REF	**RSV – Respiratory Syncytial Virus Panel Instructions**	
Σ	**RSV – Respiratory Syncytial Panel PCR Kit**	
IFU	**Information For Use**	
LDT	**Lab Development Test**	
RUO	**For Research Use Only**	
REV #	**DATE:**	**REVISION DESCRIPTION:**
REV:01	**7/21/2025**	**EDITION: V1**
REV:02	**8/31/2025**	**2ND Release**

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Definitions & Abbreviations

Abbreviation	Definition
Ct	Cycle Threshold; the PCR cycle at which fluorescence exceeds baseline.
IC	Internal Control: non-target DNA (Human RNaseP) added to monitor extraction and amplification.
PC	Positive Control; plasmid containing assay target sequences.
NC	Negative Control; reagent blank or plasmid containing IC only.
LoD	Limit of Detection; lowest concentration of target reliably detected.
QC	Quality Control; procedures ensuring run validity.
RUO	Research Use Only; not for use in diagnostic procedures (per 21 CFR 809.10(c)(2)(i)).
GLP	Good Laboratory Practice.
PPE	Personal Protective Equipment.
qPCR	Quantitative Polymerase Chain Reaction (real-time PCR).
UDG	Uracil-DNA Glycosylase; enzyme used to prevent carryover contamination.
dUTP	Deoxy Uridine Triphosphate; used with UDG to eliminate amplicon contamination.
FAM / VIC / ROX / CY5	Fluorescence channels are used for multiplex detection.

Pathogen & Resistances Target Symbols							
	Bacteria		Virus		Fungi		Resistance

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1 Product Name.

RSV – Respiratory Syncytial Virus Panel (RSV-A, RSV-B, ± coinfections).

2 Specification.

A. Product Name: ReagenX™ RSVX Multiplex PCR Kit
B. Catalog Number: RX-RSVP-001
C. Kit Sizes: 8 tests / 24 tests
D. Format: Lyophilized PCR reagents in 8-well strip tubes

3 Intended Use.

A. The **ReagenX™ RSV-X Mini Multiplex PCR Kit** is intended for the qualitative *in vitro* detection of nucleic acids from common respiratory pathogens — including Severe Acute Respiratory Syndrome Coronavirus ***2 (2019-nCoV), Influenza A virus, Influenza B virus, Respiratory Syncytial Virus (RSV), Respiratory Adenovirus, Mycoplasma pneumoniae, Human Rhinovirus, and Parainfluenza Viruses type 1, 2, and 3*** — extracted from ***nasopharyngeal or oropharyngeal swab specimens***. The assay is designed for use with patients suspected of respiratory infection, including those with suspected clustered cases, as well as other individuals requiring pathogen detection, using real-time PCR.
B. **For Research Use Only (RUO). Not for use in diagnostic procedures.** This product is not cleared or approved by the U.S. Food and Drug Administration (FDA) for diagnostic use. Performance characteristics must be established by the end-user laboratory in accordance with 21 CFR 809.10(c)(2)(i).

4 Principle of the Procedure.

A. The ReagenX™ RSV-X Mini Multiplex PCR Kit is designed with specific primers and fluorescent probes for the qualitative detection of respiratory pathogens, including Severe Acute Respiratory Syndrome Coronavirus 2 (2019-nCoV: ORF1ab gene and N gene), Influenza A virus, Influenza B virus, Respiratory Syncytial Virus, Respiratory Adenovirus, Mycoplasma pneumoniae, Human Rhinovirus, and Parainfluenza Virus types 1, 2, and 3.
B. Nucleic acids extracted from nasopharyngeal and oropharyngeal swab specimens are amplified using real-time polymerase chain reaction (qPCR) with multiple fluorescent probes, enabling simultaneous detection of pathogen-specific target sequences.
C. The Human RNaseP gene is incorporated as an internal control (IC) to verify specimen quality and to monitor nucleic acid extraction and amplification, thereby ensuring the reliability of negative results and minimizing false-negative outcomes.
D. To prevent carryover contamination, the amplification system includes a uracil-DNA glycosylase (UDG)/dUTP safeguard, which degrades residual amplified products and reduces the risk of false positives.
E. The kit is provided as a fully premixed, freeze-dried system. Each PCR tube contains Taq DNA polymerase, reverse transcriptase, UDG enzyme, RNase inhibitor, reaction buffer, dNTP/dUTP mix, Mg^{2+}, and pathogen-specific primers/probes.
F. Each strip contains 1–8 lyophilized wells, with each well targeting a designated pathogen gene region.
G. After rehydration with dissolving solution and addition of extracted nucleic acid, amplification is performed directly on a compatible real-time PCR instrument.

