

PRACTICAL EXPERIENCE AND CHALLENGES OF COVID-19 TESTING IN IRAN

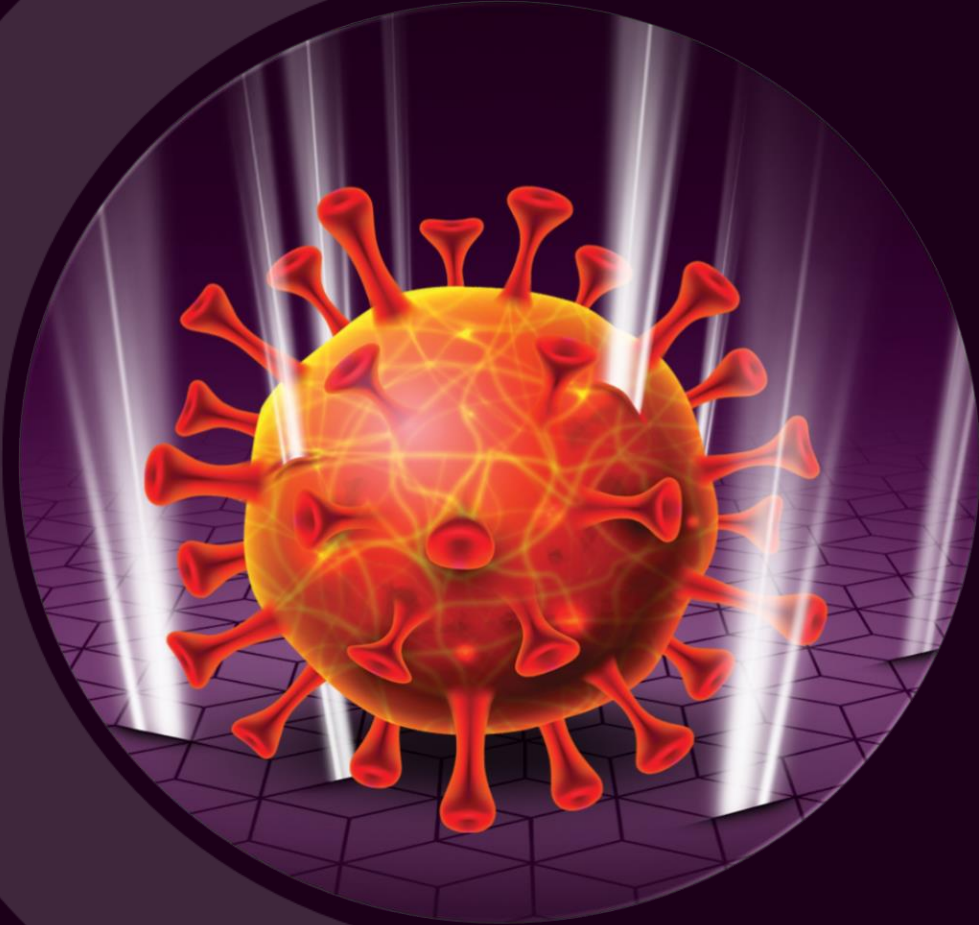
Present by:

KAYHAN AZADMANESH MD, PH.D.

PROFESSOR OF VIROLOGY DEPARTMENT

RAPID RESPONSE TEAM

PASTEUR INSTITUTE OF IRAN



Outline

- An introduction to Iran
- A brief history of COVID-19 in Iran
- Setting up the molecular tests
- Establishing the national network
- Evaluation of new kits
- Role of serological tests in Iran
- Challenges in coordinating the network

Iran

- Are: 1.648 million km²
- Population: 83 m
- Capital: Tehran
- 31 Provinces
- Diverse climate





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Overview

Explorer

Global >  Iran (Islamic Republic of)

Data last updated: 2020/5/31, 6:26pm CEST

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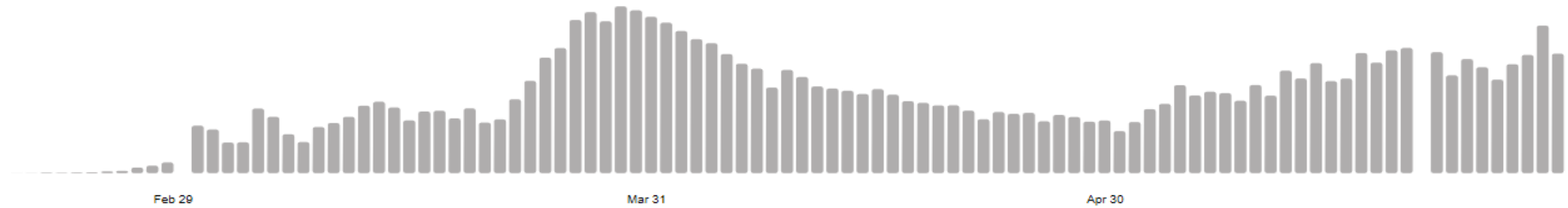
Confirmed Cases Over Time

