RT-PCT Test Quality Control

—Chen Yu Hui BGI Research

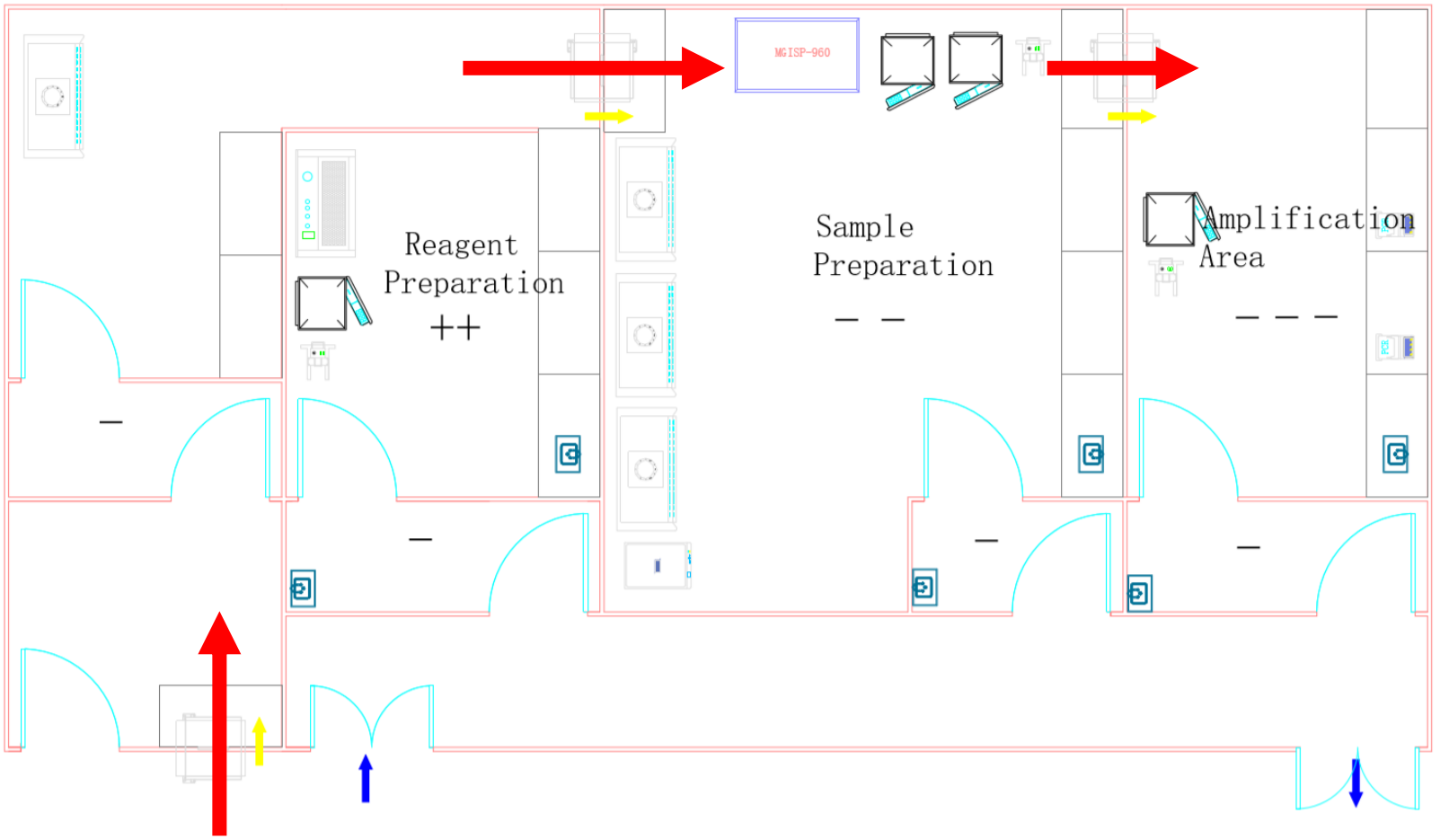
- Prior to sample handling
- Check points during experiment
- Quality control of test data

Sample Collection——Sample Pretreatment——RNA Extraction——qPCR Reaction——Result Calling

Prior to sample handling

- Laboratory layout and designated function of each area
- Equipment Maintenance
- Reagent and Consumables QC

Prior to sample handling - Laboratory layout

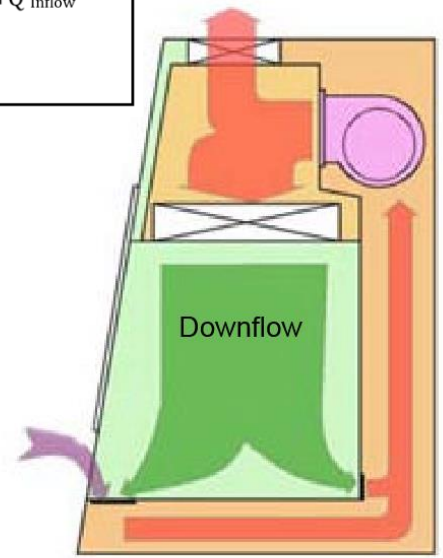


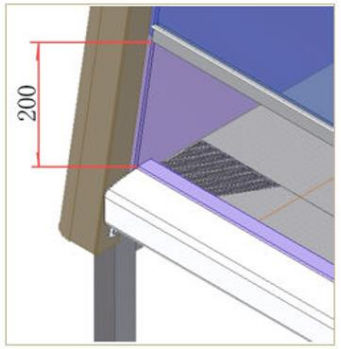
***Designated Function *Unidirectional Workflow *Proper pressure to contain nucleic acid in the expected area**