5 Pathogen Targets.

Category	Target	Example Role	Notes
Virus	2019-nCoV ORF1ab	COVID-19 detection	Pandemic pathogen
Virus	2019-nCoV N gene	COVID-19 detection	Pandemic pathogen
Internal Control	RNaseP	Extraction/Amplification control	Validity check
Virus	Influenza A	Respiratory infection	Seasonal epidemics
Virus	Influenza B	Respiratory infection	Seasonal epidemics
Virus	Respiratory syncytial virus (RSV)	Respiratory infection	Pediatric severity
Virus	Adenovirus	Respiratory infection	Common cold/respiratory
Bacteria	Mycoplasma pneumoniae	Atypical pneumonia	Antibiotic responsive
Virus	Human rhinovirus	Respiratory infection	Common cold
Virus	Parainfluenza virus type 1	Respiratory infection	Childhood infections
Virus	Parainfluenza virus type 2	Respiratory infection	Childhood infections
Virus	Parainfluenza virus type 3	Respiratory infection	Childhood infections

Table 6. Pathogen Targets

6 Warnings & Precautions.

A. ⚠ For Research Use Only (RUO). Not for use in diagnostic procedures.

B. Treat all human specimens as potentially infectious and handle them in strict accordance with the laboratory's biosafety requirements.

C. Laboratory personnel must receive professional training in:
- **Sample collection and specimen handling**
- **Reagent preparation and workflow setup**
- **PCR instrument operation**
- **Data analysis and interpretation**

Laboratory operations must comply with local, national, and international regulations governing molecular diagnostic testing.

D. The laboratory should be physically divided into separate areas to minimize contamination:
- **Reagent preparation area**
- **Sample preparation area**
- **Amplification and analysis area**

⚠ **Use dedicated equipment and consumables in each area. Cross-use of materials is strictly prohibited.**

E. E. Personal Protective Equipment (PPE) must be always worn, including:
- **Laboratory coats or gowns**
- **Gloves (powder-free, disposable)**
- **Protective eyewear (goggles or face shield)**
- **Hair covers**
- **Surgical masks or fit-tested N95 respirators**

Ensure full coverage of exposed skin to prevent direct contact with specimens or reagents.

F. In case of spills or leaks:
- **Immediately rinse exposed skin with copious amounts of water.**
- **If reagents contact wounds or mucous membranes, seek medical attention promptly and notify the appropriate health and safety office.**

G. Proper laboratory training in PCR workflows and contamination control is essential to ensure accuracy and safety. Always read and fully understand the Instructions for Use (IFU) before performing the assay.

7 Kit Components.

Components	8 tests/kit	16 tests/kit	Ingredient
Lyophilized reagent PCR tubes A	1×8 strip tubes	2×8 strip tubes	Primers and probes for ORF1ab, N gene of 2019-nCoV and human RNaseP gene, dNTP/dUTP Mix, Mg^{2+}, polymerase, Reverse Transcriptase, RNase Inhibitor, UDG Enzyme
Lyophilized reagent PCR tubes B	1×8 strip tubes	2×8 strip tubes	Primers and probes for influenza A, influenza B and respiratory syncytial virus, dNTP/dUTP Mix, Mg^{2+}, polymerase, Reverse Transcriptase, RNase Inhibitor, UDG Enzyme
Lyophilized reagent PCR tubes C	1×8 strip tubes	2×8 strip tubes	Primers and probes for adenovirus, mycoplasma pneumoniae and human rhinovirus, dNTP/dUTP Mix, Mg^{2+}, polymerase, Reverse Transcriptase, RNase Inhibitor, UDG Enzyme
Lyophilized reagent PCR tubes D	1×8 strip tubes	2×8 strip tubes	Primers and probes for parainfluenza virus type 1, 2, 3, dNTP/dUTP Mix, Mg^{2+}, polymerase, Reverse Transcriptase, RNase Inhibitor, UDG Enzyme
A tube Positive Control	400 µL ×1 tube	400 µL ×1 tube	Pseudo virus containing target genes of 2019-nCoV and human RNaseP gene
B tube Positive Control	400 µL ×1 tube	400 µL ×1 tube	Pseudo virus containing target genes of influenza A, influenza B and respiratory
C tube Positive Control	400 µL ×1 tube	400 µL ×1 tube	Pseudo virus contains target genes of adenovirus, mycoplasma pneumoniae and human rhinovirus
D tube Positive Control	400 µL ×1 tube	400 µL ×1 tube	Pseudo virus containing target genes of parainfluenza virus type 1, 2, 3
Negative Control	1 mL ×1 tube	1 mL ×1 tube	Pseudo virus containing RNaseP target genes
Dissolving Solution	1 mL ×1 tube	1 mL ×1 tube	Stabilizer

