AdvSHENTEK Mycoplasma DetectInnova Cassette

User Guide

Version: A/3
For Research Use Only
Product No.: 16031010405

Huzhou Shenke Biotechnology Co., Ltd.

(IMPORTANT: Please read this document carefully before experiment.)

1. Product Overview

The AdvSHENTEK Mycoplasma DetectInnova Cassette, designed for use with the AdvSHENTEK DetectInnova System, uses magnetic particle separation technology and real-time fluorescence PCR technology. The cassette is a closed, disposable system, which contains all necessary reagents for nucleic acid extraction, purification, amplification, and detection, enabling an efficient, all-in-one workflow from sample preparation to result analysis. After sampling or simplified preparation, the user pipettes Magnetic Particles and sample combined with Internal Control (IC) into the cassette, inserts the cassette into an AdvSHENTEK DetectInnova System, and starts a test. The entire test takes approximately 3 hours. For more information, please refer to the Operating Manual for the Mycoplasma DetectInnova System.

The kit is for Research Use Only and is not for use in diagnostic procedures or for therapeutic purposes in humans or animals.

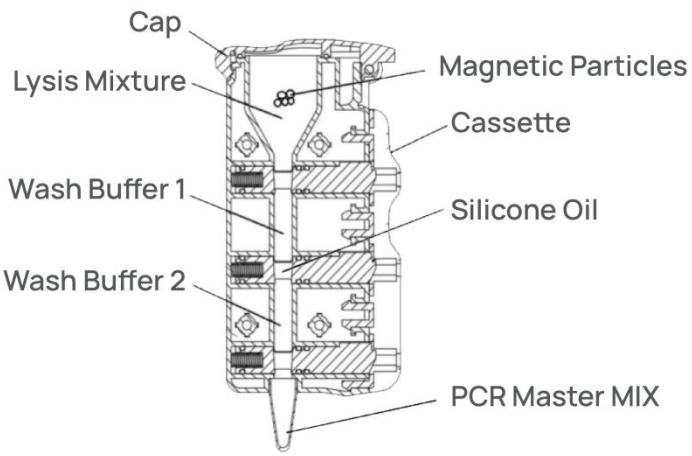

Figure 1. Schematic Diagram of the Cassette

2. Applications

The system enables the qualitative detection of adventitious agents, including *Mycoplasma, Spiroplasma, Acholeplasma, Entomoplasma, and Mesoplasma* in master and working cell banks, virus seed stocks, in-process samples, and final products, etc. It is well suited for release testing or process control testing of cell

therapy samples that may contain high cell counts (up to 10^8 cells) and cell supernatants.

3. Performance Validation

The system is validated according to USP <63>, EP 2.6.7, and JP XVIII, with a detection limit of 10 CFU/mL. It can detect approximately 200 mycoplasma-related species with high specificity. Comprehensive validation studies confirm its performance in a wide range of matrices, including non-mycoplasma species, production cells, engineered bacteria, and fungi.

The closed-system design prevents contamination and ensures that false-positive results are eliminated. Internal controls are built in to monitor the efficiency of nucleic acid extraction and the entire detection process.

4. Kit Contents and Storage

WARNING: Please review the Safety Data Sheets (SDSs) and adhere to handling instructions. Wear appropriate protective equipment, including eyewear, masks, gloves, and laboratory clothing.

Table 1. Kit Components and Storage Conditions

Reagent	Part No.	Quantity	Storage
MPDI2 Cassette*	NNF002	4 Cassettes	2-8°C
MP Internal Control	NNA067	250 µL × 1 tube	2-8°C
MP Positive Control	NNA068	250 µL × 1 tube	2-8°C
Lysis Buffer	NND066	1.25 mL × 1 tube	2-8°C
Magnetic Particles	NND065	250 µL × 1 tube	2-8°C
Saline Buffer	NND067	500 µL × 1 tube	2-8°C
Digestion Buffer	NND068	1 mL × 1 tube	2-8°C
IC Buffer	NND069	1.5 mL × 1 tube	2-8°C
Sample Buffer	NND070	10 mL × 1 bottle	2-8°C

**The cassette name on the QR code label must exactly match the corresponding test program. Scanning the QR code automatically assigns the appropriate execution program on the instrument.*

Note: *Protect the cassette from light after opening. The kit components can be stored under the specified conditions for up to 12 months. Verify the expiration date on the label.*

5. Package

4 Cassettes/Kit

6. Kit Collection, Transport and Storage

Store the kit at 2-8ºC.

Avoid placing kits near heating or cooling vents or in direct sunlight.

All kit components should be stored and used as a complete set. Do not mix components from different lot numbers. Discard any remaining kit components once all cassettes have been used.

7. Materials Required (Not included)

- Low retention, DNase-free, sterile microcentrifuge tubes (1.5 and 2.0 mL)
- Low retention, sterile filter tips (10 μL, 100 μL, 1000 μL)
- 75% ethanol

8. Related Equipment

- AdvSHENTEK DetectInnova System

  The AdvSHENTEK Mycoplasma DetectInnova Cassette is specifically designed for use with the AdvSHENTEK DetectInnova System. Turn on the DetectInnova System by switching the 'Power switch' to the 'I' position at the lower-left corner on the back of the device (Figure 2). The system will automatically initialize, and the display screen will automatically activate upon startup. First-time log in using the 'Admin' account and the default password 'A888888'.

