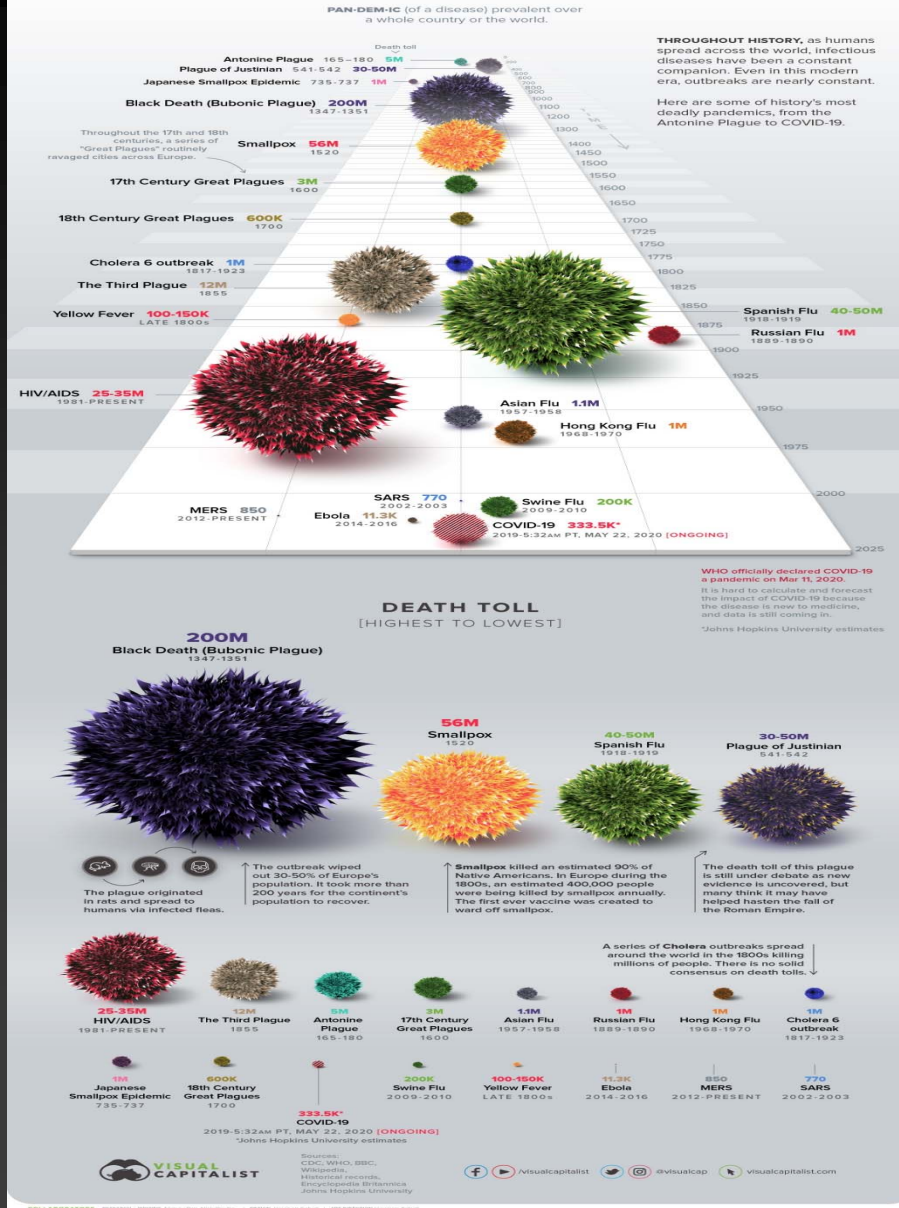


SARS-CoV-2 Genel Tanı Yöntemleri ve Biyosensörleri

EBRU SAATÇI
ERCIYES ÜNİVERSİTESİ, BİYOLOJİ BÖLÜMÜ, KAYSERİ
*sebrutr@gmail.com

KBRN, Mayıs 2021

HISTORY OF PANDEMICS



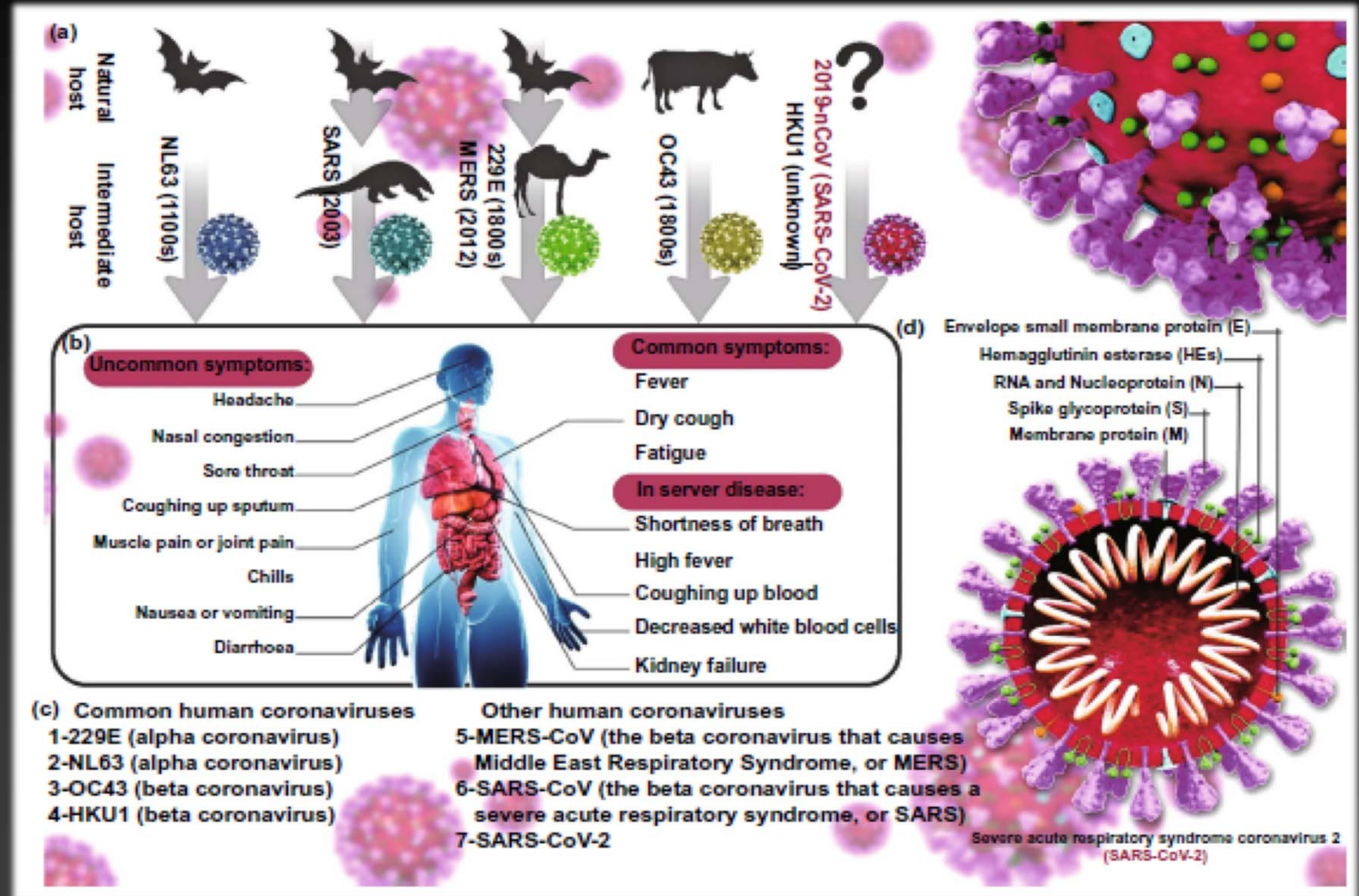
Name	Time period	Type / Pre-human host	Death toll
Antonine Plague	165-180	Believed to be either smallpox or measles	5M
Japanese smallpox epidemic	735-737	Variola major virus	1M
Plague of Justinian	541-542	Yersinia pestis bacteria / Rats, fleas	30-50M
Black Death	1347-1351	Yersinia pestis bacteria / Rats, fleas	200M
New World Smallpox Outbreak	1520 – onwards	Variola major virus	56M
Great Plague of London	1665	Yersinia pestis bacteria / Rats, fleas	100,000
Italian plague	1629-1631	Yersinia pestis bacteria / Rats, fleas	1M
Cholera Pandemics 1-6	1817-1923	V. cholerae bacteria	1M+
Third Plague	1885	Yersinia pestis bacteria / Rats, fleas	12M (China and India)
Yellow Fever	Late 1800s	Virus / Mosquitoes	100,000-150,000 (U.S.)
Russian Flu	1889-1890	Believed to be H2N2 (avian origin)	1M
Spanish Flu	1918-1919	H1N1 virus / Pigs	40-50M
Asian Flu	1957-1958	H2N2 virus	1.1M
Hong Kong Flu	1968-1970	H3N2 virus	1M
HIV/AIDS	1981-present	Virus / Chimpanzees	25-35M
Swine Flu	2009-2010	H1N1 virus / Pigs	200,000
SARS	2002-2003	Coronavirus / Bats, Civets	770
Ebola	2014-2016	Ebolavirus / Wild animals	11,000
MERS	2015-Present	Coronavirus / Bats, camels	850
COVID-19	2019-Present	Coronavirus – Unknown (possibly pangolins)	333,500 (Johns Hopkins University est. 5:32am PT, May 22, 2020)

TARİHÇE

*<https://www.visualcapitalist.com/history-of-pandemics-deadliest/>

SARS-CoV-2

İnsan koronavirüs türleri:
 229E (alfa koronavirüs),
 NL63 (alfa koronavirüs),
 OC43 (beta koronavirüs)
 HKU1 (beta koronavirüs)
 MERS-CoV (beta koronavirüs)
 SARS-CoV (beta koronavirüs)
 SARS-CoV-2 (COVID-19)



*<https://doi.org/10.1007/s40820-020-00533-y>

Testler için alınan numuneler

Table 2. Specimen collection and storage (adapted from^{7, 8, 9})

Specimen type	Collection materials	Storage temperature until testing in-country laboratory	Recommended temperature for shipment according to expected shipment time
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocculated swabs*	2-8 °C	2-8 °C if ≤5 days -70 °C (dry ice) if >5 days
Bronchoalveolar lavage	Sterile container *	2-8 °C	2-8 °C if ≤2 days -70 °C (dry ice) if >2 days
(Endo)tracheal aspirate, nasopharyngeal or nasal wash/aspirate	Sterile container *	2-8 °C	2-8 °C if ≤2 days -70 °C (dry ice) if >2 days
Sputum	Sterile container	2-8 °C	2-8 °C if ≤2 days -70 °C (dry ice) if >2 days
Tissue from biopsy or autopsy including from lung.	Sterile container with saline or VTM.	2-8 °C	2-8 °C if ≤24 hours -70 °C (dry ice) if >24 hours
Serum	Serum separator tubes (adults: collect 3-5 ml whole blood).	2-8 °C	2-8 °C if ≤5 days -70 °C (dry ice) if >5 days
Whole blood	Collection tube	2-8 °C	2-8 °C if ≤5 days -70 °C (dry ice) if >5 days
Stool	Stool container	2-8 °C	2-8 °C if ≤5 days -70 °C (dry ice) if >5 days
Urine	Urine collection container	2-8 °C	2-8 °C if ≤5 days -70 °C (dry ice) if >5 days

*Who, Laboratory testing for coronavirus disease (COVID-19) in suspected human cases, Interim guidance 19 March 2020

Test Tipleri

TEST MATERYALLERİ

- **Ayakta: Nazofaringeal ve burun swab ve balgam**
- **Hastane ortamında: alt solunum yolu örnekleri, kan, tükürük**

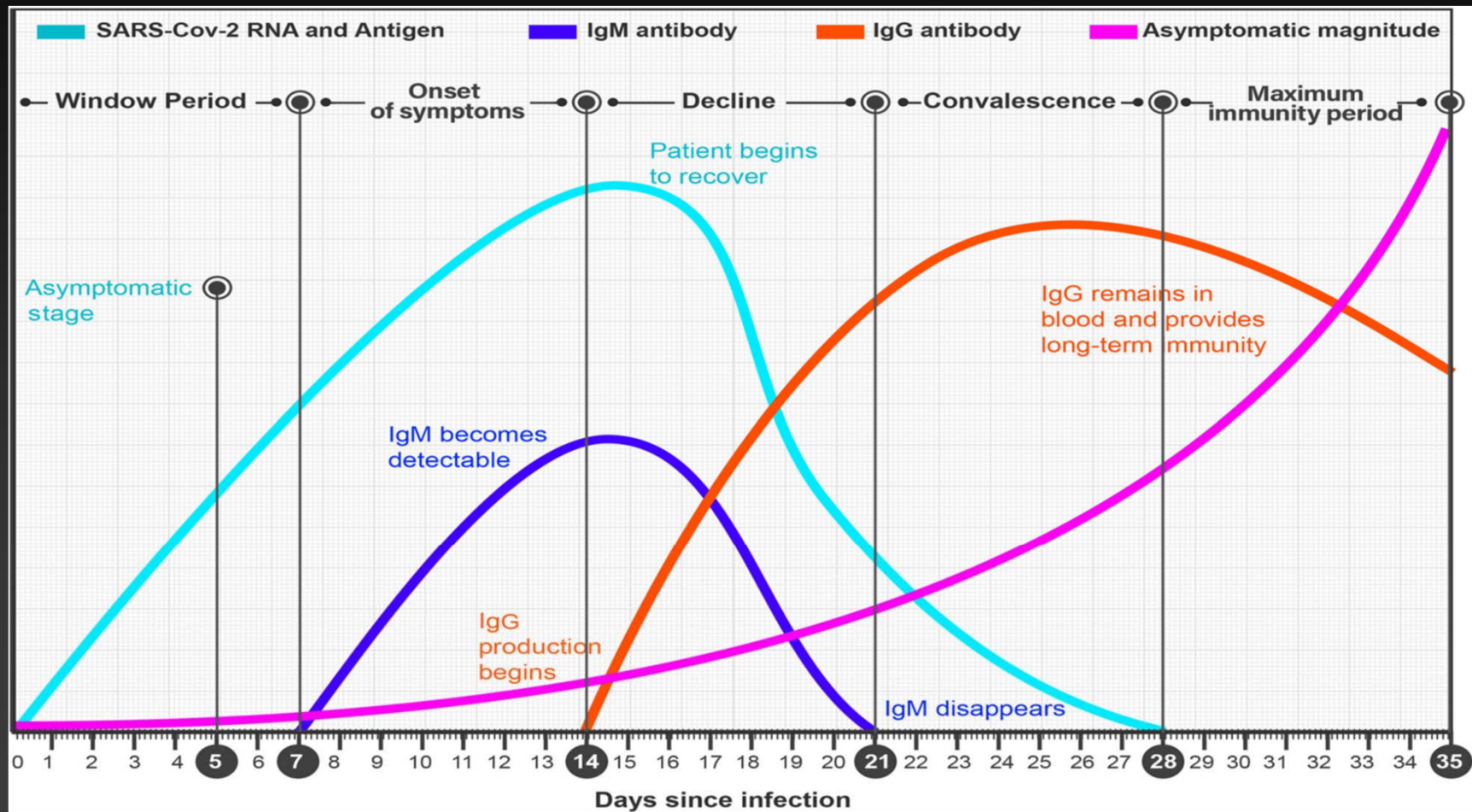
TEST ŞEKLİ

- **Nükleik asit amplifikasyon testleri (POC için uygun değil)**
- **Antijen, antikor (POC için uygun)**
- **Klinik testler (POC için uygun)**