Daily 

148,950

confirmed cases

Source: World Health Organization



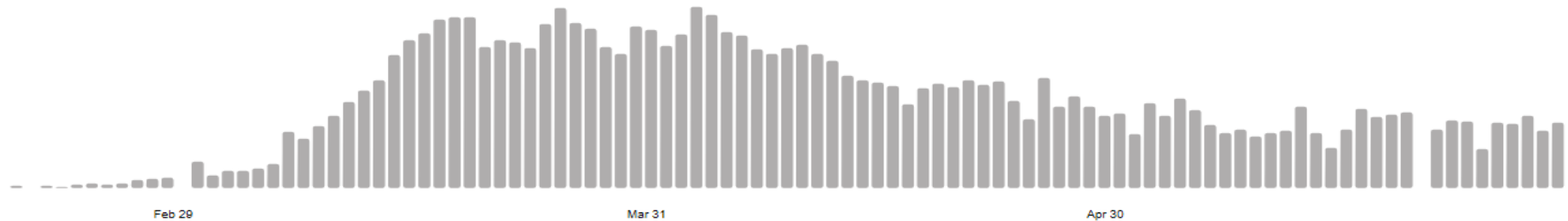
Deaths Over Time

Daily 

7,734

deaths

Source: World Health Organization



WHO Health Emergency Dashboard WHO (COVID-19) Homepage

Timeline of Development and Expansion of SARS-CoV-2 laboratory Network

Diagnostic Strategy	SARS-CoV-2 Laboratories		Date	Capacity/day
PAN CORONA RT-PCR + SARS-CoV-2 Confirmatory nested PCR/Sequencing (if necessary)	1	Pasteur Institute of Iran, Rapid Response Lab	2020-January-25	10
PAN CORONA RT-PCR + SARS-CoV-2 RT-PCR	1	Pasteur Institute of Iran, Rapid Response Lab	2020-February-5	10
WHO Primer and Probe Sets for SARS-CoV-2 RT-PCR Several Primer and Probe sets for SARS-CoV-2 RT-PCR	2	- Pasteur Institute of Iran, Rapid Response Laboratory - National Influenza Reference Laboratory	2020-February-9	50
WHO Primer and Probe Sets for SARS-CoV-2 RT-PCR	4	Pasteur Institute of Iran, Rapid Response Laboratory - National Influenza Reference Laboratory, Tehran University of Medical Sciences - Virology Research Center, Dr. Masih Daneshvari Hospital, Shahid Beheshti, University of Medical Sciences - Virology and Microbiology Molecular Diagnostic Laboratory, Arak University of Medical Sciences	2020-February-18	200
		.		
		.		
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		.		
WHO Primer and Probe Sets for SARS-CoV-2 RT-PCR Validated Commercial SARS-CoV-2 RT-PCR kits	147	laboratories all over the Country: 78 Ministry of Health and Medical Education 19 Affiliated to other Governmental Organizations 50 NGOs and Private Labs	2020-May-15	20000

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Revised in 2014

Guidance for Establishing a National Health Laboratory System

**The World Health Organization
Regional Office for Africa**

WHO-EM/LAB/390/E

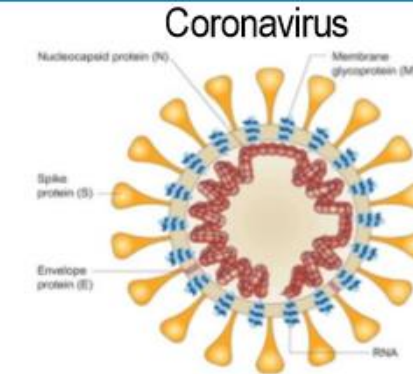
Strategic framework for strengthening health laboratory services 2016–2020



What do diagnostic tests for COVID-19 detect?

- Viral RNA detection by NAAT/RT-PCR
- COVID-19 viral antigen
- Antibodies against a COVID-19 Antigen
 - Immunoglobulin M (IgM)
 - Immunoglobulin G (IgG)
 - Immunoglobulin A (IgA)

Virus



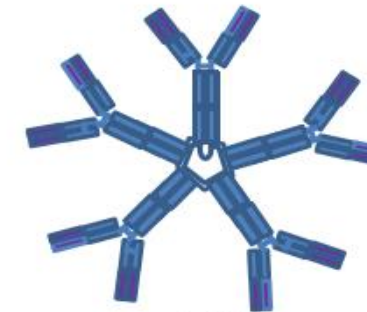
Monto, Cowling and Perds. Coronaviruses. R.A. Kaslow et al. (eds.), Viral infections in humans. https://link.springer.com/content/pdf/10.1007%2F978-1-4899-7448-8_10.pdf

Immune response

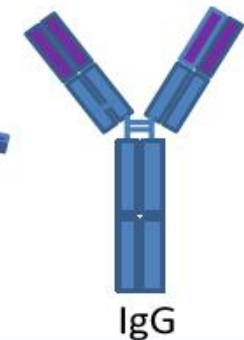
Antibodies



IgA



IgM



IgG

WHO guideline for laboratory confirmation

- To consider a case as laboratory-confirmed by NAAT in an area with no COVID-19 virus circulation, one of the following conditions need to be met:
 - A **positive NAAT result for at least two different targets** on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus using a validated assay (as at present no other SARS-like coronaviruses are circulating in the human population it can be debated whether it must be COVID-19 or SARS-like coronavirus specific);
 - OR **One positive NAAT** result for the presence of betacoronavirus, and COVID-19 virus further identified by **sequencing** partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used

The activities before finding our first cases

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for 2019-nCoV E gene assay is available via EVAg. Synthetic control for 2019-nCoV RdRp is expected to be available via EVAg from Jan 21st onward.

First line screening assay: E gene assay

Confirmatory assay: RdRp gene assay

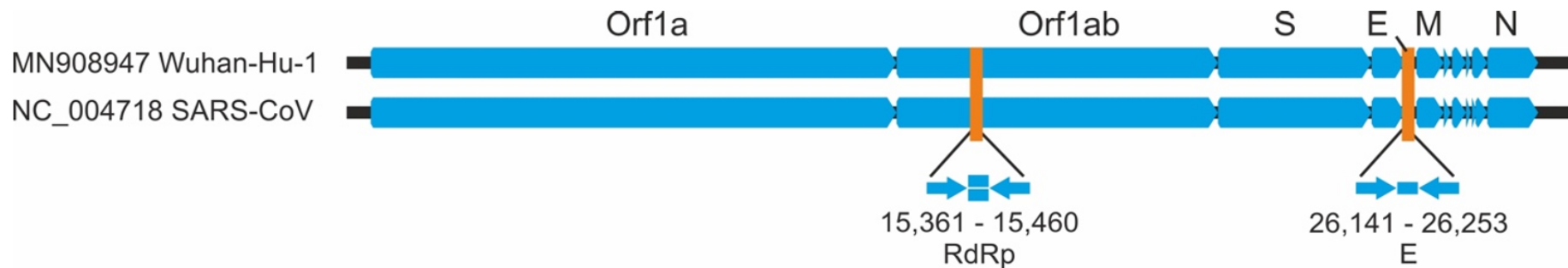


Figure 1 relative positions of amplicon targets on SARS-CoV and 2019-nCoV genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