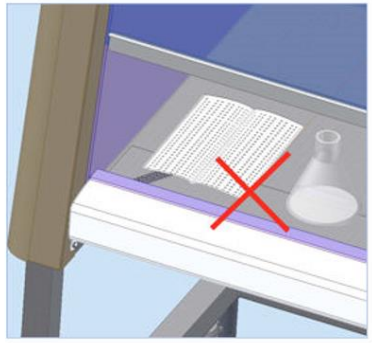
Prior to sample handling - Maintenance; Consumables QC

$Q_{Total\ volume} = Q_{Downflow} + Q_{Inflow}$

$Q_{Air\ exhaust} = Q_{Inflow}$



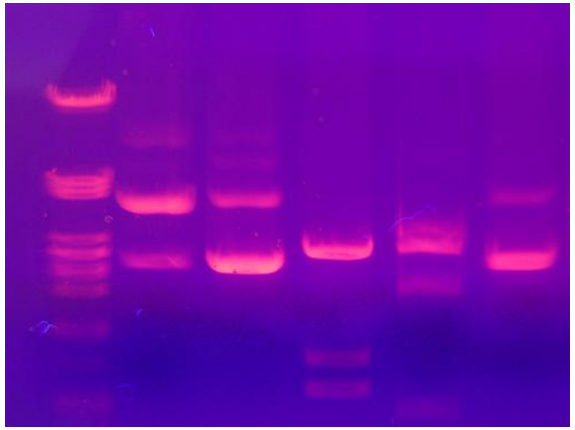




#Function #Calibration #Proper Usage Training

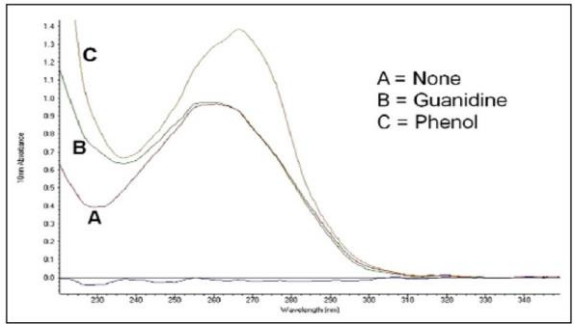
Prior to sample handling – Reagent QC & Verification

Key reagent: Nucleic Acid Extraction Kit and RT-PCT Kit



#Nucleic Acid Extraction Kit: Concentration and Purity

- Nanodrop or Qubit:A260/A280 & Electrophoresis
- Ct value: Control samples with known concentration



#RT-PCT Kit

- Sensitivity & Specificity
- Repeatability & Limit of Detection (LOD)

Conc.	Channel	1	2	3	4
10000copies/ml	FAM				
	VIC				
5000copies/ml	FAM				
	VIC				
2500copies/ml	FAM				
	VIC				

***It is suggested, at least, to have two types of RT-PCT kit for result verification**

Check points during experiment

- Sample arrival status & Sample registration
- Sample assignment into consumables
- RNA extraction & RT-PCR reaction

Check points - Sample arrival status & Sample registration

❖ **Samples should reach the lab in the box at DESIABLE TEMPERATURE**

3 Matching Sample ID

Sample No.	Name	Scannable Barcode
12345	A	A 12345
123456789	B	B 123456789
123456789-Z	C	C 123456789-Z

3 Samples arrive the lab

Provide Sample No. and Name of each sample in excel sheet to the lab BEFORE ship out the samples

HARD COPY of Information sheet with SCANNABLE barcode on the sheet is acceptable.

Sample Information/barcode provided in Hand-writing form will end up in sample rejection!!!