Table 1 Types of diagnostic approaches in COVID-19^{51,52}; - still in experimental phase, now available for research; POC – point of care

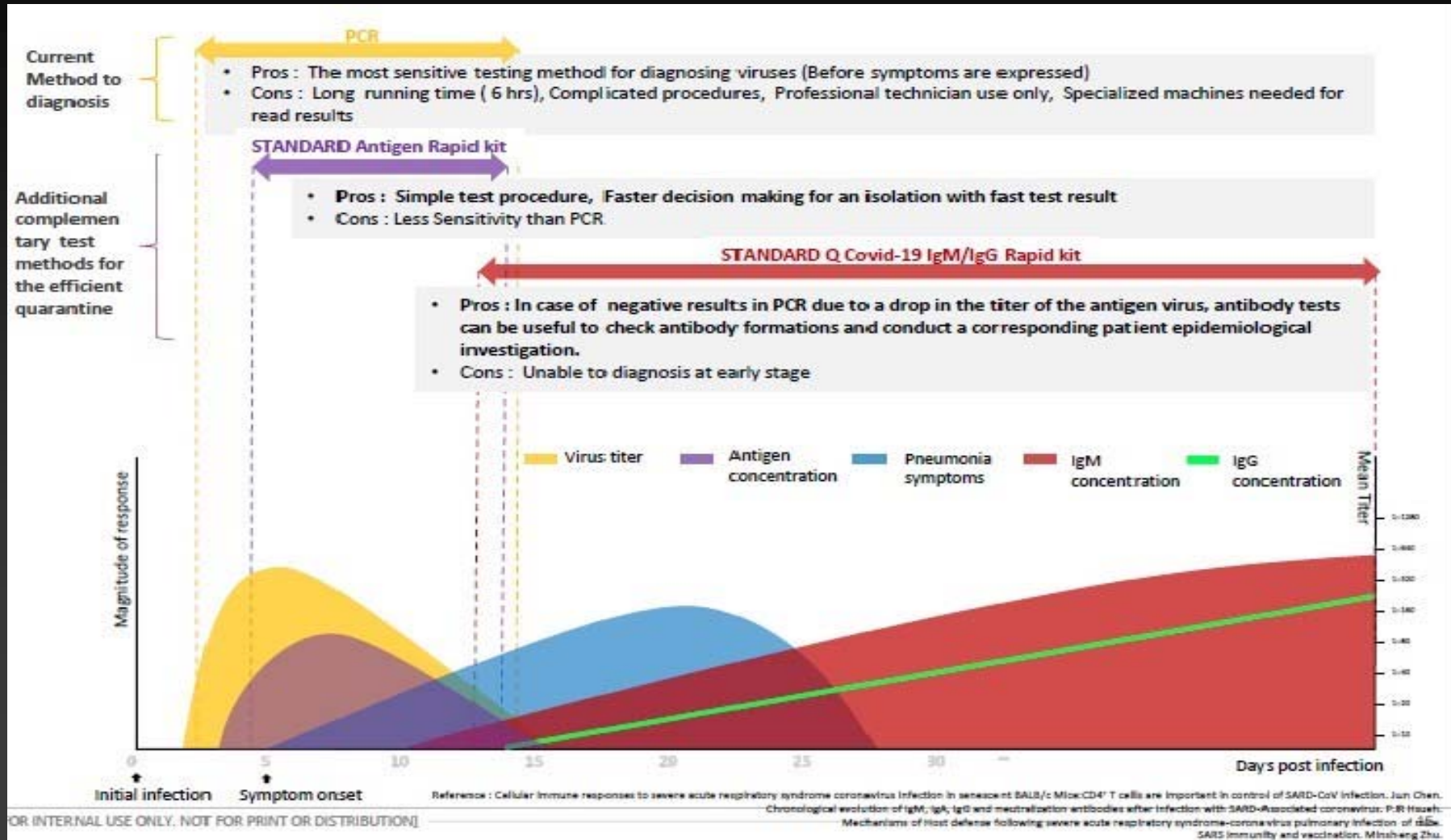
Test	Mechanism of detection	Testing material	Availability for POC	Positive Test indicates	Use of tests
Nucleic acid amplification tests (NAAT)	RT-PCR and NGS detection of genetic sequences of conserved regions for regions of the virus e.g. N, E, S and RdRP genes. Two independent sequences need to be detected	Ambulatory: nasopharyngeal swabs, sputum In hospital: sputum, endotracheal aspirate, BAL blood, feces	No; Needs to be performed in the lab	Confirms current SARS-CoV2 infection	Individual testing
Antibody based immunoassay	ELISA detecting IgM or IgG anti- SARS-CoV-2 antibodies	Serum	Yes (depending on test design)	IgM+: 3-5 days post onset IgG: past infection	Overall infection/immunity rates in a community
Antigen based immunoassay	ELISA detecting viral proteins e.g. S (spike protein) or N protein (nucleocapsid)	nasopharyngeal swabs, sputum and other lower respiratory tract secretions, BAL blood, feces.	Yes (depending on test design)	Confirms current SARS-CoV2 infection	Individual testing
Clinical tests	Clinical symptoms (fever/ cough) Epidemiological history Imaging (CT)	CT – detection of radiological features	Yes	Infection possible	Triage to identify candidates for further testing

Tanı Seyir Çizelgesi



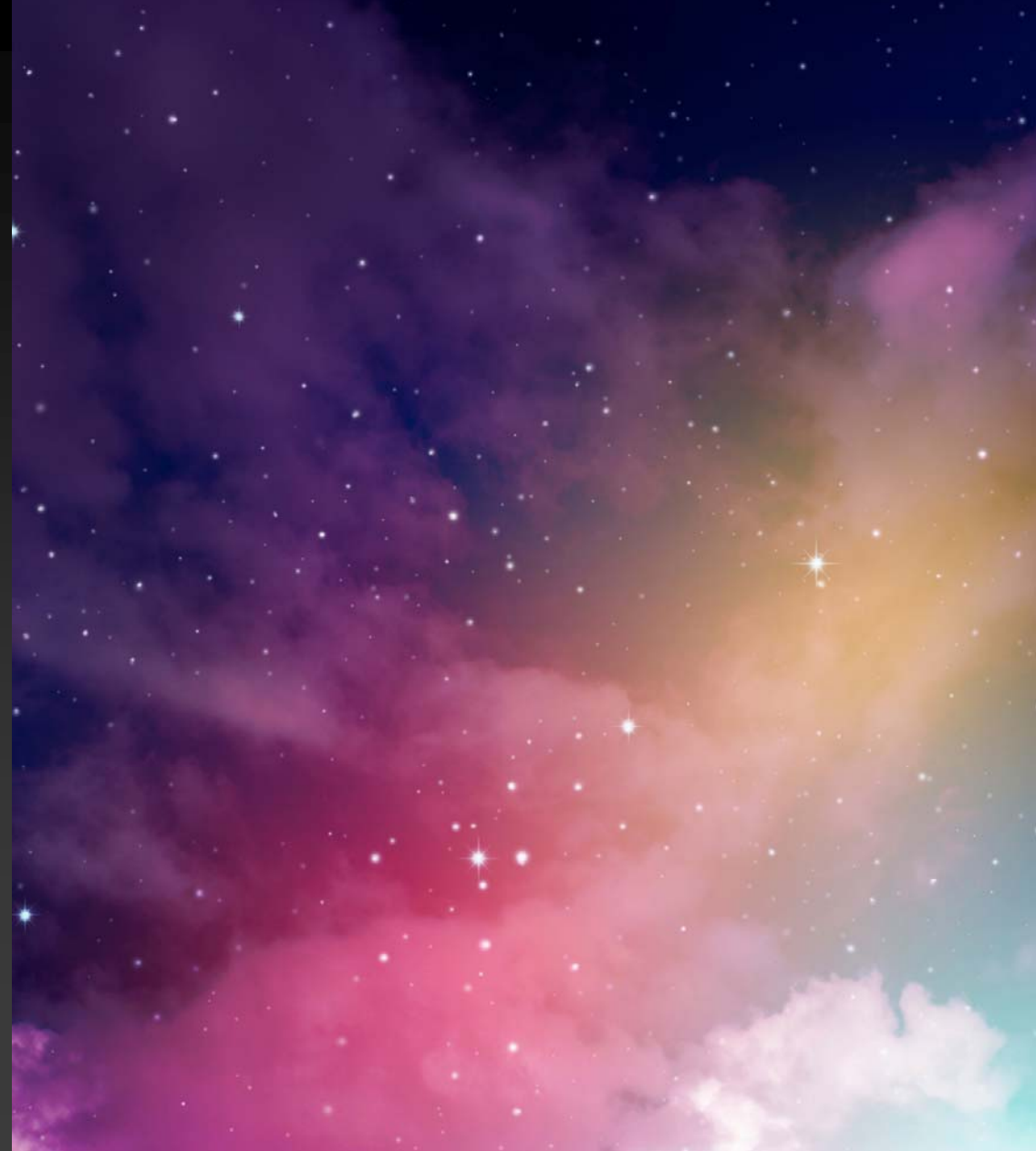
*<https://doi.org/10.1007/s40820-020-00533-y>

Test Çeşitleri ve Tanı Seyir Çizelgesi



TEST ÇEŞİTLERİ

NÜKLEİK ASİT TEMELLİ TESTLER



PCR Panel ve Primerler

- Yüksek hassasiyet ve özgüllük
 - Erken teşhis
- Fakat;
- Yüksek bütçeli
 - Test süresi uzun
 - Yanlış pozitif veya negatif:
 - . Örneklemede ve taşınımında standardizasyon eksikliği
 - . Viral yük
 - . Kontaminasyon riski

2019–Novel Coronavirus (2019–nCoV) Real-time rRT-PCR Panel Primers and Probes				
Name	Description	Oligonucleotide Sequence (5'>3')	Label ¹	Working Conc.
2019-nCoV_N1-F	2019-nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'	None	20 µM
2019-nCoV_N1-R	2019-nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'	None	20 µM
2019-nCoV_N1-P	2019-nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG TGG ACC-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'	None	20 µM
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'	None	20 µM
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'	FAM, BHQ-1	5 µM
2019-nCoV_N3-F	2019-nCoV_N3 Forward Primer	5'-GGG AGC CTT GAA TAC ACC AAA A-3'	None	20 µM
2019-nCoV_N3-R	2019-nCoV_N3 Reverse Primer	5'-TGT AGC ACG ATT GCA GCA TTG-3'	None	20 µM
2019-nCoV_N3-P	2019-nCoV_N3 Probe	5'-FAM-AYC ACA TTG GCA CCC GCA ATC CTG-BHQ1-3'	FAM, BHQ-1	5 µM
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'	None	20 µM
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'	None	20 µM
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'	FAM, BHQ-1	5 µM

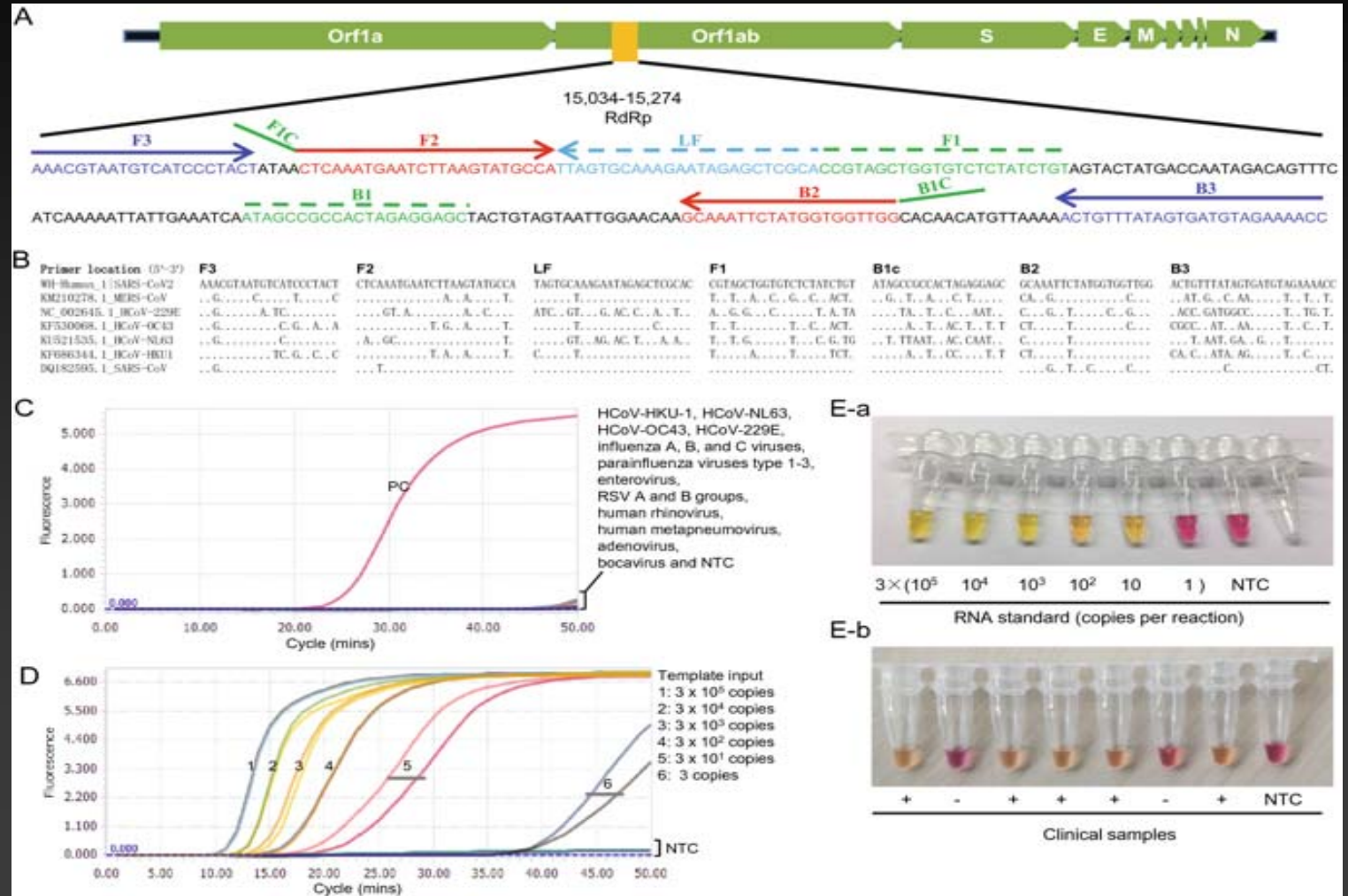
¹TaqMan® probes are labeled at the 5'-end with the reporter molecule 6-carboxyfluorescein (FAM) and with the quencher, Black Hole Quencher 1 (BHQ-1) (Biosearch Technologies, Inc., Novato, CA) at the 3'-end.

