Appendix 1 Virologic detection methods

Procedure

Multiple Virus (COVID-19/Influenza A/B) RNA Qualitative Assay on ABI QuantStudio5

(I) Preparation of amplification reagents

(i) Take out each component in the kit, equilibrate to room temperature, oscillate to mix, and centrifuge briefly.

(ii) According to the number of samples to be tested, negative control, and positive control, take the corresponding amount of reagents at a ratio of 26μL of amplification reaction solution per person and 4μL of enzyme mixture per person, mix thoroughly to form PCR Mix, and centrifuge briefly.

(iii) Dispense into PCR reaction tubes at 30μL/tube and transfer to the sample processing area.

(II) Nucleic acid extraction

(i) Take out the extraction solution 1 plate, washing solution 1 plate, washing solution 2 plate, and elution solution S plate from the kit, gently invert and mix, centrifuge briefly, and then tear off the membrane for use.

(ii) Add 300μL of the sample to be tested, negative control, and positive control to the wells of the extraction solution 1 plate in sequence.

(iii) Place the above four deep-well plates and disposable magnetic rod sets in the corresponding positions of the automatic nucleic acid extractor and run the nucleic acid extraction program.

(iv) After the program is completed, immediately remove the elution solution S plate from the instrument and seal it with a sealing film.

(III) Nucleic acid spotting

(i) Take 20 μL of sample nucleic acid, negative control and positive control from the elution solution S plate, add them to the aliquoted PCR Mix, cover the tube cap and centrifuge for a few seconds to remove bubbles, and transfer to the amplification area.

(IV) Amplification

(i) Place the reaction tube in the sample slot of the qPCR instrument, set the sample information and the following program, and then run.

(ii) Detection channel: Check FAM, VIC, ROX and CY5 fluorescence, where FAM fluorescence indicates 2019-nCoV virus, VIC fluorescence indicates influenza A virus, ROX fluorescence indicates influenza B virus, and CY5 fluorescence indicates the internal standard gene.

(iii) Amplification program: Stage 1: 50℃, 5min (Number of Cycles: 1); Stage 2: 95℃, 1min (Number of Cycles: 1); Stage 3: 95℃, 10s; 60℃, 20s (Number of Cycles: 41); fluorescence collection is performed during Stage 3 Step 2.

(V) Result analysis

(i) Set reasonable thresholds and baselines, and interpret the results using a Ct value ≤ 40 and a typical S-shaped amplification curve as the positive judgment criteria.

Influenza A Virus (H1N1/H3N2) RNA Qualitative Assay on ABI QuantStudio5

(I) Preparation of amplification reagents

(i) Take out each component in the kit, equilibrate to room temperature, oscillate to mix, and centrifuge briefly.

(ii) According to the number of samples to be tested, negative control and positive controls, take the corresponding amount of reagents in the ratio of 17 μL of amplification reaction solution per person, 2 μL of primer-probe mixture per person, and 1 μL of enzyme mixture per person, mix thoroughly to form PCR Mix, and centrifuge briefly.

(iii) Dispense into PCR reaction tubes at 20μL/tube and transfer to the sample processing area.

(II) Nucleic acid extraction

(i) Take out the extraction solution 1 plate, washing solution 1 plate, washing solution 2 plate, and elution solution S plate from the kit, gently invert and mix, centrifuge briefly, and then tear off the membrane for use.

(ii) Add 300μL of the sample to be tested, negative control, and positive controls to the wells of the extraction solution

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1 plate in sequence.

(iii) Place the above four deep-well plates and disposable magnetic rod sets in the corresponding positions of the automatic nucleic acid extractor and run the nucleic acid extraction program.

(iv) After the program is completed, immediately remove the elution solution S plate from the instrument and seal it with a sealing film.

(III) Nucleic acid spotting

(i) Take 5 μL of sample nucleic acid, negative control and positive controls from the elution solution S plate, add them to the aliquoted PCR Mix, cover the tube cap and centrifuge for a few seconds to remove bubbles, and transfer to the amplification area.

(IV) Amplification

(i) Place the reaction tube in the sample slot of the qPCR instrument, set the sample information and the following program, and then run.

(ii) Detection channel: Check FAM and VIC fluorescence, where FAM fluorescence indicates influenza A virus H1N1 2009, and VIC fluorescence indicates influenza A virus H3N2.

(iii) Amplification program: Stage 1: 50℃, 15min (Number of Cycles: 1); Stage 2: 95℃, 5min (Number of Cycles: 1); Stage 3: 95℃, 3s; 55℃, 45s (Number of Cycles: 45); fluorescence collection was performed in Stage 3 Step 2.

(V) Result analysis

(i) Set reasonable thresholds and baselines, and interpret the results with a Ct value of ≤38 and a typical S-type amplification curve as the positive judgment standard. Repeat the test when the Ct value is in the range of 38-41. If the Ct value of the repeated experiment is still in the range of 38-41 and there is a typical S-type amplification curve, it is judged as positive, otherwise it is negative.

Influenza B Virus (Victoria/Yamagata) RNA Qualitative Assay on ABI QuantStudio5

(I) Preparation of amplification reagents

(i) Take out each component in the kit, equilibrate to room temperature, oscillate to mix, and centrifuge briefly.