	Forward primer region	Reverse primer region
HCoV-NL63 :	ACACAGCTGAATCTTAAGTATGC	TGGGATTATCCCAAATGTGA
HCoV-229E :	ACTCAGTTAAATCTTAAATACGC	TGGGACTATCCTAAGTGTGA
FIPV :	ACTCAAATGAATTTGAAATATGC	TGGGACTATCCTAAGTGTGA
TGEV :	ACTCAGTTAAATCTTAAATACGC	TGGGACTATCCTAAGTGTGA
PEDV :	ACACAGCTCAACCTTAAATACGC	TGGGATTACCCAAAGTGCGA
HCoV-OC43 :	ACTCAAATGAATTTGAAATATGC	TGGGATTATCCTAAGTGTGA
BCoV :	ACTCAAATGAATTTGAAATATGC	TGGGATTATCCTAAGTGTGA
PHEV :	ACTCAAATGAATTTGAAATATGC	TGGGATTATCCAAAGTGTGA
CRCV :	ACTCAGATGAATTTGAAATATGC	TGGGATTATCCTAAGTGTGA
MHV :	ACTCAAATGAATCTTAAATATGC	TGGGACTATCCTAAATGTGA
SDAV :	ACTCAAATGAATCTTAAATATGC	TGGGACTATCCTAAGTGTGA
SARS-CoV :	ACTCAAATGAATCTTAAGTATGC	TGGGATTATCCAAAATGTGA
IBV :	ACTCAAATGAATTTAAAATATGC	TGGGATTATCCTAAGTGTGA
TCoV :	ACTCAAATGAATTTAAAATATGC	TGGGATTATCCTAAGTGTGA
	ACWCARHTVAAYYTNAARTAYGC	TGGGAYTAYCCHAARTGYGA

Fig. 1. Selection of primers for the novel pancoronavirus RT-PCR. Shown is the alignment of 14 coronaviral sequences of a conserved region of the polymerase gene. The forward (Cor-FW) and reverse (Cor-RV) primer sequences are shown at the bottom (Y=C/T, W=A/T, V=A/C/G, R=A/G, H=A/T/C, N=A/C/T/G).

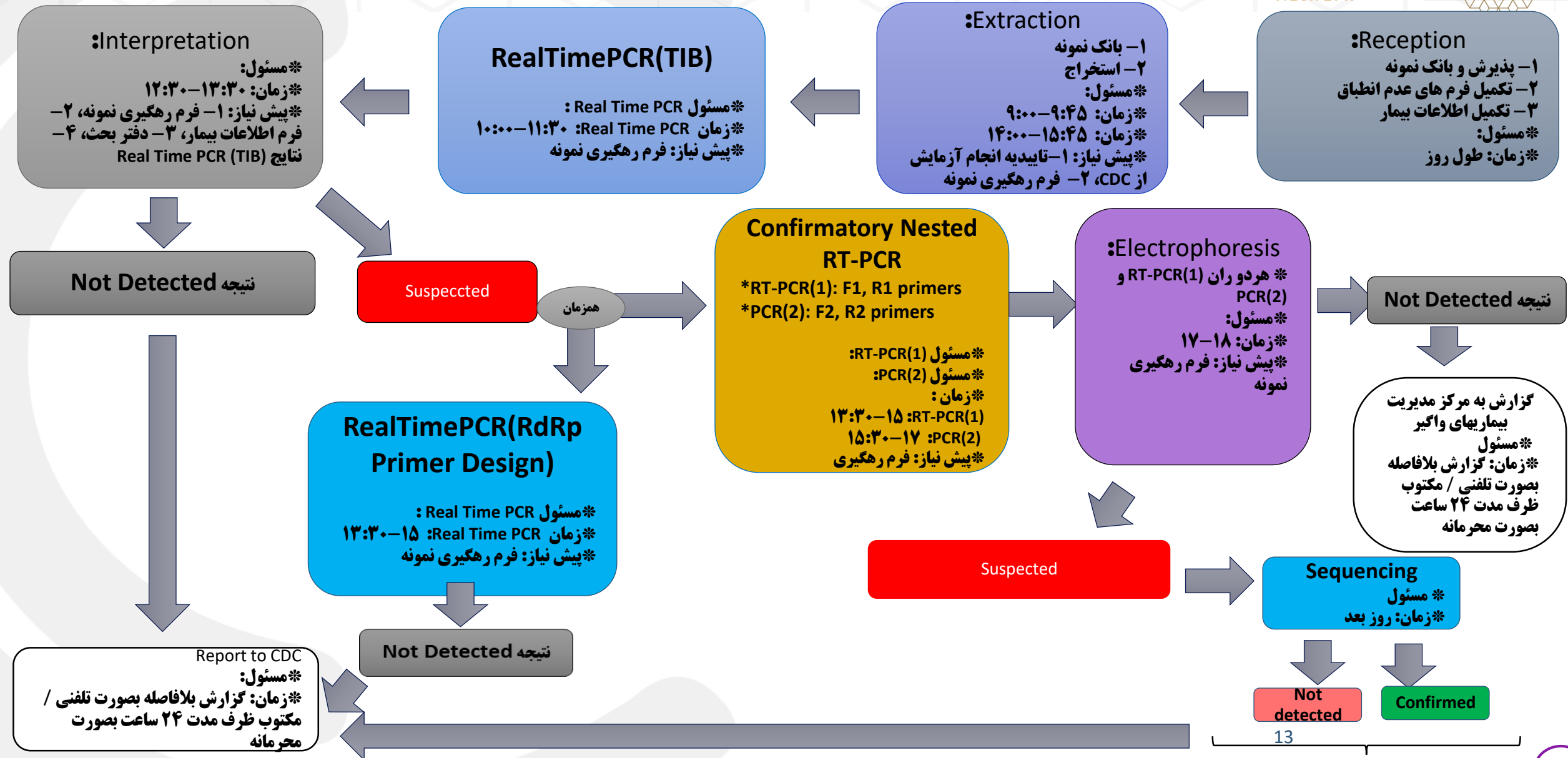
Avian Coronavirus in Wild Aquatic Birds^{▽†‡}

Daniel K. W. Chu,¹ Connie Y. H. Leung,¹ Martin Gilbert,² Priscilla H. Joyner,² Erica M. Ng,¹
Tsemay M. Tse,¹ Yi Guan,¹ Joseph S. M. Peiris,^{1,3*} and Leo L. M. Poon^{1*}

