CURRICULUM VITAE

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Qualifications :

- Medical doctor (25-08-1982- Universitas Padjadjaran)
- Clinical Pathologist (13-07-1992 – Universitas Padjadjaran)
- Doctor/S3 (12-10-2004 - Universitas Padjadjaran)
- Consultant of Tropical and Infectious Disease/Clinical Microbiology (04-11-2007)
- PhD – Radboud University Medical Center Nijmegen - The Netherlands (9-11-2009)
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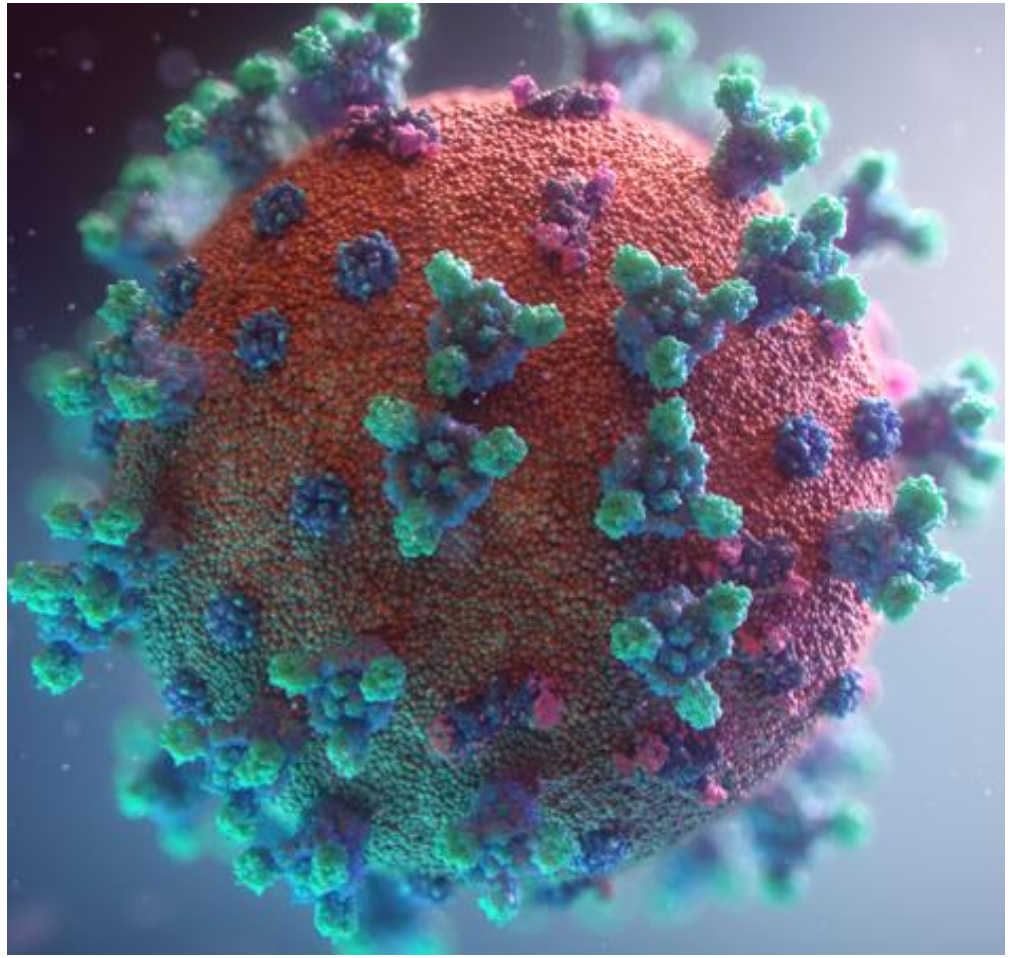

Berbagai Metoda dan Target Gen pada pemeriksaan PCR

SARS-CoV-2
penyebab
COVID-19

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Karakteristik SARS-CoV-19

- SARS-CoV-2 belongs to the broad family of viruses known as coronaviruses. It is a positive-sense single-stranded RNA (+ssRNA) virus. Other coronaviruses are capable of causing illnesses ranging from the common cold to more severe diseases such as Middle East respiratory syndrome (MERS). It is the seventh known coronavirus to infect people, after 229E, NL63, OC43, HKU1, MERS-CoV, and SARS-CoV.
- Member of the subgenus *Sarbecovirus* (Beta-CoV lineage B). Its RNA sequence is approximately 30,000 bases in length.

