Laboratory Diagnosis of COVID19

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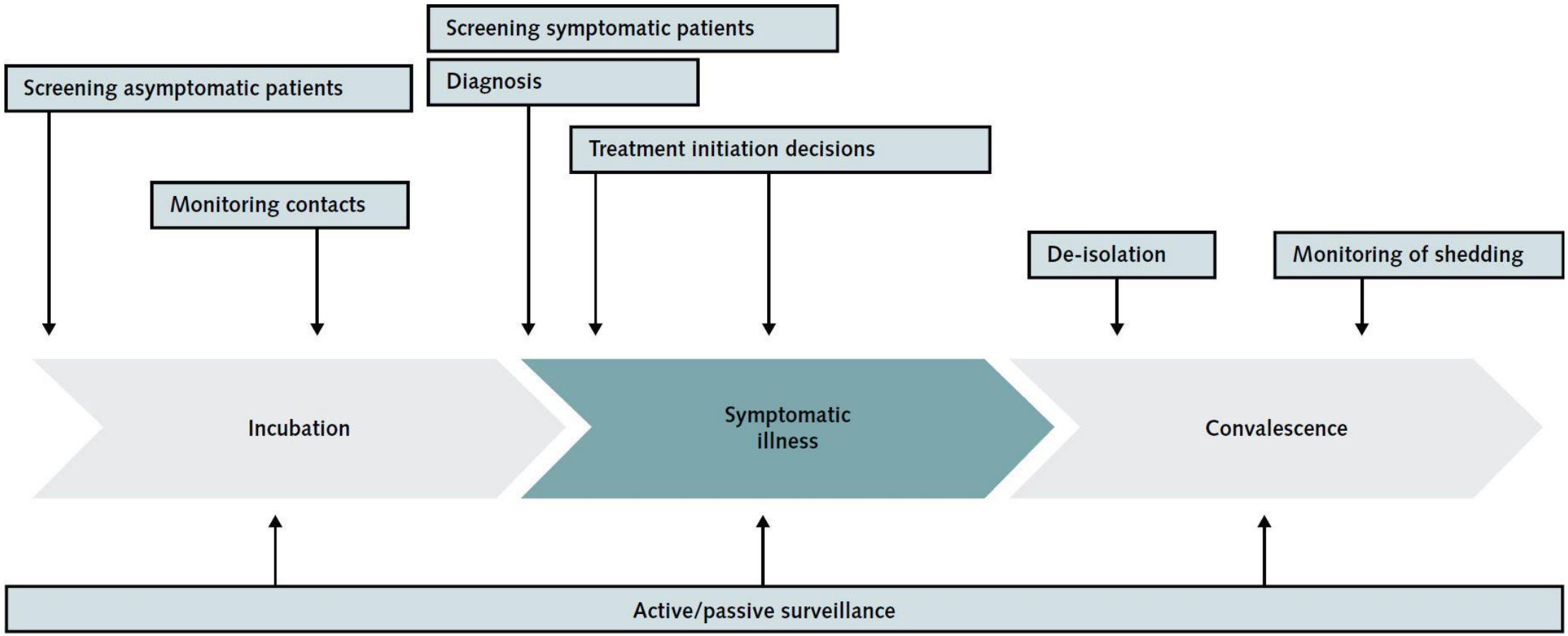
Screening symptomatic patients
Screening asymptomatic patients
Diagnosis
Treatment initiation decisions
Monitoring contacts
De-isolation
Monitoring of shedding
Incubation
Symptomatic illness
Convalescence
Active/passive surveillance

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for Wuhan virus will be provided shortly.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay
Additional confirmatory assay: N gene assay

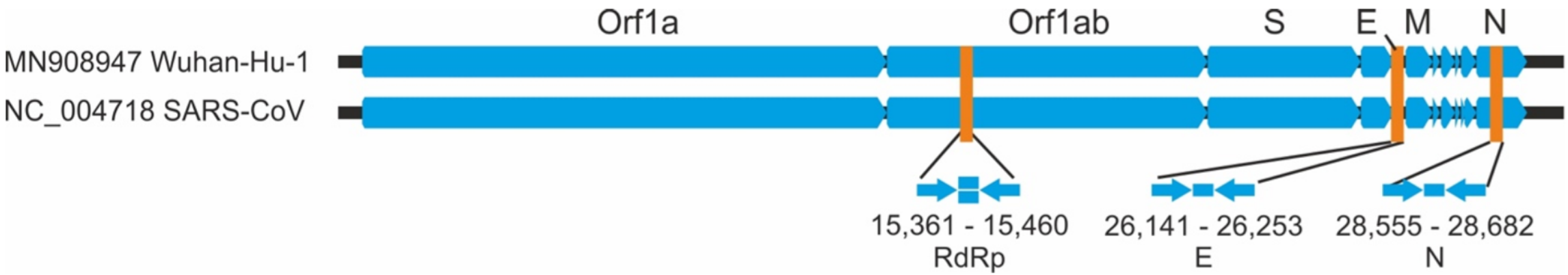

Figure 1 relative positions of amplicon targets on SARS-CoV ad Wuhan-CoV genome. N: nucleocapsid; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

