

# Results Analysis and Report Interpretation

**Zuyuan Xu PhD  
BGI Research Institute  
October 19, 2020**





# Contents

01

Overview of RT-PCR in SARS-CoV-2 Test

02

Key Parameters Used for Data Analysis

03

Report Interpretation

04

FAQ on Report Interpretation



# 01

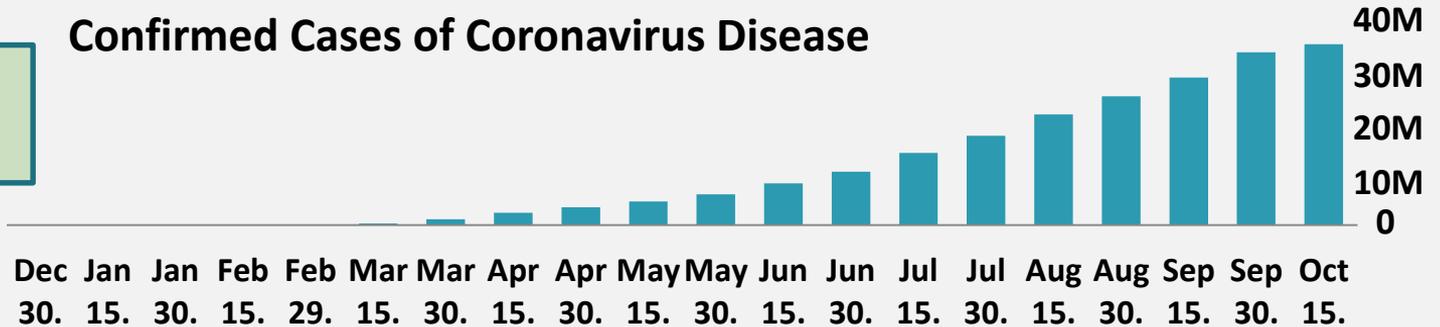
## Overview of RT-PCR in SARS-CoV-2 Test

# Number at a glance of COVID-19

**39,196,259**

Confirmed cases

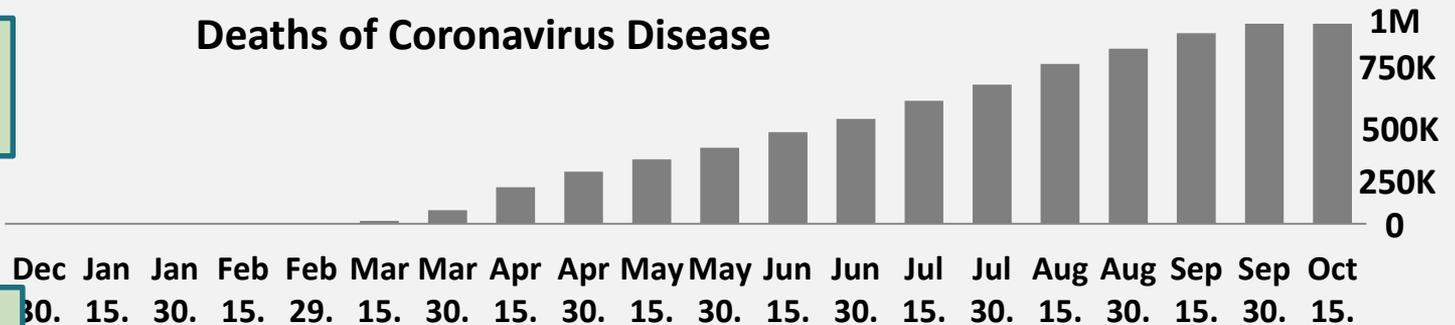
## Confirmed Cases of Coronavirus Disease



**1,101,298**

Deaths

## Deaths of Coronavirus Disease

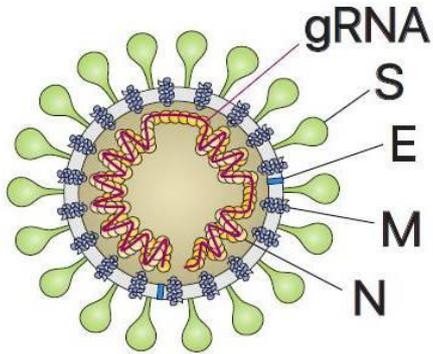
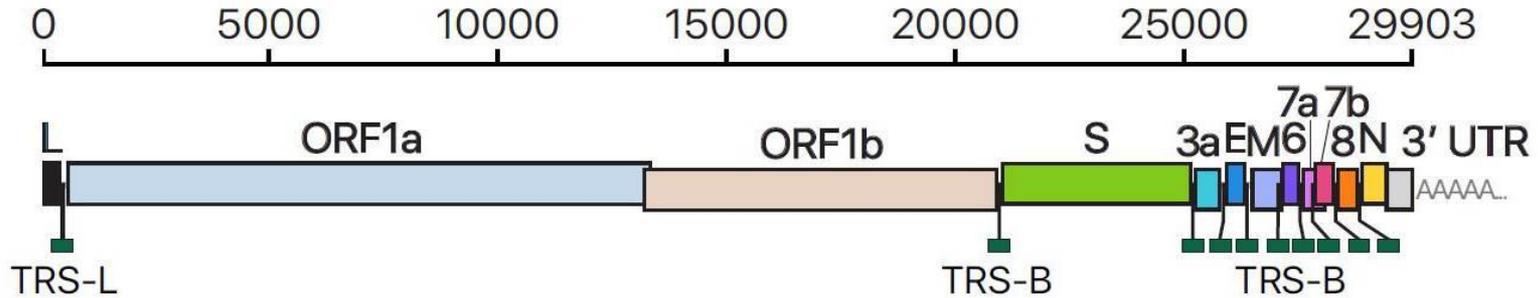