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Dimana Pengerjaan PCR SARS-CoV 2?

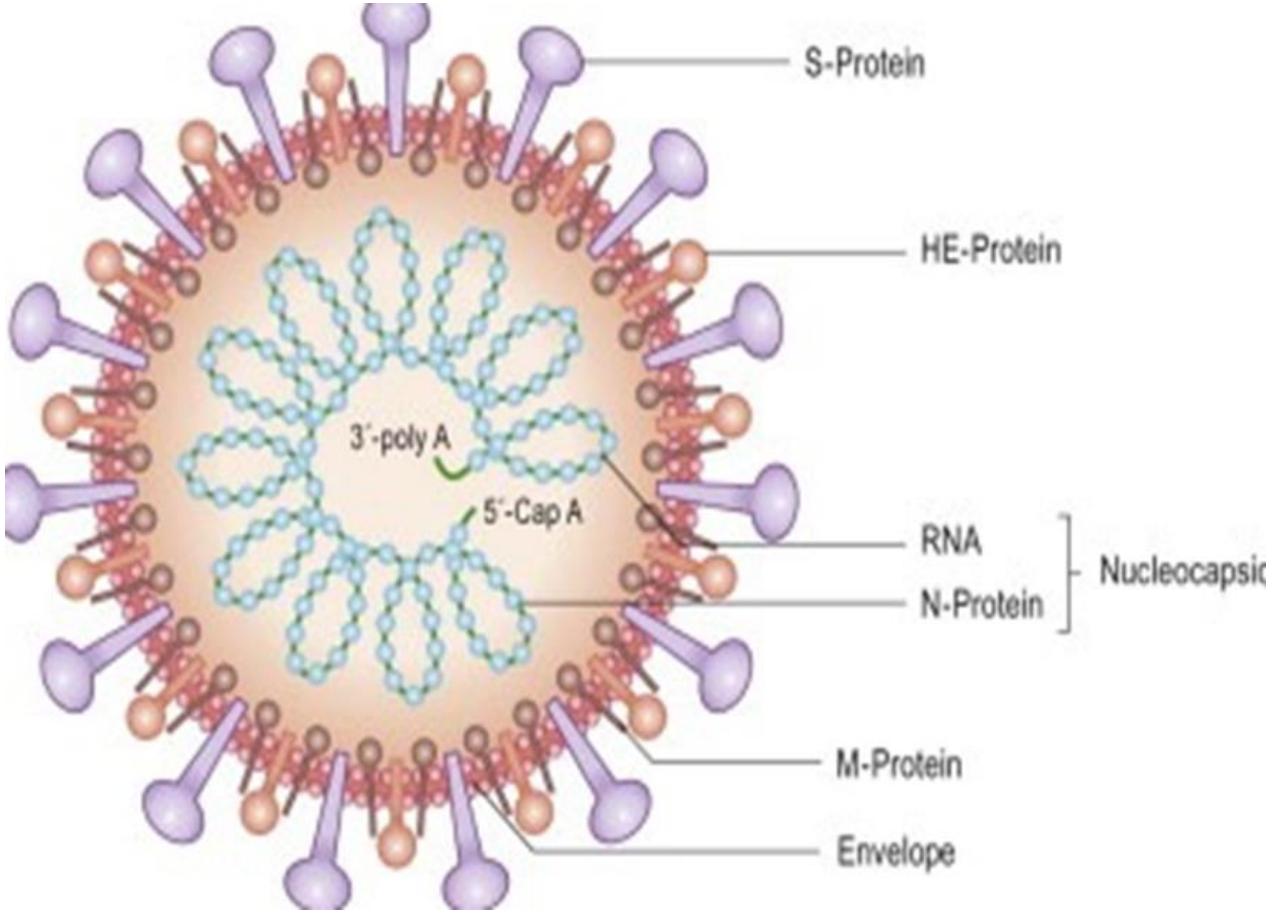
SURAT EDARAN

NOMOR HK.02.01/MENKES/234/2020

TENTANG

PEDOMAN PEMERIKSAAN UJI *REAL TIME-POLYMERASE CHAIN REACTION* (RT-PCR) SARSCoV-2 BAGI LABORATORIUM DI LINGKUNGAN RUMAH SAKIT DAN LABORATORIUM LAIN YANG MELAKUKAN PEMERIKSAAN *CORONAVIRUS DISEASE 2019* (COVID-19)