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

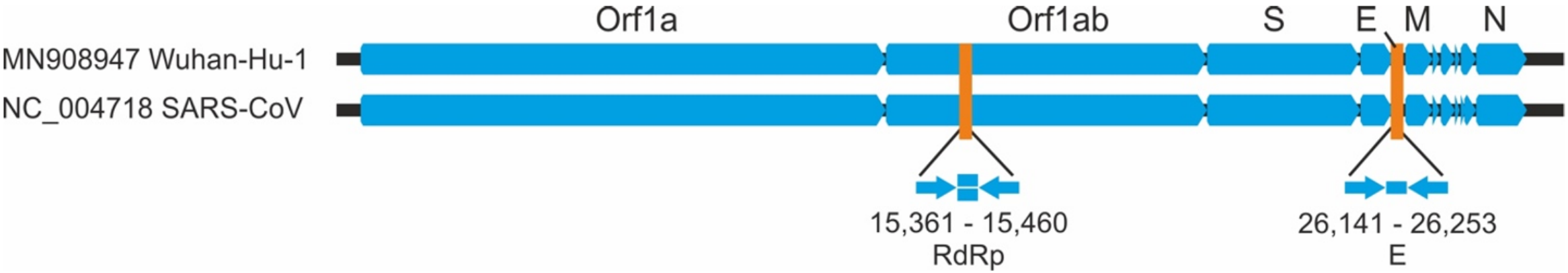

Figure 1 relative positions of amplicon targets on SARS-CoV an 2019-nCoV genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718.

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

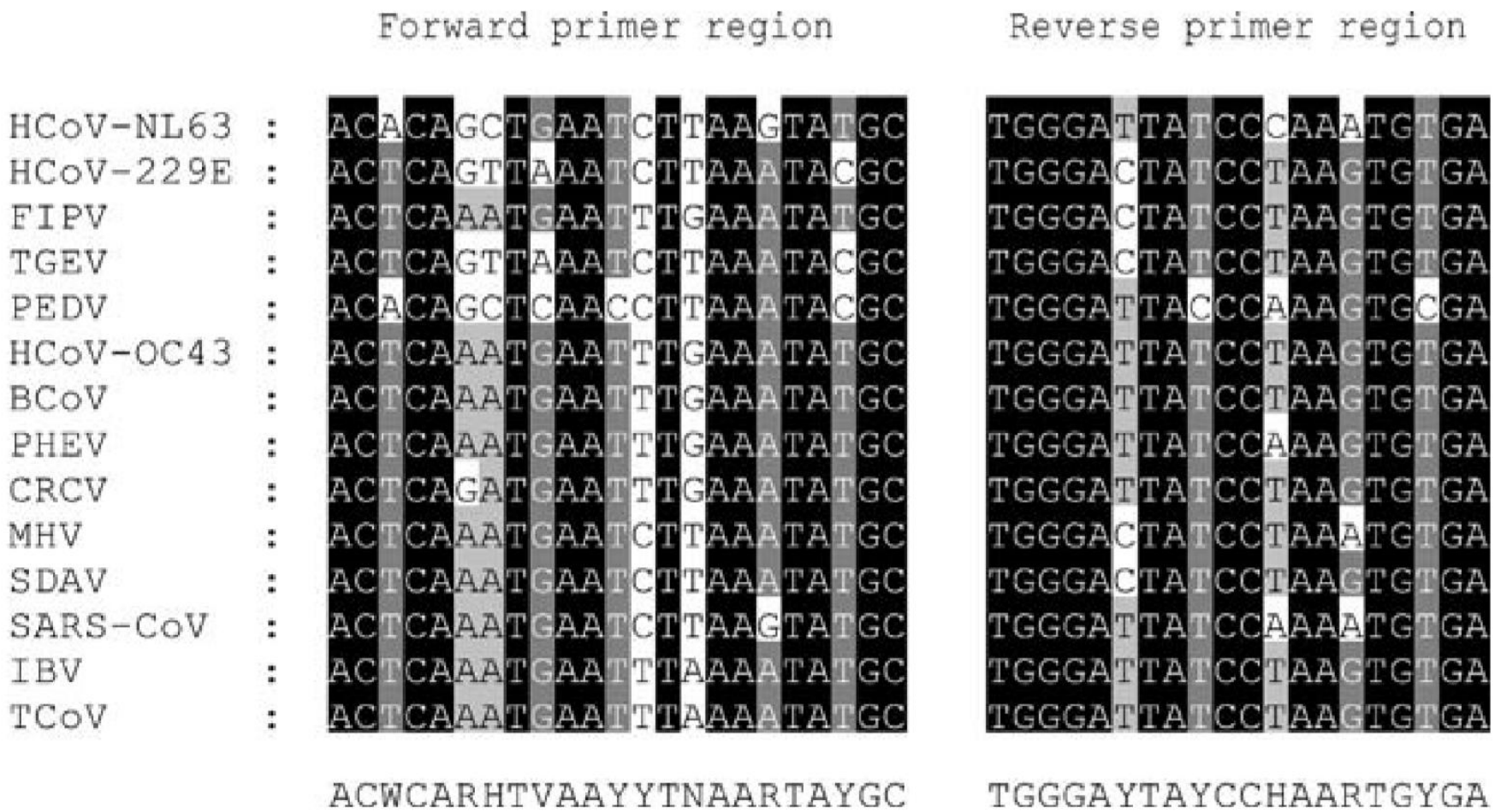

Fig. 1. Selection of primers for the novel pancoronavirus RT-PCR. Shown is the alignment of 14 coronaviral sequences of a conserved region of the polymerase gene. The forward (Cor-FW) and reverse (Cor-RV) primer sequences are shown at the bottom (Y=C/T, W=A/T, V=A/C/G, R=A/G, H=A/T/C, N=A/C/T/G).