Note: Oligonucleotide sequences are subject to future changes as the 2019–Novel Coronavirus evolves.

- E, N1, N2, N3, nsp10 ve 14 hedef genleri için

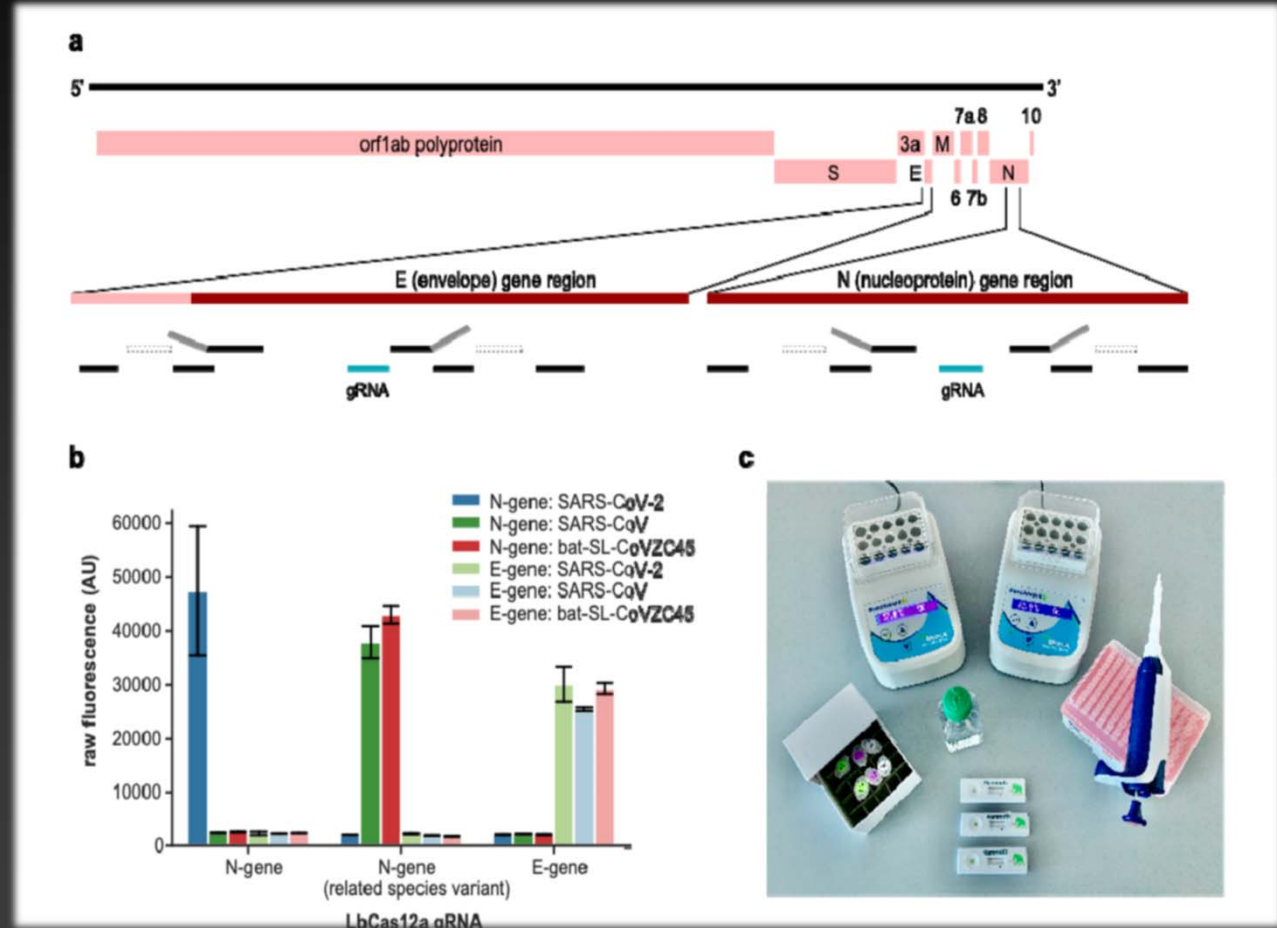
RT-LAMP (Ters Transkripsiyon Döngüsü Aracılı İzotermal Tespit)

- Test süresi 30 dk
- Yüksek hassasiyet
- Düşük bütçeli
- Thermo cycler'a ihtiyaç yok: izotermal
- Görüntüleme ve miktar belirleme kolay: Türbidimetri, floresans ve LFA gibi metotlarla görüntüleme



CRISPR-temelli DETECTR ve Lateral Flow Kombinasyonu ile SARS-CoV-2 Hızlı Testi

- a) SARS-CoV-2 genomu ve Primerleri, probları ve gRNA'ları gösteren genom haritası (Gen hedefleri N ve E geni)
- b) gRNA özgünlüğü üzerinde primerlerin ve probların görüntülenmesi. Cas12 gRNA'lar, SARS-CoV-2'yi spesifik olarak hedeflenir veya ilgili koronavirüs suşlarını geniş ölçüde tespit eder.
- c) Test kiti, İzotermal bloklar, LFIA kartujları



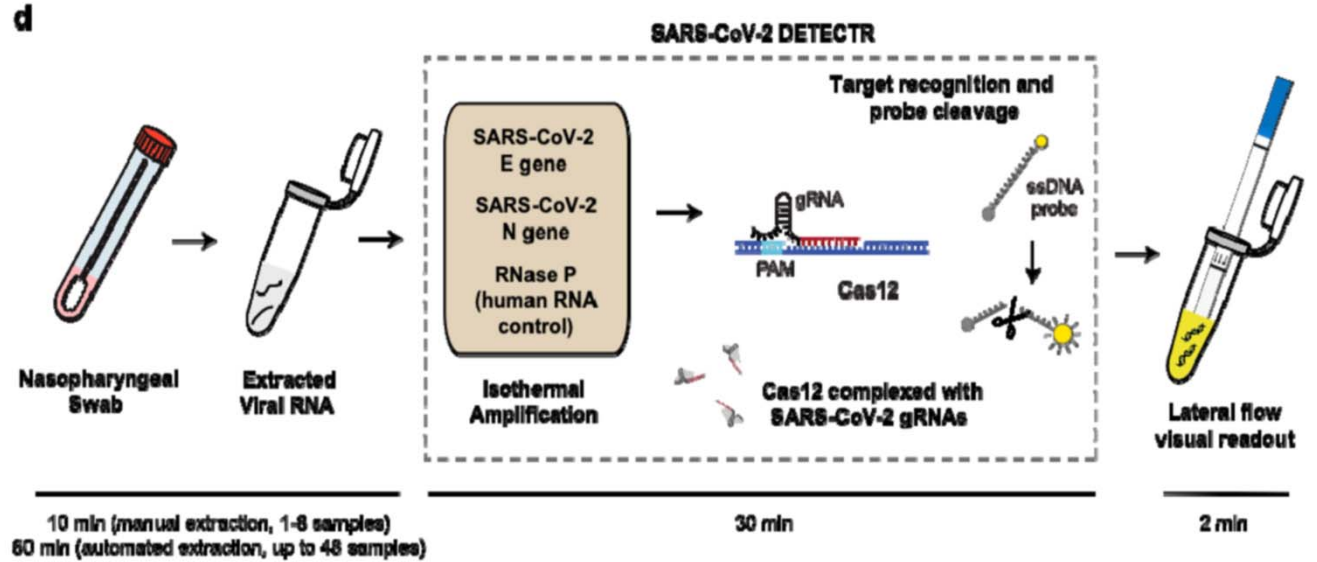
CRISPR (clustered regularly interspaced short palindromic repeats)

DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter)

*doi:10.1101/2020.03.06.20032334

CRISPR-temelli DETECTR ve Lateral Flow Kombinasyonu ile SARS-CoV-2 Hızlı Testi

- d) SARS-CoV-2 DETECTR iş akışının şeması. Geleneksel RNA ekstraksiyonu veya numune matrisi, DETECTR'ye (E ve N geni ve RNaz P için LAMP ön amplifikasyonu ve Cas12 bazlı saptama) bir girdi olarak kullanılabilir ve floresan okuyucu veya LFIA ile görselleştirilir.

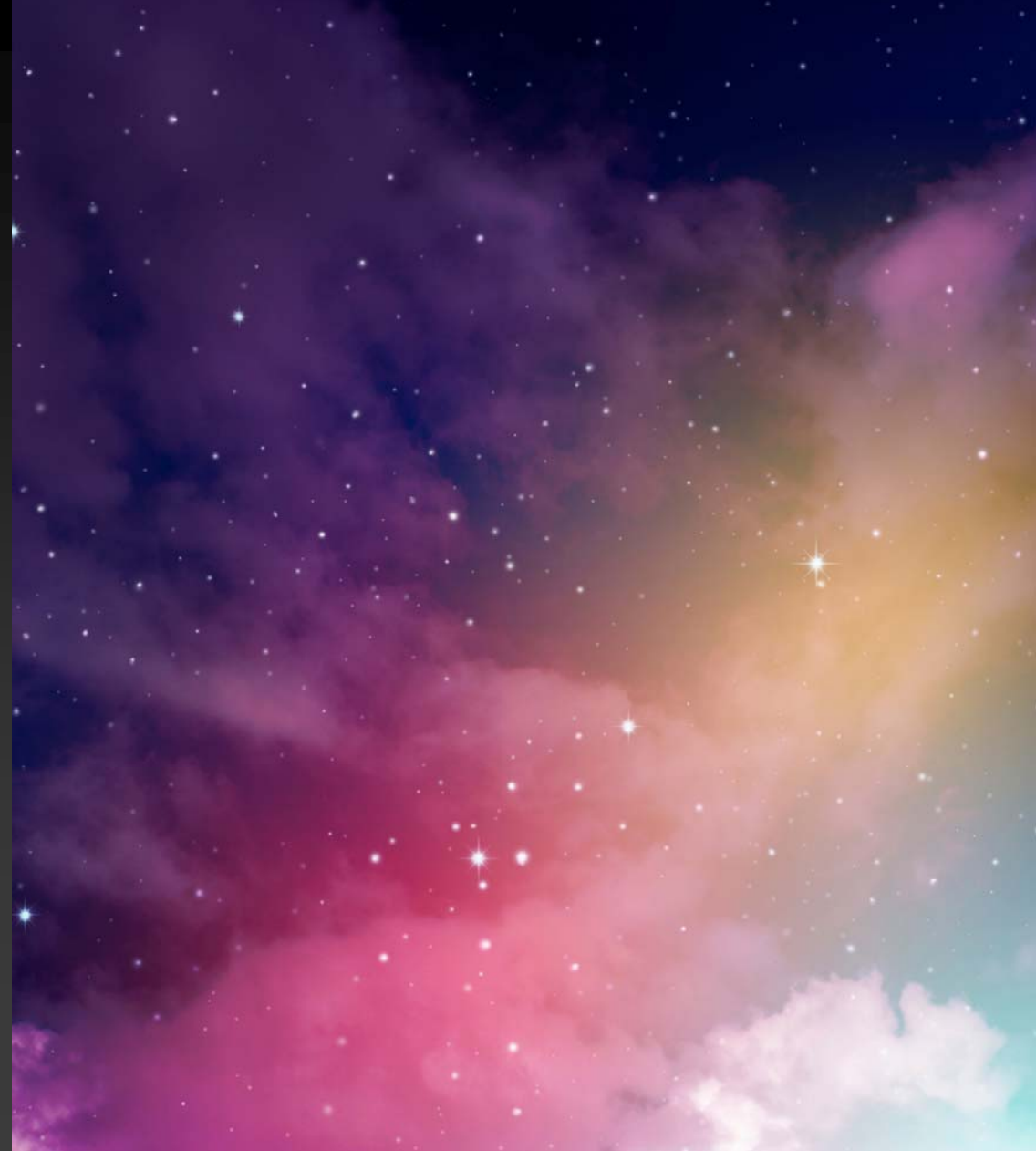


- e) Lateral Flow Strip gösterimi: Olumlu bir sonuç için en az iki SARS-CoV-2 viral geninin saptanmasını gerektirir (N ve E geni).



