Detect RT-PCR kit	Mylab	ICMR, India	E, RdRP	-	-
3	FOSUN COVID-19 RT-PCR Kit	Fosun	US-FDA	E, N, ORF1ab	300 copy/ml	$C_t < 36$ positive
4	TRUPCR SARS-CoV-2 RT-qPCR kit	Black Biotech	US-FDA	E, N, RdRP	10 copy/µl	$C_t < 35$ positive
5	TaqPath COVID-19 Combo Kit	Thermo Fisher Scientific	US-FDA	S, N ORF1ab	2 copy/µl	$C_t < 40$ positive
6	Lab Gun Real-Time PCR Kit	Lab Genomics	US-FDA	E, RdRP	20 copy/µl	$C_t < 40$ positive
7	Real-Time Fluorescent RT-PCR Kit for 2019- nCoV	BGI Genomics	US-FDA CE-IVD	ORF1ab	150 copy/µl	$C_t < 37$ positive

	Lab Gun	Fosun	BGI	Thermo Fischer Scientific	Black Bio	My Lab	Seegene
Sensitivity	93.02% (80.9%-8.5%)	95.2% (83.8%-99.4%)	100.0% (91.1%-100.0%)	100.0% (91.1%-100.0%)	100.0% (91.1%-100.0%)	88.8% (75.9% - 96.2%)	100.0% (91.1%-100.0%)
Specificity	100% (69.1%-100%)	100% (69.1%-100%)	100% (69.1%-100%)	100% (69.1%-100%)	100% (69.1%-100%)	100% (69.1%-100%)	100% (69.1%-100%)
Positive predictive value	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Negative predictive value	76.9% (52.8%-90.8%)	83.3% (56.3% -95.0%)	100.0%	100.0%	100.0%	66.6% (46.6%-82.0%)	100.0%
Accuracy	94.3%	96.15%	100.0%	100.0%	100.0%	90.9%	100.0%