**235**

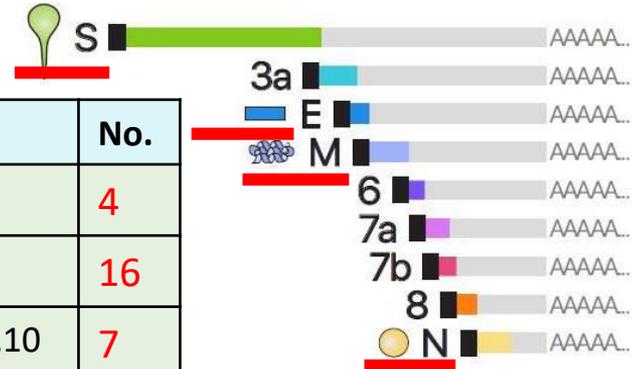
Countries, areas, or territories with cases

<https://covid19.who.int/>

# Schematic presentation of the SARS-CoV-2 genome organization



Gene Name	No.
S,E,M,N	4
nsp1-16	16
ORF3a,6,7a,7b,8,9b,10	7



Taken from Kim et al., 2020

# In Vitro Diagnostics for COVID-19

## Two types of test for COVID-19

- Viral/ Diagnostic tests
  - molecular tests eg. RT-PCR
  - antigen tests
- Antibody / Serology tests



## Advantages for RT-PCR based test kit

- Gold standard
- LOD: about 100 copies/ml
- Sensitivity: close to 100%
- Specificity: 100%

- Available for robotic molecular platforms
- High throughput batch processing of clinical specimens

# Number of targets in RT-PCR based SARS-CoV-2 test kits

## Three types of test kit

- One-target kit
- Two-target kit
- Three-target kit

## Evaluation and assay validation

- LOD
- Specificity
- Sensitivity
- Accuracy

## PCR program

Step	Tem. ° C	Duration	Number of cycles
Reverse transcription	50° C	5-20 mins	1
Taq inhibitor inactivation	95° C	1-10 mins	1
Denaturation	95° C	15 sec	40x-45x
Anneal/ extension*	55- 60° C	30 sec	

\* Fluorescence data should be collected during the anneal step.

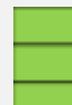
# Quality control and Plate setup

	1	2	3	4	5	6	7	8	9	10	11	12
A	■											
B	■											
C	■											
D												
E												
F												■
G												■
H												■



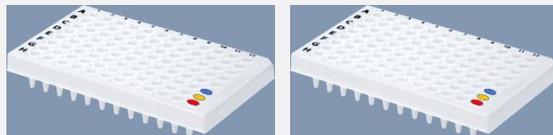
## PCR process QC

Positive control /PC, **F12**  
Negative control/ NC, **G12**  
Blank control/ NTC , **H12**



## RNA extraction QC/ Specimen preparation QC

Blank control  
Negative sample control  
Positive sample control



## Summary: RT-PCR in SARS-CoV-2 test

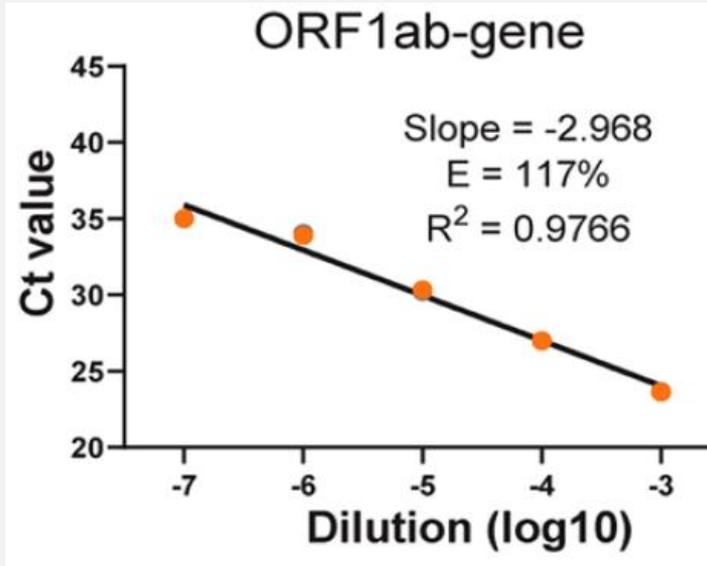
- Unique sequences from about 5/26 genes used in test reagents
- Gold standard
- Target number: one/ two/three
- Plate setup and quality control



