2019-nCoV kits Quick-Start Protocol

Notes before start:

1. Please go through Sansure User's manual before operation.
2. Do not mix components from different batches of reagents
3. **Medical personnel should wear PPE (Personal Protective Equipment) during specimen collection and test. PPE includes N95 mask, splash-proof goggles, one-piece suit, gloves and boots cover.**

1. Sample Preparation

Please inactivate the sample at 56℃ for 30 min before sample processing.

For throat swab sample(**Flocked swab** are recommended)

A. Samples stored in sterile saline or cell preservation solution(TE buffer) can be used directly.

B. For samples using other cell preservation solutions, please contact Sansure Biotech's engineers before use.

C. Sample storage: The sample to be tested can be used immediately, or stored at -20 ± 5℃ for 3 months, or stored at -70 ℃ for a long time. Avoid repeated freeze-thaw cycles. Samples should be shipped with curling and ice or foam boxes with ice seals.

D. Mix samples thoroughly with shaking (use the Vortex Mixer), then please **inactivate** the sample at 56°C for 30 minutes in a metal bath or water bath before experiment.

1.1 For Sputum sample

A. Add sterile normal saline which is 2~3 fold volume of specimen into sputum specimen collection tube (for example, 1ml sputum specimen+ 2~3ml sterile normal saline), mix them thoroughly with shaking (use the Vortex Mixer), then put it at room temperature for 30 minutes to completely liquefy the sputum. Take 1ml of the mixed solution and centrifuge at 3,000rpm for 30 seconds, then take the supernatant as the sample to be tested.

B. (For viscous sputum)Add 4% NaOH which is 2~3 fold volume of specimen into sputum specimen collection tube (for example, 1ml sputum specimen+ 2~3ml 4% NaOH), mix them thoroughly with shaking(use the Vortex Mixer), then put it at room temperature for 30 minutes to completely liquefy the sputum, take 1ml of the mixed solution and centrifuge at 12,000rpm for 3min.Then discard the supernatant, add 500ul normal saline to the tube, mix thoroughly with shaking , and then take the supernatant as the sample to be tested.

1.2 For Alveolar lavage fluid, pleural fluid sample

A. Alveolar lavage fluid, pleural and ascites fluid samples can be directly used for nucleic acid extraction.

B. ※If the sample is very viscous, add 2-3ml of normal saline to the sample, and after shaking and mixing thoroughly(use the Vortex Mixer), take 1ml of the mixed solution, centrifuge at 3,000rpm for 30 seconds, and

then save the supernatant as the sample to be tested.

1.3 For faeces sample

Take faeces specimen as big as a soybean. Add 2ml saline into faeces specimen and mix them thoroughly. Take 1ml of the mixed solution and Centrifuge it at 3,000rpm for 5 minutes. and then save the supernatant as the sample to be tested.

1.4 For anus swab

Put the anus swab to collection tube with 2mL normal saline, and mix them thoroughly. Centrifuge it at 3,000rpm for 5 minutes. Pipette 200uL of the supernatant for future use. and then save the supernatant as the sample to be tested.

1.5 Serum or plasma sample

Blood samples were collected at 2-4ml, centrifuged at 1,500 × g for 10 minutes, and then ice bathed for 3-5 minutes. and then take the serum and plasma as the sample to be tested.

2. **Preparation for PCR amplification** (PCR Master Mix prepared in reagents preparation area)

2.1 Take out each component to thaw at room temperature (20℃-25℃) (or hold them in hands for fully thawing until no ice crystal in each tube). centrifuge all the components at 2,000rpm for 10-15 seconds in case of solution on the lids and tube walls.