Table 7. → Kit Components

Notes:

1. Do not mix components from different batches.
2. Positive/Negative Controls should be used when contamination or reagent failure is suspected.
3. Treat all nasopharyngeal swabs, aspirates, or respiratory specimens as potentially infectious with airborne pathogens.
4. Perform sample preparation, reagent setup, and amplification in physically separated areas.
5. Use dedicated pipettes and filtered tips; change gloves often, especially after handling respiratory samples.
6. Do not exchange reagents from different lots.
7. Protect fluorescent reagents and PCR tubes from direct light.
8. Dispose of swabs, tubes, and other consumables in accordance with biosafety waste management (autoclaving, incineration, or chemical disinfection).
9. This kit is RUO; not approved for diagnostic purposes. Use under GLP with trained operators.

7.1 Materials Required but Not Supplied

Equipment / Material	Description	Notes
Class II Biosafety Cabinet	For safe specimen handling and contamination control	Required for molecular diagnostics
Personal Protective Equipment (PPE)	Lab coats/gowns, gloves, protective eyewear (goggles/face shield), surgical or N95 masks	To ensure operator safety
Adjustable Micropipettes	0.5–10 µL, 10–100 µL, 100–1,000 µL ranges	Calibrated, single- or multi-channel
Aerosol-Resistant Filter Tips	RNase/DNase-free	To minimize contamination risk
1.5 mL Microcentrifuge Tubes & Racks	Sterile, RNase/DNase-free	For sample and reagent handling
Benchtop Microcentrifuge (≥12,000 rpm)	For nucleic acid extraction/release	Compatible with 1.5 mL tubes
Vortex Mixer	For specimen and reagent mixing	Power ≥40W recommended
Heating Block / Water Bath (95 °C)	For nucleic acid release	Fits 1.5 mL tubes
Centrifuge Tube Holder (for vortex)	Optional; can replace manual mixing	For consistent bead-based lysis if used
Laboratory Refrigerator (4–10 °C)	For sample and reagent storage	Continuous temperature monitoring recommended
Laboratory Freezer (–20 °C)	For sample/reagent preservation	Avoid repeated freeze–thaw cycles
Metal Bath / Water Bath	For 1.5 mL centrifuge tubes at 95 °C	Used during nucleic acid release
Collection Swabs & Transport Medium – RSV / RSV+	Nasopharyngeal or oropharyngeal swabs collected in UTM/VTM	FDA-cleared/CE-marked. Bioteke Disposable Virus Sampling Swab Kits are recommended.
Real-time PCR Instrument	With FAM, VIC/HEX, ROX, CY5 channels	Compatible systems: ABI 7500, Bio-Rad CFX96, QuantStudio, SLAN-96S, BTK-96

8 Storage & Shelf Life.

A. The kit can be transported at room temperature (no more than one month).
B. It can be stored at-20℃± 5℃for one year.
C. Repeated freezing and thawing should not exceed 5 times.

9 Instruments.

A. Real-time PCR instruments such as ABI7500, Bio-Rad CFX96, SLAN-96S, QuantStudio and BTK-8

10 Specimen Collection & Handling

10.1 Specimen Types

- Nasopharyngeal swabs, oropharyngeal swabs, sputum, or bronchoalveolar lavage (BAL) fluid.
- All specimens should be collected directly into **VTM/UTM/ITM sampling tubes** in accordance with standard technical specifications.
- *Use of Bioteke Disposable Virus Sampling Swab Kits (with UTM/VTM) is recommended for optimal performance.*

10.2 Collection

- Performed by trained personnel using **sterile synthetic flocked swabs**.
- Avoid calcium alginate swabs or cotton swabs with wooden shafts, which may inhibit PCR.

10.3 F. Storage

- **2–8 °C**: up to 24 hours (≤72 hours acceptable if validated).
- **–20 °C**: up to 10 days.
- **–70 °C or below**: up to 3 months (recommended for long-term storage).
- Avoid repeated freeze–thaw cycles.

10.4 Transport

- Transport specimens in **sealed insulated containers with ice packs** at 2–8 °C if delivery is within 24–72 hours.
- For longer transport times, ship specimens **frozen on dry ice** (–70 °C).