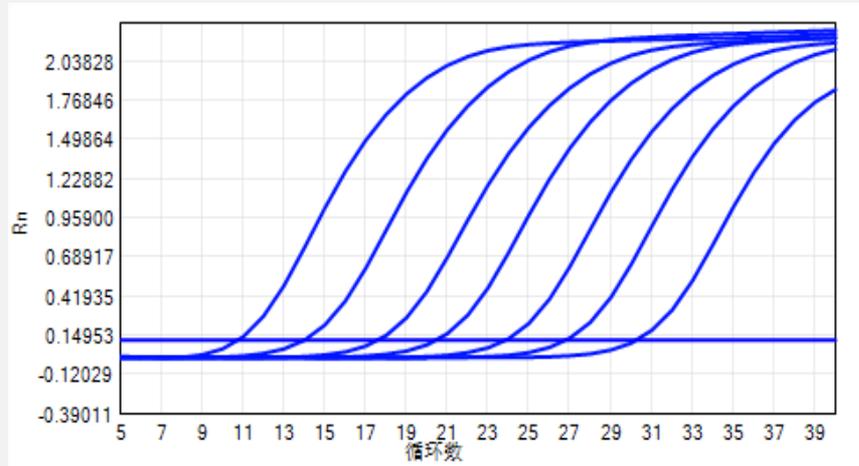
# 02

## Key Parameters Used for Data Analysis

# Standard Curve



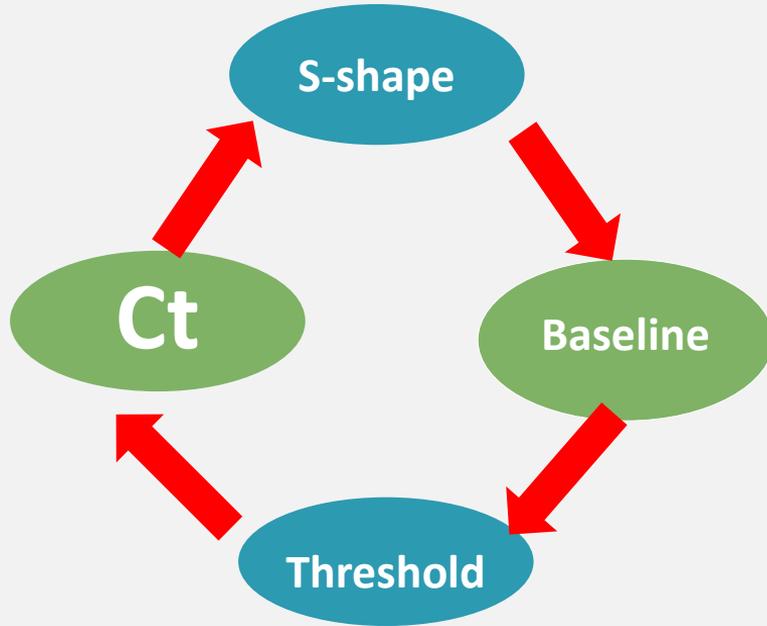
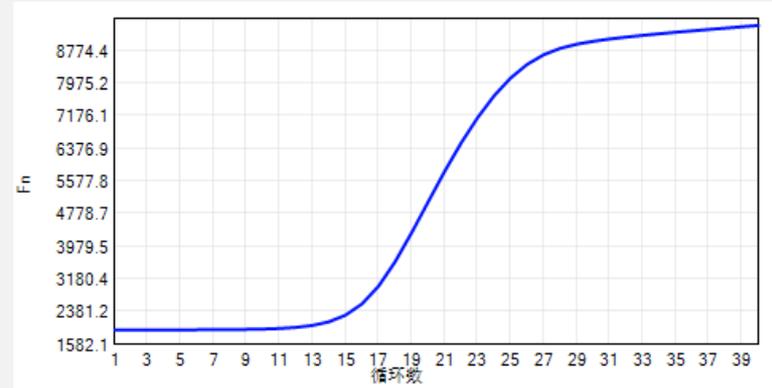
- Ct vs Log(DNA copies)