A. Persyaratan Gedung
   - Gedung BSL-2
   - Ruang Laboratorium BSL-2
B. Persyaratan BSC kelas II A2
C. Persyaratan Peralatan
D. Persyaratan SDM
E. Persyaratan Praktek Biosafety dan Biosecurity
F. Persyaratan Good Laboratory Practice



Gen yang dapat digunakan sebagai Target PCR

1. RdRP (RNA-dependent RNA polymerase)
2. N (nucleocaspid protein)
3. E (envelope protein)
4. ORF 1ab
5. S (spike protein)

Bahan Pemeriksaan

Upper respiratory specimens:

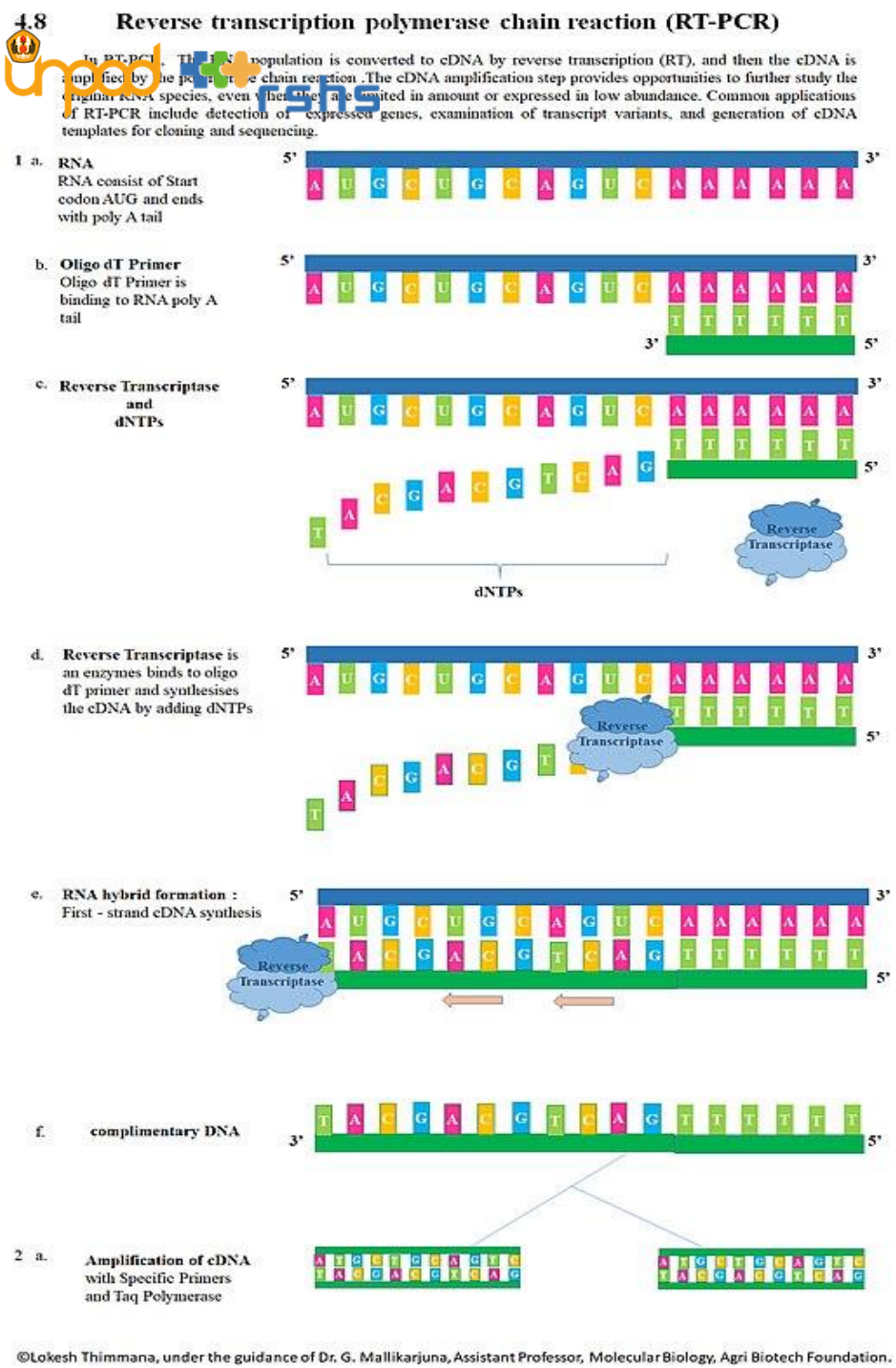
nasopharyngeal swabs,

oropharyngeal swabs,

sputum,

BAL,

tracheal aspirates)



Prinsip PCR virus RNA

(1) extraction of RNA from patient specimens,

(2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and

(3) real-time detection with 2019-nCoV specific probes.

Real-Time RT-PCR Panel for Detection 2019-Novel Coronavirus

Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases

Instructions for Use

CDC

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

[1]TaqMan® 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.

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

Graphical view of primer pairs

Detailed primer reports

Primer pair 1

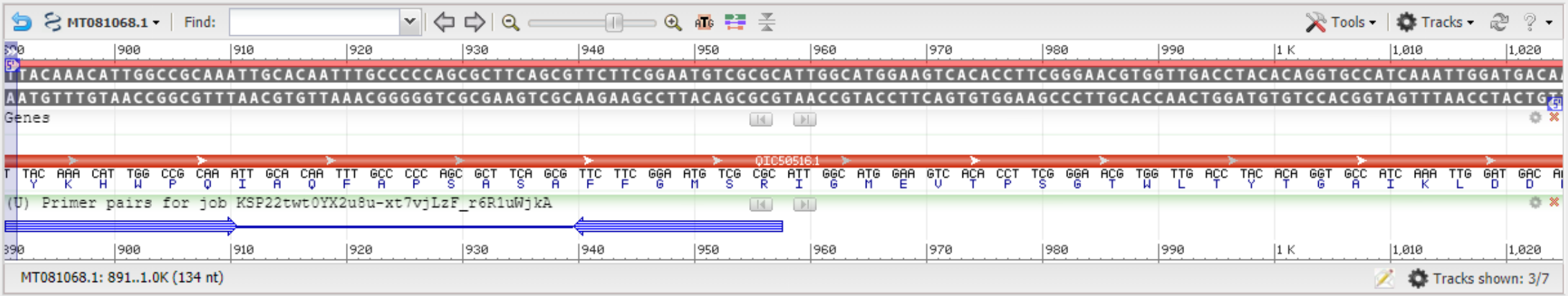
	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GACCCCAAAATCAGCGAAAT	Plus	20	14	33	56.67	45.00	2.00	2.00