Check points-Sample arrival status & Sample registration

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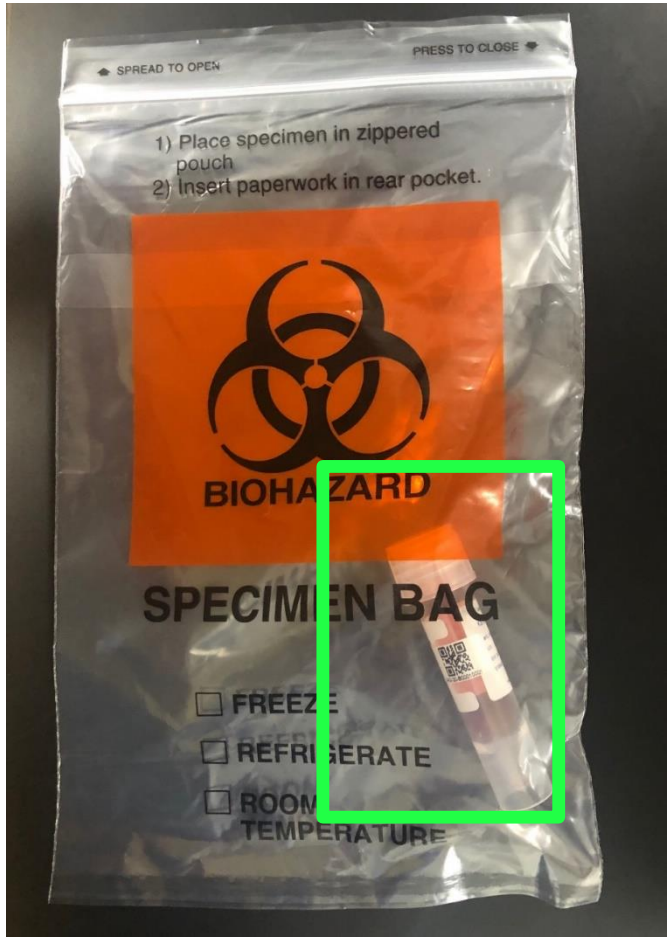

ONE TUBE, ONE BAG

*Scannable barcdoe

*Barcode paper should be ***heat-resistence***

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One sample leaks,
All samples will be rejected

Check points - Sample assignment

COVID-19检测任务单/ COVID-19 Sample Nucleic Acid Test Record

板号/Plate ID.:20200407-1　排单人Arrange Staff: HZB　排单时间/Time:　提取试剂配液编号Extraction Reagent Batch ID: 0318-E1

Sample ID	1	2	3	4	5	6	7	8	9	10	11	12
A	KA0004817	KA0003944	KA0004804	KA0004845								
	1 ☐	9 ☐	17 ☐	25 ☐	33 ☐	41 ☐	49 ☐	57 ☐	65 ☐	73 ☐	81 ☐	89 ☐
B	KA0003576	KA0003572	KA0004822	KA0004818								
	2 ☐	10 ☐	18 ☐	26 ☐	34 ☐	42 ☐	50 ☐	58 ☐	66 ☐	74 ☐	82 ☐	90 ☐
C	KA0004837	KA0003585	Blank	KA0003591								
	3 ☐	11 ☐	19 ☐	27 ☐	35 ☐	43 ☐	51 ☐	59 ☐	67 ☐	75 ☐	83 ☐	91 ☐
D	KA0004827	KA0003583	[illegible]	KA0003210								
	4 ☐	12 ☐	20 ☐	28 ☐	36 ☐	44 ☐	52 ☐	60 ☐	68 ☐	76 ☐	84 ☐	92 ☐
E	KA0004810	KA0003574	KA0004842									
	5 ☐	13 ☐	21 ☐	29 ☐	37 ☐	45 ☐	53 ☐	61 ☐	69 ☐	77 ☐	85 ☐	93 ☐
F	KA0003578	KA0004844	KA0004806									
	6 ☐	14 ☐	22 ☐	30 ☐	38 ☐	46 ☐	54 ☐	62 ☐	70 ☐	78 ☐	86 ☐	94 ☐
G	KA0004833	KA0003597	KA0004840									
	7 ☐	15 ☐	23 ☐	31 ☐	39 ☐	47 ☐	55 ☐	63 ☐	71 ☐	79 ☐	87 ☐	95 ☐
H	KA0003586	KA0003214	KA0004830									PC
	8 ☐	16 ☐	24 ☐	32 ☐	40 ☐	48 ☐	56 ☐	64 ☐	72 ☐	80 ☐	88 ☐	96 ☐