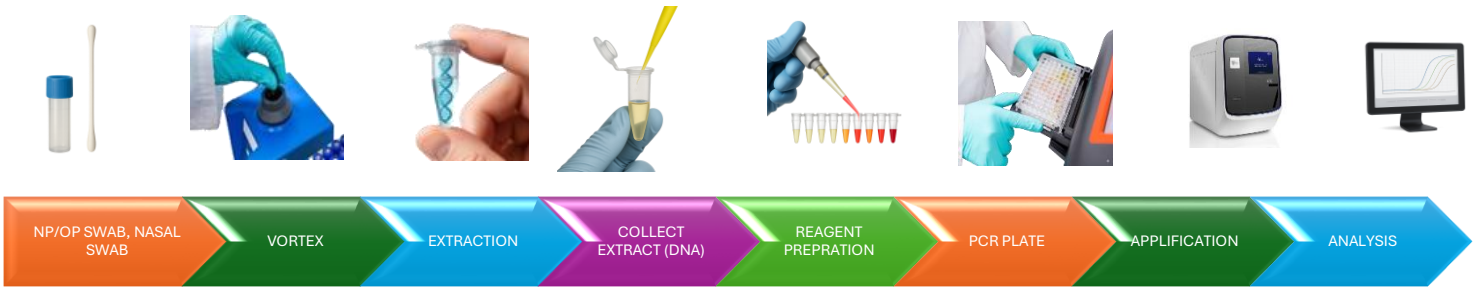
10.5 Rejection Criteria

- Specimens not collected in VTM/UTM/ITM.
- Leaking, broken, or unlabeled tubes.
- Samples stored beyond allowable stability limits.

11 Test Procedure & Protocol

11.1 Workflow

Respiratory specimen (NP/OP swab, nasal swab, sputum, BAL in VTM/UTM) → Nucleic acid extraction / release → Collect extract (RNA/DNA) → Reagent rehydration (15 µL Dissolving Solution) → PCR setup (add 5 µL extract) → Amplification (qPCR, 45 cycles) → Result analysis



According to Corresponding Requirement & Procedures

11.2 Reagent Preparation (Reagent Preparation Area)

- Take out the **nucleic acid extraction reagent** or **Nucleic Acid Release Reagent** and the components of the kit.
- Balance them at **room temperature**.
- Centrifuge liquid items briefly to collect the contents, then set aside for standby use.

11.3 Specimen Processing (Specimen Processing Area)

A. Nucleic Acid Extraction

- Take liquid samples, Negative Control, and A/B/C/D tube Positive Controls for nucleic acid extraction.
- Perform extraction according to the requirements and procedures of validated nucleic acid extraction kits or release reagents.

B. Amplification System Configuration

- Take out Lyophilized reagent PCR tubes according to the number of samples.
- Each sample corresponds to Lyophilized reagent PCR tubes A, B, C, and D.
- One strip = detection for 8 samples.
- If Negative and Positive Control tests are required, increase the number of samples by 2.

C. Stepwise Setup:

- Add 15 µL Dissolving Solution to each tube to dissolve the lyophilized powder.
- Add 5 µL nucleic acid of the extracted Negative Control / Sample to be tested / Positive Control.
- Total volume of each tube = 20 µL.
- Cap tubes tightly, mix gently by hand (do not vortex).
- Centrifuge briefly at low speed to collect liquid and remove bubbles.

⚠ Note:

- **Nucleic acid samples and Negative Control should be added into tubes A, B, C, and D respectively.**
- **A-tube Positive Control → only into tube A.**
- **B-tube Positive Control → only into tube B, and so on.**

Sample test well preparation	Tube A	Tube B	Tube C	Tube D
Dissolving solution	15 µL	15 µL	15 µL	15 µL
Extracted nucleic acid of sample	5 µL	5 µL	5 µL	5 µL

Table 11.3a. → Sample Well Preparation

Negative control test well preparation	Tube A	Tube B	Tube C	Tube D
Dissolving solution	15 μL	15 μL	15 μL	15 μL
Extracted nucleic acid of negative control	5 μL	5 μL	5 μL	5 μL

Table 11.3b. → Negative Control Well Preparation

Positive control test well preparation	Tube A	Tube B	Tube C	Tube D
Dissolving solution	15 μL	15 μL	15 μL	15 μL
Extracted nucleic acid of A tube positive control	5 μL	–	–	–
Extracted nucleic acid of B tube positive control	–	5 μL	–	–
Extracted nucleic acid of C tube positive control	–	–	5 μL	–
Extracted nucleic acid of D tube positive control	–	–	–	5 μL