阳性对照: Positive Control	阴性对照: Negative Control	
PCR-反应液: PCR-Mix	酶混合液: Enzyme Mix	样本核酸释放剂: Lysis Buffer

2.2 According to the quantity of test specimens, negative control and positive control, pipette appropriate quantity components in proportion (**PCR Master Mix:** 26μL 2019-nCoV-PCR-Mix/test+ 4μL 2019-nCoV-PCR-Enzyme

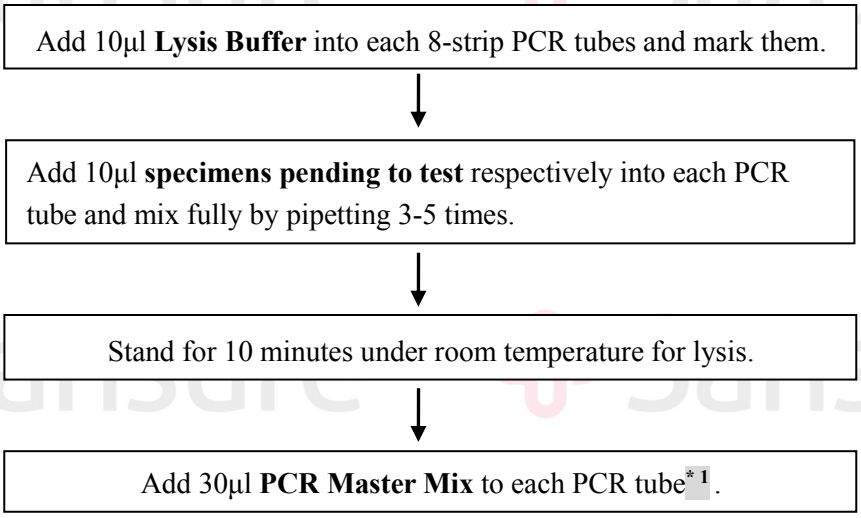
mix/test), and fully mix them into PCR-Master mix. Centrifuge them instantaneously for later use.

	1 sample	10 samples	24 samples	48 samples
2019-nCoV-PCR Mix (μL)	26	260	624	1248
2019-nCoV-PCR-Enzyme Mix (μL)	4	40	96	192
Note: The above configuration is just for your reference and to ensure enough volume of the PCR-Mastermix, more volume of the actual pipetting may be required.				

[Fresh preparation of PCR master mix for use after sample preparation is required each time; remember to mix them fully and centrifuge instantaneously (2,000rpm for 10-15 seconds) to avoid solution in the tube's wall.]