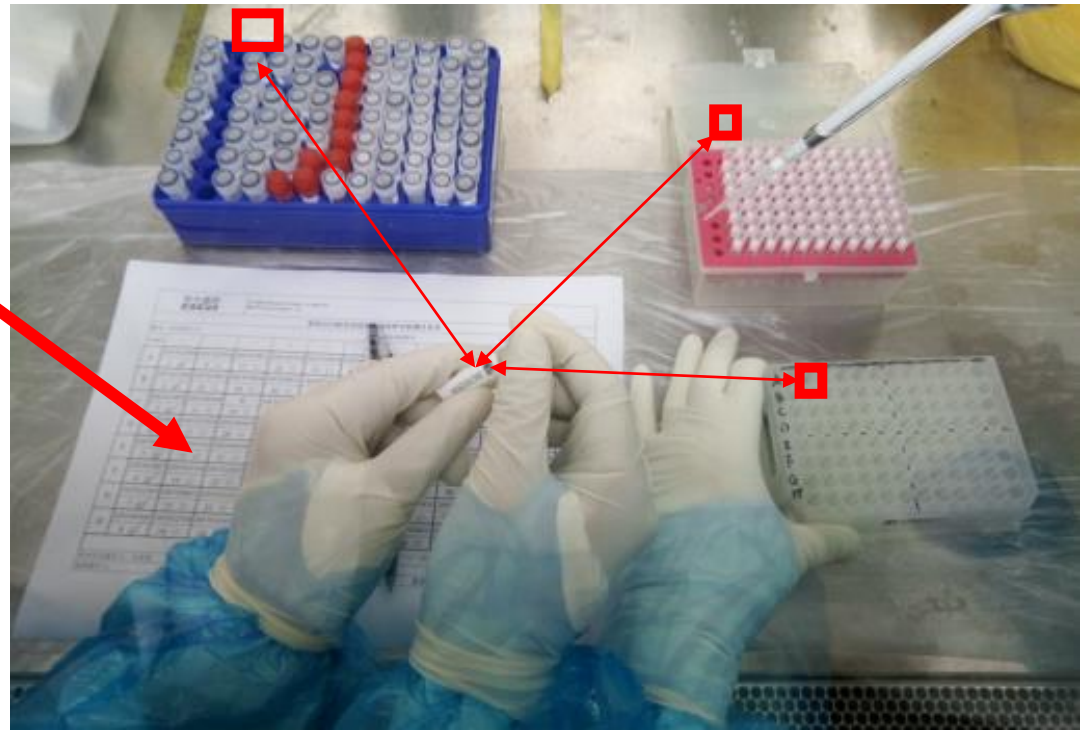
备注/Remark:　qPCR试剂配液编号/qPCR Reagant Mix Batch ID: 0318-Q1

取样操作人/Sampling Staff:　复核人/Verifier:　取样时间/Sampling Time:

提取操作人/Nucleic Acid Extraction Staff:　复核人/Verifier:　提取仪器编号/Nucleic Acid Extraction MGISP-960 No.: 6

上机操作人/qPCR Operator:　qPCR仪编号/qPCR Machine No.:　上机时间/qPCR Experiment Time:

A1	KA0004817
B1	KA0003576
C1	KA0004837
D1	KA0004827
E1	KA0004810
F1	KA0003578
G1	KA0004833
H1	KA0003586
A2	KA0003944
B2	KA0003572
C2	KA0003585
D2	KA0003583
E2	KA0003574
F2	KA0004844
G2	KA0003597
H2	KA0003214
A3	KA0004804
B3	KA0004822
C3	Blank
D3	KA0004843
E3	KA0004842
F3	KA0004806
G3	KA0004840

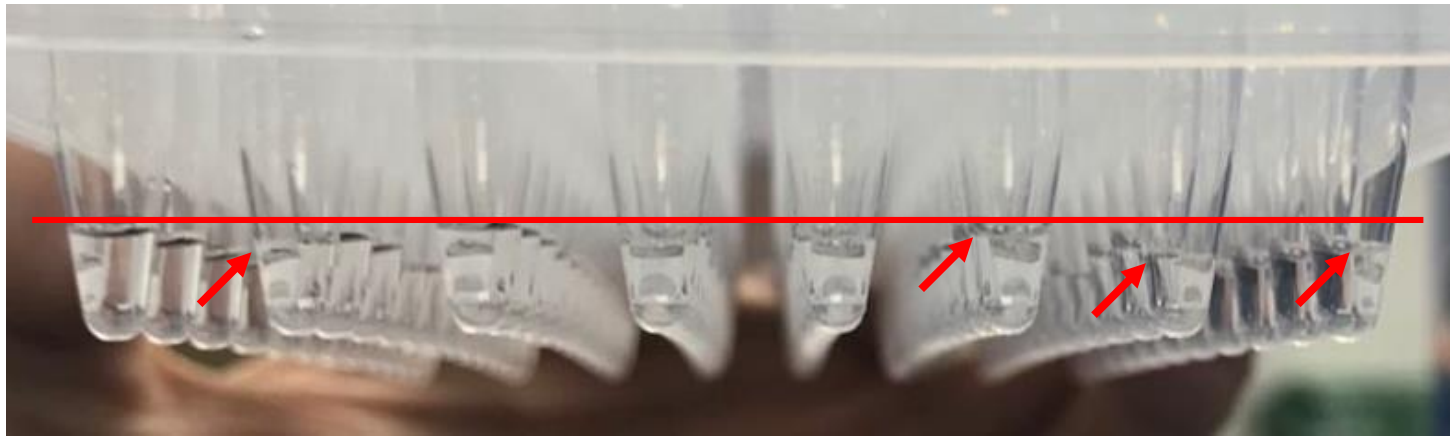


Sample layout for RNA extraction

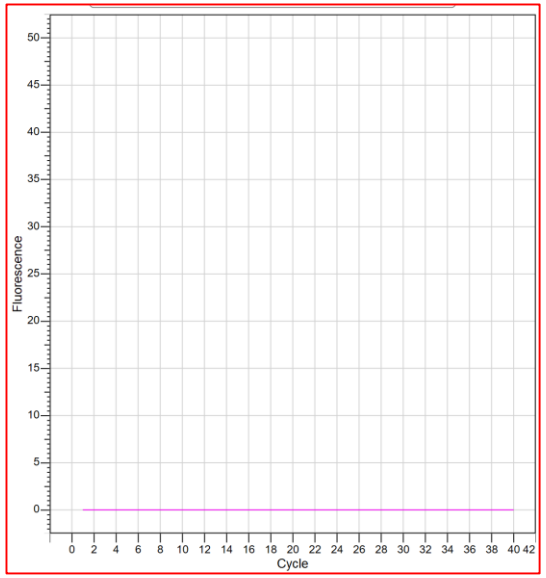
Sample loading verified by two technicians