State Key Laboratory for Emerging Infectious Diseases, Department of Microbiology and Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong Special Administrative Region, China¹; Wildlife Conservation Society, Cambodia²; and HKU-Pasteur Research Centre, Hong Kong Special Administrative Region, China³

Received 29 July 2011/Accepted 16 September 2011

a pancoronavirus nested PCR (nPCR) for the RNA-dependent RNA polymerase (RdRp) sequence. Briefly, cDNA was amplified in a first-round PCR (forward primer 5'-GGKTGG GAYTAYCCKAARTG-3' and reverse primer 5'-TGYTGTS WRCARAAAYTCRTG-3'; 40 cycles of 94°C for 20 s, 48°C for 30 s, and 72°C for 50 s). The PCR product was then amplified in a second-round PCR under amplification condition identical to those of the first-round PCR, except that a new set of primers was used in the assay (forward primer 5'-GGTTGGG ACTATCCTAAGTGTGA-3', reverse primer 5'-CCATCATC AGATAGAATCATCAT-3'). The final PCR products (440 bp) were analyzed by sequencing.



Sequences producing significant alignments

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Show

100 ▾


☒ select all 100 sequences selected
[GenBank](#)[Graphics](#)[Distance tree of results](#)

	Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-TX1/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106054.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA8/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106053.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA7/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106052.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/WH-09/human/2020/CHN, complete genome	1020	1020	100%	0.0	100.00%	MT093631.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/01/human/2020/SWE, complete genome	1020	1020	100%	0.0	100.00%	MT093571.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/61-TW/human/2020/ NPL, complete genome	1020	1020	100%	0.0	100.00%	MT072688.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/NTU02/2020/TWN, complete genome	1020	1020	100%	0.0	100.00%	MT066176.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/NTU01/2020/TWN, complete genome	1020	1020	100%	0.0	100.00%	MT066175.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/Yunnan-01/human/2020/CHN, complete genome	1020	1020	100%	0.0	100.00%	MT049951.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA6/2020, complete genome	1020	1020	100%	0.0	100.00%	MT044258.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-IL2/2020, complete genome	1020	1020	100%	0.0	100.00%	MT044257.1
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<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WI1/2020, complete genome	1020	1020	100%	0.0	100.00%	MT039887.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 isolate HZ-1, complete genome	1020	1020	100%	0.0	100.00%	MT039873.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-521/2020 RNA, complete genome	1020	1020	100%	0.0	100.00%	LC522975.1
<input checked="" type="checkbox"/>	Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-501/2020 RNA, complete genome	1020	1020	100%	0.0	100.00%	

 Feedback




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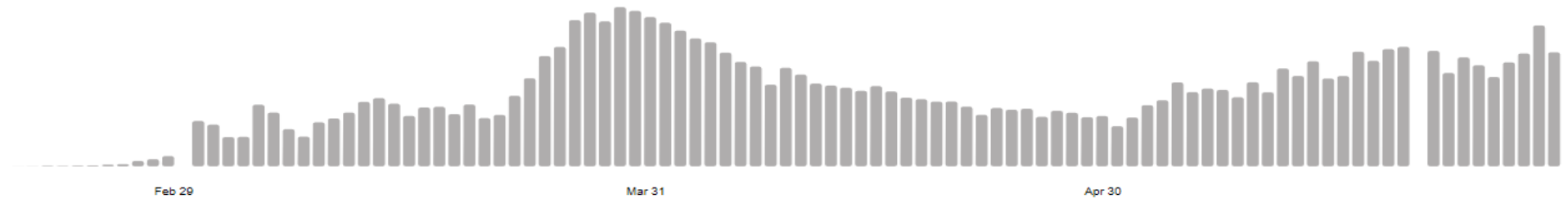
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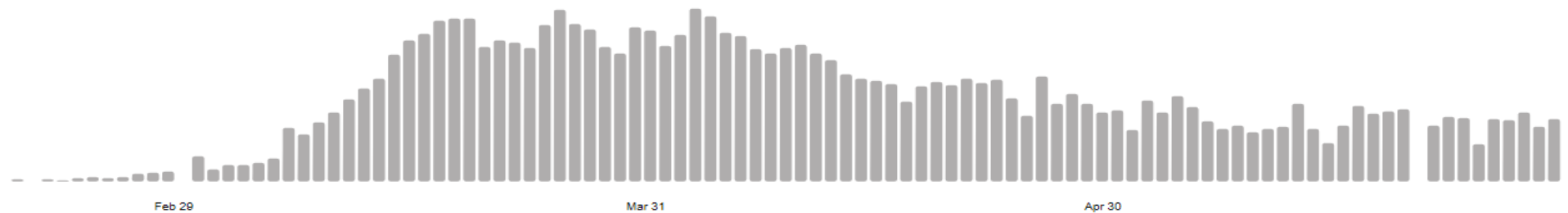
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Strategic framework for strengthening health laboratory services 2016–2020



Strategic Goals:

- Strengthen leadership and governance of the national laboratory systems;
- Strengthen the organization and management of the national laboratory systems towards quality;
- Establish sustainable, sufficient and competent human resources for laboratory service delivery;
- Ensure safe and secure laboratory environments;
- Promote effective laboratory referral networking (in-country and among countries) and enhance coordination; and
- Promote rational and evidence-based use of laboratory services

Establishing the network

- After confirming the first cases, and finding out that the disease has been spread out of the first foci, the MOH established a Coronavirus Laboratory Committee to synchronize and manage the laboratory efforts:
 - Pasteur Institute of Iran
 - Department of Health Reference Laboratories
 - Department of Control Reference labs of FDO
 - Deputy of health of the MOH
 - The procurement body of the MOH

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Establishing the network II:

- Regional influenza and HIV labs were recruited
- Safe and secure sample transport method was already in place
- 3 questionnaires were sent to these labs, focusing on:
 - Personnel quality and training
 - Biosafety and Biosecurity measures in place
 - Laboratory facility available
- A brief refreshment training in PII
- RT-PCR reagents for 100 tests were provided to each lab
- The results file of the first 3 runs were analyzed in parallel, focusing on:
 - Cts of + control, samples and ICs
 - Shape of graphs
 - Interpretation of the results

Establishing the network III:

- A series of samples were sent to PII to be re-tested
- HRL granted the permission to run the test to the lab
- In the third round of expansion, the regional lab became in charge of inspection and training, but the results are still analyzed in PII and the HRL grants the permission.
- 2 data collection web-based system have been exploited
- A national EQAP has been also established to monitor the performance of the labs

- Current number of approved labs : more than 200, about 150 are active
- A what's app group is formed to facilitate the communication among them
- Horizontal and vertical communication, technical assistance and sample exchange are actively promoted among the labs.