3. Sample Treatment (Handled in "specimen processing region")

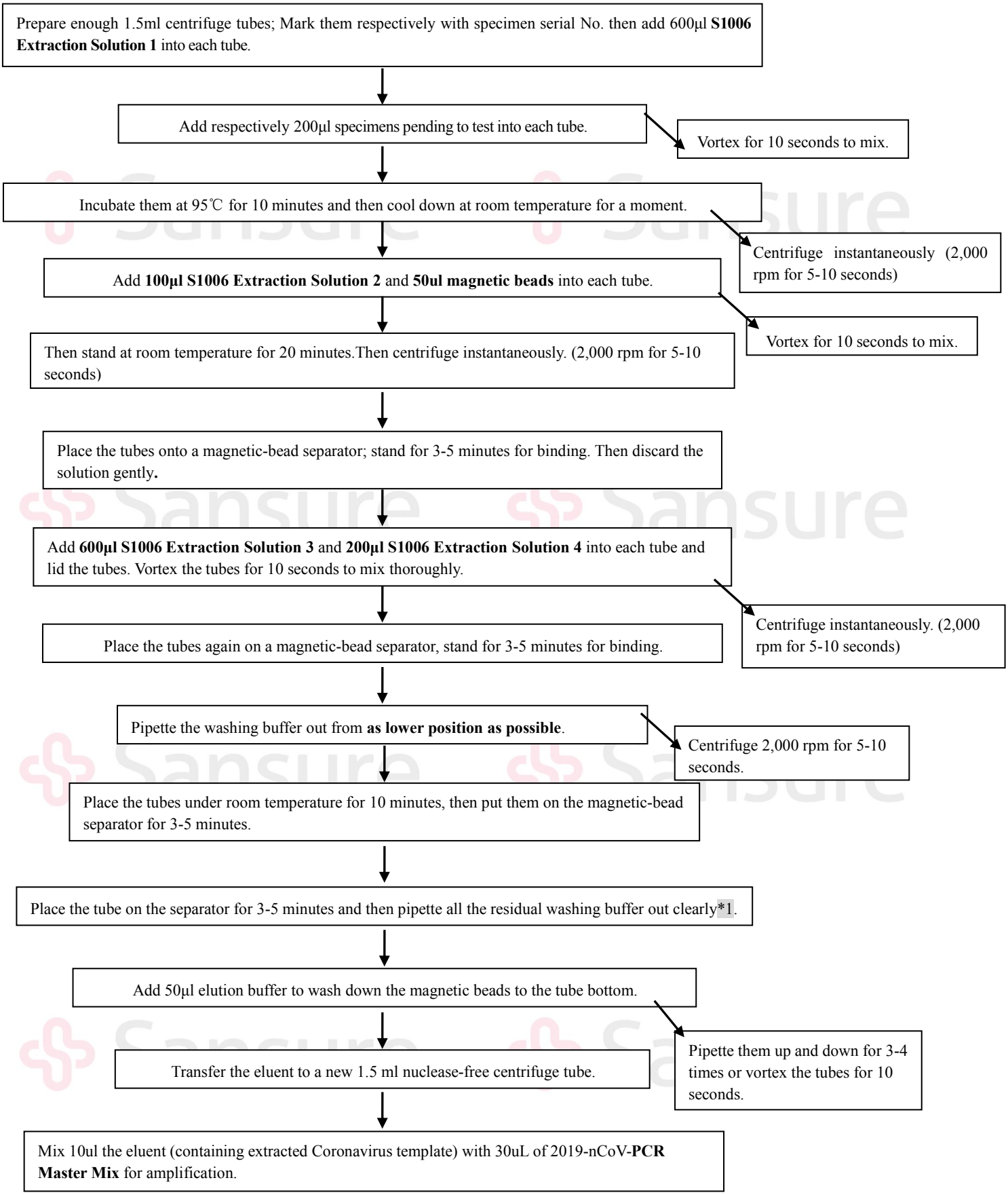
3.1 One tube extraction(Suitable for samples stored with normal saline and Sansure cell preservation solution, matching with S1014E Sample Release Reagent)



Ps. The positive control and Negative control should be synchronous treated with specimens.

*1 mix thoroughly with shaking or pipetting, then centrifuge 2,000 rpm for 10-15 seconds.