Reverse primer	TCTGGTTACTGCCAGTTGAATCTG	Minus	24	85	62	60.80	45.83	7.00	5.00
Product length	72								

Products on intended targets

>MT081068.1 Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/HS_194/human/2020/CHN nucleocapsid phosphoprotein (N) gene, complete cds

Name	Description	Oligonucleotide Sequence (5'>3')	Label[1]	Working Conc.
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

Graphical view of primer pairs



Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	TTACAAACATTGGCCGCAAA	Plus	20	891	910	57.11	40.00	5.00	5.00
Reverse primer	GCGCGACATTCCGAAGAA	Minus	18	957	940	58.53	55.56	5.00	2.00
Product length	67								

Name	Description	Oligonucleotide Sequence (5'>3')	Label[1]	Working Conc.
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

Graphical view of primer pairs

Detailed primer reports

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	GGGAGCCTTGAATACACCAAAA	Plus	22	408	429	58.84	45.45	4.00	0.00
Reverse primer	TGTAGCACGATTGCAGCATTG	Minus	21	479	459	59.87	47.62	5.00	3.00
Product length	72								

Interpretasi

2019-nCoV rRT-PCR Diagnostic Panel Results Interpretation

2019 nCoV_N1	2019 nCoV_N2	2019 nCoV_N3	RP	Result Interpretation[a]
+	+	+	±	2019-nCoV detected
If only one, or two, of three targets is positive			±	Inconclusive Result
-	-	-	+	2019-nCoV not detected
-	-	-	-	Invalid Result