Check points - RNA extraction & RT-PCR reaction

Sample volume matters!

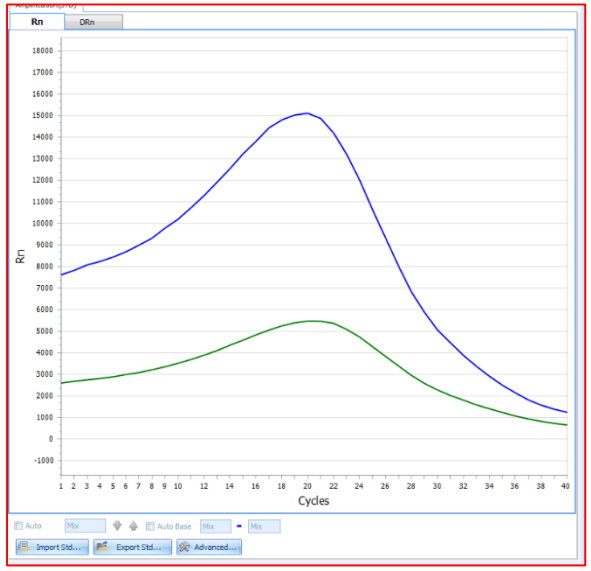


Miss adding of reagent or sample



NO Signal Captured

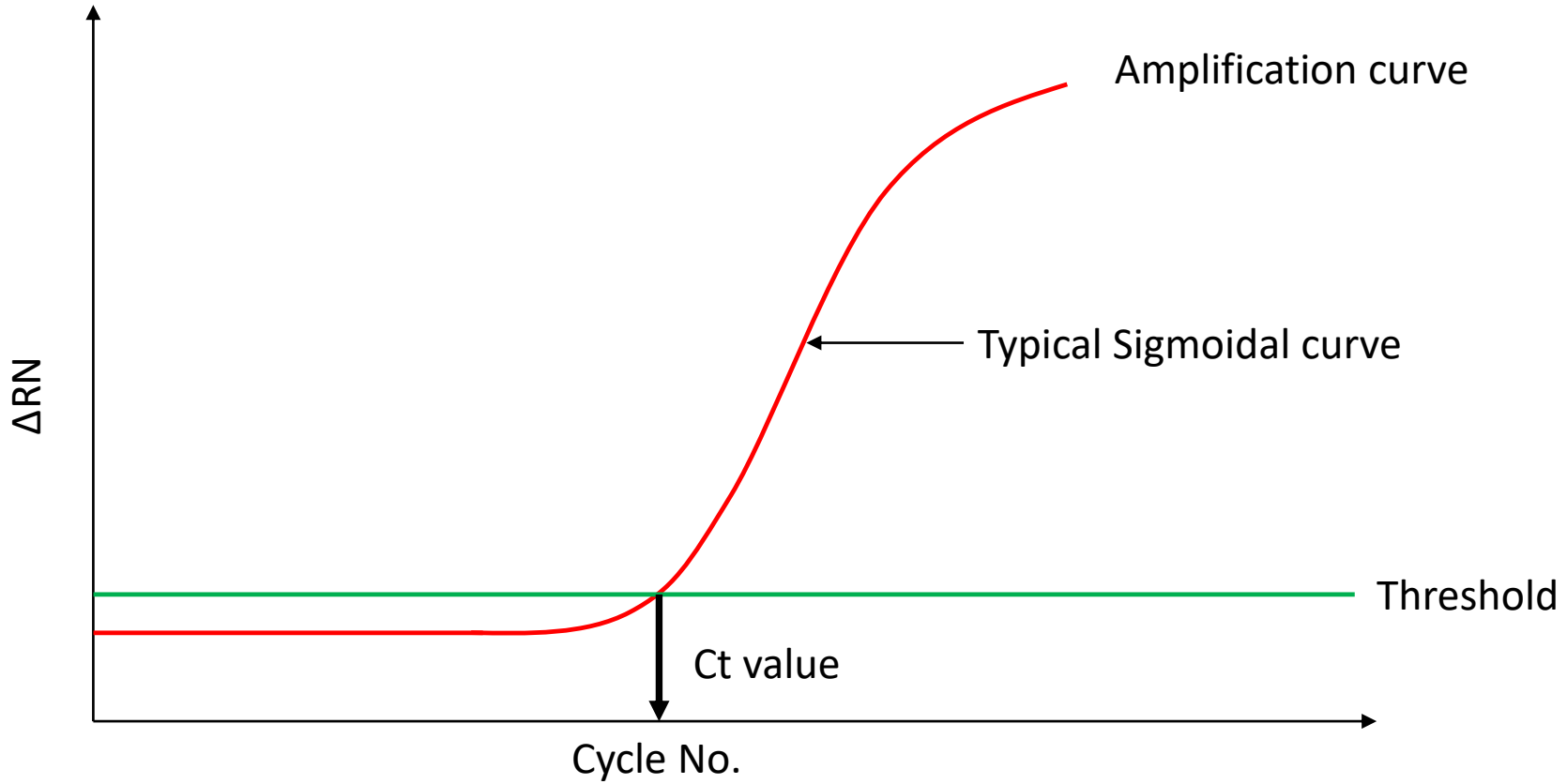
Evaporation



Unusual Amplification Plot

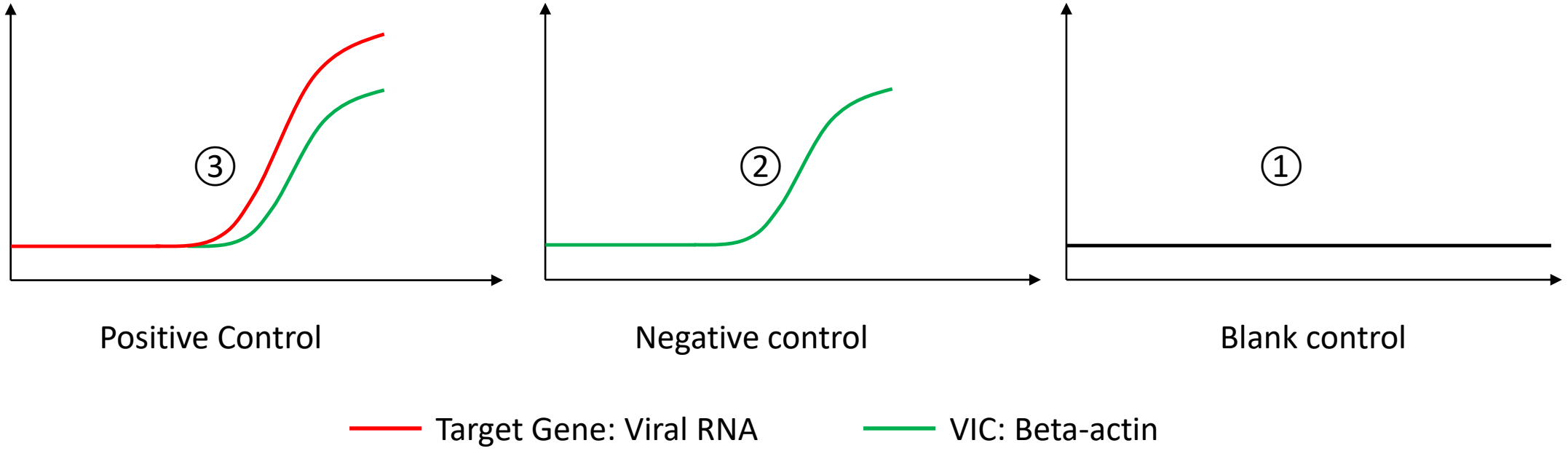
Quality control of test data - Key Concepts

- **Baseline**: The signal level during the initial cycles of PCR, usually cycles 3 to 15, in which there is little change in fluorescent signal.