Supply of kits

- All new PCR kits are evaluated in PII as an accredited lab by the FDO
- Suitable kits for the network are chosen to be purchased by the MOH
- The extraction kits, sample collection kits and other consumables were primarily left to the Medical Universities, but later were assisted by the lab committee.

Evaluation of new PCR kits

- New kits became available during February - March – April
- These kits detect multiple targets in SARS2-CoV genome, plus control genes
- The first series were all compared with Tib Mol Biol primer-probe set
- Some were chosen for scaling up the lab network, based on:
 - Multiple targets
 - Internal control
 - Good performance
 - Price
- Emergency Use Authorization

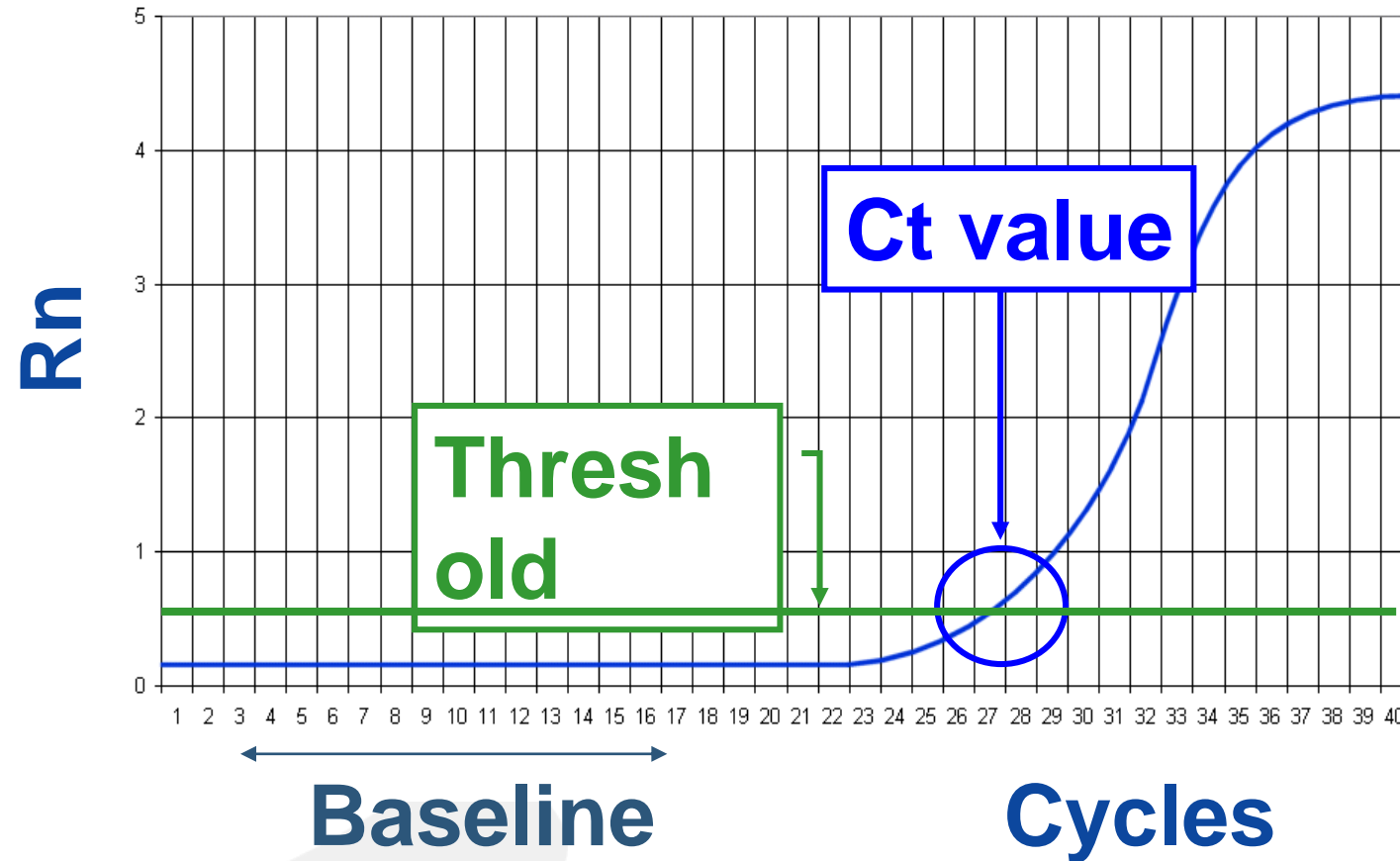
Strategic Goals:

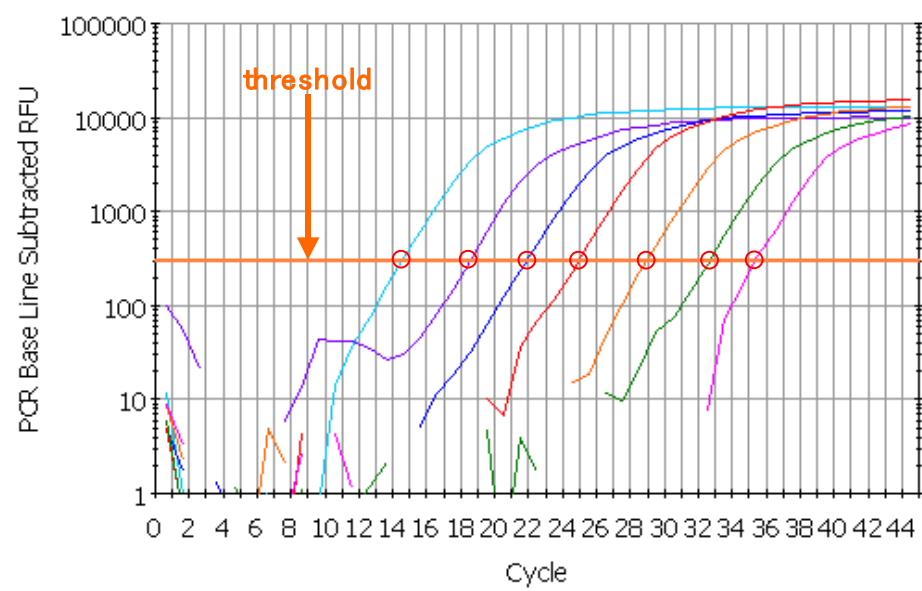
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 - OR **One positive NAAT** result for the presence of betacoronavirus, and COVID-19 virus further identified by **sequencing** partial or whole genome of the virus as long as the sequence target is larger or different from the amplicon probed in the NAAT assay used