Automation: cobas 6800 instrument



Hologic Panther

NeuMoDx SARS-CoV-2 assay

RT-PCR-based rapid diagnostic tests

Provides results in about 45 min, with a 5-min hands-on specimen processing time

ISOTHERMAL AMPLIFICATION TECHNOLOGIES

Isothermal amplification technologies

Reverse transcription-recombinase polymerase amplification

Transcription-mediated amplification

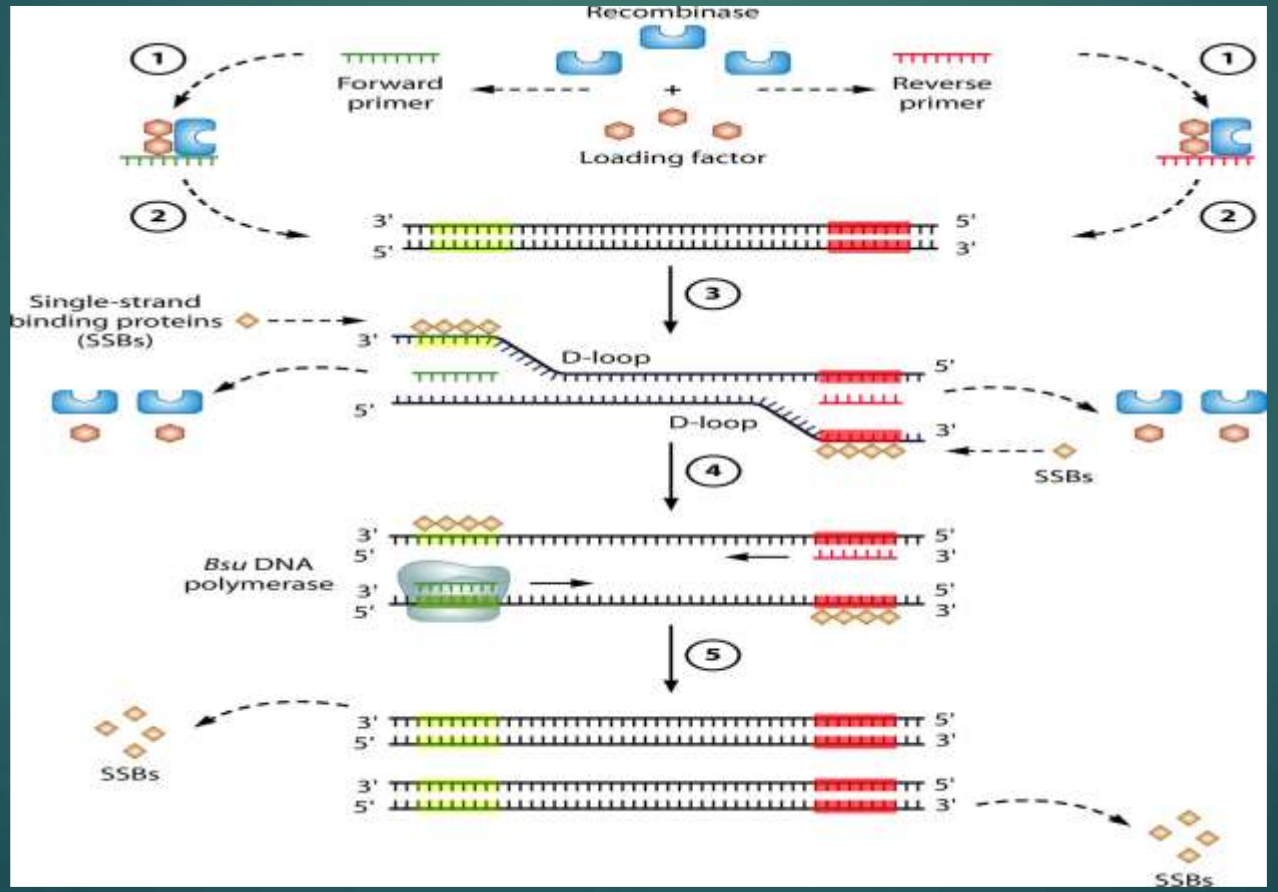
Nicking enzyme-assisted reaction

Reverse transcription–loop-mediated isothermal amplification

CRISPR-Cas technology

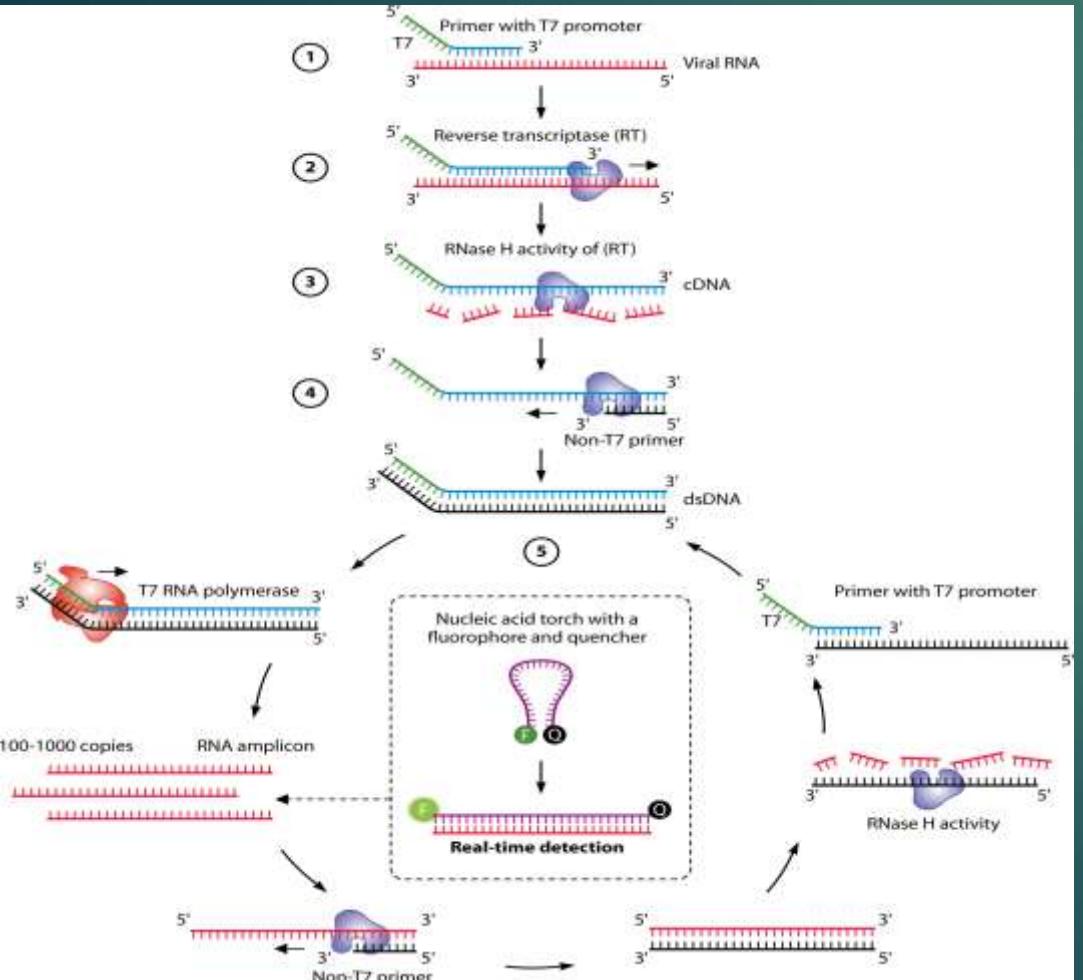
Reverse transcription-recombinase polymerase amplification

Approximately 4 copies/reaction in a 10-min reaction



Transcription-mediated amplification

Aptima SARS-CoV-2 assay showed similar analytical sensitivity, with LoDs ranging between 62.5 and 125 copies/ml



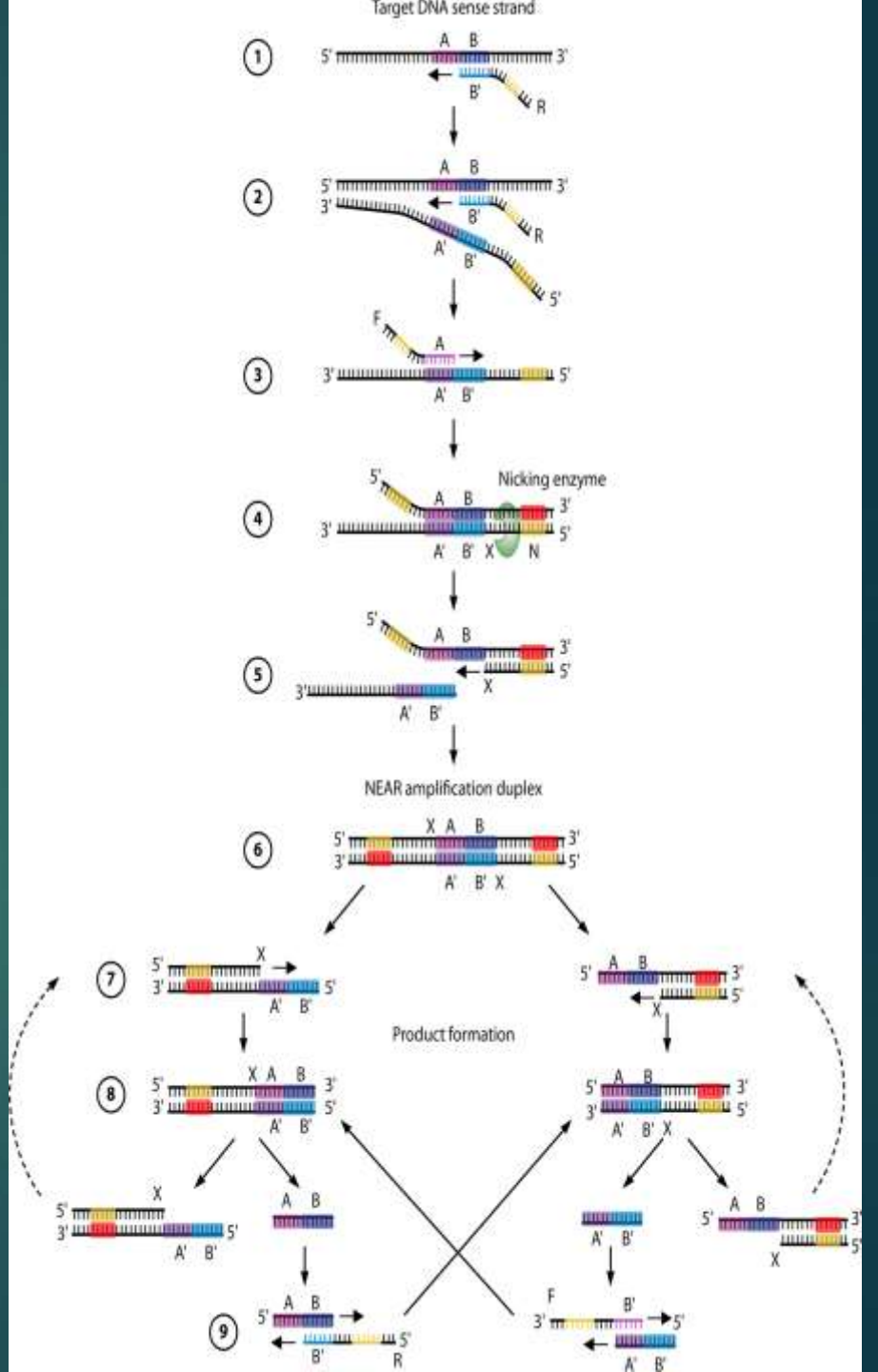
Nicking enzyme-assisted reaction

Abbott Diagnostics

Specificity/negative percent agreement (NPA) near 100%

but relatively poor sensitivity/positive percent agreement (PPA) of between 48% and 70%