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Avian Coronavirus in Wild Aquatic Birds[▽]†‡

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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 WRCARAAYTCRTG-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

Download | Manage Columns | 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
Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-TX1/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106054.1
Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA8/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106053.1
Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA7/2020, complete genome	1020	1020	100%	0.0	100.00%	MT106052.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/WH-09/human/2020/CHN, complete ge	1020	1020	100%	0.0	100.00%	MT093631.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/01/human/2020/SWE, complete genon	1020	1020	100%	0.0	100.00%	MT093571.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS0CoV-2/61-TW/human/2020/ NPL, complete g	1020	1020	100%	0.0	100.00%	MT072688.1
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Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/Yunnan-01/human/2020/CHN, complet	1020	1020	100%	0.0	100.00%	MT049951.1
Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-CA6/2020, complete genome	1020	1020	100%	0.0	100.00%	MT044258.1
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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Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-WI1/2020, complete genome	1020	1020	100%	0.0	100.00%	MT039887.1
Severe acute respiratory syndrome coronavirus 2 isolate HZ-1, complete genome	1020	1020	100%	0.0	100.00%	MT039873.1
Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-521/2020 RNA, complete genom	1020	1020	100%	0.0	100.00%	LC522975.1
Severe acute respiratory syndrome coronavirus 2 2019-nCoV/Japan/TY/WK-501/2020 RNA, complete genom	1020	1020	100%	0.0	100.0	

Severe acute respiratory syndrome coronavirus 2 isolate 2019-nCoV/USA-TX1/2020, complete genome

Sequence ID: MT106054.1 **Length:** 29882 **Number of Matches:** 1

Range 1: 15334 to 15885 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1020 bits(552)	0.0	552/552(100%)	0/552(0%)	Plus/Plus

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Sbjct  15334  ATTATGGCCTCACTTGTTCTTGCTCGCAAACATACAACGTGTTGTAGCTTGTCACACCGT  15393