Concept of Threshold and Ct Value



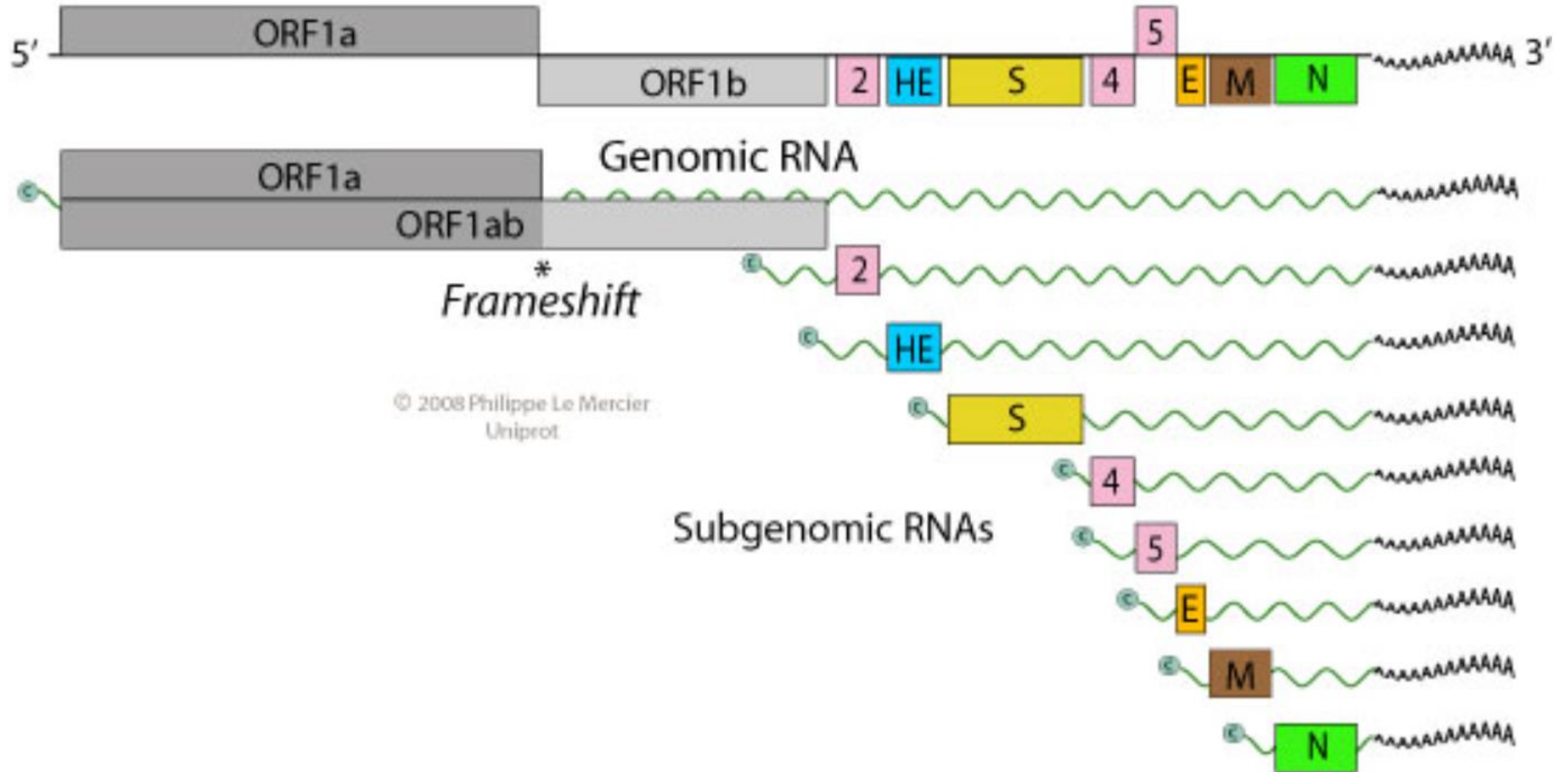


Correlation Coefficient: 0.999 Slope: -3.488 Intercept: 39.204 $Y = -3.488 X + 39.204$