- **Threshold**: A statistically significant increase over the calculated baseline signal. Usually sets the threshold at 10 times the standard deviation of the fluorescence value of the baseline.
- **Ct**: Cycle number at which the fluorescent signal of the reaction crosses the threshold. So no cross, no Ct.



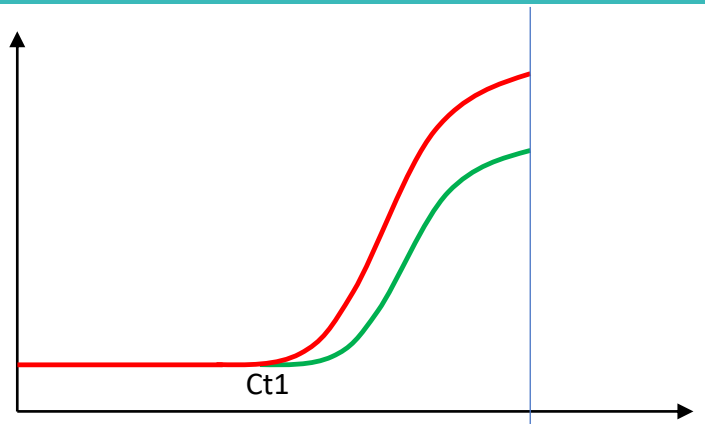
Quality control of test data – Control samples