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Query  121    TCACTATATGTTAAACCAGGTGGAACCTCATCAGGAGATGCCACAACTGCTTATGCTAAT  180
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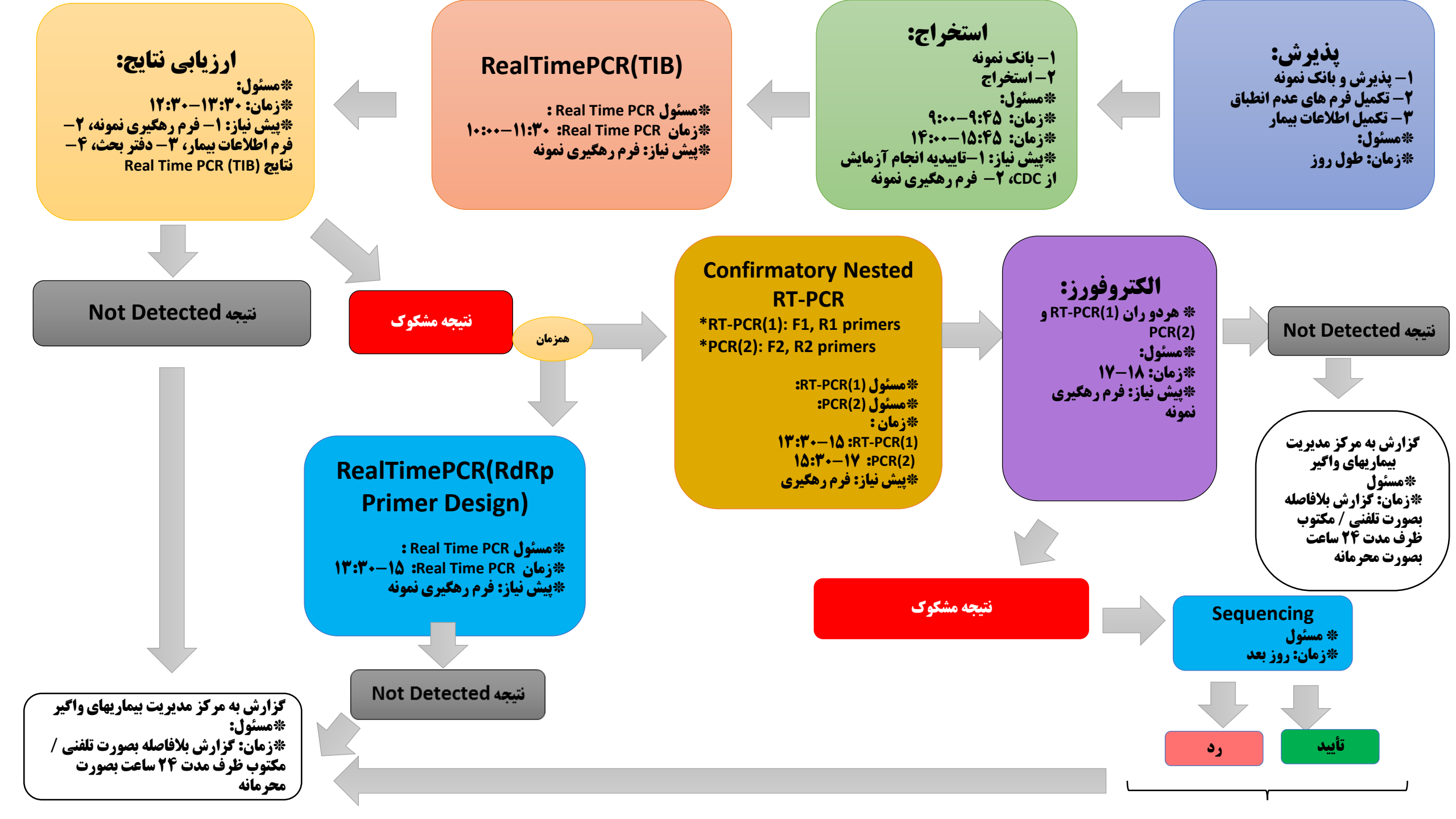
پذیرش:
۱– پذیرش و بانک نمونه
۲– تکمیل فرم های عدم انطباق
۳– تکمیل اطلاعات بیمار
*مسئول:
*زمان: طول روز
استخراج:
۱– بانک نمونه
۲– استخراج
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*پیش نیاز: ۱–تاییدیه انجام آزمایش از CDC، ۲– فرم رهگیری نمونه
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*زمان Real Time PCR: ۱۰:۰۰–۱۱:۳۰
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ارزیابی نتایج:
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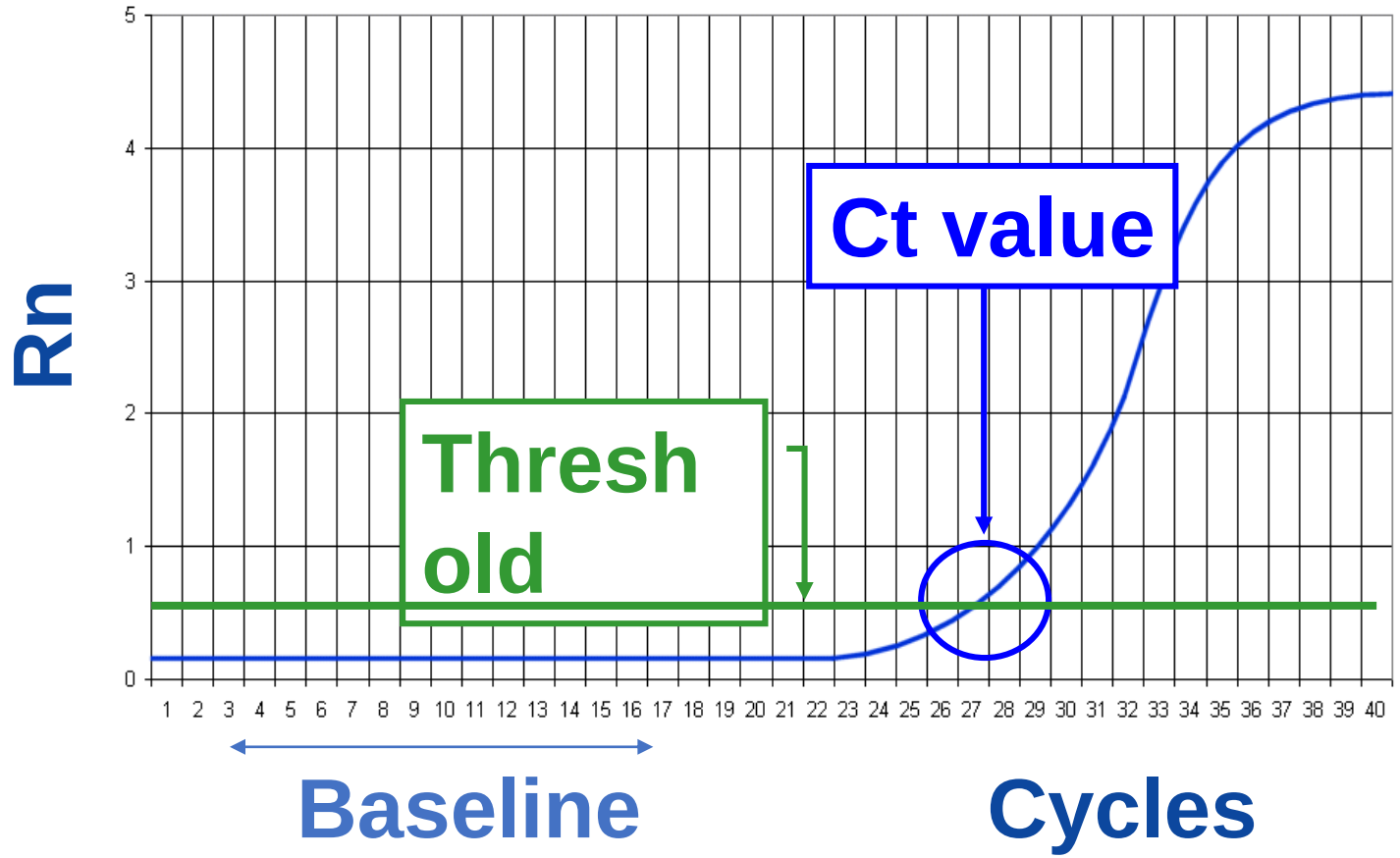
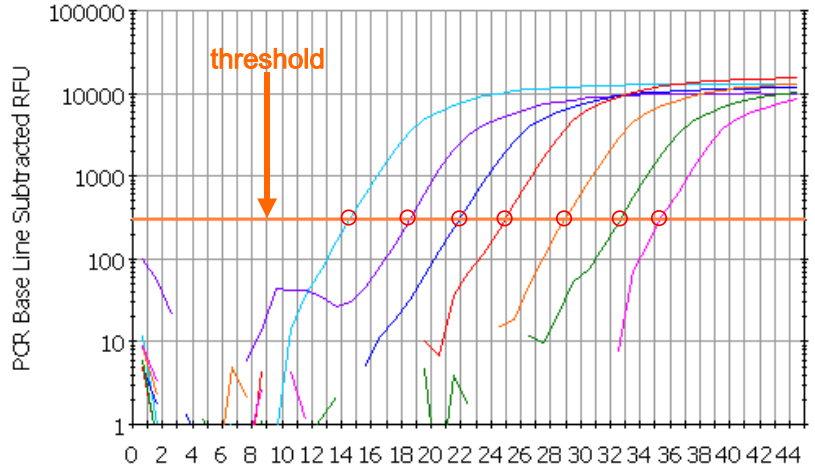
*زمان: ۱۲:۳۰–۱۳:۳۰