- Sample is diluted 10-fold 7-8 times

# Key Parameters used for Data Analysis

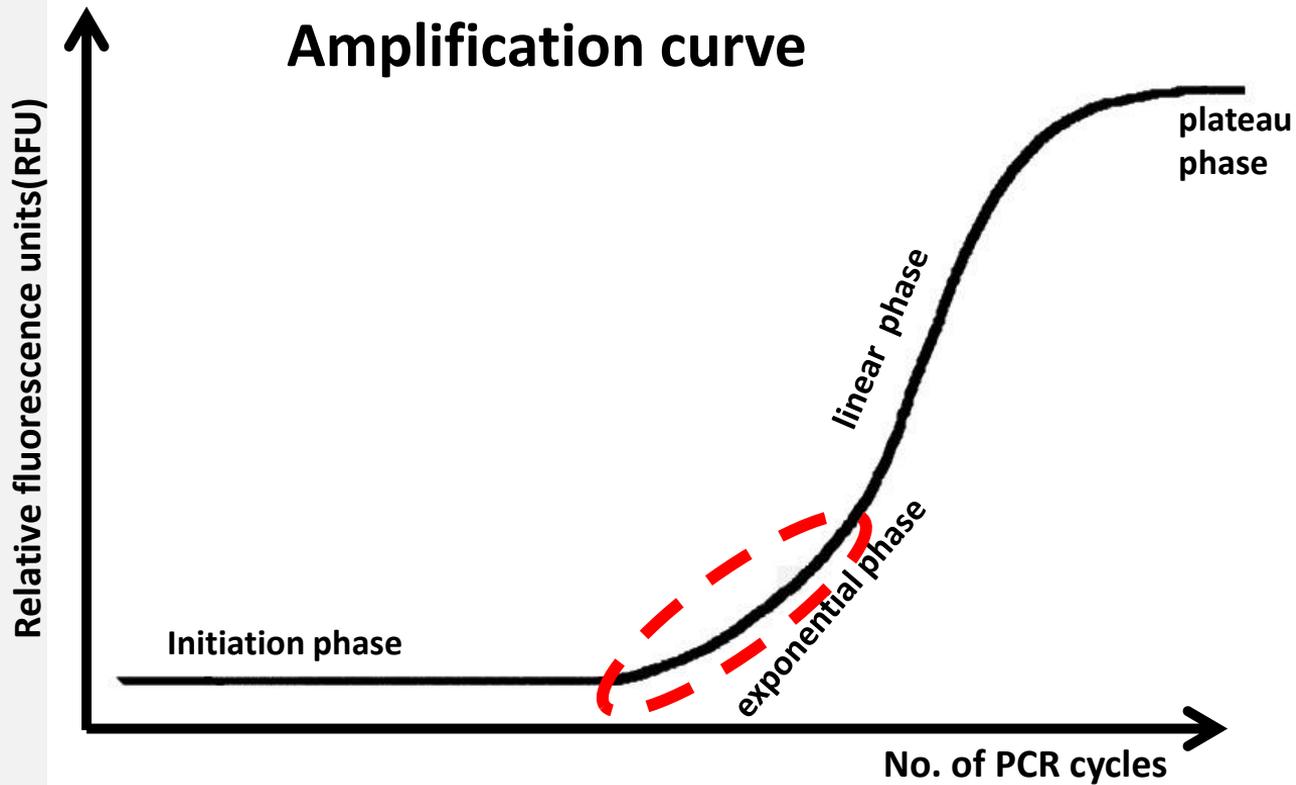
Linear mode



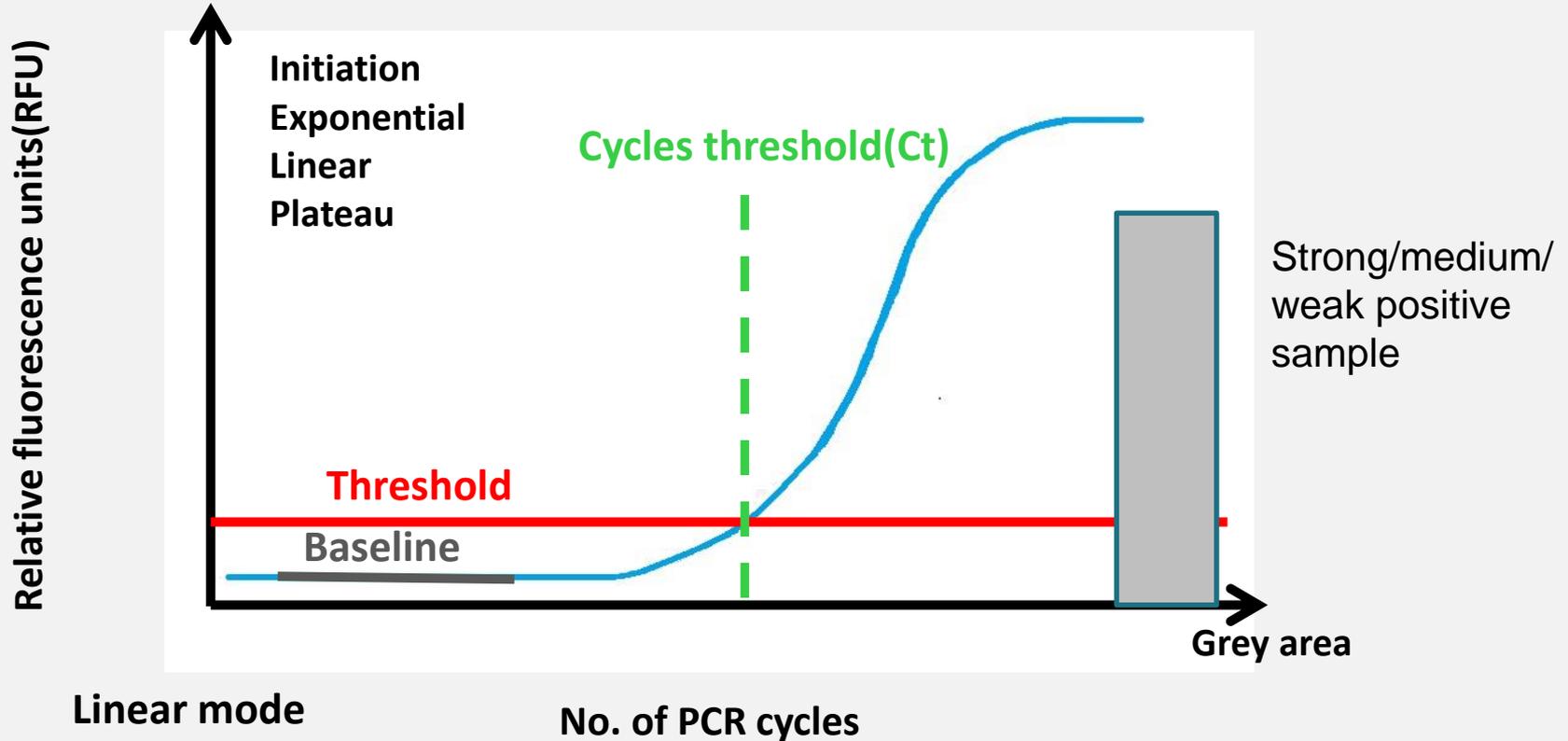
Quality Control

Internal QC

External QC



## Two lines ,Ct and Grey Area



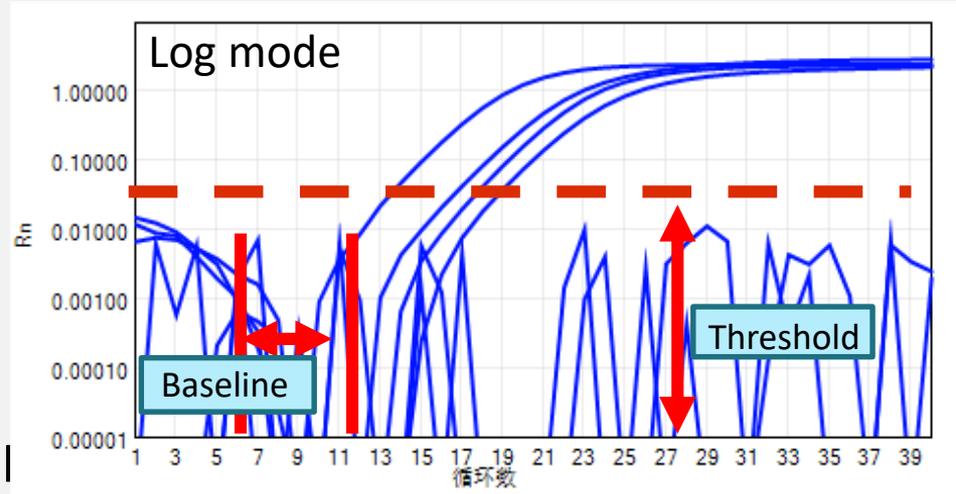
# Setting baseline and threshold

## Baseline

- for ABI7500 :  
cycle 3 -15
- for SLAN-96P :  
cycle 6 -12.

## Threshold

- Each fluorescent channel should be set separately.
- The blank control.
- Just above the maximum level of blank control curve.