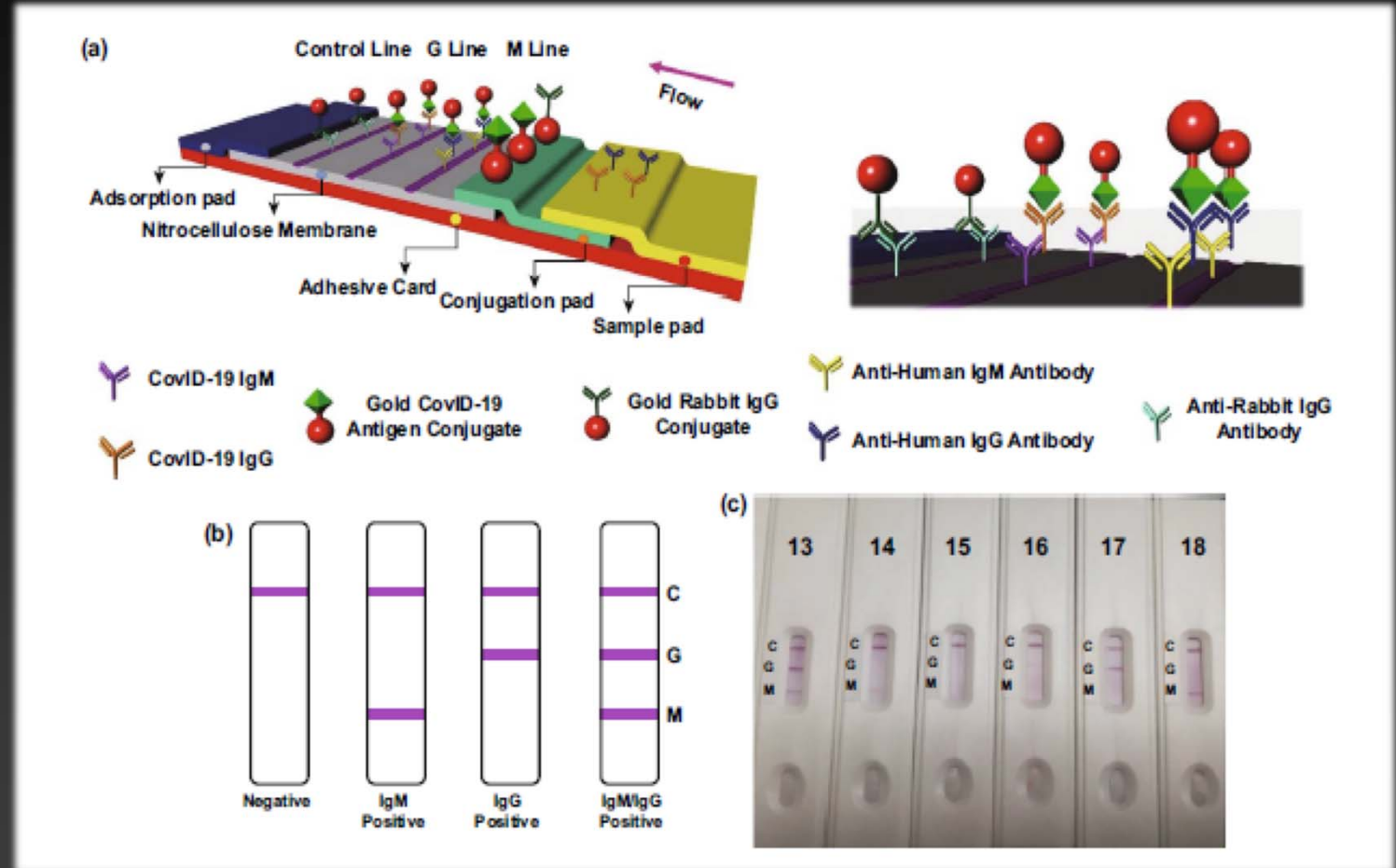
TEST ÇEŞİTLERİ

POC TESTLERİ



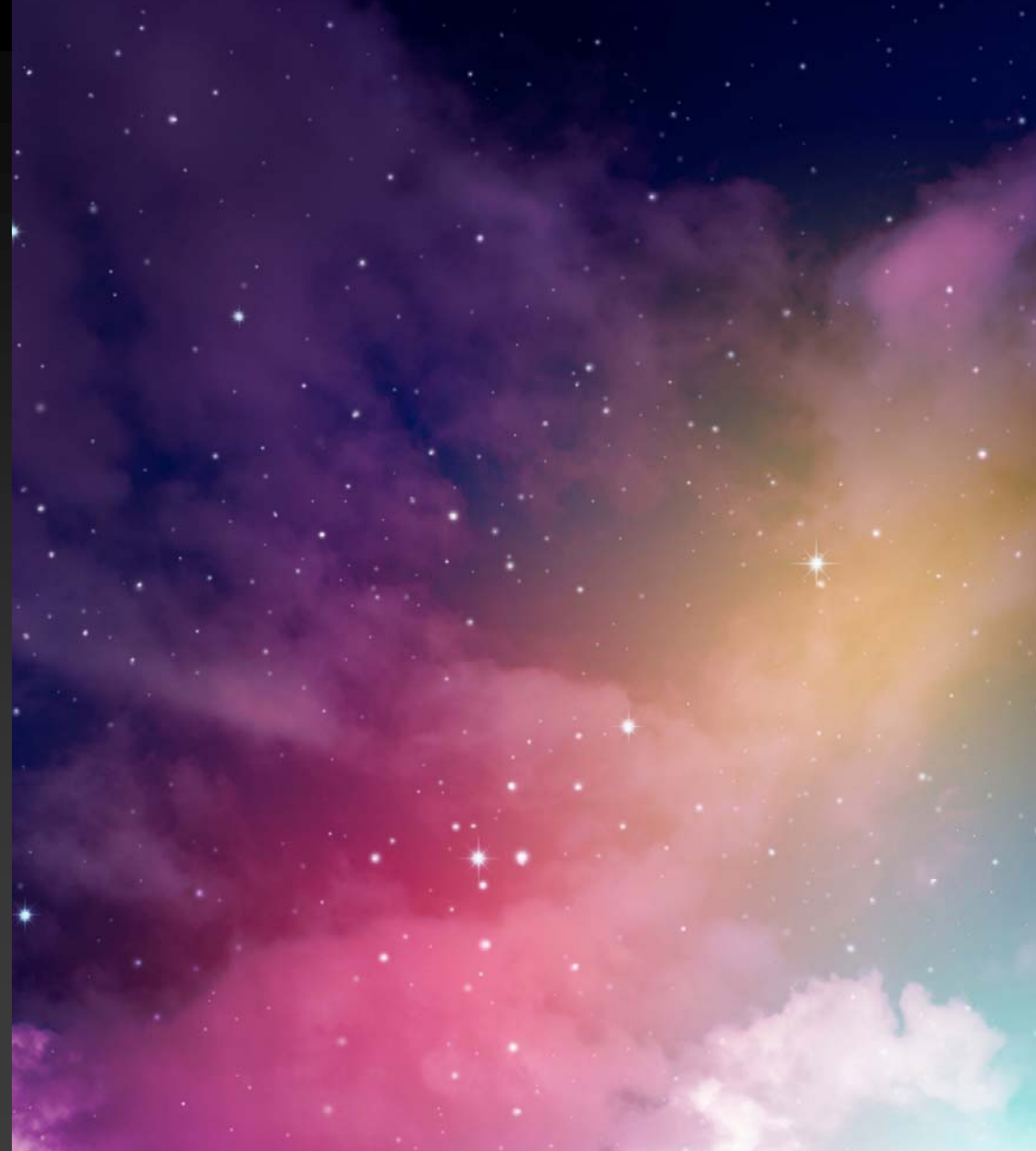
SARS-CoV-2 antikorları için geliştirilen LFIA

- Metot: Lateral flow strip test
- Taşıyıcı: Altın nanopartiküller
- Antijen: S1 veya N protein
- Hasta kanında COVID-19 IgM ve IgG testi
- Kalitatif ve kantitatif analiz
- Bulaşım sonrası 6. ve 10. günden sonra
- Hassasiyet düşük



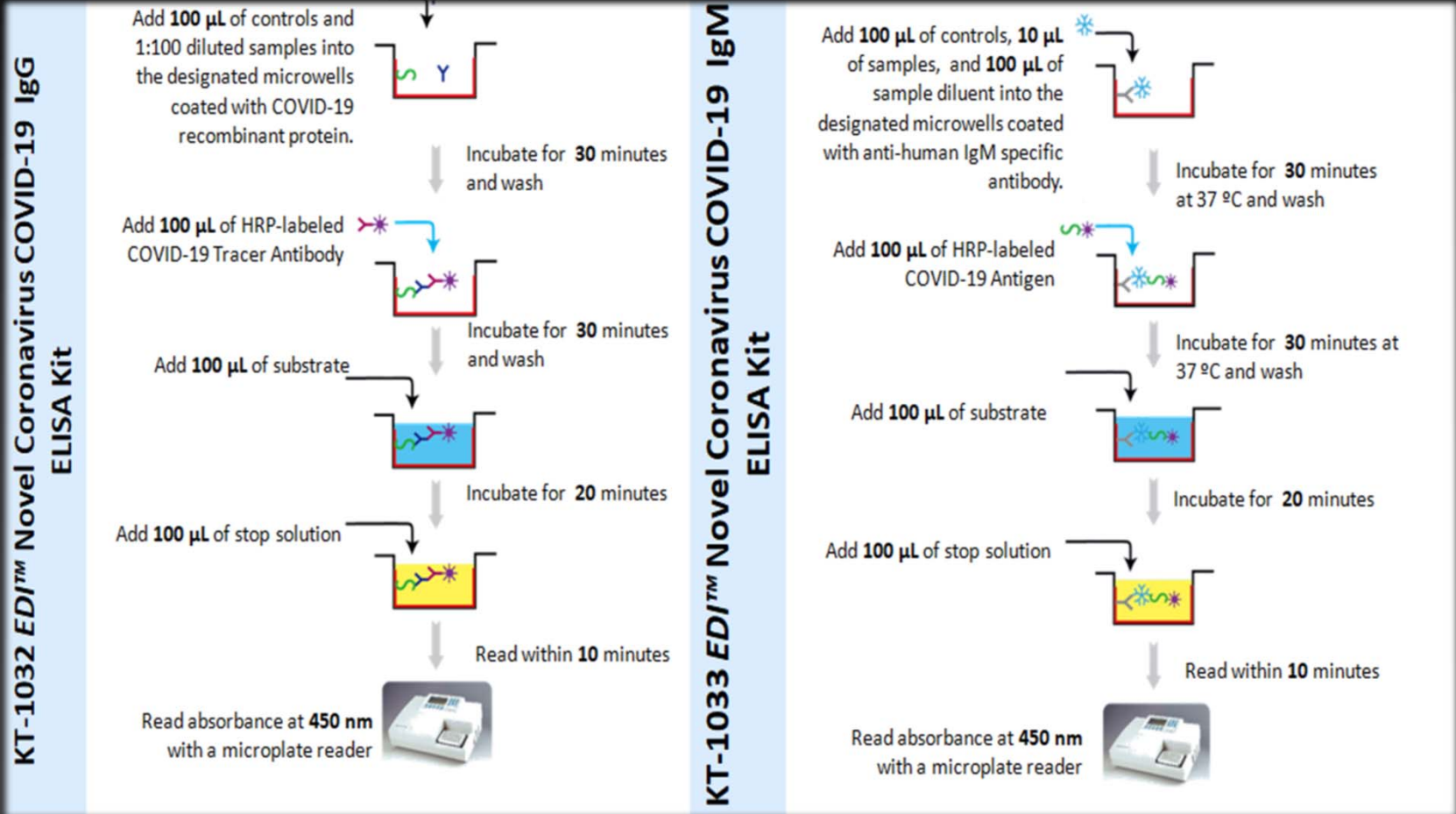
TEST ÇEŞİTLERİ

ELİZA



Sars-CoV-2 Serolojik Testler

- Rekombinant antijen (S veya N protein) temelli
- Covid-19 Ig G veya IgM ölçümleri
- Benzer şekillerde Covid-19 IgA'sı içinde ölçümler bulunmaktadır.



IgG Titrasyonu ile İnhibisyona Dayalı Kantitatif ELİZA

PRINCIPLE OF THE ASSAY

The newly identified 2019 novel coronavirus (SARS-CoV-2) has posed a serious threat to human health.. It is helpful to develop the Anti SARS-CoV-2 neutralizing antibody titer serologic assay kit to test the level of protective neutralizing antibody in serum.

This assay kit employs a competitive ELISA format and is uniquely suitable for rapid high-throughput detection of the titer of Anti-SARS-CoV-2 neutralizing antibody in serum / plasma. Briefly, we provide you with a biotinylated Human ACE2 protein, a SARS-CoV-2 Spike protein RBD, an Anti-SARS-CoV-2 neutralizing antibody (human IgG1) (as Reference), and Streptavidin-HRP reagent.

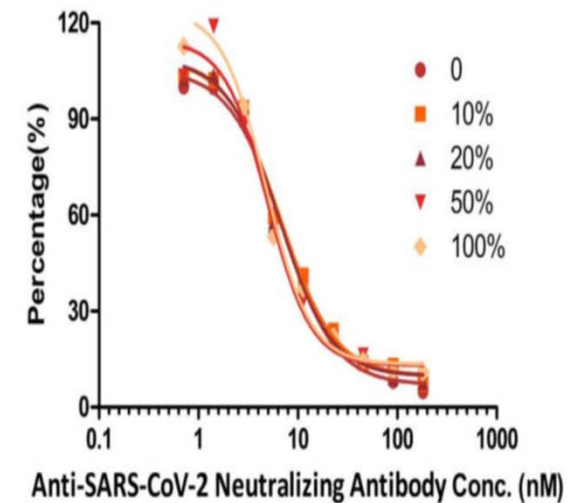
Your experiment will include 4 simple steps:

- a) Coat the plate with SARS-CoV-2 Spike protein RBD.
- b) Add 50ul biotinylated human ACE2 to coated plate.
- c) Add the reference in kit or your samples from serum or plasma of interest.
- d) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the titer of your samples to inhibit Spike protein RBD: ACE2 binding will be determined by comparing OD readings among different experimental groups.

METHOD VERIFICATION

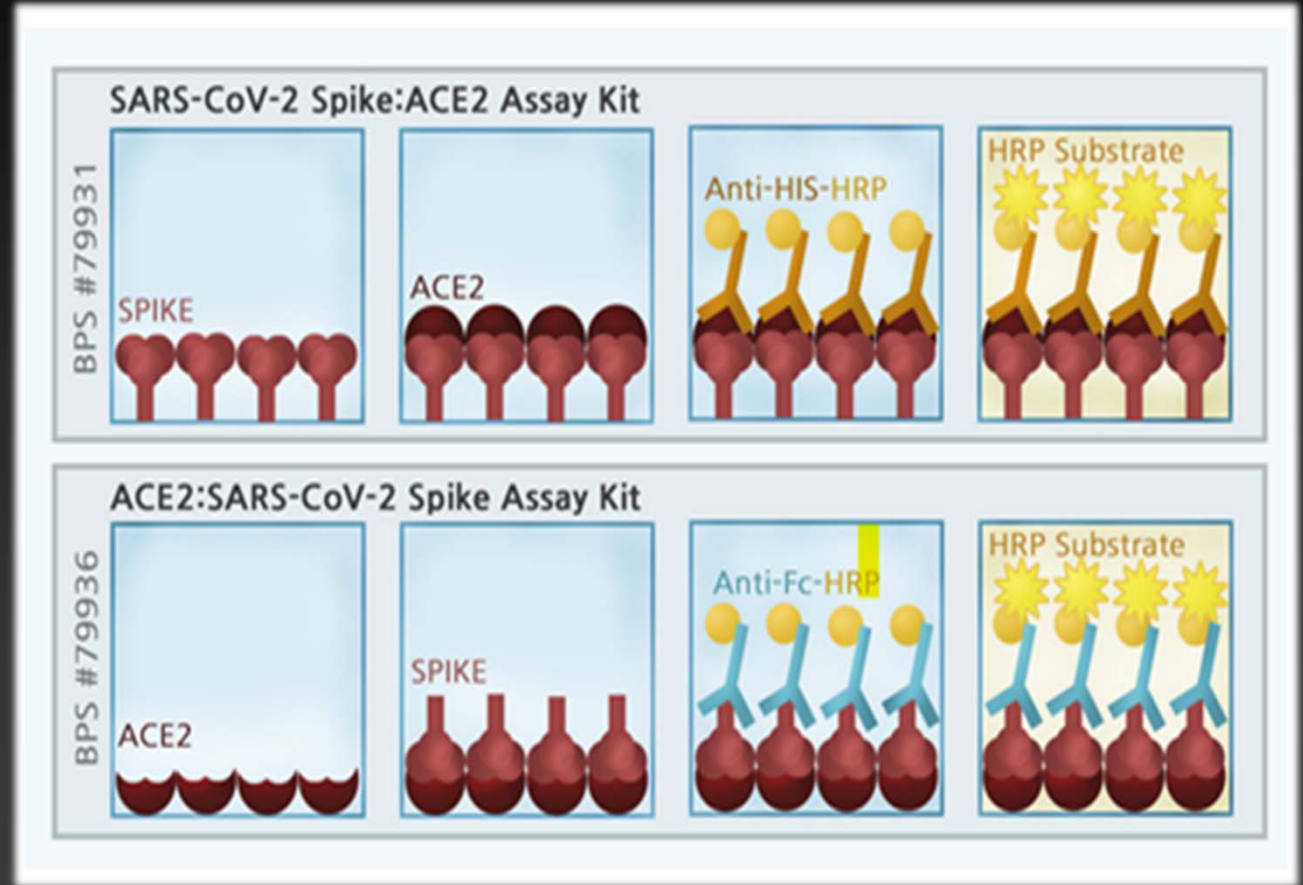
Determination of Anti-SARS-CoV-2 Neutralizing Antibody titer in different concentrations of human serum.