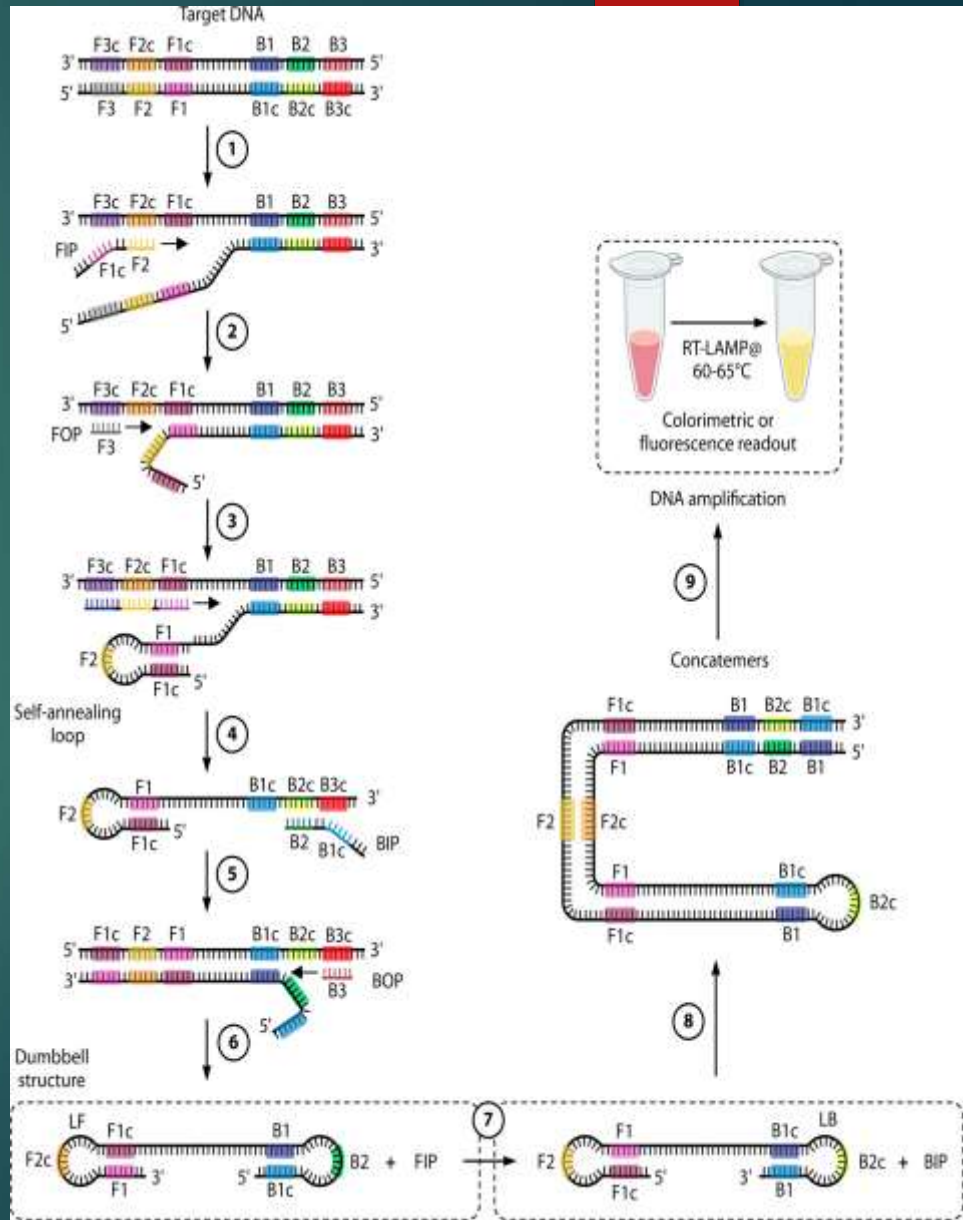
While other studies showed a high specificity/NPA (;100%) as well as high sensitivity/PPA values above 90%



Reverse transcription–loop-mediated isothermal amplification

Real-time SARSCoV-2 RT-LAMP (Variplex; Amplex Diagnostics, German

The analytical sensitivity of most RT-LAMP assays was found to be in the range of 100 to 200 copies per reaction sensitivity of 80% and a specificity of 73%