Laboratory-Safe Detection Protocol for SARS-CoV-2

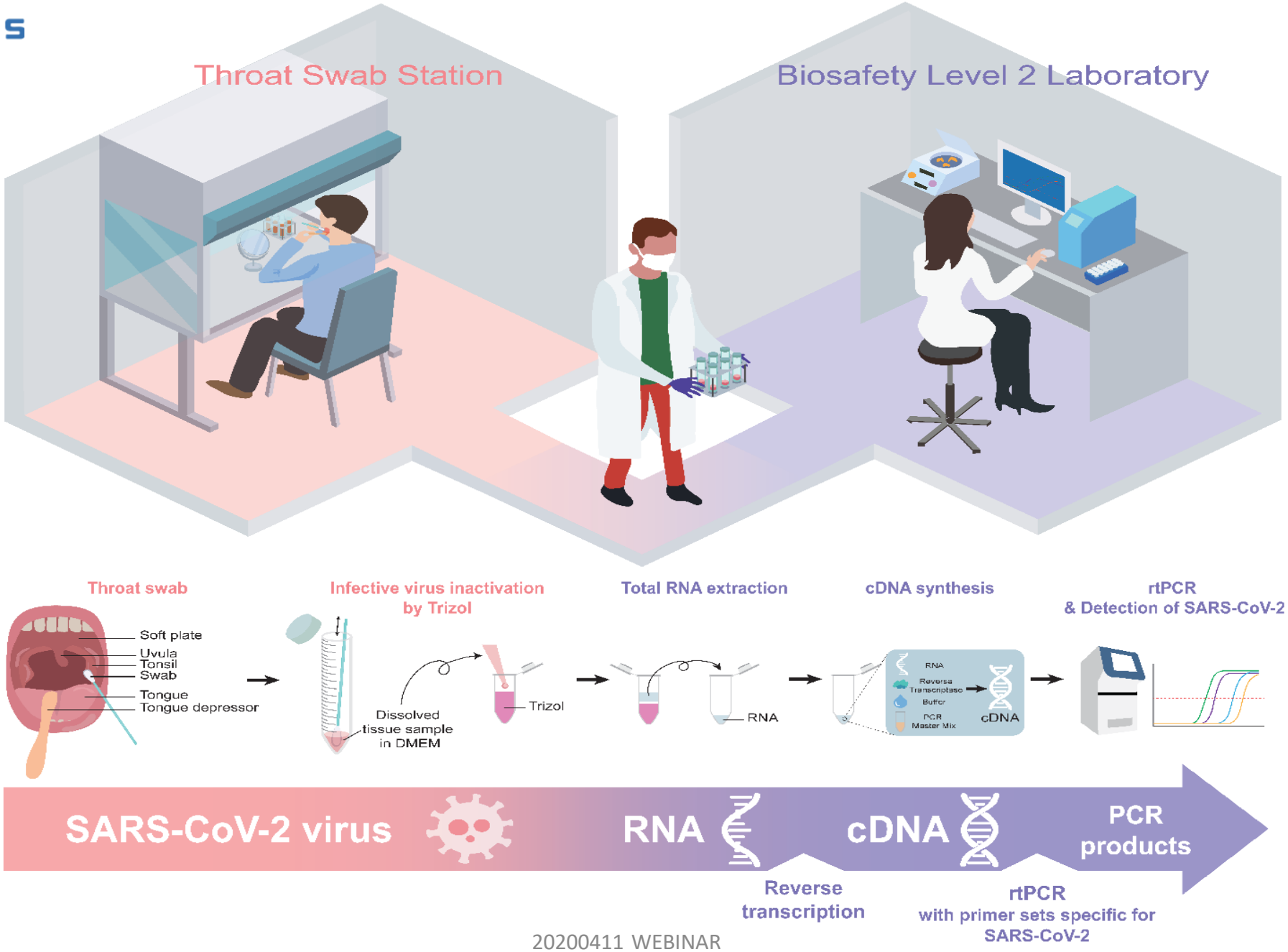


Fig. 2. Schematic diagram of the low-cost, laboratory-safe protocol for SARS-CoV2.