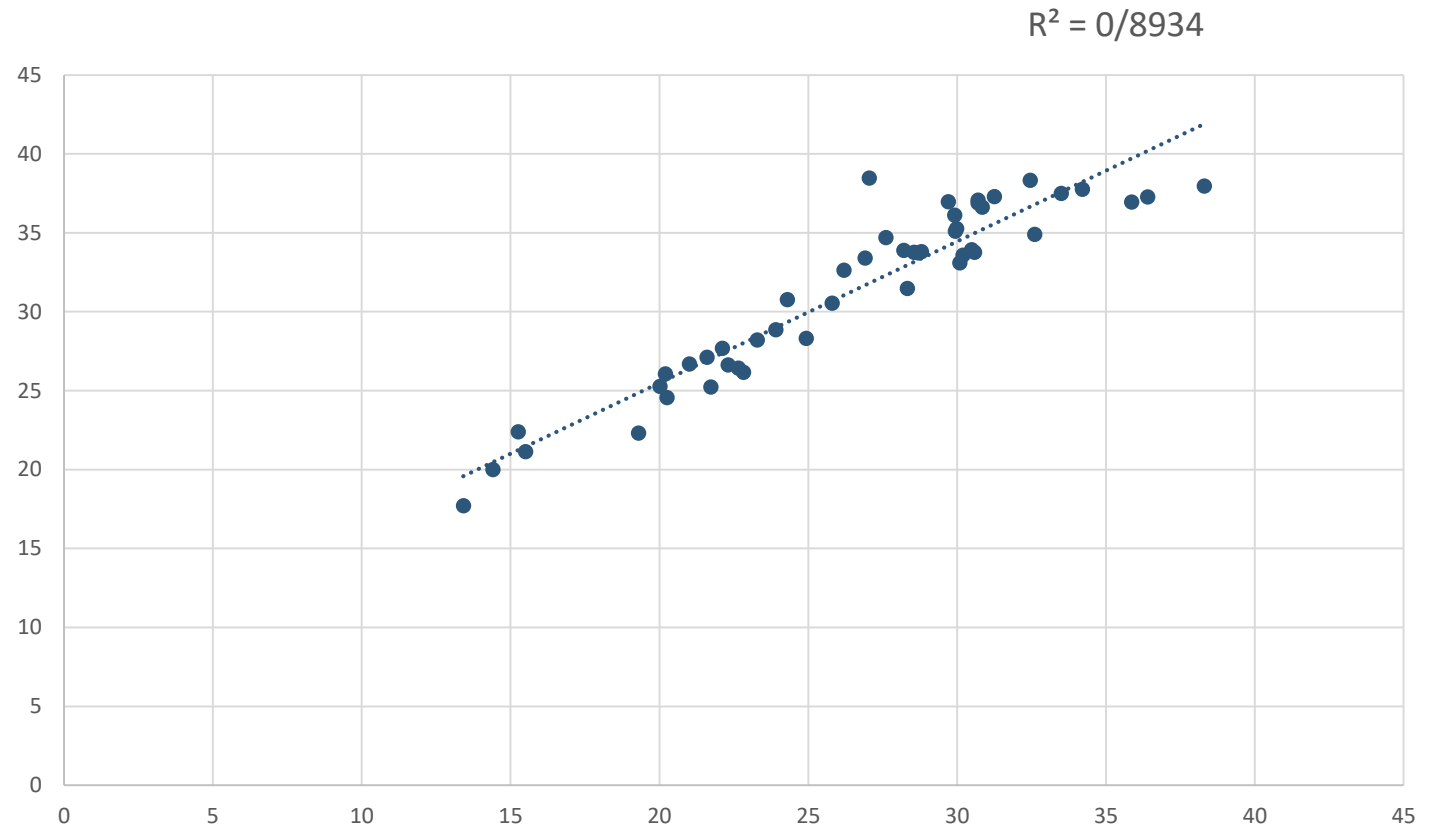
Unknowns
Standards



PCR Standard Curve: Data 27-Jan-03 1233ileff.opd



sample code	E gene	RdRp gene
113	28.32	31.47
114	27.05	38.47
117	35.92	ND
119	28.55	33.76
120	28.21	33.89
123	31.25	37.28
125	32.3	ND
126	29.98	35.27
127	36.4	37.26
128	26.2	32.63
129	34.2	37.76
130	38.3	37.95
131	30.7	37.06
132	28.7	33.72
133	25.8	30.53
135	30.84	36.61
137	22.3	26.63
138	21.6	27.12
140	29.7	36.96
142	33.5	37.5
143	33.79	ND
144	35	ND
147	32.45	38.32
148	15.25	22.38
149	33.3	ND
150	20.2	26.05
151	35	ND
156	26.9	33.39
157	32.3	ND
158	14.4	20
160	21	26.69



SENSITIVITY

$$\text{Sensitivity} = P[+ \text{ test} \mid + \text{ disease}] = TP / (TP + FN)$$

		DISEASE STATUS	
		+	-
TEST RESULT	+	TP	FP
	-	FN	TN

SENSITIVITY

$$\text{Sensitivity} = P[+ \text{ test} \mid + \text{ disease}] = \text{TP} / (\text{TP} + \text{FN})$$

		DISEASE STATUS	
		+	-
TEST RESULT	+	75	2
	-	25	98

Real time PCR:
Sensitivity : 75%
Specificity: 98%

Positive Predictive Value : 0.1% prevalence

$$PPV = P[+ \text{ disease} \mid + \text{ test}] = TP / (TP + FP)$$

		DISEASE STATUS	
		+	-
TEST RESULT	+	75	2000
	-	25	98000

$$PPV = 75/2075$$

Positive Predictive Value : 25% prevalence

$$PPV = P[+ \text{ disease} \mid + \text{ test}] = TP / (TP + FP)$$

		DISEASE STATUS	
		+	-
TEST RESULT	+	75	6
	-	25	294

$$PPV = 75/81$$

WHO stand on how many genes to test (as of March 19):

- In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which, for example, screening by rRT-PCR of a single discriminatory target is considered sufficient
- However, since now we have moved to broader screening and testing, now we prefer 2 target multiplex tests.

Serological tests

- These tests detect the human immunologic responses against the virus:
 - IgM
 - IgG
 - IgA
- Target antigen ?
 - N
 - S
 - ...
- ELISA is the method of choice.
- Rapid tests

NEWS • 21 APRIL 2020

The researchers taking a gamble with antibody tests for coronavirus

Despite uncertainties, some scientists are betting that blood tests will help end lockdowns and get people back to work.