CRISPR-Cas technology

SHERLOCK

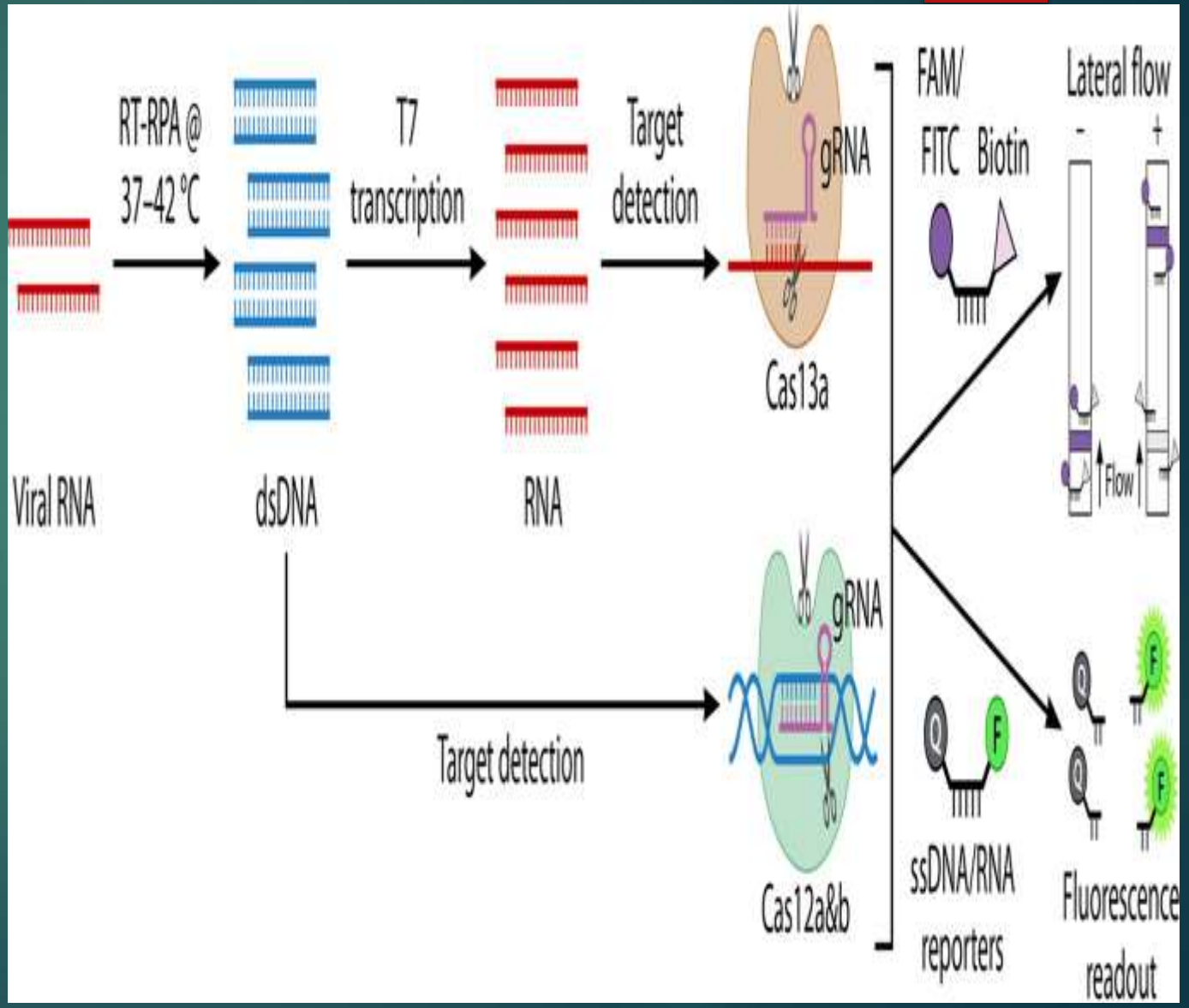
CREST

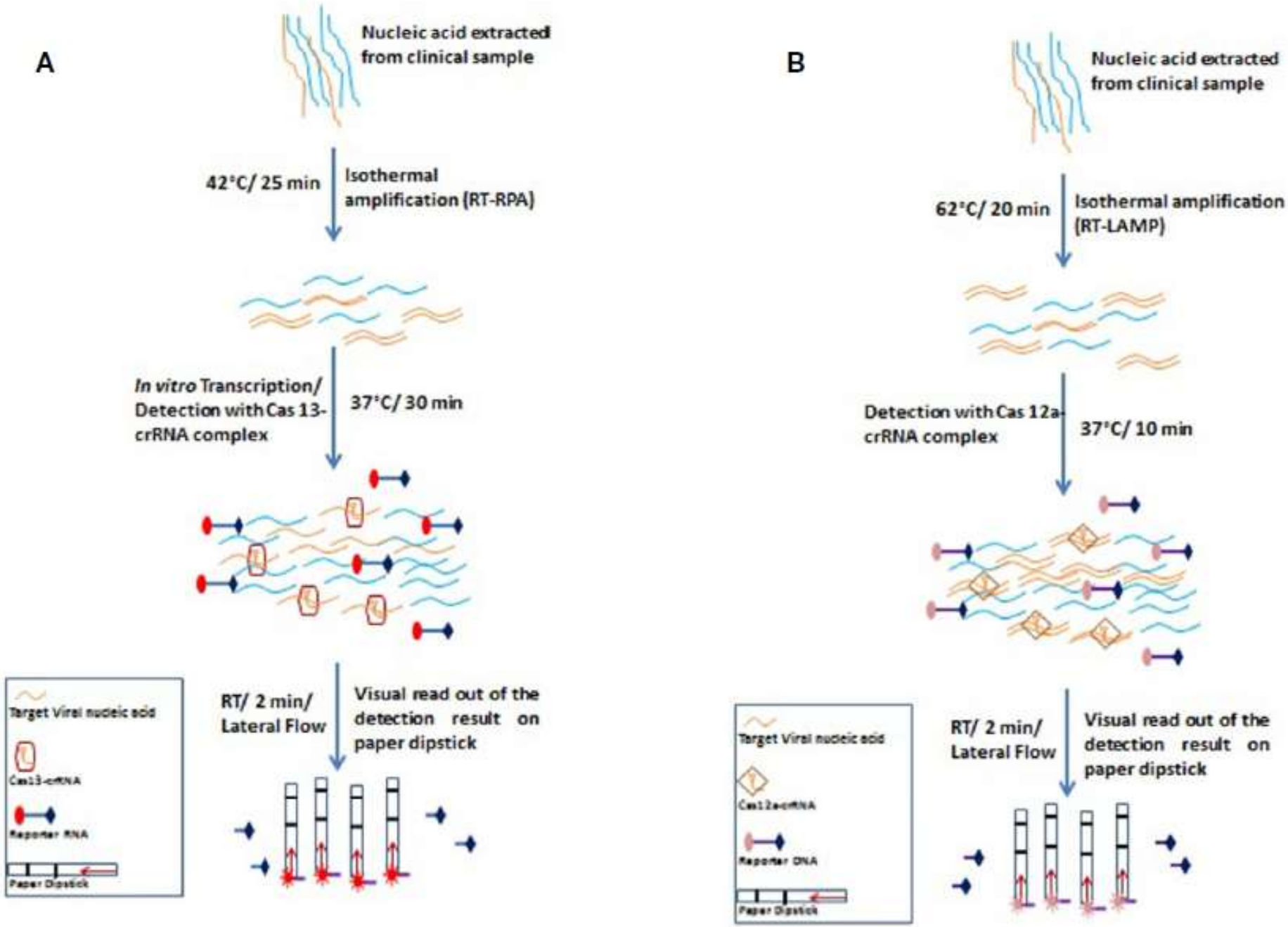
DETECTR

AIOD-CRISPR

RISPR/Cas12a-NER

CONAN




A
Nucleic acid extracted from clinical sample
42°C/ 25 min
Isothermal amplification (RT-RPA)
In vitro Transcription/ Detection with Cas 13-crRNA complex
37°C/ 30 min
RT/ 2 min/ Lateral Flow
Visual read out of the detection result on paper dipstick
Target Viral nucleic acid
Cas13-crRNA
Reporter RNA
Paper Dipstick
B
Nucleic acid extracted from clinical sample
62°C/ 20 min
Isothermal amplification (RT-LAMP)
Detection with Cas 12a-crRNA complex
37°C/ 10 min
RT/ 2 min/ Lateral Flow
Visual read out of the detection result on paper dipstick
Target Viral nucleic acid
Cas12a-crRNA
Reporter DNA
Paper Dipstick

False negative results

Inappropriate sample collection

Inappropriate extraction workflow

Inappropriate Real Time-PCR workflow

Inappropriate sensitivity of the assays used

Low amounts of viral RNA (e.g., early or late in COVID-19 disease).

scientific reports

OPEN

Predictors of negative first SARS-CoV-2 RT-PCR despite final diagnosis of COVID-19 and association with outcome

Jean-Baptiste Lascarrou[1,2], Gwenhael Colin[2,3], Aurélie Le Thuaut[4], Nicolas Serck[5], Mickael Ohana[6], Bertrand Sauneuf[7], Guillaume Geri[8], Jean-Baptiste Mesland[9], Gaetane Ribeyre[10], Claire Hussenet[11], Anne Sophie Boureau[12] & Thomas Gille[13,14]

By multivariate analysis, two factors were independently associated with a lower risk of a first false-negative test,

headache

and fatigue/malaise

BIOSENSORS FOR VIRAL RNA DETECTION

Biosensors for viral RNA detection

Biosensors are highly promising substitutes for the conventional RT-PCR diagnostic techniques due to their simplicity, cost-effectiveness, rapid detection, and high sensitivity and specificity

- Electrochemical biosensors
- Optical biosensors

Electrochemical biosensors

Biorecognition cues triggered by the hybridization reactions result in assessable impedimetric, potentiometric,chronoamperometric, or voltammetric signals

Coronavirus subgroup	Analyte	Assay method	Detection time	Concentration range	Limit of detection	Tested sample
SARS-CoV	Oligonucleotide	Electrochemical (SWV)	2 h 10 min	0.01–1.01 nM	6 pM	Spiked oligonucleotide solutions
SARS-CoV	Oligonucleotide	Electrochemical (CA)	3 h 25 min	–	8 pM	Spiked oligonucleotide solutions
		Electrochemical (CV)	1 h 30 min		0.5 nM	
SARS-CoV	Oligonucleotide	Electrochemical (CV)	2 h 20 min	2.5–50 pM	2.5 pM	Spiked oligonucleotide solutions
SARS-CoV	Oligonucleotide	Electrochemical (SWV)	3 h	0.1–10 nM	–	Spiked oligonucleotide solutions

Coronavirus subgroup	Analyte	Assay method	Detection time	Concentration range	Limit of detection	Tested sample
SARS-CoV	Oligonucleotide	Optical (colorimetry)	5 min	–	< 100 fmol	Spiked oligonucleotide solutions
SARS-CoV	RNA	Optical (SPR)	~2 h	–	2 nM	Throat swab samples
MERS-CoV	RNA	Optical (fluorescence)	30 min	–	0.1 pM	Pseudo-serum samples
MERS-CoV	Oligonucleotide	Optical (colorimetry)	–	20–1000 nM	1.53 nM	Spiked oligonucleotide solutions
MERS-CoV	Oligonucleotide	Optical (colorimetry)	10 min	–	1 nM	Spiked oligonucleotide solutions
SARS-CoV-2	Oligonucleotide	Optical (LSPR and PPT)	~14 min	–	0.22 pM	Spiked oligonucleotide solutions