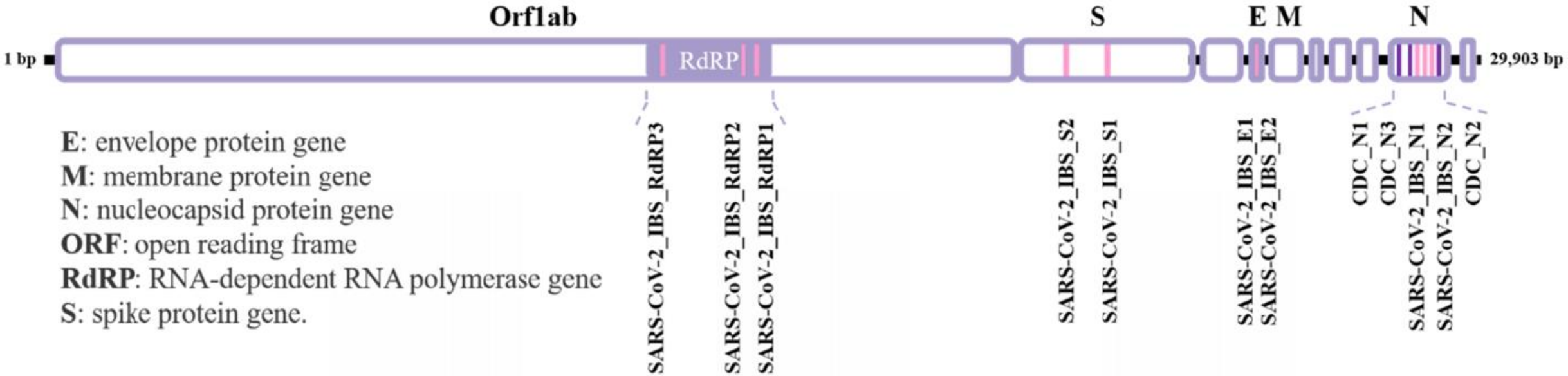


Fig. 5. The domain map of SARS-CoV-2 form the sequence obtained from the first patient in Republic of Korea. The location of each primer set is indicated at corresponding sequence. The primer set from CDC is targeting nucleocapsid protein gene (N) to detect SARS-CoV-2. In-house (IBS) designed primer sets for detecting SARS-CoV-2 target RNA-dependent RNA polymerase gene (RdRP), spike protein gene (S), envelope protein gene (E) and N.

Joungha Wo_Low-cost and Laboratory-safe Detection Protocol for COVID-19

Assay / Use	Oligonucleotide ID	Sequence (5'–3')	Comment
RdRP gene	RdRP_SARSr-F2	GTGARATGGTCATGTGTGGCGG	use 600 nM per reaction
	RdRP_SARSr-R1	CARATGTTAAASACACTATTAGCATA	use 800 nM per reaction
	RdRP_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for Wuhan-CoV, will not detect SARS-CoV use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe, will detect Wuhan virus, SARS-CoV and bat-SARS-related CoVs use 100 nM per reaction and mix with P2
E gene	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	use 400 nM per reaction
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	use 400 nM per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	use 200 nM per reaction
N gene	N_Sarbeco_F1	CACATTGGCACCCGCAATC	use 600 nM per reaction
	N_Sarbeco_R1	GAGGAACGAGAAGAGGCTTG	use 800 nM per reaction
	N_Sarbeco_P1	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	use 200 nM per reaction