(ii) According to the number of samples to be tested, negative control and positive controls, take the corresponding amount of reagents in the ratio of 17 μL of amplification reaction solution per person, 2 μL of primer-probe mixture per person, and 1 μL of enzyme mixture per person, mix thoroughly to form PCR Mix, and centrifuge briefly.

(iii) Dispense into PCR reaction tubes at 20μL/tube and transfer to the sample processing area.

(II) Nucleic acid extraction

(i) Take out the extraction solution 1 plate, washing solution 1 plate, washing solution 2 plate, and elution solution S plate from the kit, gently invert and mix, centrifuge briefly, and then tear off the membrane for use.

(ii) Add 300μL of the sample to be tested, negative control, and positive controls to the wells of the extraction solution 1 plate in sequence.

(iii) Place the above four deep-well plates and disposable magnetic rod sets in the corresponding positions of the automatic nucleic acid extractor and run the nucleic acid extraction program.

(iv) After the program is completed, immediately remove the elution solution S plate from the instrument and seal it with a sealing film.

(III) Nucleic acid spotting

(i) Take 5 μL of sample nucleic acid, negative control and positive controls from the elution solution S plate, add them to the aliquoted PCR Mix, cover the tube cap and centrifuge for a few seconds to remove bubbles, and transfer to the amplification area.

(IV) Amplification

(i) Place the reaction tube in the sample slot of the qPCR instrument, set the sample information and the following program, and then run.

(ii) Detection channel: Check FAM and VIC fluorescence, where FAM fluorescence indicates influenza B virus Yamagata, and VIC fluorescence indicates influenza B virus Victoria.

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(iii) Amplification program: Stage 1: 50℃, 15min (Number of Cycles: 1); Stage 2: 95℃, 5min (Number of Cycles: 1); Stage 3: 95℃, 3s; 55℃, 45s (Number of Cycles: 45); fluorescence collection was performed in Stage 3 Step 2.

(V) Result analysis

(i) Set reasonable thresholds and baselines, and interpret the results with a Ct value of ≤40 and a typical S-type amplification curve as the positive judgment standard. Repeat the test when the Ct value is in the range of 40-43. If the Ct value of the repeated experiment is still in the range of 40-43 and there is a typical S-type amplification curve, it is judged as positive, otherwise it is negative.

Table S1 Elimination criteria

Elimination Criteria
On the data review meeting before the research database is locked, the Principal Investigator, the Sponsor and the relevant responsible persons of the statistical department will determine whether individual cases are eliminated. In any of the following cases, a patient should be comprehensively determined as eliminated from or included in the analysis population based on the factors such as the patient's degree of trial completion and the reason for withdrawal, and the specific classification criteria for each analysis population will be further defined in the Statistical Analysis Plan (SAP).
(1) The participant is found not to meet the inclusion criteria or meet the exclusion criteria after inclusion.
(2) There is no record about the participant after randomization.
(3) The participant has not used the investigational drug.
(4) The effectiveness and safety cannot be determined because the case has used the contraindicated drug prescribed by the protocol.
(5) The subject shows poor compliance, and uses less than 80% or more than 120% of the prescribed drug dose during the trial.
(6) The close contact has not lived with the index cases for 7 days or more during the trial.

Table S2 Study schedule of specific assessments

Item	Screening period	Treatment period		Follow-up period	
Visit cycle	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4
Visit time	-2–0 day	Day 3 (±1 day)	Day 5 (±1 day)	Day 9 (±1 day)	Day 30 (±3 days)
Basic medical history					
Index cases and close contacts informed consent	●				
Rapid detection of influenza virus antigen in index cases	●				
Detection of influenza virus nucleic acid in index cases	●				
Detection of novel coronavirus antigen in index cases	●				
Detection of novel coronavirus antigen in close contacts	●	●	●		
Detection of novel coronavirus nucleic acid in close contacts	●	●	●		
Demographic data of index cases and close contacts	●				
General clinical data of index cases and close contacts	●				
Effectiveness evaluation (for close contacts)					
Influenza symptom score	▲	▲	▲	▲	
Temperature measurement (armpit)	▲	▲	▲	▲	
Rapid detection of influenza virus antigen	▲				
Influenza virus nucleic acid test	●	●	●	●	
Safety and other assessments (for close contacts)					
Vital signs	●	●	●	●	
Physical examination	●		●		
Urine pregnancy test (women of childbearing age only)	●				
Blood routine examination	●		●		
Routine urine test	●		●		
Biochemical examination of liver and kidney functions	●		●		
Resting electrocardiogram	●		●		
Adverse events/serious adverse events/adverse reactions	●	●	●	●	●
Other work					
Record the time of starting drug delivery	●				
Record the end time of medication			●		
Recording of concomitant medications	●	●	●*	●	
Issue diary cards	●	●	●		
Recover diary cards		●	●	●	
Issue investigational drugs	●				
Recover surplus investigational drugs and diary cards			●		
Economic data collection		●	●	●	●
Completion of eCRFs	●	●	●	●	●

● A black dot indicates a mandatory survey.

▲ Record once during screening examination and before random enrolling. If screening examination and random enrolling are on the same day, it is only necessary to record once; Record it before 22:00 every night.

* Record the medication during influenza in the index cases by telephone interview.

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