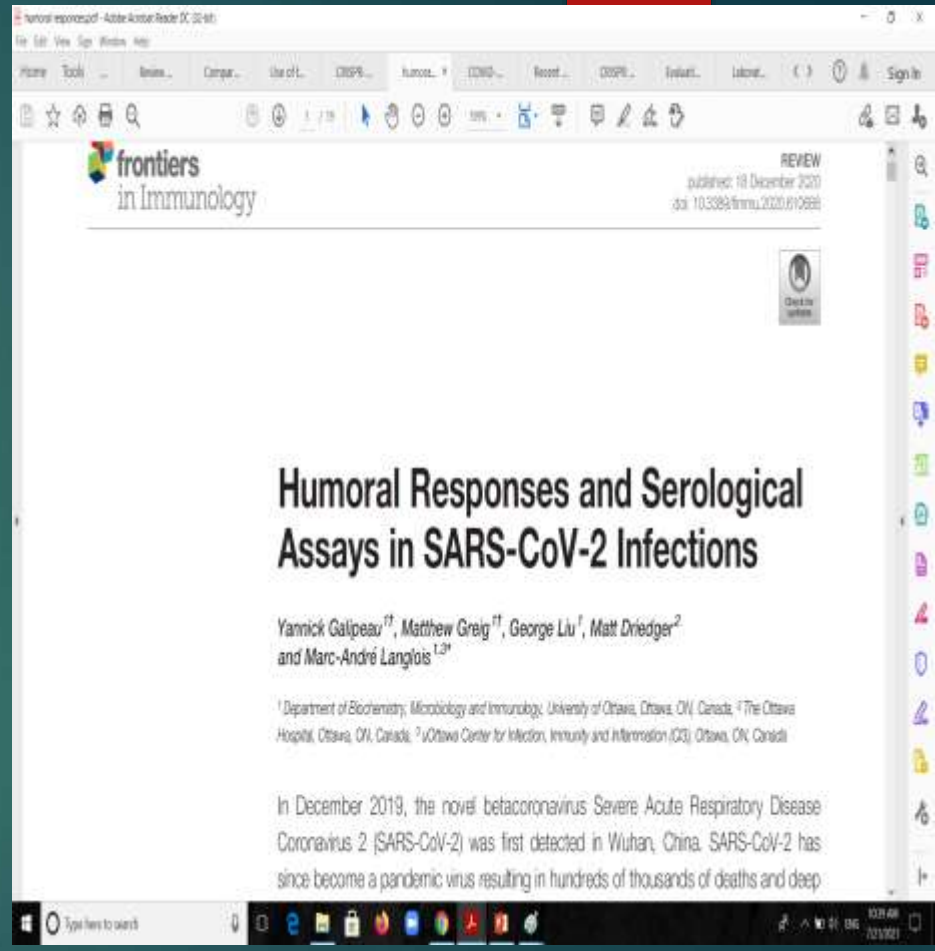
SARS-COV-2 ANTIGEN DETECTION

SARS-CoV-2 Antigen Detection

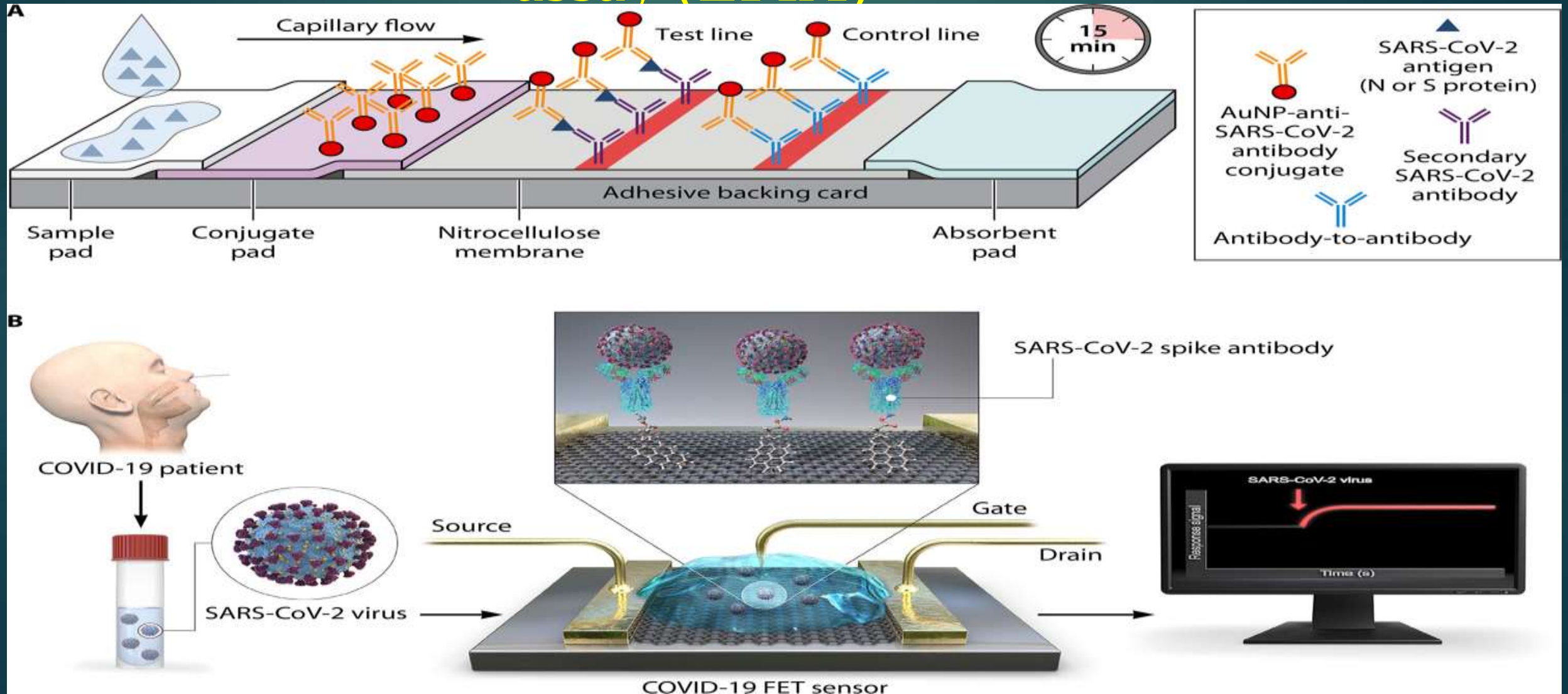
ELISA

Chemiluminescence immunoassays (CLIAs)

Lateral flow immunochromatographic assay (LFIA).



Lateral flow immunochromatographic assay (LFIA)



The average sensitivity of Ag-RDTs was found to be 56.2% (95% CI, 29.5% to 79.8%)

The average specificity was 99.5% (95% CI, 98.1% to 99.9%)

In comparison, NAAT-based RDTs had an average sensitivity of 95.2% (95% CI, 86.7% to 98.3%) and an average specificity of 98.9% (95% CI, 97.3% to 99.5%)

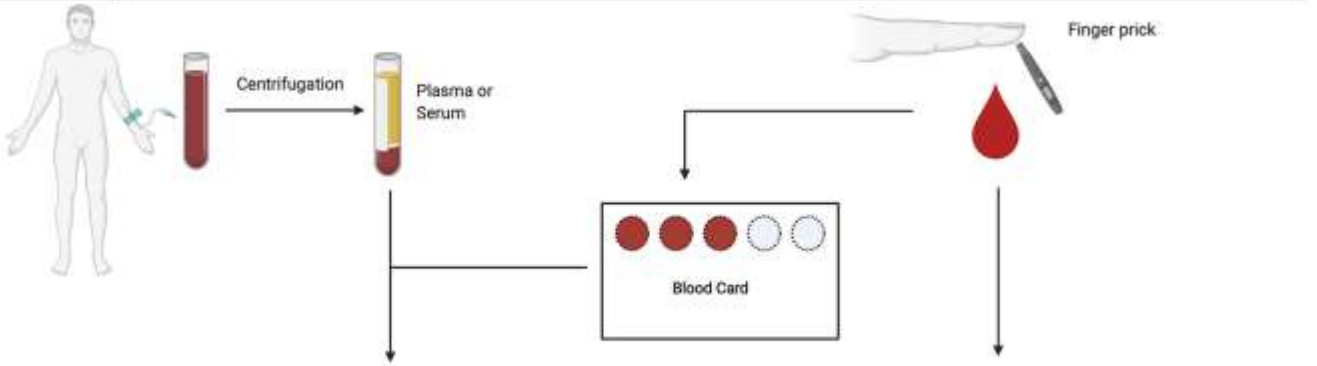
Device or assay (manufacturer)	Methods	Target antigen protein	Specimen type(s)	Authorized detection window (dpo)	Authorized settings	Time (min)	LoD ($TCID_{50}$/ml)[b]
BinaxNOW COVID-19 Ag card home test[c] (Abbott Diagnostics, USA)	LFIA, visual readout	N	NS, ANS	7	Home, H, M, W	15	140.6
CareStart COVID-19 antigen test (Access Bio, USA)	LFIA, visual readout	N	NPS	5	H, M, W	10	800
QuickVue SARS antigen test[c] (Quidel Corporation, USA)	LFIA, visual readout	N	ANS	5	H, M, W	10	7,570
Veritor system for rapid detection of SARS-CoV-2 (BD, USA)	LFIA, instrument readout	N	NS	5	H, M, W	15	140
Sofia 2 Flu + SARS antigen FIA[c] (Quidel Corporation, USA)	LFIA, IFA, instrument readout	N	NS, NPS	5	H, M, W	15	91.7
LumiraDx SARS-CoV-2 Ag test[c] (LumiraDx Ltd., UK)	Microfluidics, IFA, instrument readout	N	NS	12	H, M, W	12	32
Clip COVID rapid antigen test[c] (Luminostics Inc., USA)	LFIA, ILA, instrument readout	N	ANS	5	H, M, W	30	88
Ellume COVID-19 home test[c] (Ellume Limited, Australia)	LFIA, IFA, smartphone readout	N	MTS	NA	Home, H, M, W	15	6,309