Table 11.3c → Positive Control Well Preparation

11.4 Run Setup Check List

Item	Requirement	Verified
Reagents equilibrated	All kit components at room temp; probes protected from light	☐
Strip assignment	One 8-well strip per sample; wells 1–8 assigned in order	☐
Reaction volume	15 μL Dissolving Solution + 5 μL nucleic acid = **20 μL** total	☐
Controls included	≥1 Positive Control and 1 Negative Control per run	☐
Pipetting	Always use aerosol-resistant filter tips; avoid vortexing after reagent rehydration.	☐
Spin-down	Brief centrifugation to remove bubbles	☐
Instrument channels	**FAM / VIC(HEX) / CY5** enabled (per Tube A–D mapping)	☐
Program	50 °C 2 min → 95 °C 2 min → **45 ×** (95 °C 10 s; 60 °C 30 s)	☐
Data settings	Baseline Start 3–15; End 5–20; threshold above NC curve	☐

Table 11.4 → Run Setup Check List

11.5 Notes on Instrument Loading

- **ABI7500, CFX96, QuantStudio, SLAN-96S**:
  - The prepared 8-strip tubes can be directly transferred to the amplification detection area.
- **BTK-8**:
  - The mixture from 8-strip tubes should be transferred into the chip wells.
  - Hold pipette at 90° vertical, use aerosol barrier tips, pipette into the center, stop at the first stop to avoid bubbles.
  - Seal wells with chip membrane before transferring to amplification area.

11.6 Storage of Remaining Tubes

- Each lyophilized reagent strip contains 8 test tubes.
- If the number of samples + controls is not a multiple of 8, unused tubes may be cut off with scissors, sealed, and stored at –20 ℃ ± 5 ℃ for next use.

11.7 PCR Amplification (Detection Area)

A. Put the reaction tubes into PCR instrument and set the names of each reaction well in the corresponding order.
B. Select fluorescence channels and targets corresponding to different tubes according to the following table:

Channel	Tube A	Tube B	Tube C	Tube D
FAM	2019-nCoV ORF1ab gene	Influenza A virus	Respiratory adenovirus	Parainfluenza virus type 1
VIC	2019-nCoV N gene	Influenza B virus	Mycoplasma pneumoniae	Parainfluenza virus type 2
CY5	Human RNaseP gene (IC)	Respiratory syncytial virus	Human rhinovirus	Parainfluenza virus type 3

Table: 11.7 → PCR Amplification (Detection Area)

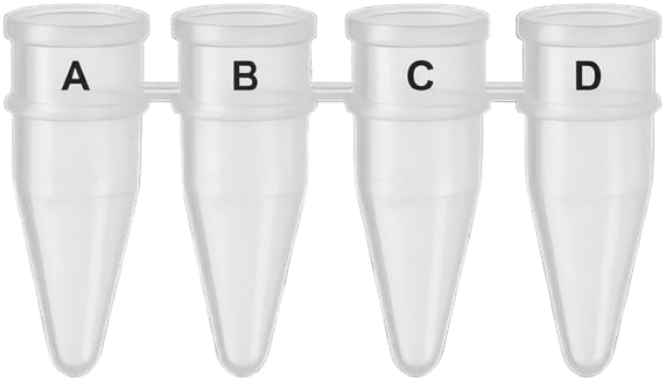
11.8 PCR Cycling Program

Steps	Temperature	Time	Cycles
1. Reverse Transcription	50 ℃	10 min	1
2. Pre-denaturation	95 ℃	2 min	1
3. Denaturation	95 ℃	10 s	45
3. Annealing, extension, fluorescence acquisition	58 ℃	30 s	

Table: 11.8a → Amplification Program of Common Real-Time PCR Instrument

Steps	Temperature	Time	Cycles
1. Reverse Transcription	50 ℃	5 min	1
2. Pre-denaturation	95 ℃	2 min	1
3. Denaturation	95 ℃	5 s	45
3. Annealing, extension, fluorescence acquisition	58 ℃	14 s	