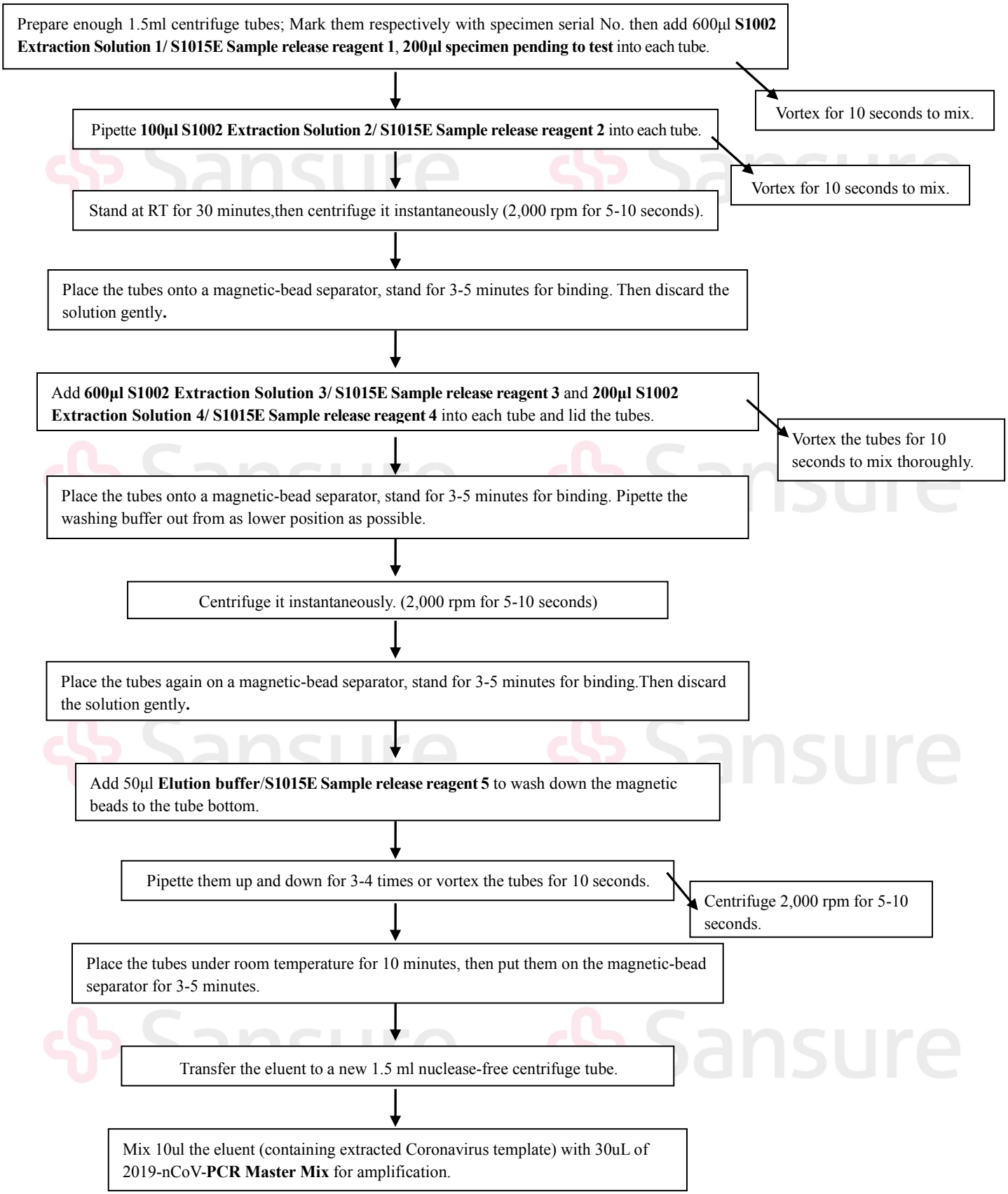
3.2 Magnetic beads extraction(Suitable for preservation solution without guanidine, matching with S1006E)



Ps. The positive control and Negative control should be synchronous treated with specimens.

*1 Care should be taken not to touch the brown stuff (magnetic beads) on the side wall.