*پیش نیاز: ۱– فرم رهگیری نمونه، ۲– فرم اطلاعات بیمار، ۳– دفتر بحث، ۴– نتایج Real Time PCR (TIB)
Not Detected نتیجه
نتیجه مشکوک
همزمان
Confirmatory Nested RT-PCR
*RT-PCR(1): F1, R1 primers
*PCR(2): F2, R2 primers
*مسئول RT-PCR(1):
*مسئول PCR(2):
*زمان :
RT-PCR(1): ۱۳:۳۰–۱۵
PCR(2): ۱۵:۳۰–۱۷
*پیش نیاز: فرم رهگیری
الکتروفورز:
* هردو ران RT-PCR(1) و PCR(2)
*مسئول:
*زمان: ۱۷–۱۸
*پیش نیاز: فرم رهگیری نمونه
Not Detected نتیجه
گزارش به مرکز مدیریت بیماریهای واگیر
*مسئول
*زمان: گزارش بلافاصله بصورت تلفنی / مکتوب ظرف مدت ۲۴ ساعت بصورت محرمانه
RealTimePCR(RdRp Primer Design)
*مسئول Real Time PCR :
*زمان Real Time PCR: ۱۳:۳۰–۱۵
*پیش نیاز: فرم رهگیری نمونه
Not Detected نتیجه
نتیجه مشکوک
Sequencing
* مسئول
*زمان: روز بعد
رد
تأیید
گزارش به مرکز مدیریت بیماریهای واگیر
*مسئول:
*زمان: گزارش بلافاصله بصورت تلفنی / مکتوب ظرف مدت ۲۴ ساعت بصورت محرمانه