- Positive Control: To check whether Viral RNA can be detected
- Blank control: To monitor the contamination level or background noise level
- Negative control: To check whether beta-actin gene can be amplified successfully without Viral RNA signal



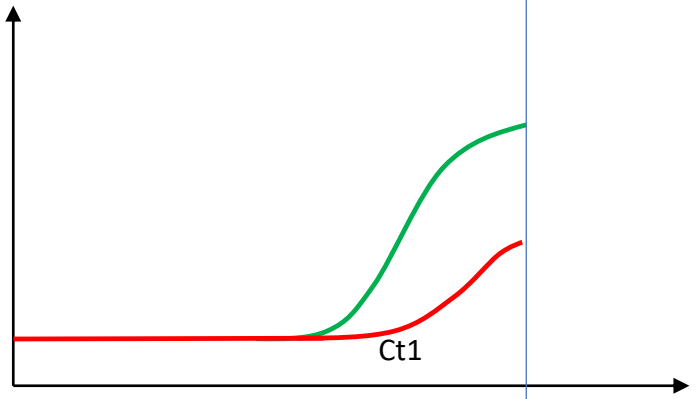
❖ Both viral RNA and beta-actin DNA/RNA should be extracted and then amplified during qPCR reaction, which realizes the quality control of nucleic acid extraction. No beta-actin gene amplification or unusual amplification plot will lead to retest.