Table: 11.8b → Amplification Program of BTK-8

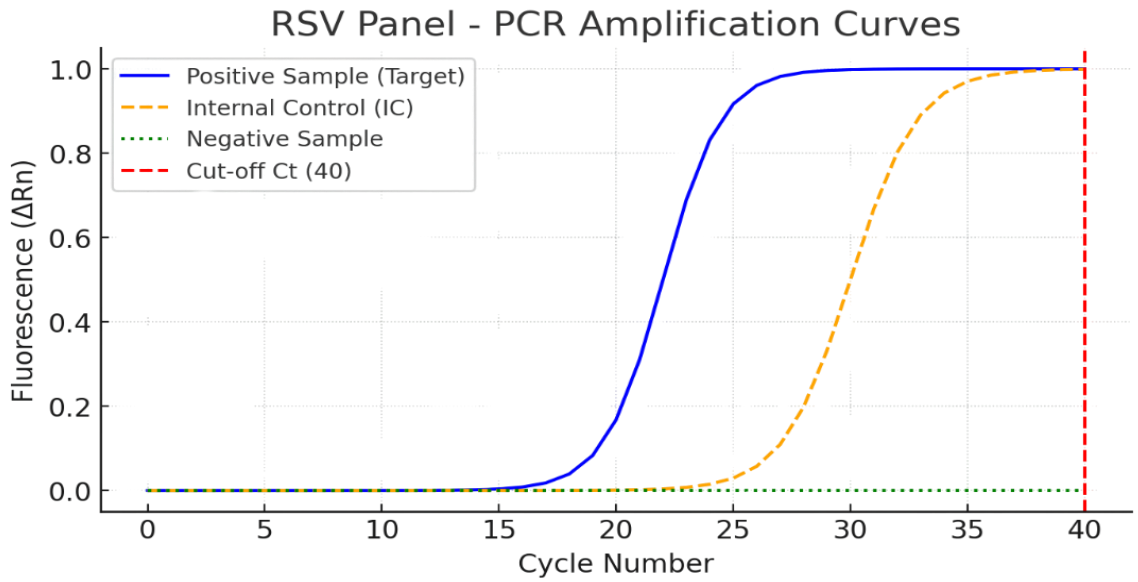


11.9 Results Analysis (refer to Instrument User Manual)

After the reaction, the results will be saved automatically.

A. Common real-time PCR instruments:

- Adjust the Start value and End value of the Baseline:
  - Start value: **3–15**
  - End value: **5–20**
- The amplification curve of the Negative Control should be straight or remain below the threshold line.
- Analyze the amplification curves of different detection targets separately, using the corresponding Negative Control.
- Threshold line setting principle:
- The threshold line should be placed just above the highest point of the Negative Control curve and above the fluorescence background of the sample.



B. BTK-8 instruments:

- Analysis is performed using the default program.
- Click “Analysis” to automatically obtain the results.
- Adjust parameters as needed to meet the requirements of Section 5: Quality Control.
- Review the detection results in the Report window.

11.10 Quality Control

The results of negative control and positive control in each tube and channel shall conform to the following table:

Channel	Negative Control	Positive Control
Tube A		
FAM	No Ct or Ct > 40	Ct ≤ 38 with normal amplification curve
VIC	No Ct or Ct > 40	Ct ≤ 38 with normal amplification curve
CY5	Ct ≤ 38 with normal amplification curve	Ct ≤ 38 with normal amplification curve
Tube B/C/D		
FAM	No Ct or Ct > 40	Ct ≤ 38 with normal amplification curve
VIC	No Ct or Ct > 40	Ct ≤ 38 with normal amplification curve
CY5	No Ct or Ct > 40	Ct ≤ 38 with normal amplification curve

Table 11.10 → Quality Control

12 Test Results Interpretation

12.1. Interpretation of Ct value of target gene detection:

Target Gene	Tube and Channel	Channel	Negative (–)	Positive (+)
2019-nCoV ORF1ab gene	Tube A	FAM	No Ct or Ct > 40	Ct ≤ 40
2019-nCoV N gene	Tube A	VIC	No Ct or Ct > 40	Ct ≤ 40
Human RNaseP gene (Internal control)	Tube A	CY5	No Ct or Ct > 40	Ct ≤ 40
Influenza A virus	Tube B	FAM	No Ct or Ct > 40	Ct ≤ 40
Influenza B virus	Tube B	VIC	No Ct or Ct > 40	Ct ≤ 40
Respiratory syncytial virus	Tube B	CY5	No Ct or Ct > 40	Ct ≤ 40
Respiratory adenovirus	Tube C	FAM	No Ct or Ct > 40	Ct ≤ 40
Mycoplasma pneumoniae	Tube C	VIC	No Ct or Ct > 40	Ct ≤ 40
Human rhinovirus	Tube C	CY5	No Ct or Ct > 40	Ct ≤ 40
Parainfluenza virus type 1	Tube D	FAM	No Ct or Ct > 40	Ct ≤ 40
Parainfluenza virus type 2	Tube D	VIC	No Ct or Ct > 40	Ct ≤ 40
Parainfluenza virus type 3	Tube D	CY5	No Ct or Ct > 40	Ct ≤ 40

Table 12.1 → Interpretation of Ct value of Target Gene Detection:

12.2. Interpretation of Specimen

Since tube A contains the detection of internal control, the results of tube A need to be determined before all results are determined.

- If tube A gets valid results, the whole test is valid, and then the results of other tubes B, C and D can be continued to be interpretated.
- If tube A produces invalid result, the whole test is invalid, and the sample needs to be sampled and tested again.