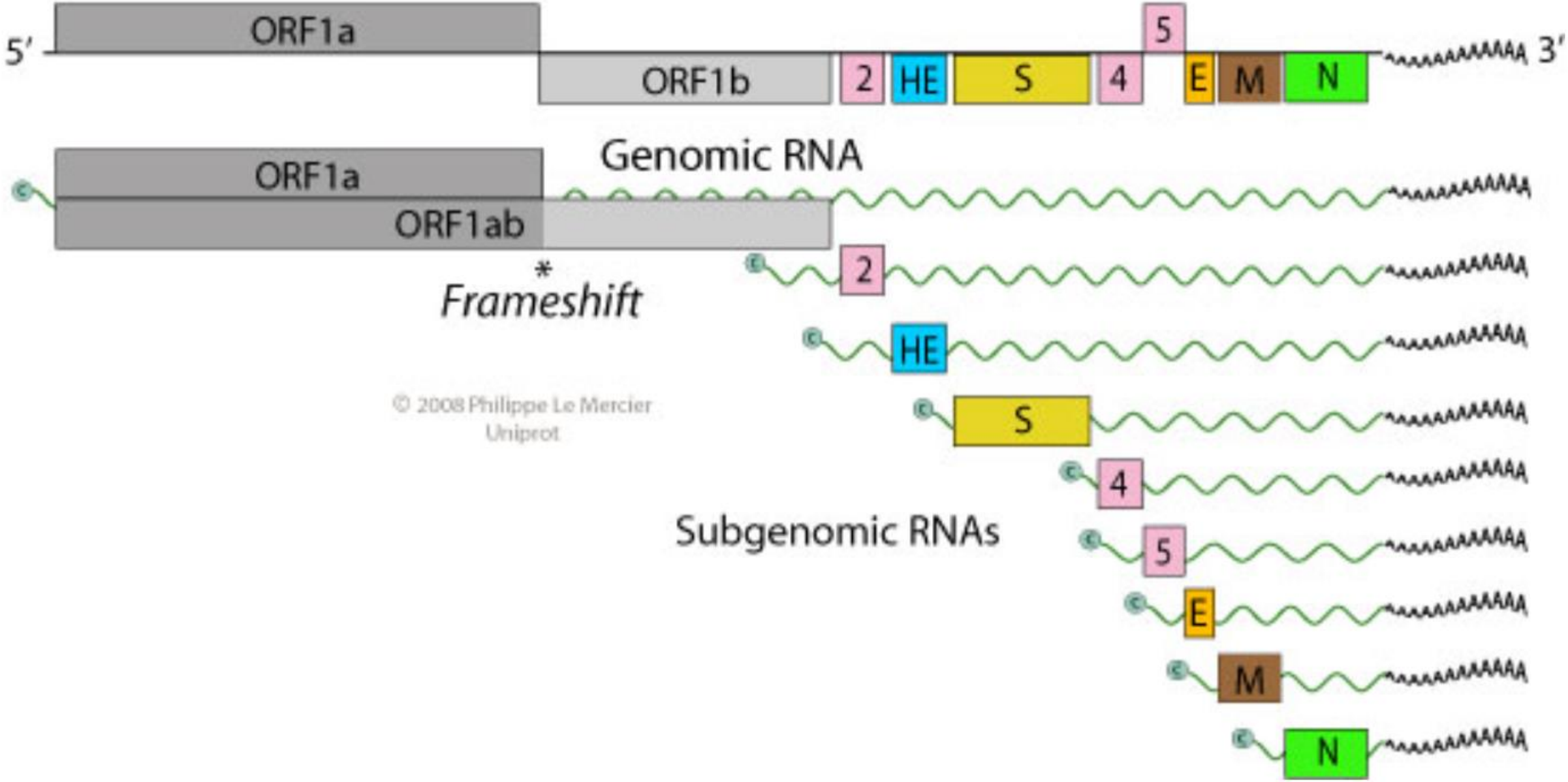
Concept of Threshold and Ct Value





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

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


5′
ORF1a
ORF1b
2
HE
S
4
5
E
M
N
3′
Genomic RNA
ORF1a
ORF1ab
*
Frameshift
© 2008 Philippe Le Mercier
Uniprot
2
HE
S
4
Subgenomic RNAs
5
E
M
N

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
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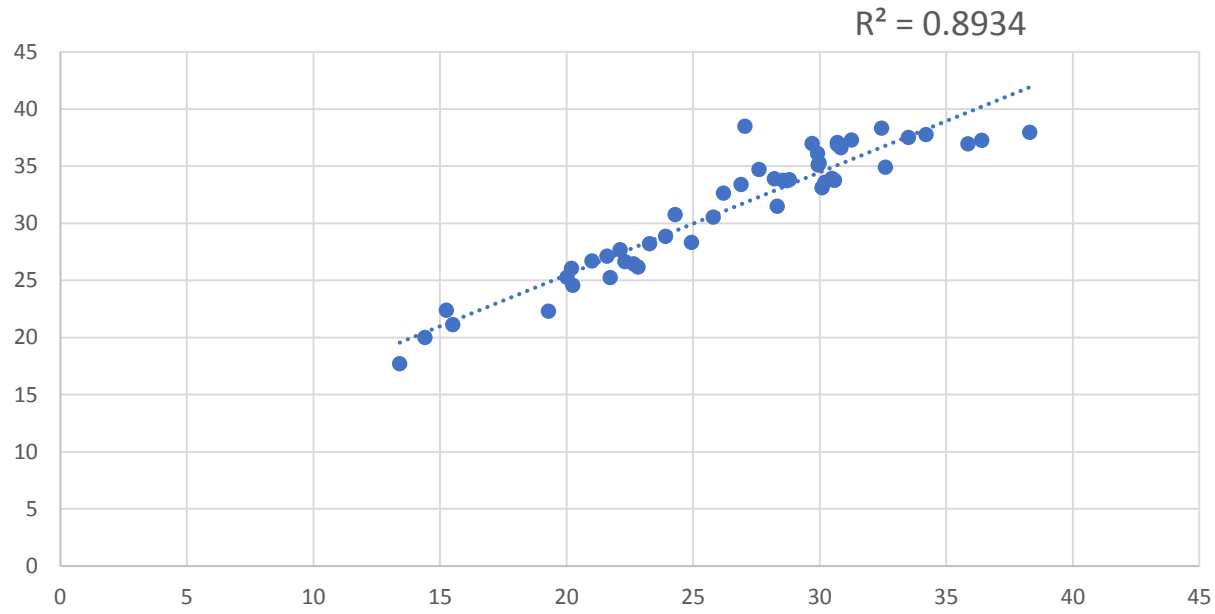
151	35	ND
156	26.9	33.39
157	32.3	ND
158	14.4	20



SENSITIVITY

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

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

SENSITIVITY

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

TEST RESULT	DISEASE STATUS +	DISEASE STATUS -
+	995	9
-	5	991

Positive Predictive Value : 0.1% prevalence

PPV=P[+ disease | + test]= TP / (TP+FP)

TEST RESULT	DISEASE STATUS +	DISEASE STATUS -
+	995	9000
-	5	991000

PPV= 995/9995

Positive Predictive Value : 10% prevalence

PPV=P[+ disease | + test]= TP / (TP+FP)

TEST RESULT	DISEASE STATUS +	DISEASE STATUS -
+	995	90
-	5	9910

PPV= 995/1085

WHO position on how many genes to test-2 (as of March 19):