Quality control of test data – Example of result with Ct value

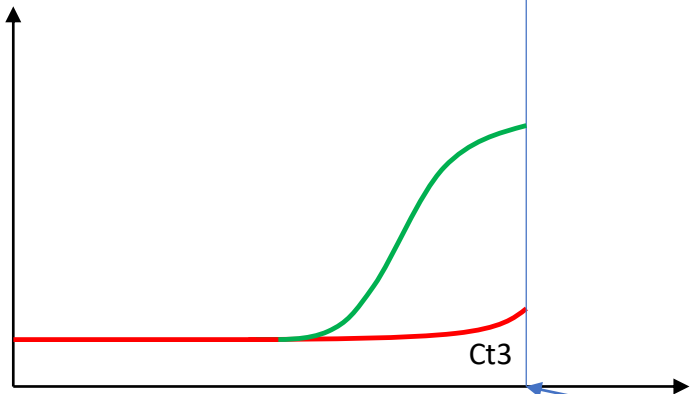


Strong Test Positive result

Ct1<Ct2<Ct3

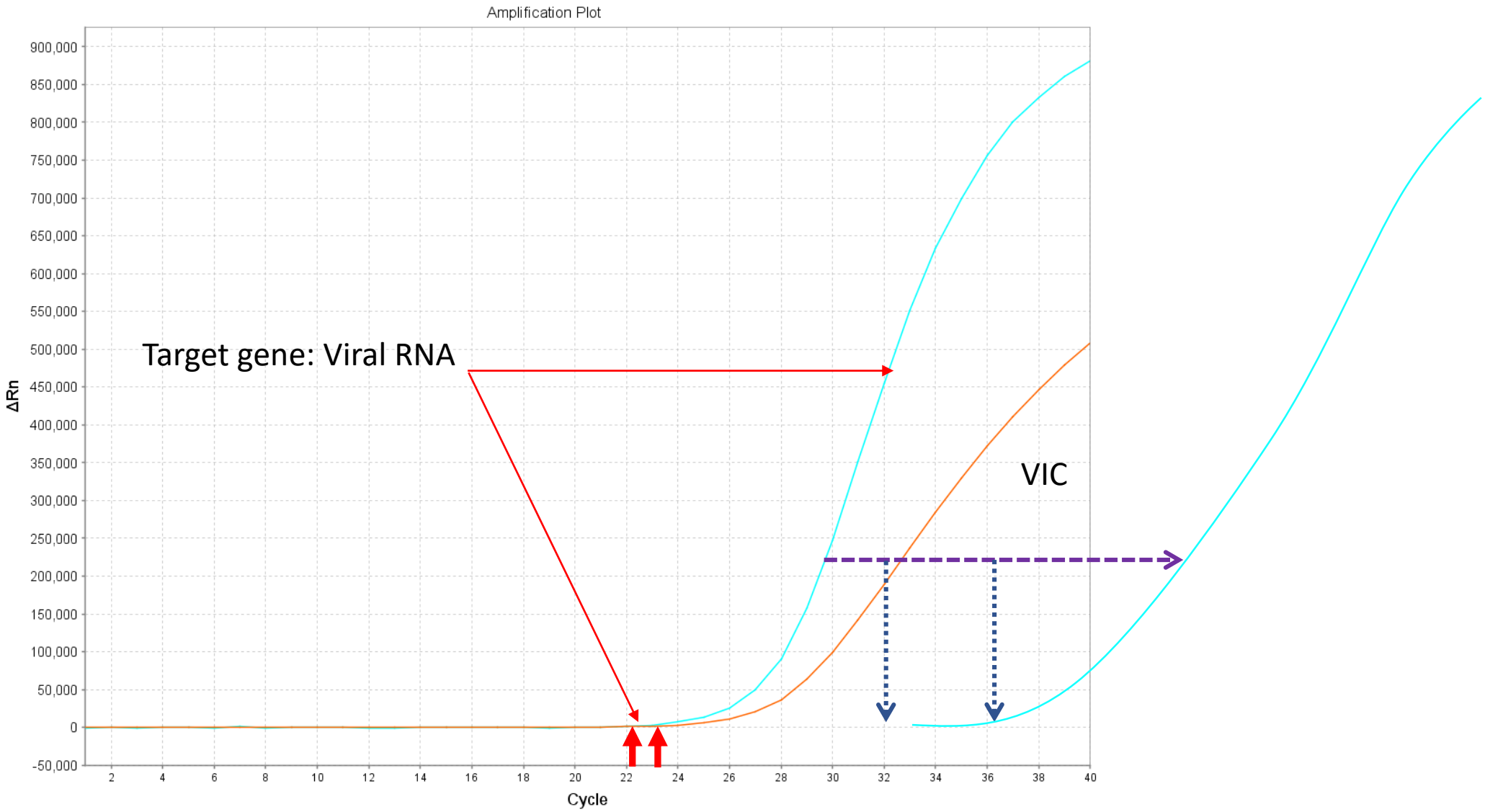


Weak Test Positive result



Suspicious Test Positive result:
With out “S” curve

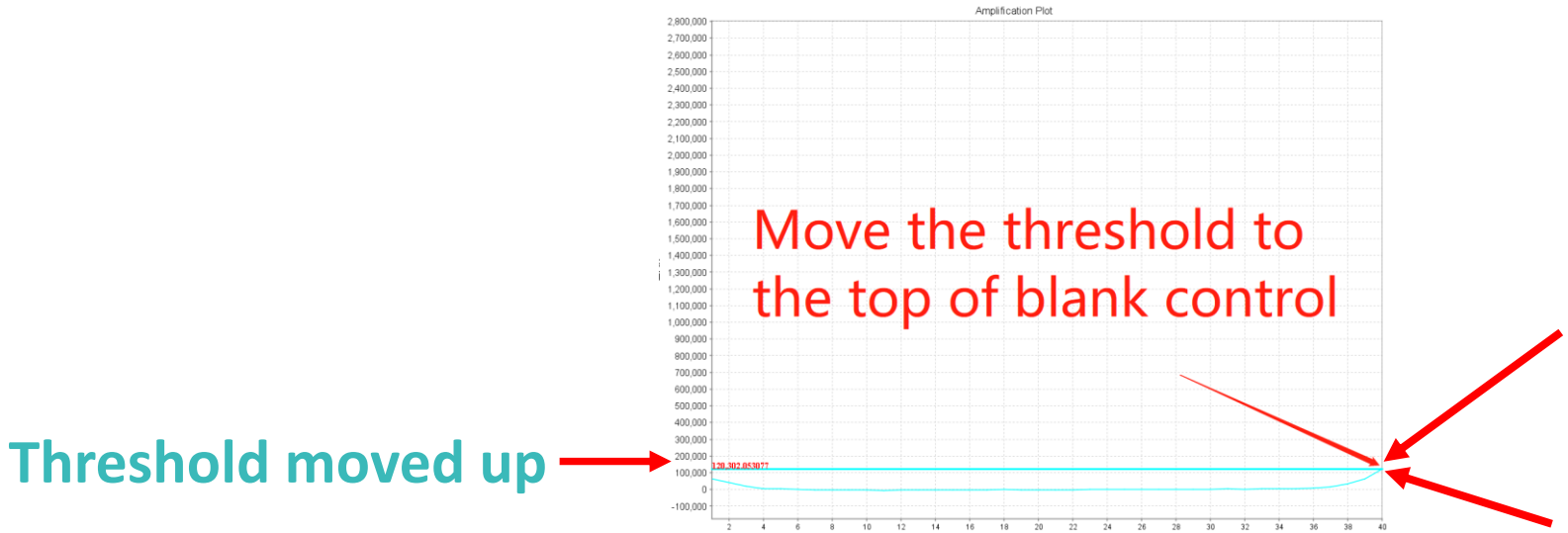
Quality control of test data – Example of result with Ct value



Why non-specific amplification in blank control?

1) Primer design is not perfect causing primer dimer formation leading to amplification in the end of PCR cycles;
2) Dye in the reagent kit partly degrades evidently in the end of cycles;
3) Primer in the kit is too concentrated, thus generating non-specific peaks for blank controls;
4) Slight contamination during sample mixing;
5) Lab environment is not clean enough with accumulated DNA/RNA aerosol;
6) 7500 is not well calibrated;
……

- Always, *repeating the test and * thoroughly clean the laboratory area and related equipment will alleviate this situation. Separating RNA extraction and reagent mixing in different Bio-safety cabinets is suggested.
- Manually move the threshold to cover the noise and reanalyse Ct value for the rest of sample is suggested before data interpretation.



THANK YOU!