Serological Immunological Methods for SARS-CoV-2 Detection

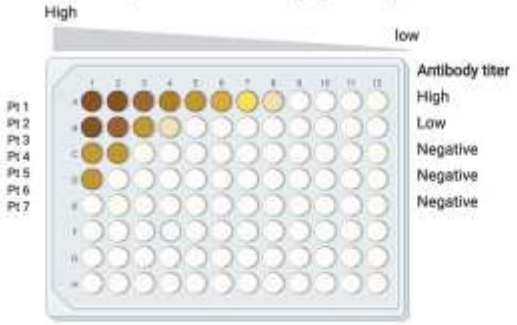
Serological Immunological Methods for SARS-CoV-2 Detection

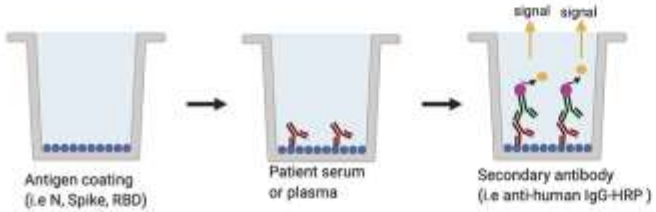
- Chemiluminescence immunoassay
- Lateral flow immunoassays
- Neutralization assays

Serological assays

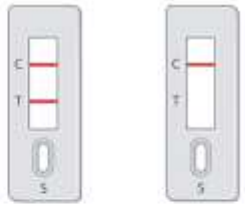


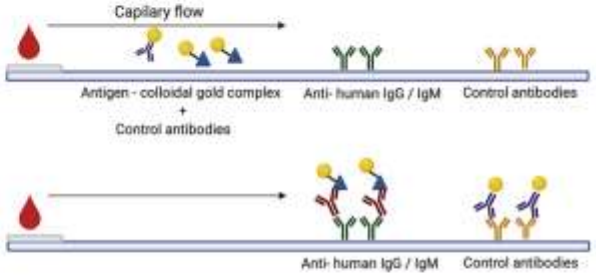
Quantitative assay (ELISA)



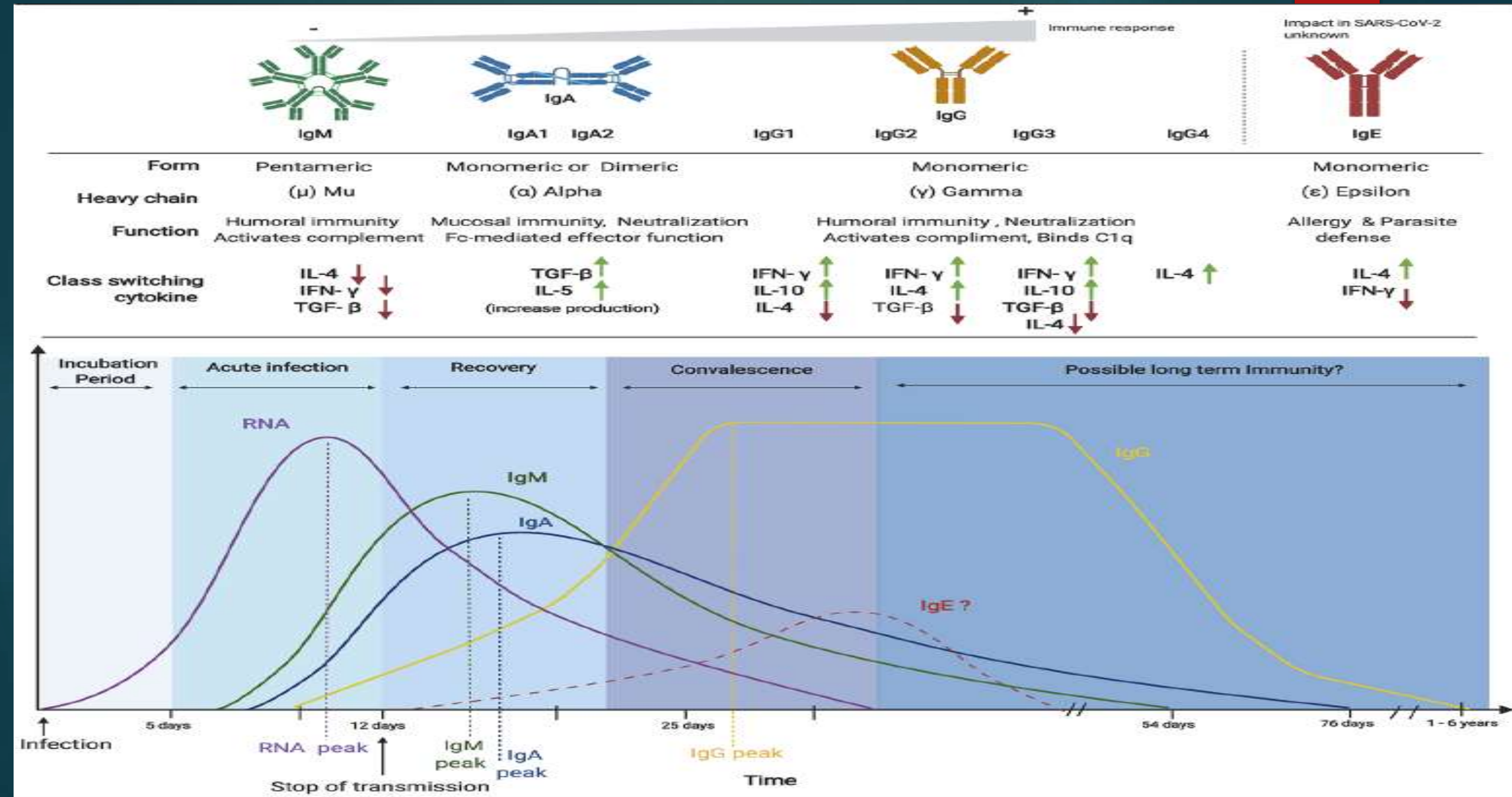


Binary assay (LFA)





	Quantitative assay (ELISA)	Binary assay (LFA)
Readout	Quantitative titer	Positive or negative
Usage	Laboratory assay	At home, clinic testing , POC
High throughput	Yes	No
Reliability	High / Moderate	Low
Cost	Low / Moderate	High