## Summary: Key parameters

- Baseline
- Threshold line

Setting can be manual or automated

- Ct
- S-shape with Exponential phase

- External QC
- Internal QC in Specimen

Amplification plot of each specimen is different

They are different in each test kit



03

## Report Interpretation



Take time to look at data

Sample info, channel, Ct  
Blank control, positive control

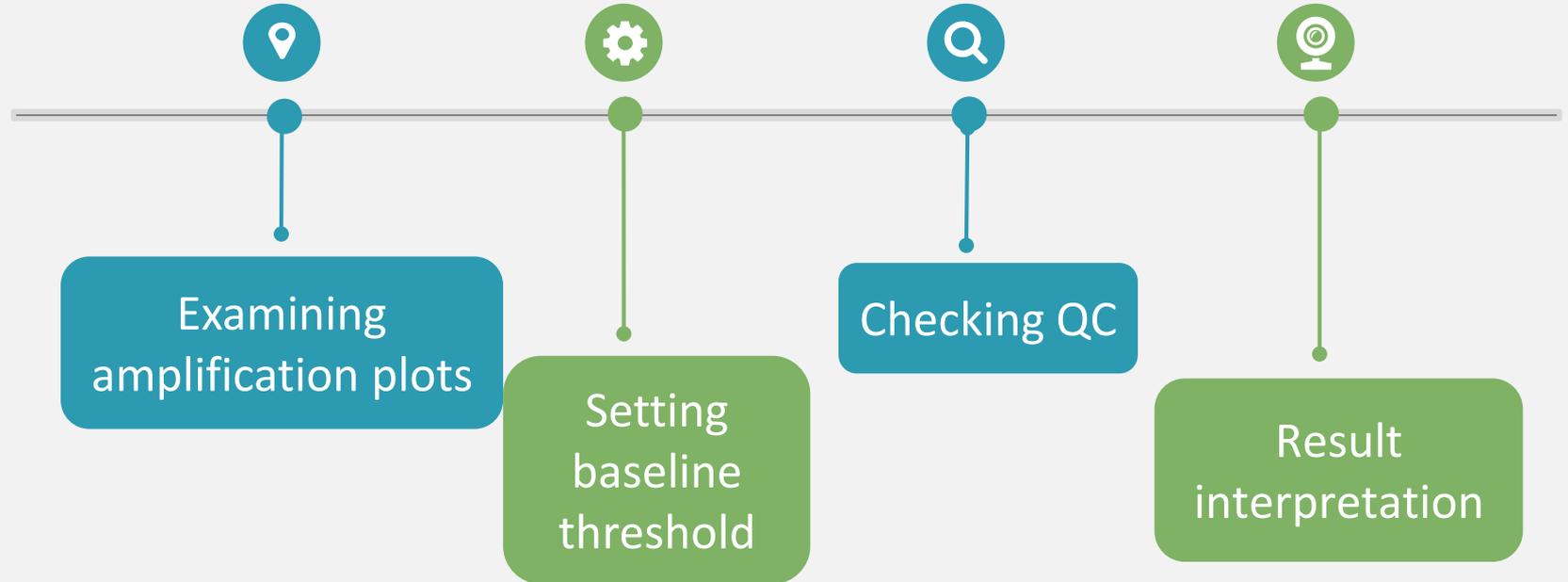
Project Name	SZ20200815-15					
Starting time	2020-08-15 22:19:22					
Ending time	2020-08-15 23:53:28					
Project type	Quantitative/Qualitative					
Tested by						
Checked by						
Remarks						
Mode A	Yes					
Mode B	Yes					
HotCover Use	Yes					
Temperature	Tem. Control mode					
Signal Scan	Signal, Scan					
Well Number	Pathogen	Specimen	Channel	Dye	Target	Ct
A1	SARS-CoV-2		1	FAM	FAM	39.02
A1	SARS-CoV-2		2	HEX	HEX	22.57
B1	SARS-CoV-2		1	FAM	FAM	NoCt
B1	SARS-CoV-2		2	HEX	HEX	19.56
C1	SARS-CoV-2		1	FAM	FAM	NoCt
C1	SARS-CoV-2		2	HEX	HEX	23.36
D1	SARS-CoV-2		1	FAM	FAM	NoCt

Take time to look at data

Sample info, channel, Ct  
Blank control, positive control

37	G11	SARS-CoV-2	1	FAM	FAM	NoCt	
38	G11	SARS-CoV-2	2	HEX	HEX	21.69	
39	H11	SARS-CoV-2	1	FAM	FAM	NoCt	
40	H11	SARS-CoV-2	2	HEX	HEX	23.30	
41	A12	SARS-CoV-2	1	FAM	FAM	NoCt	
42	A12	SARS-CoV-2	2	HEX	HEX	21.68	
43	B12	SARS-CoV-2	1	FAM	FAM	39.01	
44	B12	SARS-CoV-2	2	HEX	HEX	27.40	
45	C12	SARS-CoV-2	1	FAM	FAM	NoCt	
46	C12	SARS-CoV-2	2	HEX	HEX	26.87	
47	D12	SARS-CoV-2	1	FAM	FAM	NoCt	
48	D12	SARS-CoV-2	2	HEX	HEX	22.68	
49	E12	SARS-CoV-2	1	FAM	FAM	NoCt	
50	E12	SARS-CoV-2	2	HEX	HEX	22.11	
51	F12	SARS-CoV-2	1	FAM	FAM	NoCt	NC
52	F12	SARS-CoV-2	2	HEX	HEX	26.03	
53	G12	SARS-CoV-2	1	FAM	FAM	28.34	PC
54	G12	SARS-CoV-2	2	HEX	HEX	27.75	
55	H12	SARS-CoV-2	1	FAM	FAM	NoCt	
56	H12	SARS-CoV-2	2	HEX	HEX	NoCt	NTC

# Data analysis in RT-PCR-based SARS-CoV-2 testing



# Quality control criteria

External QC, Internal QC

Test kit: SARS-CoV2 **one**-target  
detection with threshold Ct **38**

		<b>ORF1ab FAM</b>	<b>Internal C. VIC/HEX</b>
<b>External QC</b>	Blank control/NTC	Ct NA	Ct NA
	Positive Control/PC	S-shape	S-shape
Ct ≤32		Ct ≤32	
<b>Internal QC</b>	<b>In Specimen</b>	Any value	S-shape
			≤32

The requirements of QC above should be met in each test, otherwise invalid and retest

# Testing result interpretation

SARS-CoV2 **one**-target detection kit with threshold Ct **38**

ORF1ab FAM	Internal C. VIC/HEX	Interpretation	Action
S-shape Ct≤38	S-shape Ct≤32	<u>Positive SARS-CoV-2</u>	Report results to healthcare provider.
Not in S-shape Ct is NA	S-shape Ct≤32	SARS-CoV-2 <u>Not detected</u>	Report results to healthcare provider. Consider testing for other viruses.
S-shape, Ct>38	S-shape Ct≤32	SARS-CoV-2 <u>Inconclusive</u>	Repeat test. POSITIVE If the repeat result remains similar, or NEGATIVE.
If quality control (NTC, NC, PC ) requirements are <b>Not</b> met		<u>Invalid result</u>	Repeat test. If the repeat result remains invalid, consider collecting a new specimen