Neutralizing titer of Anti-SARS-CoV-2 Neutralizing Antibody, Human IgG1 (Cat. No. SAD-S35) measured by Anti-SARS-CoV-2 neutralizing antibody titer serologic assay kit (Cat. No. TAS-K003) in different concentrations of human serum (CV < 30%).

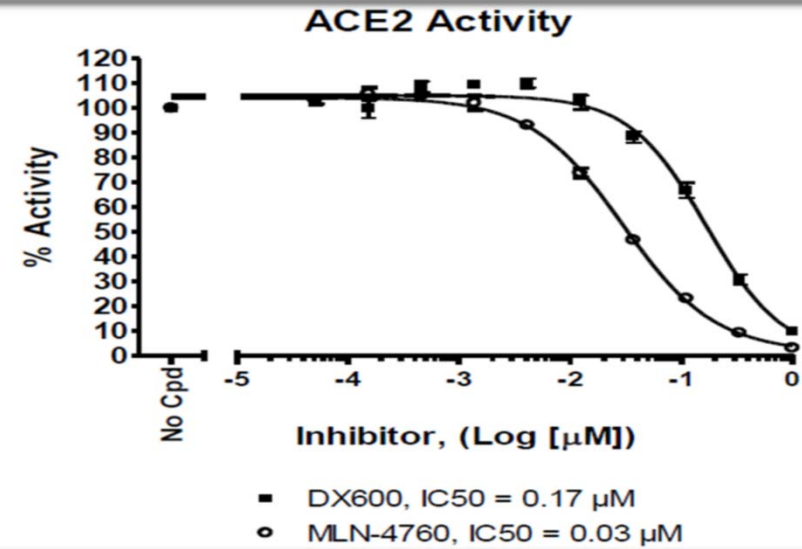
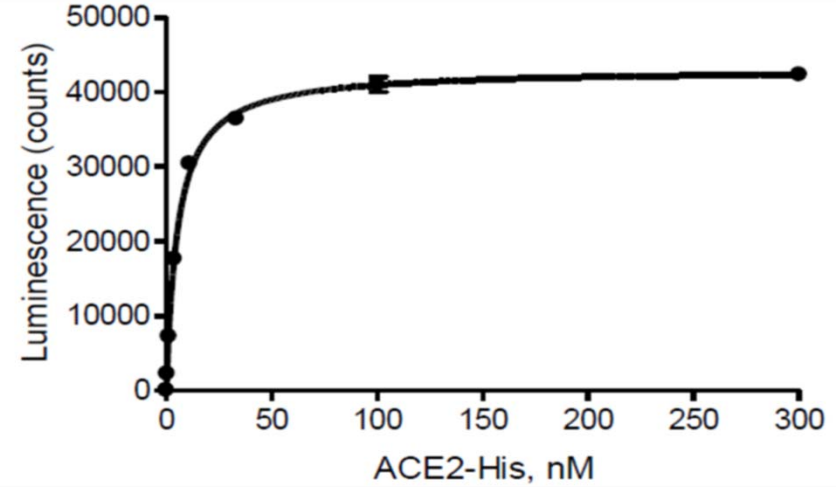
İlaç arařtırmaları için COVID-19 inhibitörü tarama test kitleri

- Plakalara ACE2-His tag veya SARS-CoV-2 Spike-Fc tag ile immobilizasyon
- Daha sonra, karşılık gelen ligandlar (ACE2 veya Spike) ile inkübasyon (inhibitörler bu inkübasyon sırasında ekleniyor)
- HRP-etiketli anti-his veya anti-Fc antikoları ile muamele ve substrat eklenmesi
- Kemilüminesans, fluorometrik veya kolorimetrik okuma



İlaç arařtırmaları için COVID-19 inhibitörü tarama test kitleri

- Şekil 1, inhibitörsüz ortam, Spike proteine ACE2 (nM) bağlanması
- Şekil 2, farklı konsantrasyonlarda ACE2 inhibitörü (μM) bulunan ortamda ACE2 aktivitesinin %'de olarak azalması

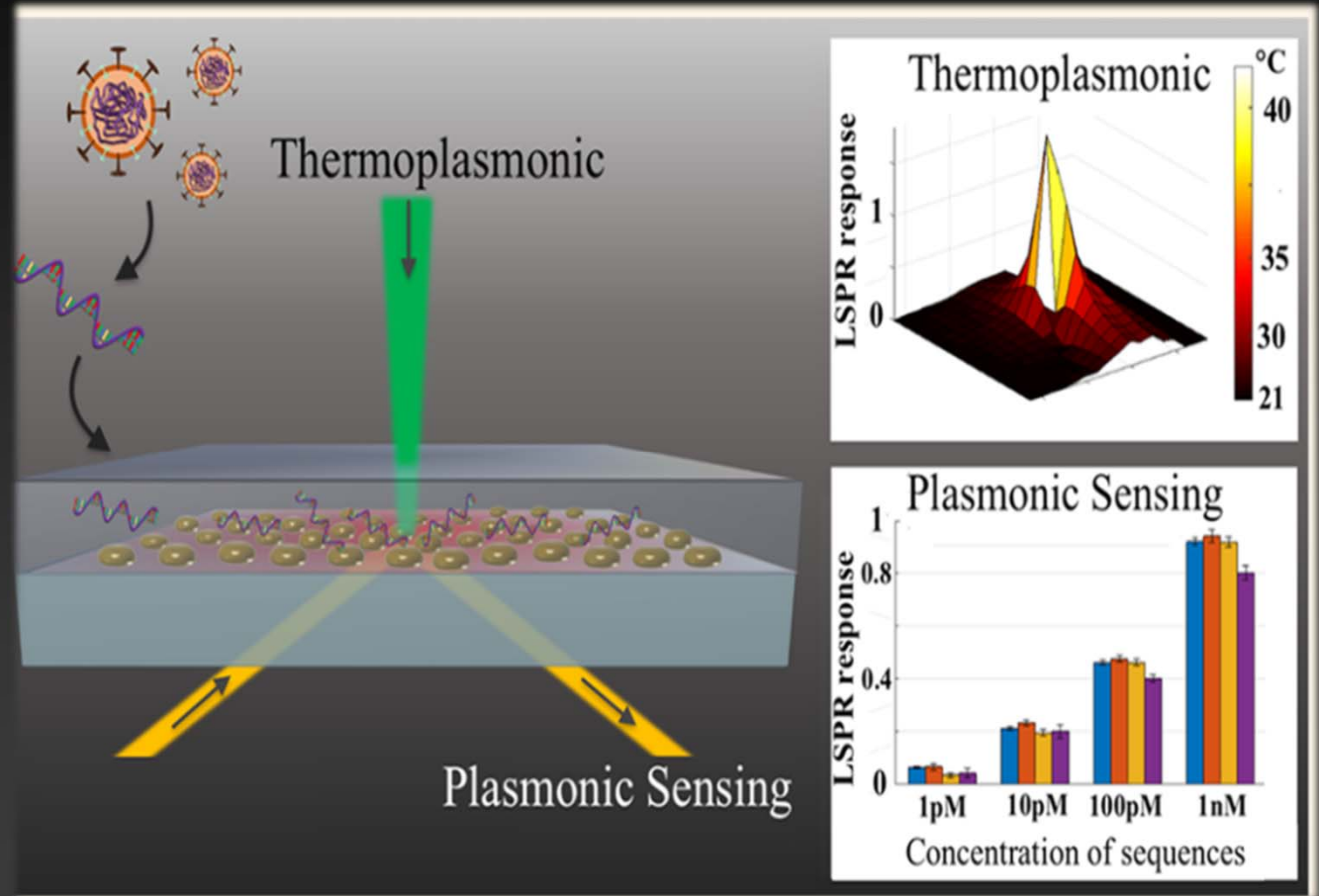


SARS-CoV-2 Tanısında Biyosensörler



SARS-CoV-2 için Çift Fonksiyonlu Plazmonik Fototermal Biyosensörü

- Plazmonik fototermal (PPT) efekti ve lokal yüzey plazmon rezonans (LSPR) algılama transdüksiyonunu birleştiren çift fonksiyonlu plazmonik biyosensör
- Tamamlayıcı nükleik asit reseptörleri ile işlevselleştirilmiş iki boyutlu altın nanopartiküller ile SARS-CoV-2 nükleik asit hibridizasyonu yoluyla seçilen sekansların hassas tespiti
- Seçilen SARS-CoV-2 sekanslarına karşı 0.22 pM konsantrasyonuna kadar düşük bir tespit limiti ile yüksek bir hassasiyet
- Klinik COVID-19 tanısı için alternatif ve umut verici bir çözüm



Field-Effect Transistör Tabanlı Biyosensör Kullanarak İnsan Nazofaringeal Swab Örneklerinde SARS-CoV-2 Hızlı Tespiti

- Klinik örneklerde, Field-effect transistör (FET) tabanlı biyosensör
- Sensör, FET'in grafen tabakalarının SARS-CoV-2 spike proteinine karşı spesifik bir antikor ile kaplanmasıyla direkt virüs deteksiyonunu içermektedir.
- 1 fg/mL hassasiyet

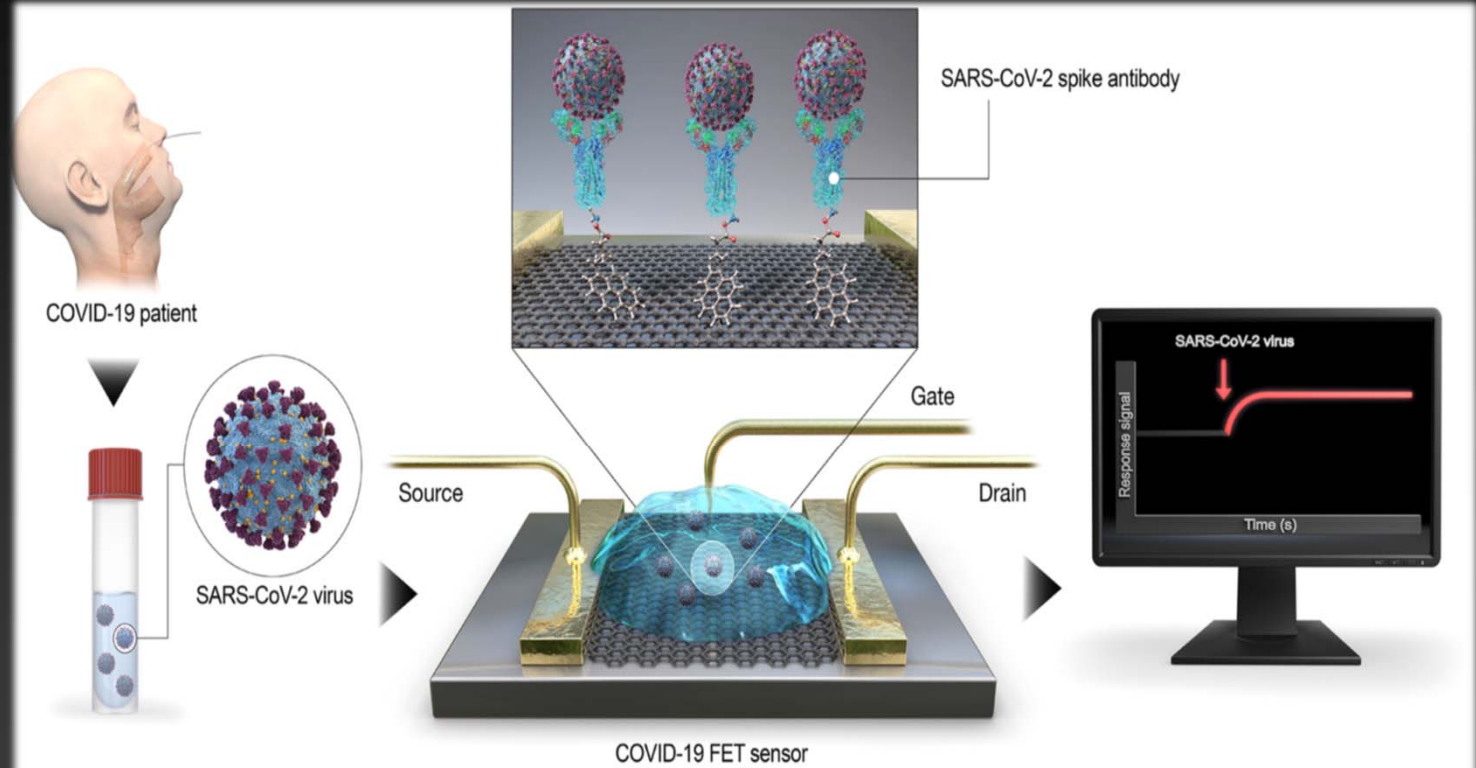
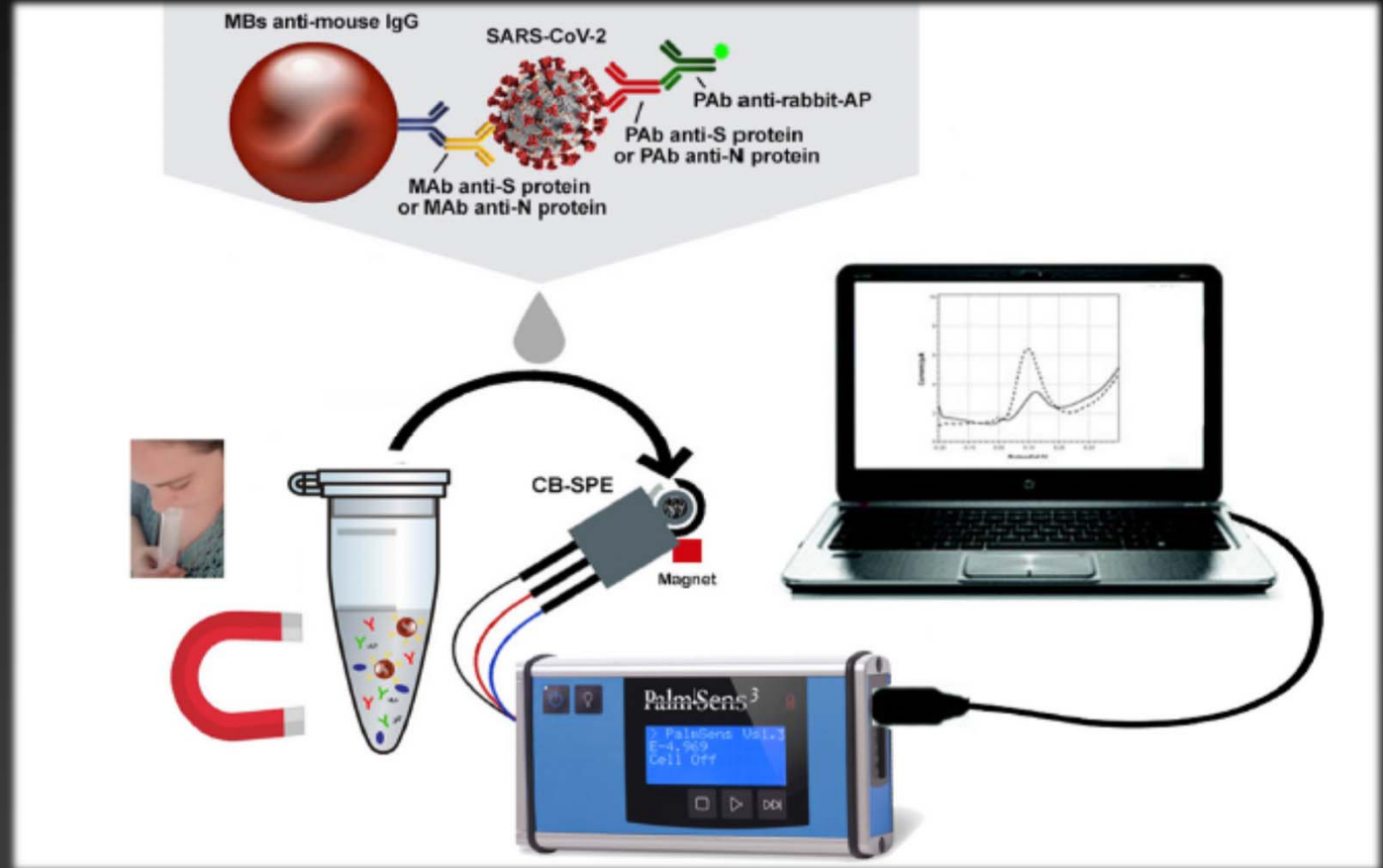