Amy Maxmen



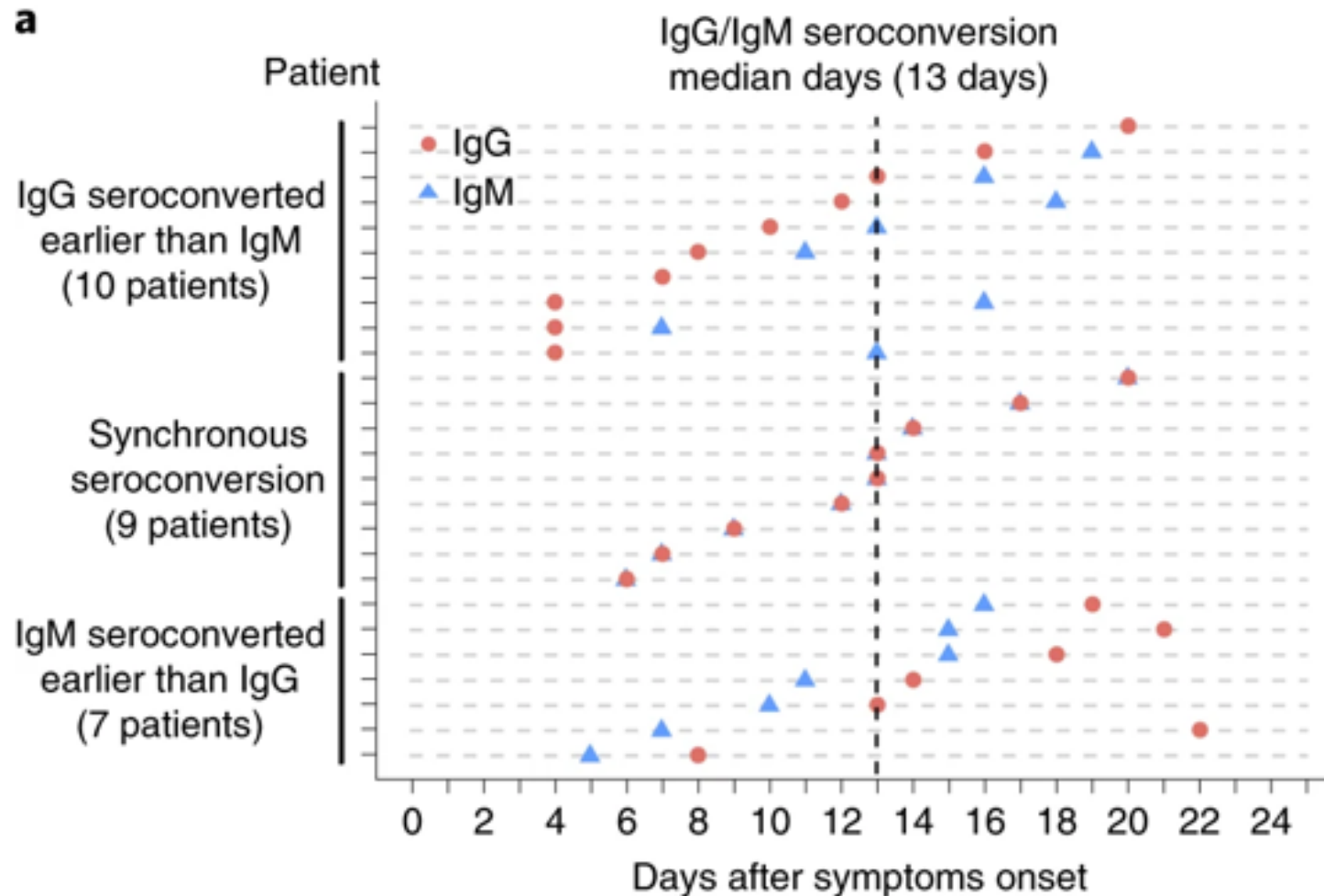
Our evaluations on ELISA kits

- Sensitivity:
 - about 80% in hospitalized patients
 - Less in outpatients
- Specificity:
 - More than 90%
- Indication of use is under evaluation:
 - Epidemiological surveys
 - Health care workers
 - Plasmapheresis?

Where WHO stands about serologic tests

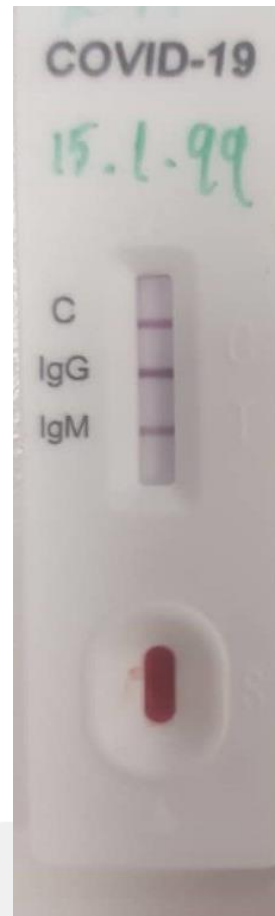
- Serological surveys can aid investigation of an ongoing outbreak and retrospective assessment of the attack rate or extent of an outbreak. In cases where NAAT assays are negative and there is a strong epidemiological link to COVID-19 infection, paired serum samples (in the acute and convalescent phase) could support diagnosis once validated serology tests are available. Serum samples can be stored for these purposes.

From: Antibody responses to SARS-CoV-2 in patients with COVID-



Long et al 2020 Nature Medicine
<https://www.nature.com/articles/s41591-020-0897-1>

Our Evaluations on Rapid tests



Our Evaluations on Rapid tests

- Sensitivity:
 - More than 70% in hospitalized patients
 - About 50% in outpatients
- Specificity: more than 90%
- Indication of use?

Position of WHO on rapid serologic tests

- Based on current data, **WHO does not recommend the use of antibody-detecting rapid diagnostic tests for patient care but encourages the continuation of work to establish their usefulness in disease surveillance and [epidemiologic research](#).**

Stand of US-FDA on serologic tests

- As stated in Section IV.D of the FDA's [*Policy for Diagnostic Tests for Coronavirus Disease-2019*](#), the FDA does not intend to object to the development and distribution by commercial manufacturers, or development and use by laboratories, of serology tests to identify antibodies to SARS-CoV-2, where the test has been validated, notification is provided to FDA,

- and information along the lines of the following is included in the test reports:
 - **This test has not been reviewed by the FDA.**
 - Negative results do not rule out SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
 - Results from antibody testing should not be used as the sole basis to diagnose or exclude SARS-CoV-2 infection or to inform infection status.
 - Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

Rapid tests

- Immunologic based:
 - Ab
 - Ag
- Molecular based
 - Isothermal Amplification

Future challenges for the labs

- Dimensions depend on the needs of health system
- Returning to work criteria are a current question.
- Other methods of scaling up the testing service are being investigated:
 - Pooling samples
- New technologies are being developed.
- Sample collection is still a big challenge.
- Data collection and LIS is still a major challenge
- Logistics

Thank you for your attention

MSTF Laboratory Network



www.intlabsnet.com

info@intlabsnet.com