# Quality control

External QC, Internal QC

Test kit: SARS-CoV2 **two**-target  
detection with threshold Ct **40**

		<b>ORF1ab FAM</b>	<b>N gene Cy5</b>	<b>Internal C. VIC/HEX</b>
<b>External QC</b>	Blank control/ NTC	Ct NA	Ct NA	Ct NA
	Positive Control/PC	S-shape	S-shape	S-shape
		Ct ≤35	Ct ≤35	Ct ≤35
<b>Internal QC</b>	<b>In Specimen</b>	Any value of Ct for two genes		S-shape
				Ct ≤35

The requirements of QC above should be met in each test, otherwise invalid and retest

# Testing result interpretation

SARS-CoV2 **two**-target detection kit with threshold Ct **40**

ORF1ab FAM	N gene Cy5	Internal C VIC/HEX	Interpretation	Action
S-shape Ct≤40	S-shape Ct≤40	S-shape Ct≤35	<u>Positive SARS-CoV-2</u>	Report results to healthcare provider.
both target curves are Not in S-shape, Ct is NA or Ct>40		S-shape Ct≤35	<u>SARS-CoV-2 Not detected</u>	Report results to healthcare provider. Consider testing for other viruses.
Only one target curve is Not in S-shape, Ct is NA or Ct>40		S-shape Ct≤35	<u>SARS-CoV-2 Inconclusive</u>	Repeat test. POSITIVE If the repeat result remains similar, or NEGATIVE
If quality control (NTC,NC,PC) requirements are <b>Not</b> met			<u>Invalid result</u>	Repeat test. If the repeat result remains invalid, consider collecting a new specimen

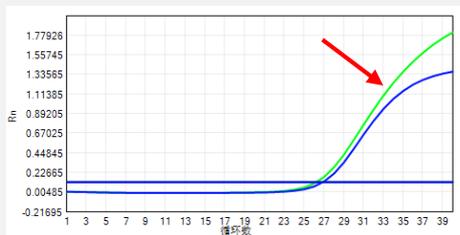
# Testing result interpretation

Test kit: SARS-CoV2 **three target** detection kit

ORF1ab	N gene	S gene	Internal Control	status	result	Action
NEG	NEG	NEG	NEG	Invalid	NA 	Repeat. If the repeat result remains invalid, consider collection a new specimen
NEG	NEG	NEG	POS	valid	SARS-COV-2 Not Detected 	Report results to healthcare provider. Consider testing for other virus
<b>Only one SARS-CoV2 target=POS</b>			POS or NEG	valid	SARS-COV-2 inconclusive 	Repeat. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
<b>Two or three SARS-CoV2 targets =POS</b>			POS or NEG	valid	Positive SARS-CoV-2 	Report results to healthcare provider and appropriate public health authorities

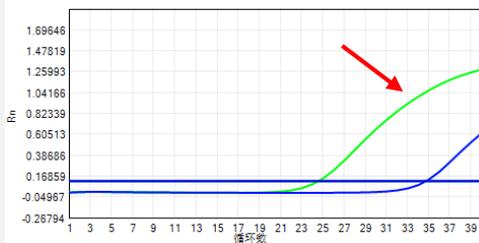
QC requirements should be met firstly

# Sample test results and interpretation



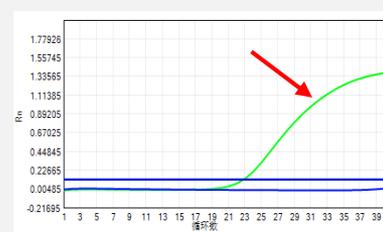
Positive control

FAM	<span style="color: blue;">■</span>	26.87
HEX	<span style="color: green;">■</span>	26.20



Positive

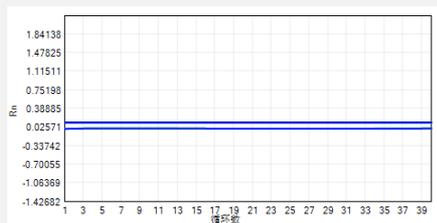
FAM	<span style="color: blue;">■</span>	34.69
HEX	<span style="color: green;">■</span>	24.52



Not detected

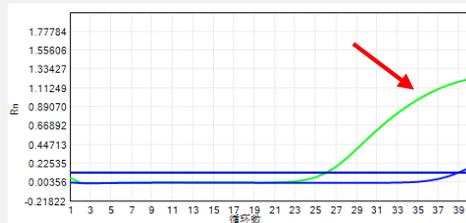
FAM	<span style="color: blue;">■</span>	NA
HEX	<span style="color: green;">■</span>	22.8