Figure 1. Schematic diagram of COVID-19 FET sensor operation procedure. Graphene as a sensing material is selected, and SARS-CoV-2 spike antibody is conjugated onto the graphene sheet *via* 1-pyrenebutyric acid *N*-hydroxysuccinimide ester, which is an interfacing molecule as a probe linker.

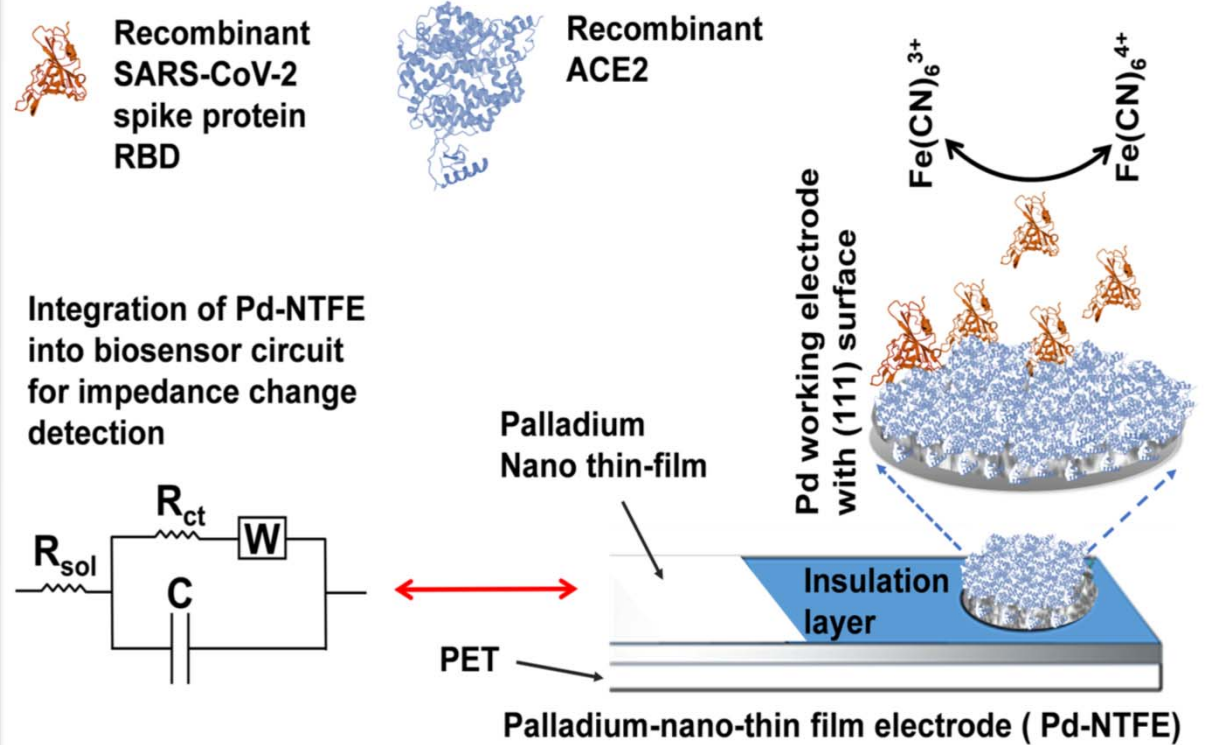
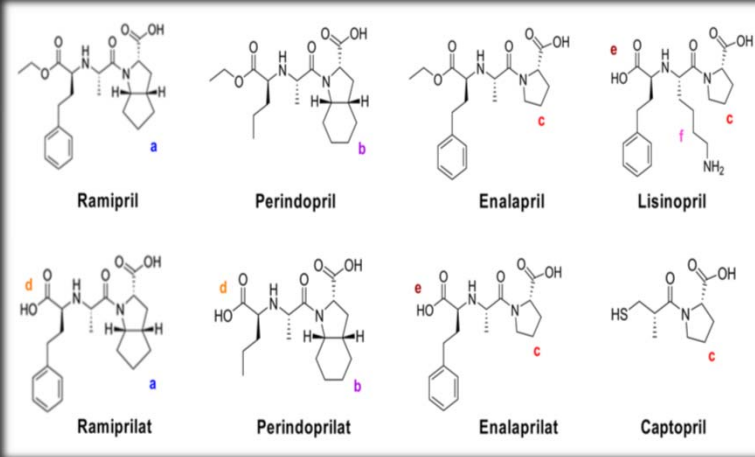
Tükürükte SARS-CoV-2 tespiti için güvenilir ve minyatürleştirilmiş elektrokimyasal immünosensör

- Karbon bazlı ekran baskılı elektrotlarla birleştirilmiş manyetik boncuklar,
- Elektrokimyasal ölçüm,
- Manyetik boncuklar ve immünojenetik etiket olarak alkalin fosfatazlı ikincil antikor kullanılarak Spike (S) veya Nükleokapsid (N) tespiti
- Enzimatik yan ürün 1-naftol,
- RT-PCR ile karşılaştırma



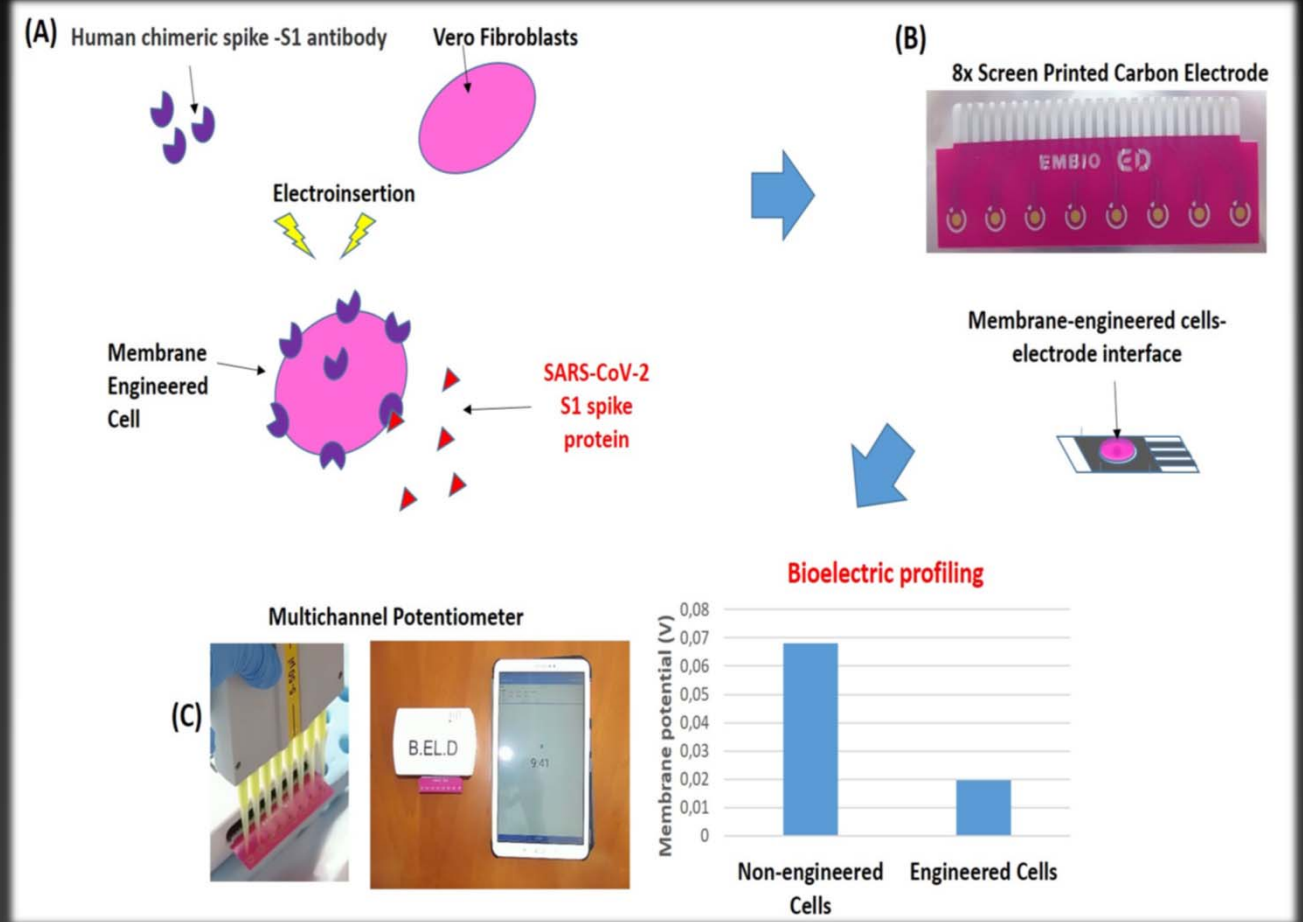
SARS-CoV-2 inhibitörlerinin hızlı taranması için esnek elektrokimyasal empedans spektroskopisi tabanlı biyoalgılama platformu

- pg düzeyinde inhibisyon ölçme imkanı sağlıyor.
- inhibitörler: Hipertansiyon tedavisinde kullanılan blokörler



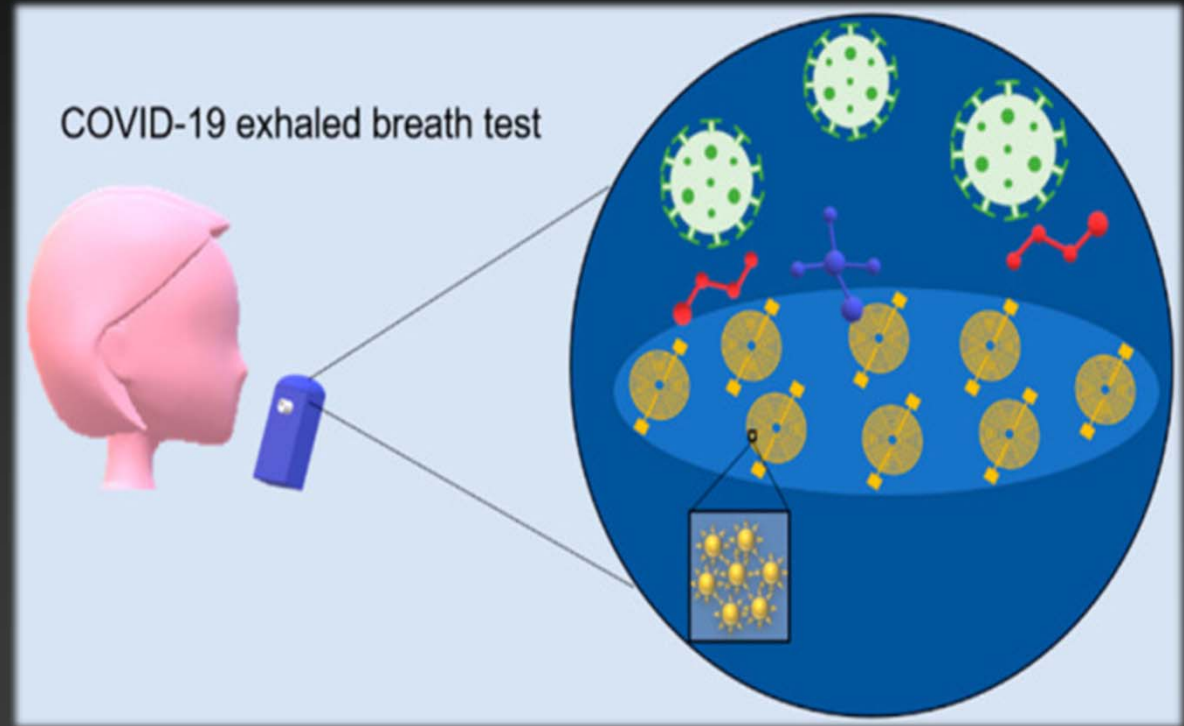
SARS-CoV-2 S1 Spike Protein Antijeninin Doğrudan Tespiti için Taşınabilir, Hücre Tabanlı Biyosensör

- Biyosensör, insan spike S1 antikorunu taşıyan membran mühendisliği ile yapılmış memeli hücrelerine dayanmaktadır.
- Proteinin membrana bağlı antikora bağlanması (Electroinsertion) ile ölçülen bir membran potansiyel farkı ile sinyal (Volt) alınmaktadır.
- 1 fg / mL'lik bir tespit limiti
- 3 dakikada sonuç
- SARS-CoV-2 N proteinine karşı çapraz reaktivite yoktur.
- Akıllı telefon / tablet ile çalıştırılan portatif bir okuma cihazı dahil, kullanıma hazır bir platform olarak yapılandırılmış.