Target Gene	Tube A valid results				Tube A invalid result
2019-nCoV ORF1ab gene	+	+	–	–	–
2019-nCoV N gene	+	–	+	–	–
Human RNaseP gene (Internal control)	+/–	+/–	+/–	+	–
Interpretation	2019-nCoV Positive	2019-nCoV suspected		2019-nCoV Negative	Invalid

Table 12.2 → Specimen Interpretation (Tube A valid vs invalid)

Note:

1. When the 2019-nCoVsuspected result is obtained, the sample shall be tested again. If the repeated test result is still positive for single gene, the sample is confirmed as 2019-nCoV positive.
2. Under the condition that the results of tube A are valid, the results of other pathogens of tube B, C and D shall be interpretated according to the following table:

12.3. Specimen Interpretation Grid – Pathogen-Specific Results

Target Gene	1	2	3	4	5	6	7	8	9
Influenza A virus	+	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–
Influenza B virus	+/–	+	+/–	+/–	+/–	+/–	+/–	+/–	+/–
Respiratory syncytial virus	+/–	+/–	+	+/–	+/–	+/–	+/–	+/–	+/–
Respiratory adenovirus	+/–	+/–	+/–	+	+/–	+/–	+/–	+/–	+/–
Mycoplasma pneumoniae	+/–	+/–	+/–	+/–	+	+/–	+/–	+/–	+/–
Human rhinovirus	+/–	+/–	+/–	+/–	+/–	+	+/–	+/–	+/–
Parainfluenza virus type 1	+/–	+/–	+/–	+/–	+/–	+/–	+	+/–	+/–
Parainfluenza virus type 2	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+	+/–
Parainfluenza virus type 3	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+/–	+
Interpretation	Influenza A Virus Positive	Influenza B Virus Positive	Respiratory Syncytial Virus Positive	Respiratory adenovirus Positive	Mycoplasma pneumoniae Positive	Human rhinovirus Positive	Parainfluenza virus type 1 Positive	Parainfluenza virus type 2 Positive	Parainfluenza virus type 3 Positive

Table 12.3 → Pathogen-Specific Expanded Grid

Note:

1. 1.Result interpretation does not exclude the occurrence of positive results of mixed infection with multiple pathogens. For example, if the test result is positive for influenza A virus and rhinovirus, it indicates that the sample is mixed infected with influenza A virus and rhinovirus. 2.If result of tube A is 2019-nCoV negative, and all the other pathogens results of tube B\C\D are negative, the sample is interpretated to be negative for all respiratory pathogens detected by this kit

13 Assay Limitations

A. The detection results of this kit are intended for clinical reference only and should not be used as the sole basis for diagnosis or treatment decisions. The clinical management of patients should always be determined in combination with clinical signs, symptoms, history, and other diagnostic information.

B. Test performance may be affected by factors such as specimen collection, handling, storage, and transportation.
- Improper collection may yield insufficient nucleic acids, causing false negatives.
- Cross-contamination during specimen handling may cause false positives.

C. C. This kit targets conserved regions of the genomes of respiratory pathogens. However, mutations or sequence variations in these regions may cause target failure, leading to potential false negatives.

14 Performance Characteristics

14.1 Limit of Detection (LoD):

A. The limit of detection was established at approximately **1,000 copies/mL** for each viral and bacterial target using quantified pseudo virus or plasmid controls

14.2 Precision: (Repeatability & Reproducibility)

A. Coefficient of variation (**CV ≤ 5%**) was observed across intra-assay and inter-assay runs, instruments, and operators.

14.3 Accuracy: (vs. Reference Method)

A. Testing of contrived positive and negative samples demonstrated **100% agreement** with sequencing results or alternate PCR assays.

14.4 Specificity: (Cross-reactivity & Interference

A. No cross-amplification was observed with unrelated respiratory pathogens, including **MERS-CoV, enteroviruses, EBV, CMV,** and common bacterial flora.
B. Seasonal coronaviruses (**229E, OC43, NL63, HKU1**) showed no significant cross-reactivity.
C. Potential interference may occur in the presence of **blood, mucus, or antibiotics**.