1



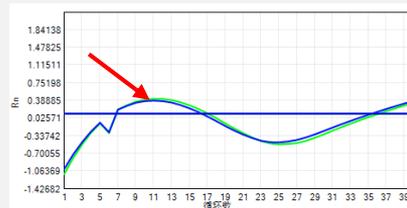
Blank control

FAM	<span style="color: blue;">■</span>	NA
HEX	<span style="color: green;">■</span>	NA



Inconclusive

FAM	<span style="color: blue;">■</span>	38.96
HEX	<span style="color: green;">■</span>	26.06



Invalid

FAM	<span style="color: blue;">■</span>	35.37
HEX	<span style="color: green;">■</span>	36.16

# Summary

## Data Analysis Workflow

External QC + Internal QC  
in Specimen Requirements

met

NOT met

retest

Results	One-gene kit	Two or three-gene kit	Action
<b>Positive</b>	Target Ct ≤ cutoff IC Ct ≤ cutoff	All targets Ct ≤ cutoff IC Ct ≤ cutoff	Report
<b>Negative</b>	Target Ct NA IC Ct ≤ cutoff	All targets Ct NA IC Ct ≤ cutoff	Report
<b>Inconclusive</b>	Target Ct grey area IC Ct ≤ cutoff	One target Ct grey area IC Ct ≤ cutoff	Retest
<b>Invalid</b>	IC Ct > cutoff or NA	IC Ct > cutoff or NA	Retest



# 04 FAQ on Report Interpretation

Q/A #1

Q: Is a two or three-target SARS-CoV-2 test kit better than a one-target's in a diagnostic test?

or

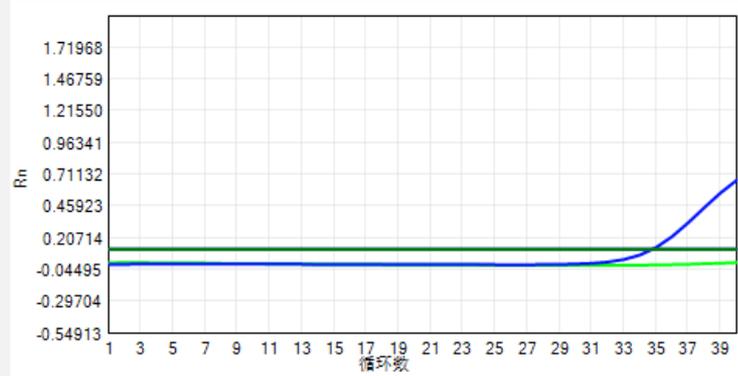
Which kind of test kit should be used in SARS-CoV-2 detection, one-, two- or three-target kit?

A: No. A good test kit means it is high sensitive and high specific. It does not depend on how many targets it has.

## Q/A# 2

Q: How to figure it out if blank control is out of order?

FAM		<b>34.76</b>
HEX		<b>NA</b>



A:

- Contamination, such as Aerosol
- Material and Reagents

Q/A# 3

Q: What should we do if ratio of false negative results is high?

A:

- Clinical specimen collection , storage and shipping
- RNA extraction control: PC/NC/NTC

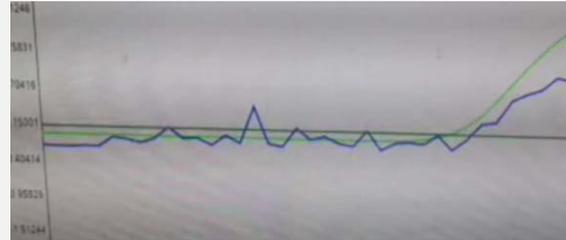
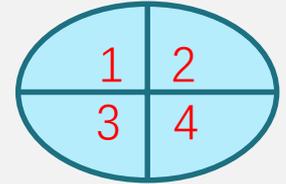
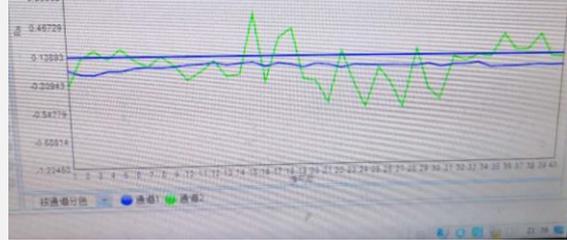
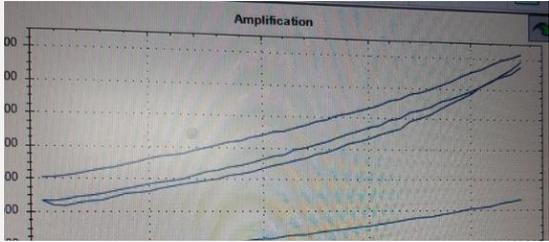
In addition, a false negative result may occur

if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.

if an excess of DNA/RNA template is present in the reaction, extracted RNA can be tested at 2 or more dilutions (e.g., 1:10 and 1:100) to verify result.

Q/A# 4

Q: Why does amplification curve look weird?



- 1 Linear shape
- 2 No amplification
- 3 No plateau phase
- 4 Curve Not smooth

A: Improve skills of experiment performances

# Summary

Generation of RT-PCR data in SARS-CoV2 test is **DECEPTIVELY** simple, the challenge is to analyze and interpret the data.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.