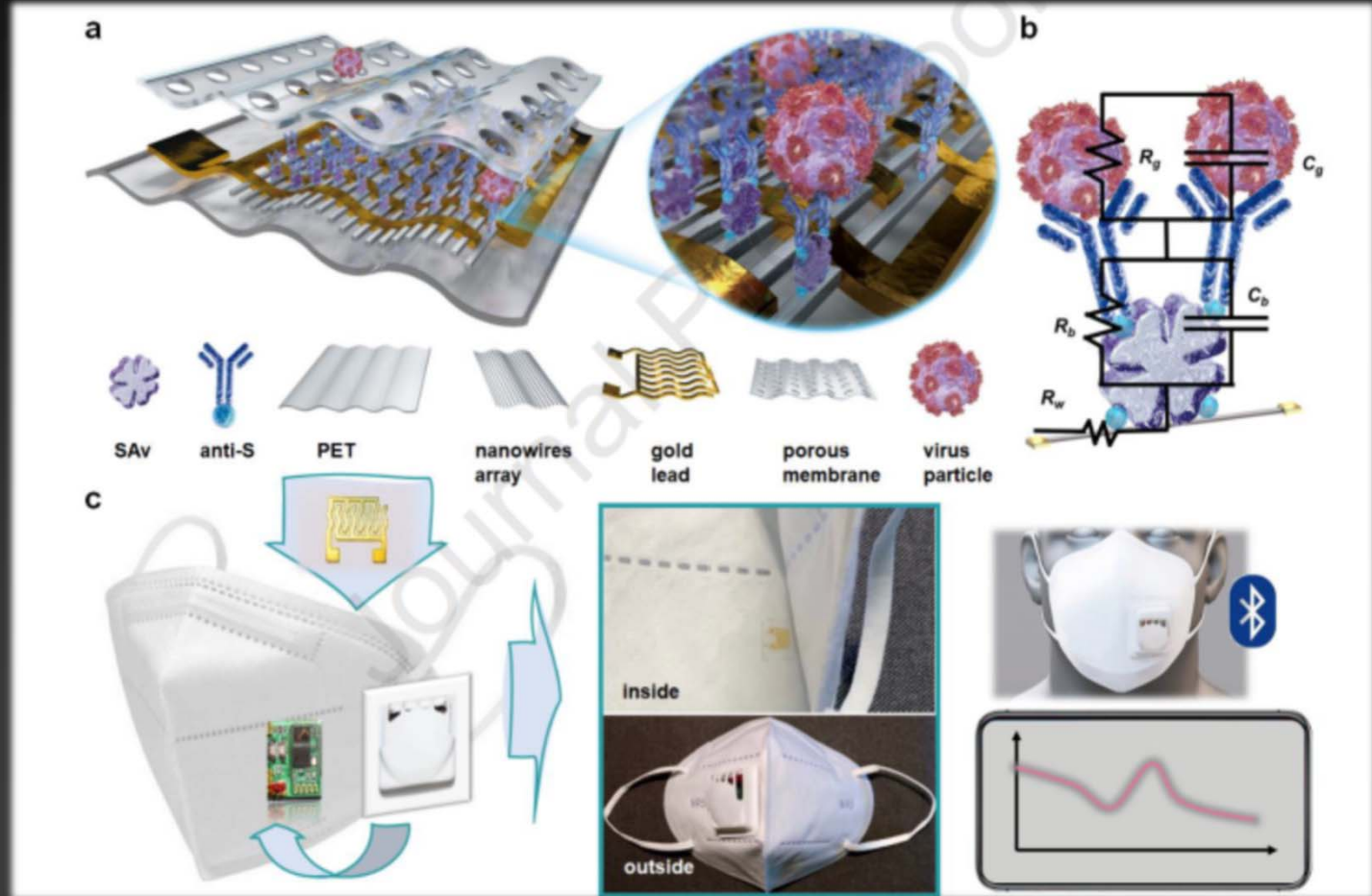
Nefeste COVID-19 Tespiti için Nanomateryal Tabanlı Sensör

- Risk altındaki veya mevcut bir COVID-19 enfeksiyonu bireylerde Sars-CoV-2 tespitinde ve izlenmesinde invazif olmayan bir yaklaşım
- Ekshale edilen nefesten hastalığa özgü biyobelirteçleri tespit edebilen ve böylece hızlı ve doğru tanıya olanak tanıyan çoklanmış algılama özelliklerine sahip nanomateryal tabanlı bir hibrit sensör dizisinden oluşan gelişmiş bir nefes cihazı
- Hassasiyet: %100
- Özgüllük: %90



Doğrudan Ekshale Edilen Koronavirüs Aerosol Taraması için Yüksek Yoğunluklu İletken Nanotel Plaka ile Entegre Akıllı Yüz Maskesi

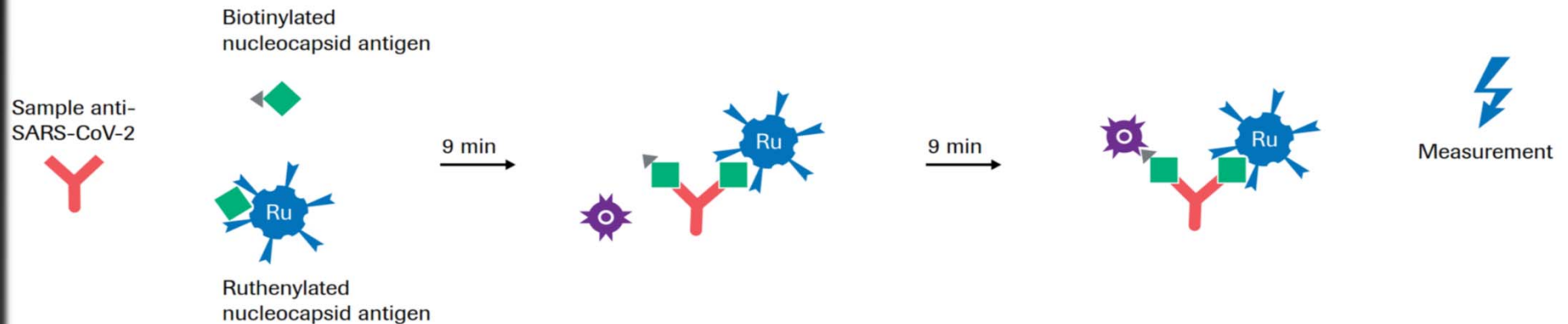
- Yüksek yoğunluklu iletken nanotel dizisine dayalı immüno-sensör (Anti-Spike)
- Minyatürleştirilmiş bir empedans devresinin ve kablosuz iletişim birimlerinin yerleştirildiği akıllı bir yüz maskesi (bluetooth)
- POC sistem
- İnsan nefesinde koronavirüs "spike" proteini ve tam virüs aerosol tespiti için
- 7 pfu / mL kadar düşük viral konsantrasyon tespiti
- Sadece 5 dakikada



Total Ig M ve G için Roche SARS-CoV-2 Elecsys Immunoassay

Electro-chemiluminescence immunoassay (ECLIA)

Test principle: double-antigen sandwich assay (testing time: 18 minutes)³³



Step 1 (9 minutes)

20 μL * / 12 μL ** of the patient sample are incubated with a mix of biotinylated and ruthenylated nucleocapsid (N) antigen. Double-antigen sandwich immune complexes are formed in the presence of corresponding antibodies.

* **cobas e** 411 analyzer and **cobas e** 601/602 modules

** **cobas e** 801 module

Step 2 (9 minutes)

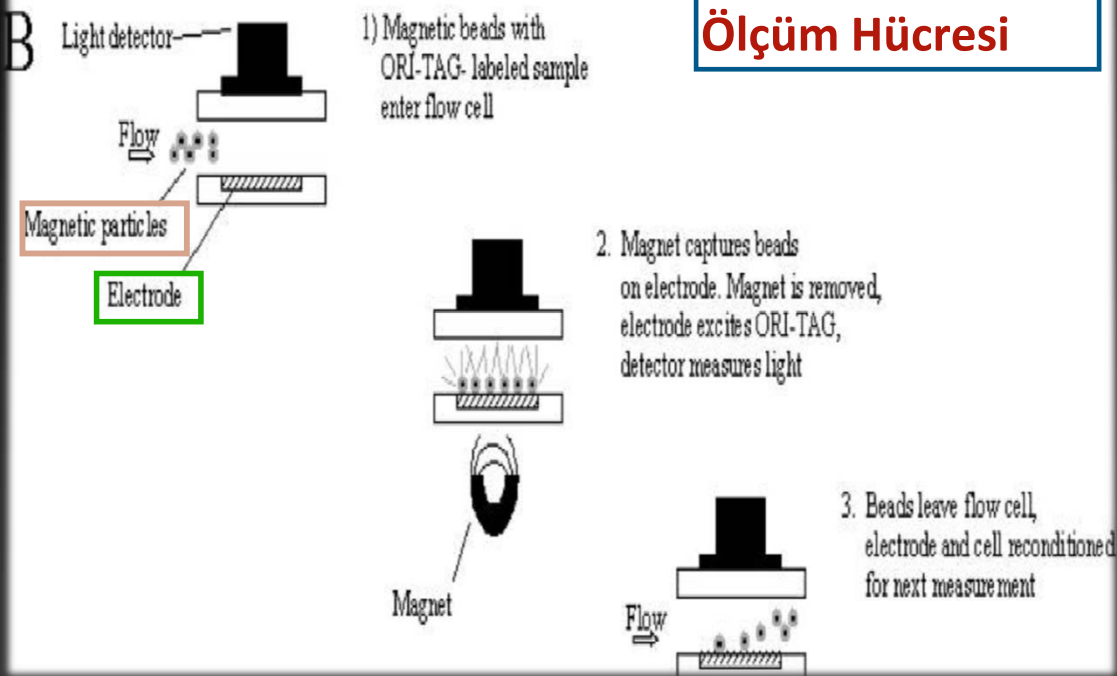
After addition of streptavidin-coated microparticles, the DAGS complexes bind to the solid phase via interaction of biotin and streptavidin.

Step 3 (measurement)

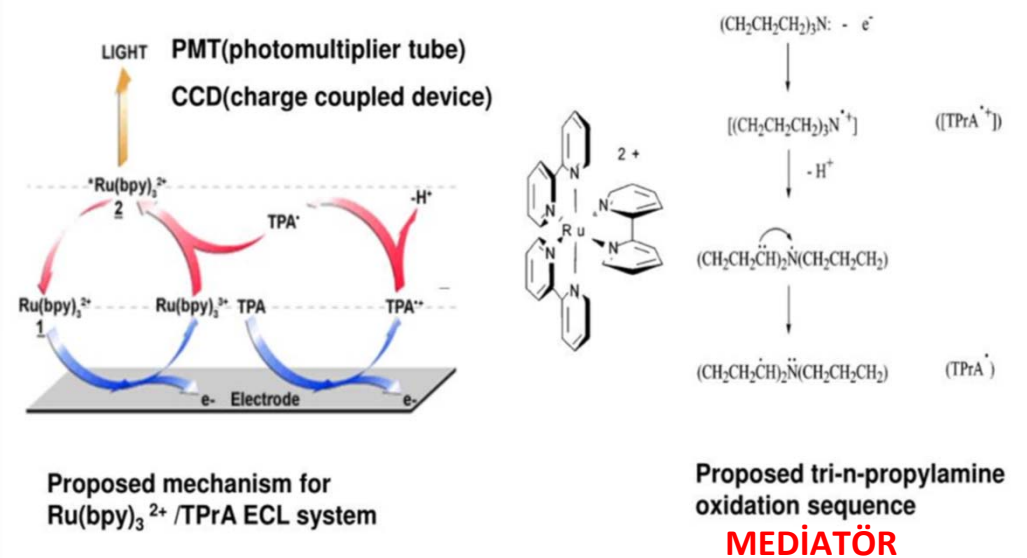
The reagent mixture is transferred to the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are subsequently removed. Electrochemiluminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield increases with the antibody titer.