TEST CEPAT MOLEKULER

Xpert® Xpress SARS-CoV-2

Instructions for Use

For Use Under an Emergency Use Authorization (EUA) Only

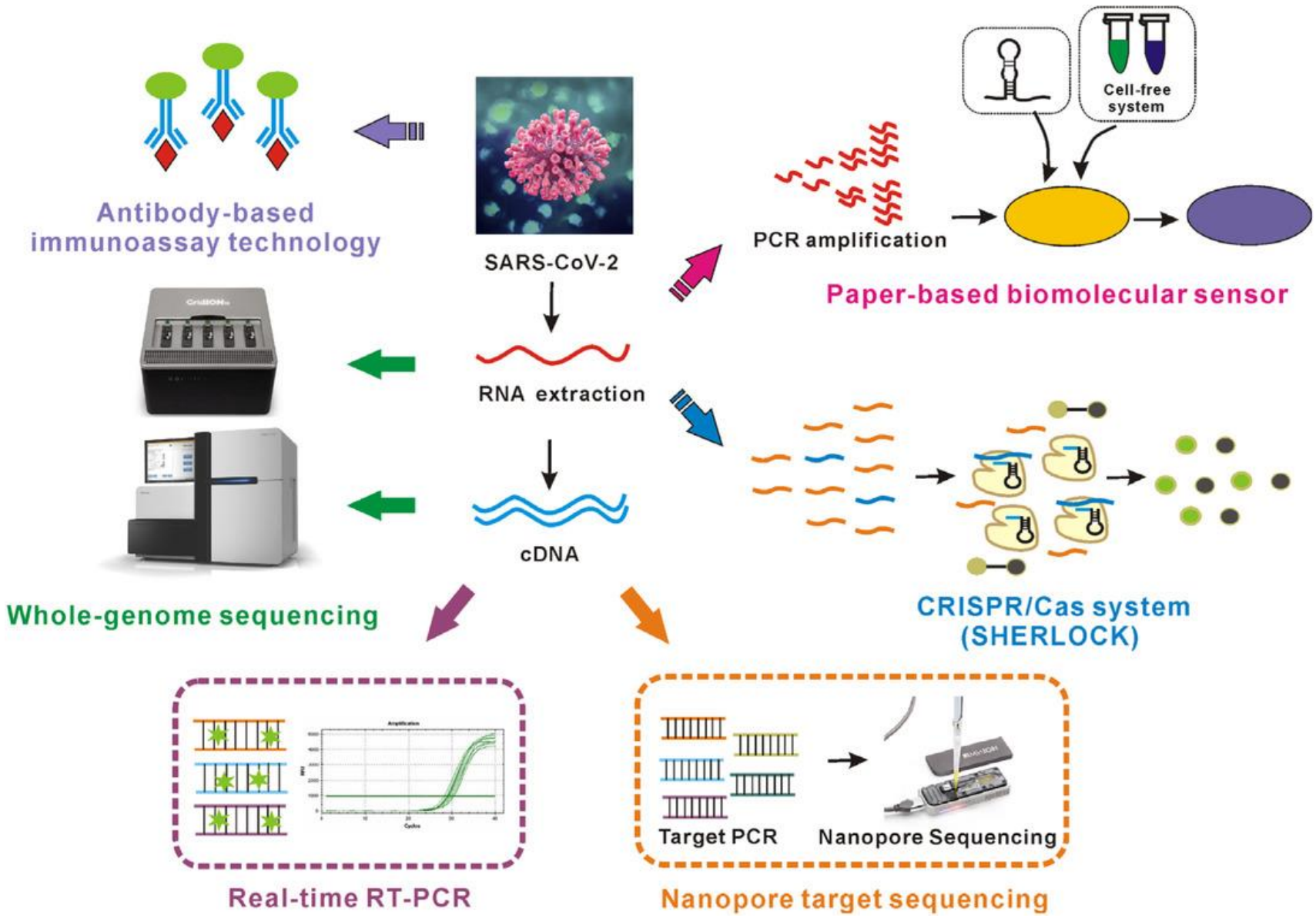
℞only

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+	+/-
SARS-CoV-2 POSITIVE	+	-	+/-
SARS-CoV-2 PRESUMPTIVE POSITIVE	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

Penggunaan Kit Vs In-house Assay

	Kit RealTime Komersil	In-House Assay
Penggunaan	Lebih mudah, hanya 1 reagen *(User friendly workflow*)	Relatif lebih sulit , lebih dari 1 reagen (primer, mastermix, probe)
Uji Klinis, Sensitivitas , Spesifitas	Sudah dilakukan (>95%)	Perlu optimasi dan uji klinis
Ketersediaan	Terkadang harus indent	Terkadang beberapa komponen harus indent
Hasil Pemeriksaan	Mudah diintrepetasi (*automatic data analyzer)*	Interpretasi manual
QC Pemeriksaan	Berupa Internal Control ditambah saat ekstraksi sampel (*whole process validation)*	Gen RP-ase manusia
Gen Target	RdRP, N, E	Dapat ditentukan sendiri
Jumlah sampel per running	72- 90 sampel (*multiplex realtime PCR)*	CDC : 16 sampel/running (*singleplex realtime PCR*)
Carry over-contamination	Relatif kecil (penggunaan *UDG system)*	Mungkin terjadi, belum menggunakan UDG



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