-
+
Immune response
Impact in SARS-CoV-2 unknown
IgA
IgG
IgM
IgA1
IgA2
IgG1
IgG2
IgG3
IgG4
IgE
Form
Pentameric
Monomeric or Dimeric
Monomeric
Monomeric
Heavy chain
(μ) Mu
(α) Alpha
(γ) Gamma
(ε) Epsilon
Function
Humoral immunity
Activates complement
Mucosal immunity, Neutralization
Fc-mediated effector function
Humoral immunity , Neutralization
Activates compliment, Binds C1q
Allergy & Parasite defense
Class switching cytokine
IL-4
IFN- γ
TGF- β
TGF-β
IL-5
(increase production)
IFN- γ
IL-10
IL-4
IFN- γ
IL-4
TGF-β
IFN- γ
IL-10
TGF-β
IL-4
IL-4
IL-4
IFN-γ
Incubation Period
Acute infection
Recovery
Convalescence
Possible long term Immunity?
RNA
IgM
IgA
IgG
IgE ?
Infection
5 days
12 days
25 days
54 days
76 days
1 - 6 years
RNA peak
IgM peak
IgA peak
IgG peak
Stop of transmission
Time

NEUTRALIZATION ASSAYS AND THEIR IMPORTANCE

Involve live authentic SARS-CoV-2 viruses produced in cell culture and therefore require all procedures to be carried outin a Biosafety Level 3 (BSL3)

Murine leukemia virus (MLV) or vesicular stomatitis virus (VSV) pseudotyped with the SARS-CoV-2 S glycoprotein

Purified ACE2 to determine the effect of neutralizing antibodies on the ACE2- Spike interaction without the requirement of live cells or viruses.

cPass SARS-CoV-2 from GenScript Biotech: pan-Ig neutralizing antibodies using the SARS-CoV-2 RBD as the viral antigen for antibody capture

ELISA-based competition binding assay against

Additional non- RBD epitopes elsewhere on the S protein have also been shown to neutralize the virus when targeted by antibodies

Percent identity of amino acid sequences between human CoVs and SARS-CoV-2.

	Alphacoronavirus		Betacoronavirus			
	229E	**NL63**	**OC43**	**HKU1**	**MERS**	**SARS**
Full spike	31.4	29.8	30.2	29.5	34.8	76.0
S1 Domain	31.2	25.0	23.8	23.7	28.3	60.3
S2 Domain	35.0	33.1	42.3	41.2	43.6	90.0
RBD	24.1	27.8	23.8	29.4	21.7	73.1
Nucleoprotein	28.4	32.6	34.6	33.9	49.7	90.5

Percent similarity of amino acid sequences between human CoVs and SARS-CoV-2.

	Alphacoronavirus		Betacoronavirus			
	229E	**NL63**	**OC43**	**HKU1**	**MERS**	**SARS**
Full spike	61.8	60.0	57.9	58.0	65.7	91.5
S1 Domain	62.5	51.4	51.7	55.6	61.9	84.2
S2 Domain	66.5	66.3	72.7	72.7	73.9	98.1
RBD	59.3	59.3	54.7	67.6	56.0	88.9
Nucleoprotein	57.9	65.1	62.1	65.1	75.4	97.2

Alignments between amino acid sequences of all seven human coronavirus were done for the Full spike, spike domains S1, S2, and RBD and the nucleoprotein (N).

Due to statistical realities, test results are less accurate for an individual than for a population, if the test is less than 100% accurate, and if the disease is of low prevalence

This has to do with the predictive value, also referred to as the ‘base rate fallacy,’ which takes into account both test performance and the background population prevalence of disease

In other words, the more prevalent a disease, the higher the predictive value for a positive test

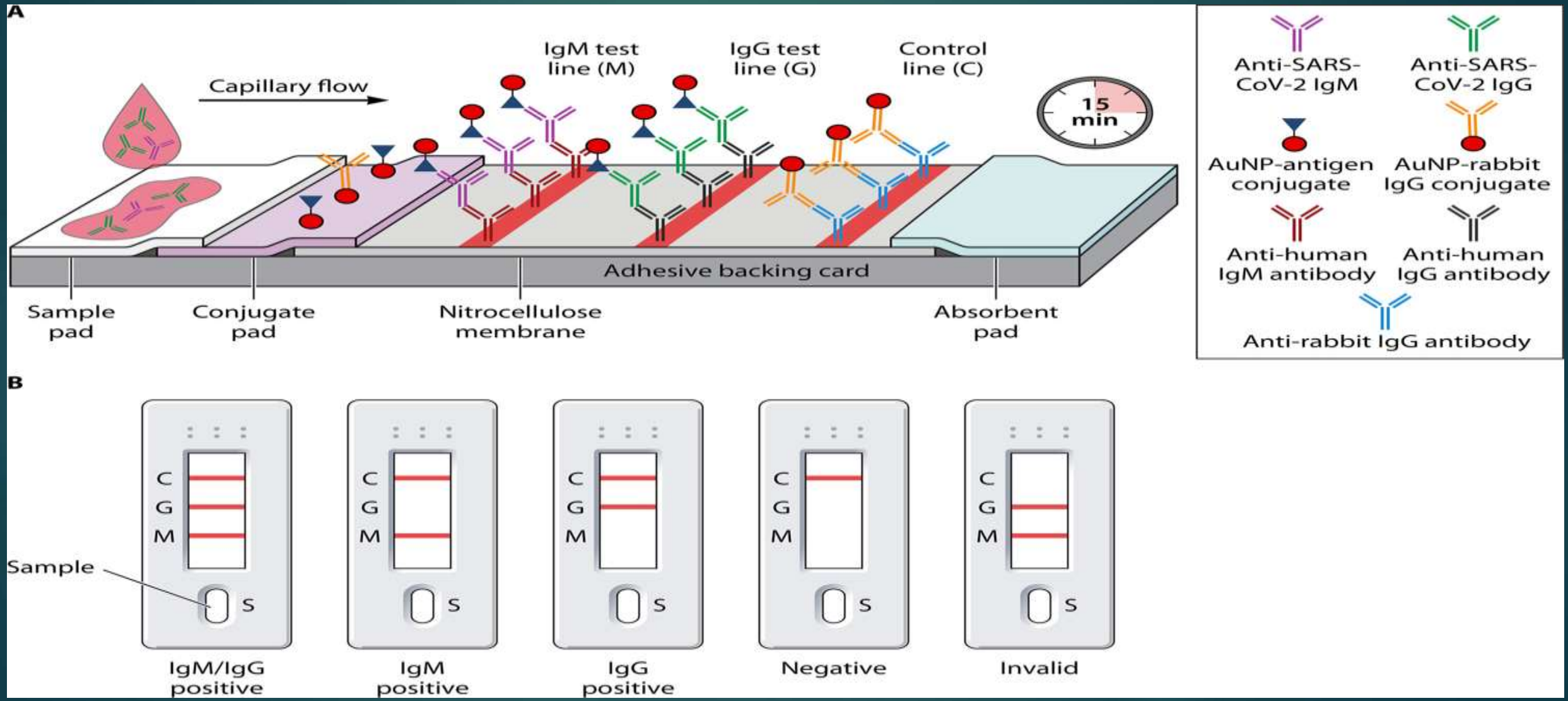
Current and Potential Uses for Antibody Tests for Individuals

Identifying and Managing Patient Care after Recovery from COVID-19 Donating Convalescent Plasma

Vaccine Selection

A Tool for Public Health, Not Individuals

Chemiluminescence immunoassay



Fluorescent microparticle immunoassays

Fluorescent nanoparticle immunoassay (FMI)-based single-molecule array assay that consisted of a mixture of four types of dye-encoded beads coupled to S, S1, S2, and N proteins and used for the detection and differentiation of SARS-CoV-2 IgM, IgG, and IgA Antibodies