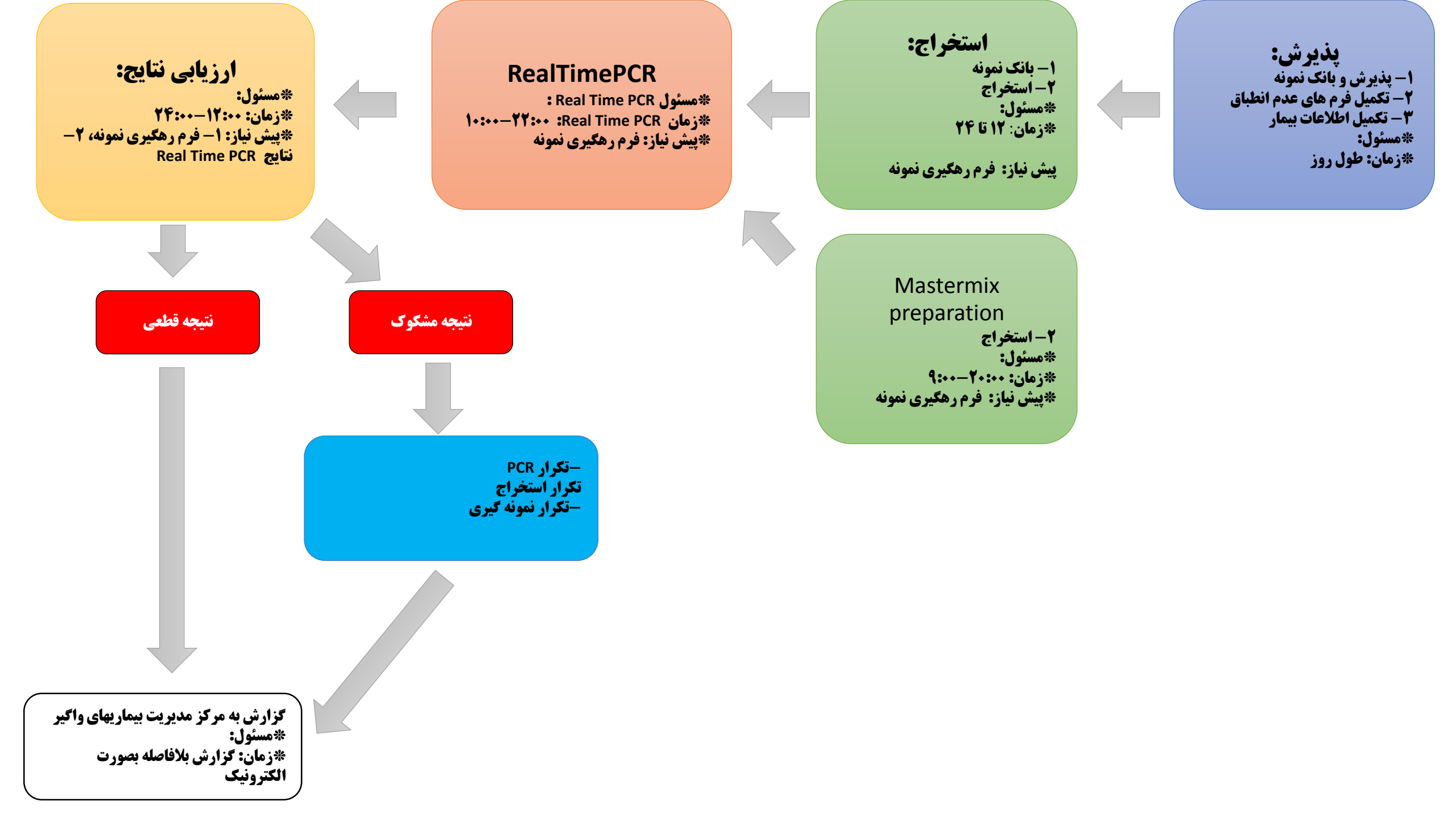
- Laboratory-confirmed case by NAAT in areas with established COVID-19 virus circulation:
- In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which, for example, screening by rRT-PCR of a single discriminatory target is considered sufficient

The US-FDA position on how many genes to test: (as of April 21)

- Based on evidence that has become recently available, and with the increased spread of COVID-19, FDA believes an appropriately validated *single* viral target SARS-CoV-2 assay could provide acceptable performance.

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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۲– استخراج
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*زمان: ۱۲ تا ۲۴
پیش نیاز: فرم رهگیری نمونه
RealTimePCR
*مسئول Real Time PCR :
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ارزیابی نتایج:
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*پیش نیاز: ۱– فرم رهگیری نمونه، ۲– نتایج Real Time PCR
Mastermix preparation
۲– استخراج
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*پیش نیاز: فرم رهگیری نمونه
نتیجه مشکوک
نتیجه قطعی
–تکرار PCR
تکرار استخراج
–تکرار نمونه گیری
گزارش به مرکز مدیریت بیماریهای واگیر
*مسئول:
*زمان: گزارش بلافاصله بصورت الکترونیک

Serological tests

- These tests detect the human immunologic responses against the virus:
  - IgM
  - IgG