*<https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html>

Total Ig M ve G için Roche SARS-CoV-2 Elecsys Immunoassay

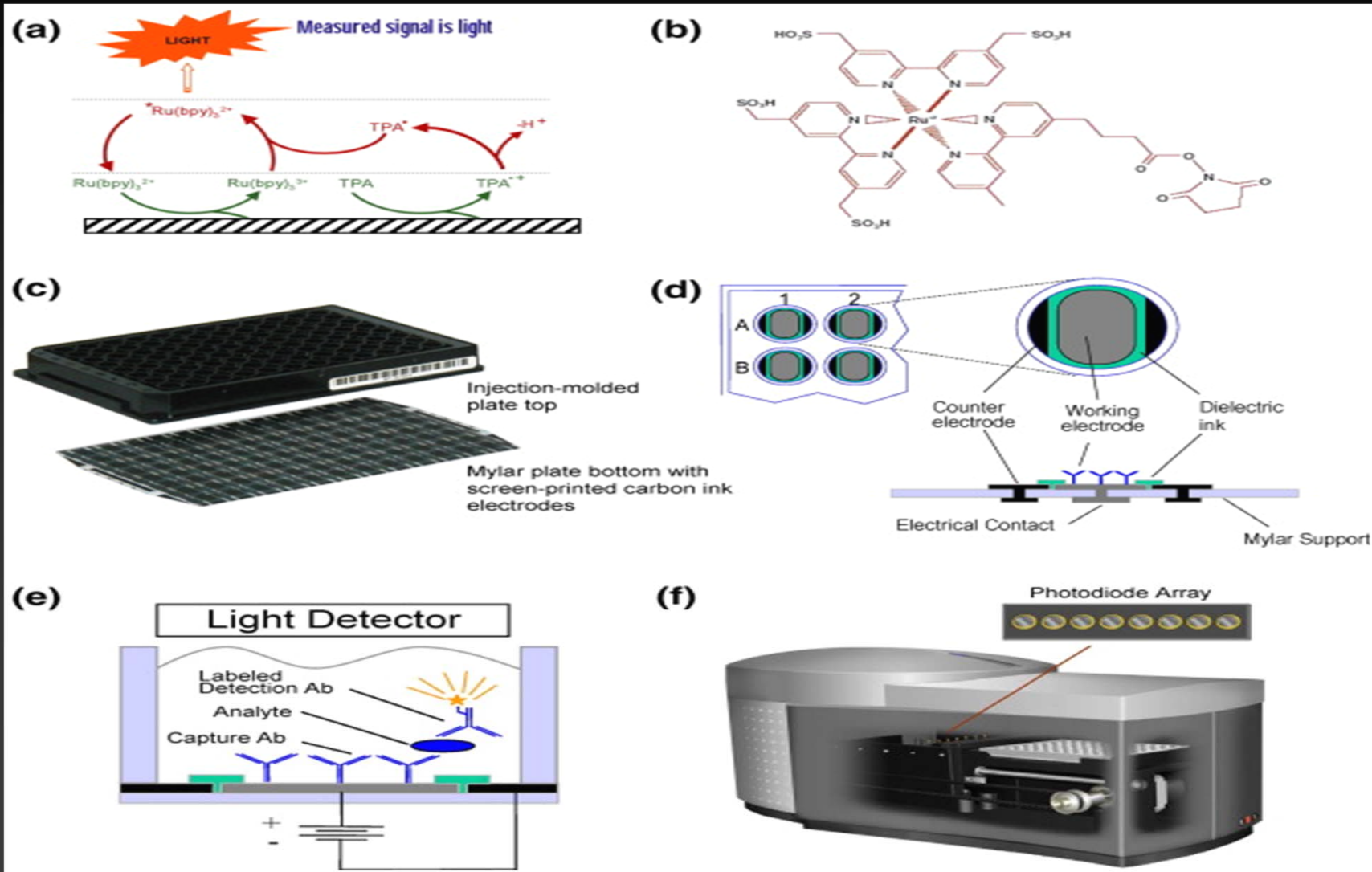


Electrochemiluminescence Process



<http://www.bioveris.com/technology.htm>, Richter, Chem. Rev.; 2004; 104(6); 3003-3036

Total Ig M ve G için Roche SARS-CoV-2 Elecsys Immunoassay



Total Ig M ve G için Roche SARS-CoV-2 Elecsys Immunoassay

Clinical sensitivity³³

A total of 204 samples from 69 symptomatic patients with a PCR confirmed SARS-CoV-2 infection were tested with the Elecsys® Anti-SARS-CoV-2 assay. One or more consecutive specimens from these patients were collected after PCR confirmation at various time points.

Days post PCR confirmation	N	Sensitivity (95 % CI*)
0 – 6 days	116	65.5 % (56.1 – 74.1 %)
7 – 13 days	59	88.1 % (77.1 – 95.1 %)
≥14 days	29	100 % (88.1 – 100 %)

* confidence interval

Clinical specificity³³

A total of 5,272 samples (from diagnostic routine, blood donors, a common cold panel, and a coronavirus panel*) obtained before December 2019 were tested with the Elecsys® Anti-SARS-CoV-2 assay.

Cohort	N	Reactive	Specificity % (95 % CI)
Diagnostic routine	3,420	7	99.80 % (99.58 – 99.92 %)
Blood donors	1,772	3	99.83 % (99.51 – 99.97 %)
Common cold panel	40	0	100 % (91.19 – 100 %)
Coronavirus panel	40	0	100 % (91.19 – 100 %)
Overall	5,272	10	99.81 % (99.65 – 99.91 %)

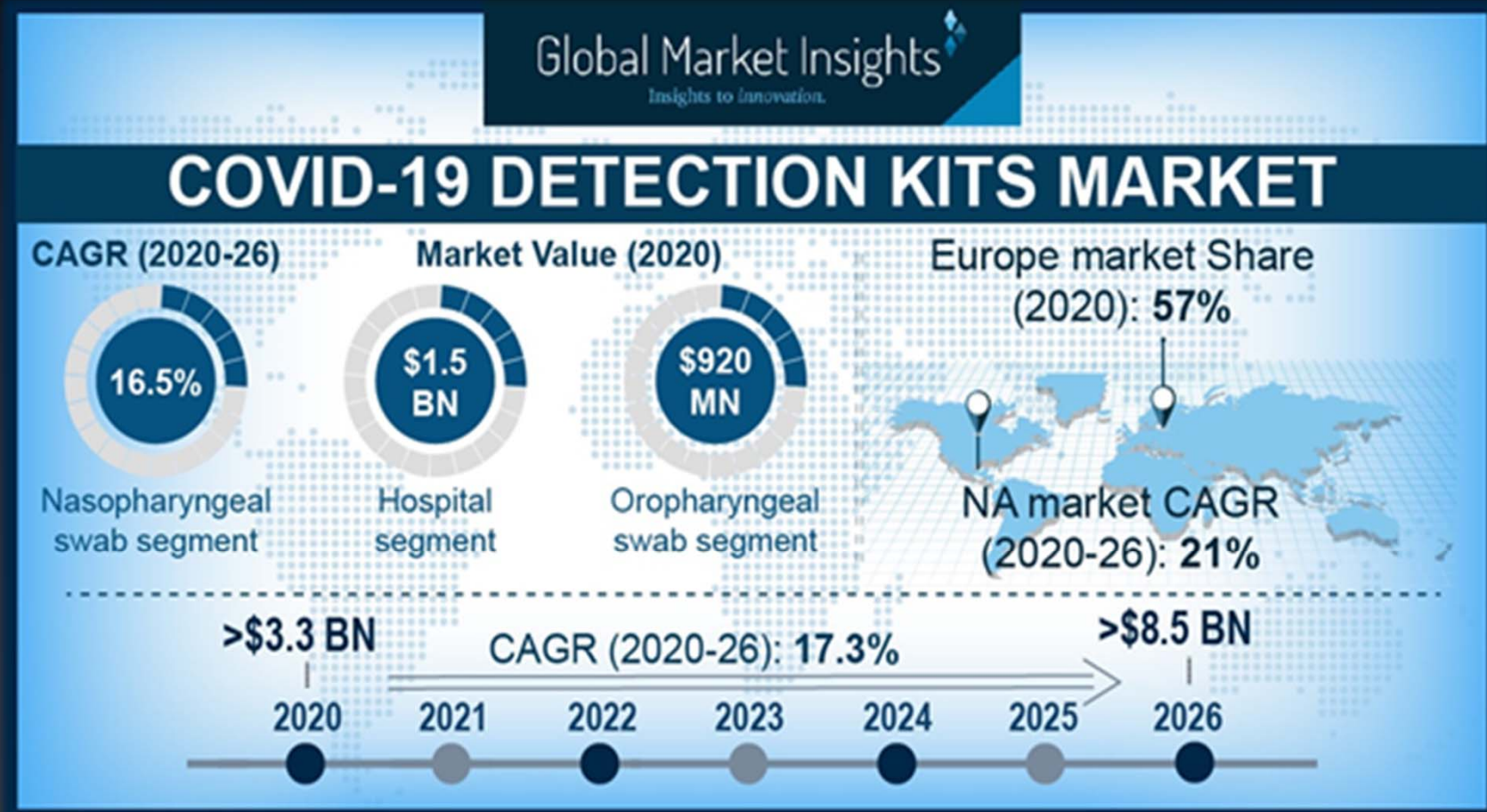
* 40 potentially non-reactive samples from individuals with recent infection with coronavirus HKU1, NL63, 229E or OC43, confirmed by PCR

*<https://diagnostics.roche.com/global/en/products/params/elecsys-anti-sars-cov-2.html>

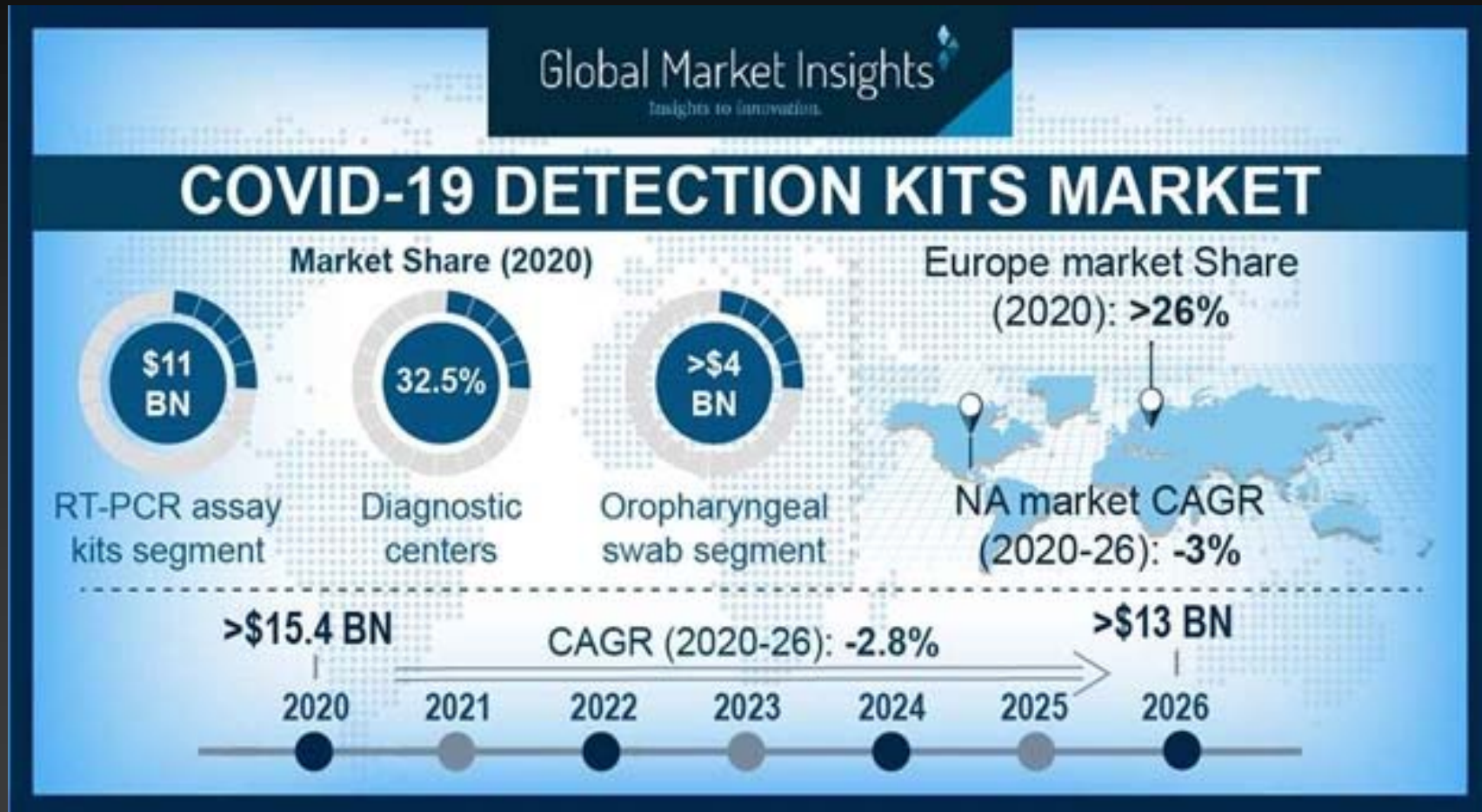
SARS-CoV-2 Testlerinin Piyasa Deęer ve Payları



COVID-19 Test Kitleri Global Pazar Payı



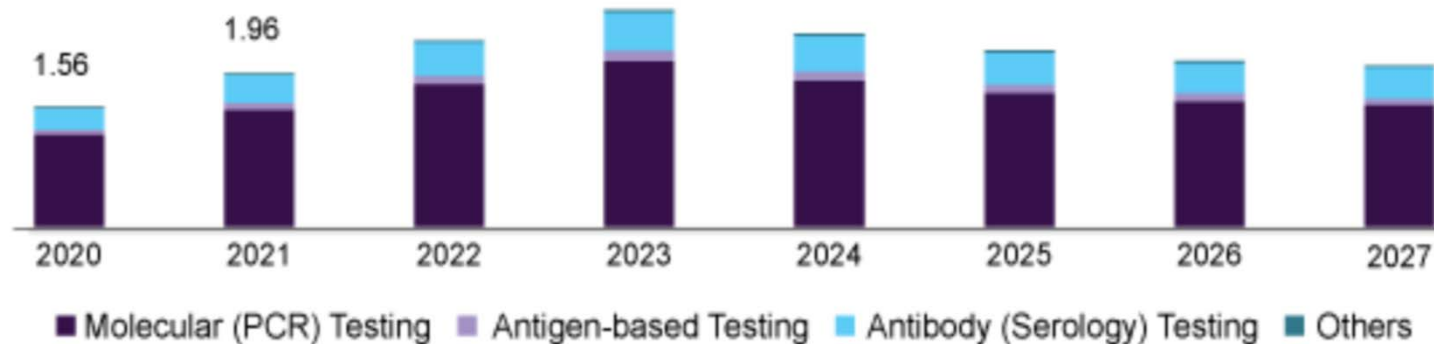
COVID-19 Test Kitleri Global Pazar Payı



COVID-19 Test Materyalleri Pazar Payı

Key RT-PCR Commercial Platforms		
Manufacturer	Products	Throughput, turnaround-time
Roche	Cobas	High, 3-8 hours
Cepheid	GeneExpert	Medium, 45mins
Biomerieux	BioFire	Low, 45mins
Qiagen	QIAstat	Low, 60mins
Thermo Fisher Scientific	ABI7500	Low, 4 hours

Canada COVID-19 diagnostics market size, by test type, 2020 - 2027 (USD Billion)



Source: www.grandviewresearch.com

*<https://www.grandviewresearch.com/industry-analysis/covid-19-diagnostics-market>

PCR, POC, LFIA ve Hibrit Sistemler

				Preferable
				Acceptable
				Not preferable
				Hybrid systems*
IDTechEx Research	qRT-PCR(device)	POC MDx (device)	LFAs (device)	
Sensitivity	> 97%	> 95%	50-70% (antigenic) 70-95% (serological)	> 80%
Specificity	~ 100%	> 99%	70%-99%	> 95%
Limit of detection (copies / mL)	100-1,000	125-500	N/A	100-10,000 depends on technologies
Test time (min)	> 120	5-45	10-30	30-90
Sample-to-answer time	Average 4-6 days	Average ~ 1h	~ 30 min	> 1 h, depends on technologies
Price (\$ per test)	< 5	10 - 150 Average around 40	0.5 - 5	Depends on technologies
Price (\$ per platform)	15K- 90 K	Average 5K-10K	< 1K	500 - 5K Depends on technologies
Technology maturity	Mature	Emerging	Mature	Many are under development

* The performances for hybrid systems are highly depending on the detection method and device design. The one using lateral flow assays can be significantly different from the one using DNA sequencing or CRISPR Cas based detection .

TEŐEKKÜRLER

KBRN, Mayıs 2021