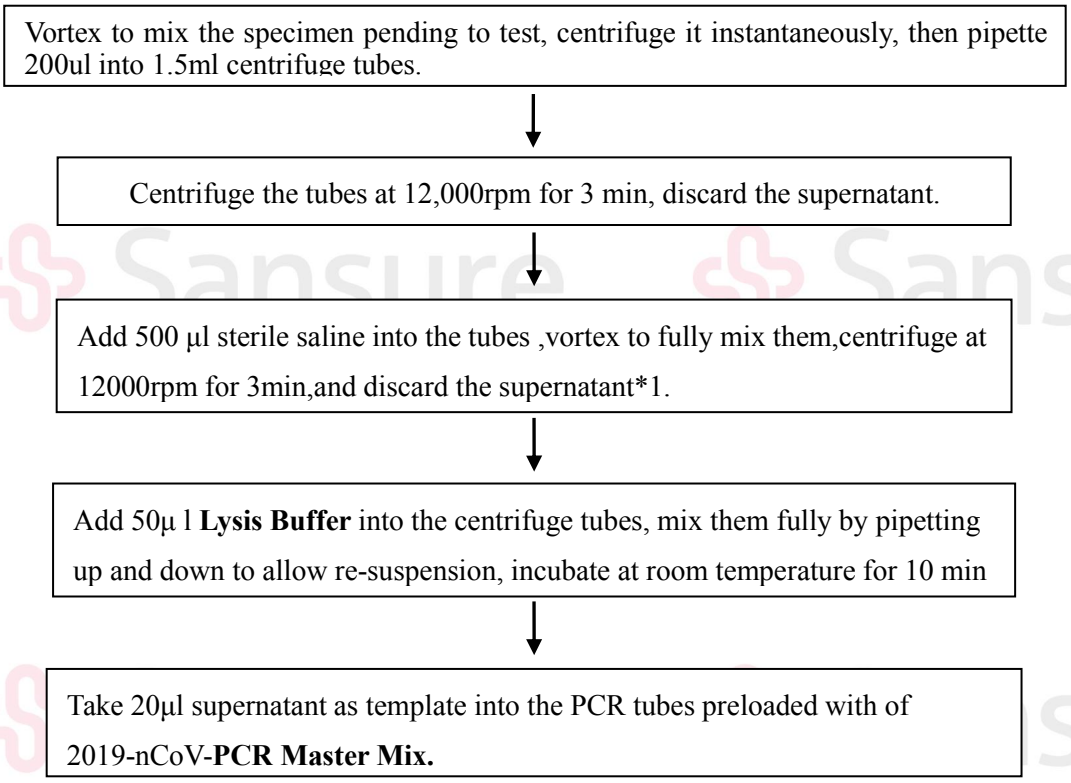
3.3 Magnetic beads extraction(Suitable for preservation solution without guanidine, matching with S1002/S1015E)



Ps. The positive control and Negative control should be synchronous treated with specimens.

*1 Care should be taken not to touch the brown stuff (magnetic beads) on the side wall.

3.3 Concentrated Washing Method



*1: Don't pipet the precipitation, retain about 20-50ul of the liquid.

4. PCR Amplification (Processing in the amplification and analysis area) Refer to user manual of each instrument for the settings)

3.1 Place PCR reaction tubes into the specimen wells of the amplification device. Set up Positive Control, Negative Control and unknown specimen in the corresponding sequence and input sample information.

3.2 Select PCR test channel :

a.Select FAM channel(ORF-lab region) and ROX (N gene) to detect the nucleic acid of 2019-nCoV virus;

b.Select CY5 channel to test internal control.

3.3 Recommend Cycle parameter setting:

Steps		Temperature	Time	Cycles
1	Reverse Transcription	50 ℃	30 minutes	1
2	cDNA pre-denaturation	95 ℃	1 minute	1
3	Denaturation	95 ℃	15 seconds	45
	Annealing, extension and fluorescence collection*	60 ℃	30 seconds *	
4	Instrument cooling	25 ℃	10 seconds	1

5. Interpretation of Test Results

5.1 If the FAM and ROX channel both detected a typical S-type amplification curve and the Ct ≤ 40, CY5 channel desplayed amplification curve and the Ct ≤ 40; report the 2019-nCoV virus is positive.

5.2 If there is typical S-shape amplification curve detected at FAM or ROX channel, and Ct ≤ 40. But another one without amplification curve, and amplification curve which is detected at CY5 channel, Ct ≤ 40, please retest it. If the result is consistent with previous result, report the 2019-nCoV virus is positive.

5.3 If the FAM, ROX and CY5 channel does not detect typical S-type amplification curve (NoCt), or Ct > 40, it means that the sample concentration is too low or there is an inhibitory reaction of interfering substances, the test result of the sample is invalid, the reason should be found and excluded, and the sample should be re-sampled for repeated experiments (if the test result of repeated tests is still invalid, please contact Sansure Biotech Inc.)

Note: For virus cultures, internal control test results are not required.