- 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

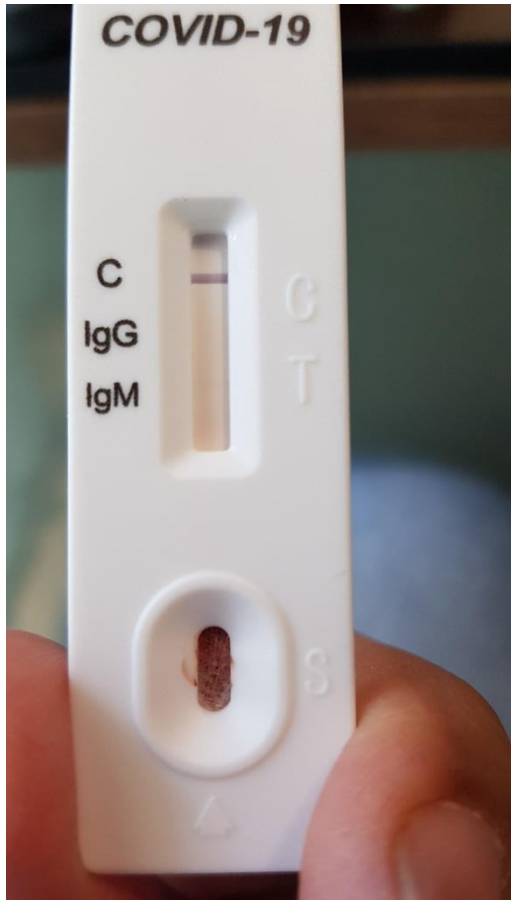
Our evaluations on ELISA kits

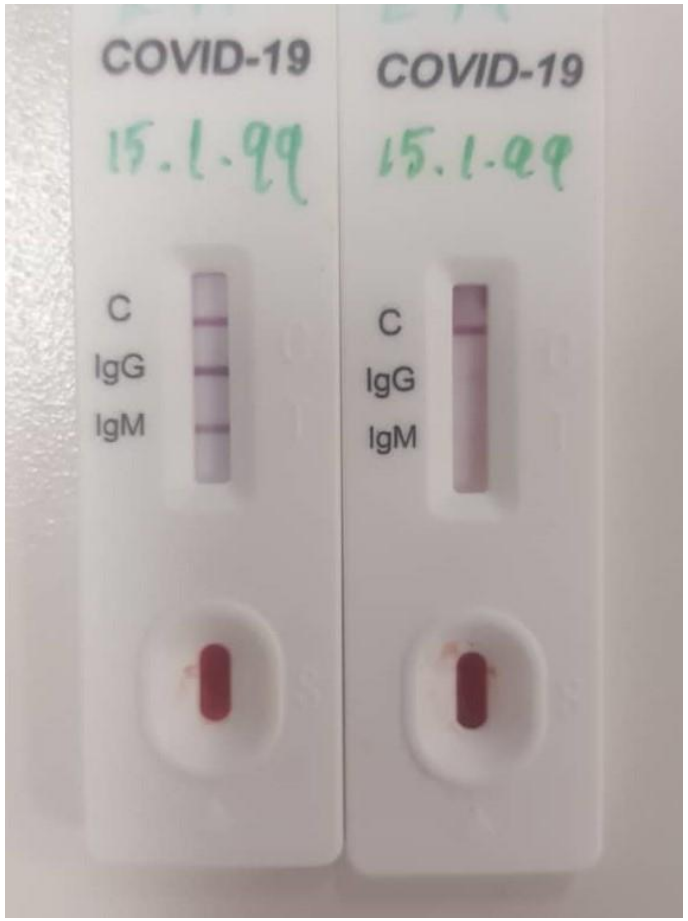
- Sensitivity:
  - about 80% in hospitalized patients
  - Less in outpatients
- Specificity:
  - More than 90%
- Indication of use is under evaluation:
  - Epidemiological surveys
  - Health care workers
  - Plasmapheresis?

Position of WHO on 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.

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**.

Position 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

Tests for SARS-CoV-2/COVID-19 and Potential Uses

Type of Test	Measure	Value	Beneficiary
Nucleic acid amplification test for viral RNA *(nasopharyngeal swab, oropharyngeal swab, sputum, bronchoalveolar lavage fluid, others)*	Current infection with SARS-CoV-2	• Inform individual of infection status so they can anticipate course of illness and take action to prevent transmission • Inform patient management and actions needed to prevent transmission • Inform actions needed to prevent transmission	• Individual • Healthcare or long-term care facility • Public health
Antibody detection	Past exposure to SARS-CoV-2	• Detect susceptible individuals (antibody negative) and those previously infected • Identify individuals with neutralizing antibodies • Facilitate contact tracing and surveillance	• Identify those potentially immune to SARS-CoV-2 (if tests can detect protective immunity, individuals could be returned to work) • Healthcare facilities: Experimental therapy • Public health

Robin Patel et al. mBio 2020; doi:10.1128/mBio.00722-20

The Laboratory Scale up

- Establishing a national laboratory network to provide technical and reagent support, standardization of protocols and collect the results nationally
  - A reference Lab (Pasteur Institute of Iran)
  - More than 100 labs currently active
  - The required testing capacity was estimated to be around 100 tests/day/million people for the first phase (mostly hospitalized patients)
  - The required testing capacity is estimated to be around 250 tests/day/million people for the public health and hospital-based interventions
  - Private medical labs

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.
- Logistics