When tested on a set of 81 plasma samples, the technique showed 86% sensitivity and 100% specificity for the samples during the first week after symptom onset and 100% sensitivity and specificity for the samples taken after the first week after symptom onset

BIOMARKERS IN COVID-19

Biomarkers in COVID-19

frontiers in Pediatrics

REVIEW
published: 30 March 2021
doi: 10.3389/fped.2020.607647

Biomarkers in COVID-19: An Up-To-Date Review

BIOMARKERS FOR COVID-19

White blood cell (WBC) counts

markers for inflammatory Conditions CRP

Procalcitonin [PCT]

Interleukin 6

Tests for anticoagulation

Indicators of tissue damage (alanine aminotransferase [ALT], aspartate aminotransferase [AST], lactate dehydrogenase [LDH], and creatine kinase [CK])

None of these tests are sensitive or specific for COVID-19

BIOMARKERS FOR COVID-19

21 studies encompassing 14,126 COVID-19 cases and 56,585 non-COVID-19 cases, only three markers showed sensitivity and specificity values of 50%:

A decrease in the lymphocyte count

Increases in CRP

Increases in IL-6

Patients with critical illness have high plasma levels of inflammatory makers, and elevated levels of d-dimer and lymphopenia have been associated with an increased risk of death

Lymphocytopenia

(i) direct viral invasion and lysis as lymphocytes express the ACE2 receptor on their surface

(ii) lymphocyte apoptosis induced by interleukins

(iii) reduced lymphocyte turnover due to the “cytokine storm” induced atrophy of lymphoid organs

(iv) reduced lymphocyte proliferation due to lactic acidosis

Lymphocytes

- Approximately 7 to 14 days from the onset of symptoms, appearance of significant lymphopenia
- Lymphopenia on admission (defined as lymphocyte count 1,100 cells/μl) is associated with three-fold risk of poor outcome, in younger as compared to older patients
- Lymphocyte counts were lower in patients with ARDS, severe disease requiring ICU care, and in non-survivors

Neutrophils

Patients requiring admission to the ICU had higher percentage and absolute number of neutrophils

Monocytes and basophils are also decreased akin to lymphocytes and eosinophils.

Eosinophils

A low percentage of eosinophils in airway and serum

Eosinophil-derived neurotoxin (EDN-1) can be a potential biomarker of COVID pneumonia

Platelets

- Both thrombocytopenia and thrombocytosis have been observed.
- However severe thrombocytopenia and bleeding are uncommon
- Thrombocytopenia was shown to correlate with other coagulation parameters and increased risk of mortality

Composite Hematological Markers

IL-2 levels correlated positively with the other cytokines and negatively with lymphocyte number

An elevated IL-2R to lymphocytes ratio was discriminative of severe and critical illness

In fact this ratio was superior to other markers for differentiation of critical illness

The ratio was significantly decreased in recovered patients, but further increased in patients who deteriorated, thus correlating with the outcome

A high neutrophil to- lymphocyte ratio (NLR) at admission can be a good surrogate marker for diagnosis of COVID-19

A rising NLR can also be used as a prognostic marker for predicting poor outcomes

Another prognostic marker the lymphocyte-to-CRP ratio (LCR), used in several types of cancers, may also be helpful

A rise in the NLR and decline in LCR correlates with the severity of COVID-19pecifically, a low LCR at presentation was seen to predict ICU admission and need for invasive ventilation.

Procalcitonin

CRP was elevated in 60.7% of patients, procalcitonin (PCT) in 5.5%,

Lactate dehydrogenase (LDH) in 41% of patients

A cut off of >10 mg/L and >0.5 ng/ml for CRP and PCT respectively have been shown to be predictors of poor outcome

A retrospective study showed that a CRP level of 26 mg/L could serve as a cut-off to predict progression to severe disease

Elevated PCT values were associated with a nearly 5-fold higher risk of severe infection

Cytokines

More than half of admitted patients were found to have elevated IL-6 levels

Higher baseline IL-6 correlated with severity, bilateral interstitial lung involvement and other acute inflammatory markers

IL 6 is good to monitor therapeutic response. Other pro-inflammatory cytokines (IL-1b, IL-2, IL-8, IL-17, G-CSF, GMCSF, IP-10, MCP-1, CCL3, and TNFa) are significantly increased in patients with severe disease

Coagulative Parameters

Coagulopathy in COVID-19 differs from the usual disseminated intravascular coagulation, in having a high fibrinogen, normal or mildly prolonged prothrombin time and activated partial thromboplastin time, platelet count >100 × 103/ml, but no significant bleeding

D-dimer levels have prognostic value and correlate with disease severity and in-hospital mortality

A level of >2.0μg/ml on admission could predict mortality

Cardiac Biomarkers

LDH CK Creatinine kinase-muscle/-brain activity (CK-MB)

Myoglobin (Mb) Cardiac troponin I (cTnI)

Alpha-hydroxybutyrate dehydrogenase (a-HBDH)

Aspartate aminotransferase (AST)

N-terminal of the prohormone brain natriuretic peptide (NT-proBNPAmong these LDH, CK, a-HBDH, and

On the other hand, CK-MB, cTnI, Mb, and NT-proBNP are more myocardial injury specific and increased to varying degrees, especially in severe and critical illness

Higher levels were associated with higher mortality

- .

Serum Albumin

Hypoalbuminemia in critically ill patients is multifactorial:

Increased capillary permeability

Decreased protein synthesis

Increased turnover

Decreased serum albumin total mass

Increased volume of distribution

Increased expression of vascular endothelial growth factor

Mean serum albumin on admission was 3.50 g/dl and 4.05 g/dl in severe and non-severe COVID-19, respectively

LDH

- About 40% of patients presented with increased LDH levels.
- Elevated LDH has been associated with a higher risk of ARDS, need for intensive care and mortality

Time period	Biomarkers
<7 days	• Total leucocyte count & lymphocyte count normal or slightly low • ↑ LDH, ↑ AST, ↑ ALT, ↑ CK, ↑ CK-MB – may be early markers of severe disease and mortality
7–14 days	• Total leucocyte count & lymphocyte count progressively fall to reach nadir at 8–9 days • Thrombocytopenia may occur • ↑ IL-6, IL-10, IL-1RA, MCP-1
>14 days	• Increasing total leucocyte count, lymphocyte & platelet count predict recovery while reducing counts predict mortality

Specific role	Biomarkers
Diagnosis	Leukopenia Lymphopenia High NLR ratio ↑ LDH ↑ AST
Assessment of severity	Lymphopenia Lymphocyte subsets – ↓CD4+, CD8+, B, NK cells, ↑ plasma cells ↑ NLR & ↓ LCR ↑ IL-2R/Lymphocytes ratio ↑ IL-6 ↑ CRP, PCT ↑ Ferritin ↑ LDH ↑ D-dimers ↑ Specific cardiac biomarkers – CK-MB, CTnT, Mb, NT-proBNP
Response to therapy	↓ CRP ↓ IL-6, IL-10, TNF-alpha, IL-2R
Prognosis	IL-6 Ferritin LDH CRP, PCT Lymphocyte count NLR LCR Platelet count Specific cardiac biomarkers – CK-MB, CTnT, Mb, NT-proBNP
MIS-C	CBC – ↓ platelets ↑ CRP, ESR, PCT ↓ Albumin ↑ Specific cardiac biomarkers – CK-MB, CTnT, NT-proBNP ↑ IL-6, IL-10, TNF-alpha ↑ D-dimers


#سفر
ممنوع

Thanks for your attention