14.5 Inclusivity: (Pathogen Strain Coverage)

A. The assay detects the following pathogens across multiple lineages and circulating strains:
- **Parainfluenza virus types 1, 2, and 3**

2019-nCoV (ORF1ab, N gene) **Influenza A virus, Influenza B virus** **Respiratory Syncytial Virus (RSV)**	**Respiratory Adenovirus** **Mycoplasma pneumoniae** **Human Rhinovirus**	**Human RNaseP (internal control)**

15 Attention

A. ⚠ For Research Use Only (RUO). Not for use in diagnostic procedures
B. Transport conditions: The kit must be transported at ≤ 37 °C. If ambient temperature exceeds 37 °C, use insulated containers with ice packs to maintain temperature.
C. Always use sterile, DNase-free and RNase-free consumables (tubes, pipette tips) during testing to prevent contamination.
D. To avoid RNase/DNase contamination, all procedures must be performed in a Class II biosafety cabinet while wearing appropriate personal protective equipment (PPE): disposable gloves, lab coats/gowns, protective eyewear, and surgical or N95 masks.
E. In case of accidental contact with skin or mucous membranes, rinse immediately with plenty of flowing water. If irritation persists, seek medical advice.
F. Before use, ensure all liquid reagents are completely thawed at room temperature, mixed thoroughly, and centrifuged briefly (≥8,000 rpm for a few seconds) to collect contents.
G. After use, all packaging, consumables, and waste liquids must be disposed of as regulated medical waste, following institutional and local biosafety regulations.
H. RSV: Handle all respiratory swabs (NP/OP), sputum, or BAL fluid as potentially infectious. Follow institutional biosafety guidelines for respiratory pathogens.

16 Troubleshooting

Problem	Possible Cause	Solution
Signal in Negative Control	Contamination (amplicons, aerosols)	Clean workspace, replace reagents/consumables
No IC amplification	RNA degradation or inhibitors	Re-extract; use RNase-free conditions; dilute sample 1:5
High Ct / weak signal	Low viral load; poor swab quality	Recollect sample; ensure proper transport/storage
Positive control fails	PC degraded or program error	Use new kit; verify RT-PCR cycling (including 50 °C RT step)
Irregular fluorescence	Tube sealing or optical issues	Re-spin tubes; reseal; check instrument optics
Channel cross-talk	Spectral overlap between fluorophores	Adjust baseline/thresholds; re-run if needed
Multiple invalid samples	Workflow contamination; RNase contamination	Recheck biosafety workflow; enforce RNase-free technique

Table 16. → Troubleshooting Guide

17 References

A. Clinical management of severe acute respiratory infection when novel coronavirus (nCOV) infection is suspected-Interim guidance, 2020.
B. General Office of the National Health Commission of the People's Republic of China, Office of State Administration of Traditional Chinese Medicine, Diagnosis and Treatment Protocol for COVID-19(Trail Verion 7).
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D. Centers for Disease Control and Prevention. Respiratory Syncytial Virus Infection (RSV). Retrieved at https://www.cdc.gov/rsv/index.html.
E. Centers for Disease Control and Prevention. Human Parainfluenza Viruses (HPIVs). Retrieved from https://www.cdc.gov/parainfluenza/index.html .
F. Wu J . Etiological and epidemiological features of acute respiratory infections in China[J]. Nature Communications.

18 Manufacturer & Distributor

Manufacturer
Bioteke Corporation (Wuxi) Co., Ltd.
Zone A, Floor 4, No. 1719-5, Huishan Avenue,
Huishan Economic Development Zone, Wuxi, Jiangsu, 214174, China
Web: www.bioteke.cn | Email: info@bioteke.cn

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1800 West Hawthorne Lane – STE P
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19 Regulatory Statement

This product is labeled **For Research Use Only (RUO)** in accordance with **21 CFR 809.10(c)(2)(i)**. It is not intended for use in diagnostic procedures. The end-user laboratory is responsible for establishing performance characteristics.

20 Symbols

	Refer to the Manufacturer	REF	UTO Panel PCR Kit	LDT	Lab Development Test
	Distributor Med X Reagent Solutions	IVD	Diagnostic Use	RUO	For Research Use Only
IFU	INFORMATION FOR USE	LOT	Batch code Use-by date	EX/REP	Authorized representative in the European Community
CE	CE mark of conformity				Keep away from Sunlight
	Do not use if package is Temperature limit		Use by date:		Temperature Limit Damaged
COLA	Commission On Laboratory Accreditation	CLIA Certified	Med X Diagnostics is CLIA Certified Diagnostic Lab	CMS	Center of Medicare & Medicaid
	DNA strand (molecular diagnostics		Caution (general hazard warning)	FDA REGISTERED	The Food and Drug Administration Registered
	In vitro diagnostic medical device (⚠ remove if RUO only)		Consult instructions for use		Keep dry
	Date of manufacture For In Vitro		Temperature limit (e.g., –20 °C storage)		Contains sufficient for <n> tests
EU	Authorized representative in the European Community		Keep away from sunlight		Control standard (if supplied)
+ / −	Positive control / Negative control		Keep upright		Do not use if package is damaged
	(Hourglass)		Recyclable packaging		Flammable reagent (if applicable)