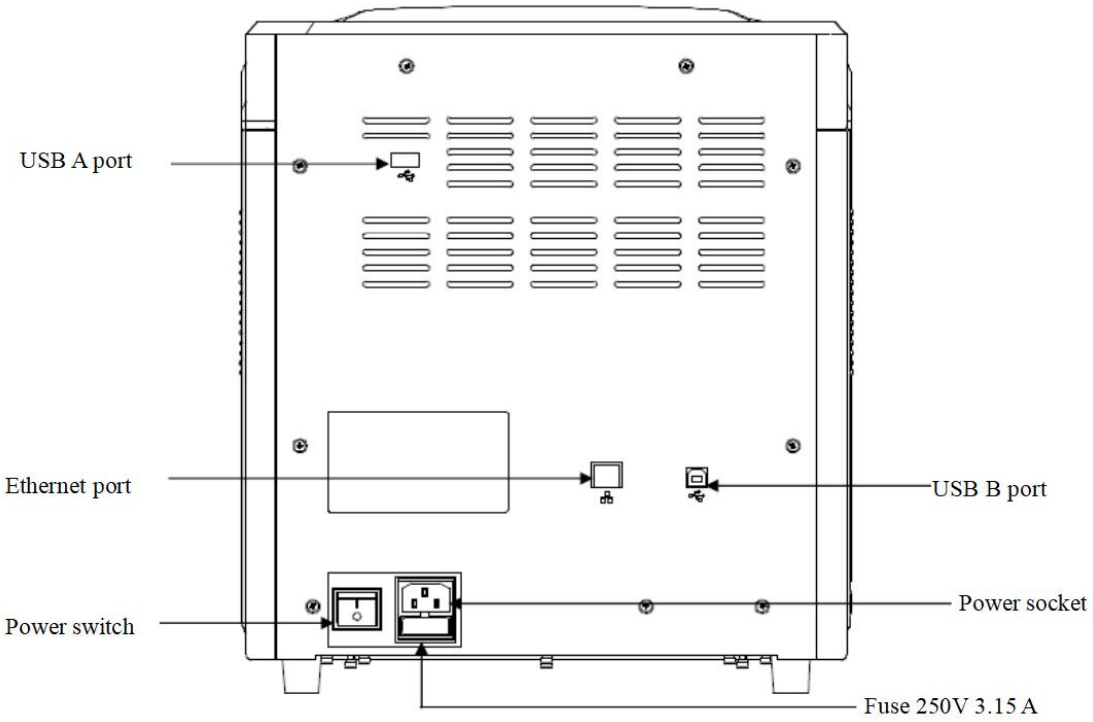

Figure 2. Instrument Rear Panel Overview

Upon successful login, the system interface will be displayed as shown in Figure 3.

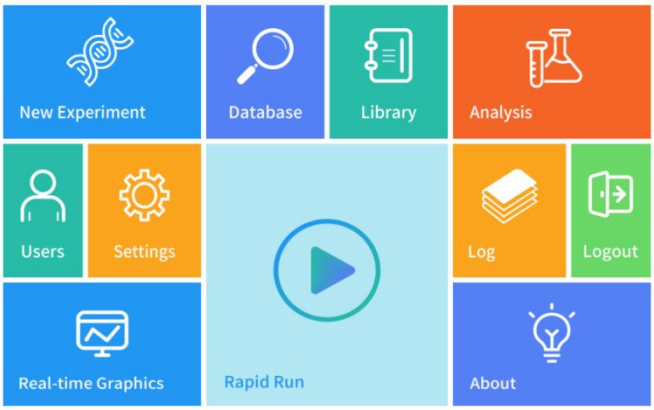

Figure 3. System Interface Displayed After Login

For detailed information on all functional buttons on the main interface, please refer to the **Operating Manual for the Mycoplasma DetectInnova Program**. Below is a brief description of the main functions:

1) **Rapid Run**: Execute assays directly using Mycoplasma DetectInnova cassettes.

2) **New Experiment**: Create a new program either by Manual Setting or by scanning the QR codes. To ensure instrument–reagent compatibility, only authorized accounts are permitted to create new protocols.

   Before performing the first test using the AdvSHENTEK DetectInnova Mycoplasmas Cassette, please refer to Chapter 10: Program Loading to load the standard program.

   For custom program development, please contact Technical Service for assistance in configuring the program parameters through Manual Setting.

3) **Database**: View, import, export, search, and back up generated experimental data.
4) **Library**: Create, edit, delete, save, and execute assay programs.
5) **Analysis**: Access program titles, detection times, sample information, and experimental results for review.
6) **Users**: Manage user accounts; only authorized personnel can add, edit, or delete accounts.
7) **Settings**: Authorized users can configure basic instrument settings, gain settings, running modes, and perform software upgrades.
8) **Log**: View operation and alarm logs, with options to export, search, and back up log files.
9) **Log Out**: Safely log out from the current user account.
10) **Real-time Graphics**: Monitor fluorescence curves, temperature curves, melting curves, running status, running data, and perform various operations in real time.
11) **About**: View the software version, instrument model, instrument serial number, operation guide, and other system information.

9. Related Equipment (If Applicable)

- Benchtop microcentrifuge
- High-speed refrigerated centrifuge

- Heating block with block inserts, for use with 1.5 mL or 2 mL tubes
- Vortex mixer
- Pipettes (10 µL, 100 µL, 1000 µL)
- Laminar flow hood or biosafety cabinet

10. Program Loading

***Note**: If the program is pre-installed in the instrument, skip this step.*

Before performing the initial test, the required 'MPDI2' program must be loaded into the instrument, follow the steps below to load the program:

- Time setting: Select '**Settings**' on the main interface, then select '**Basic Settings**' (Figure 4). Adjust the device date and time to local time via the Date and Time fields (Figure 5). ('Admin' account access only.)

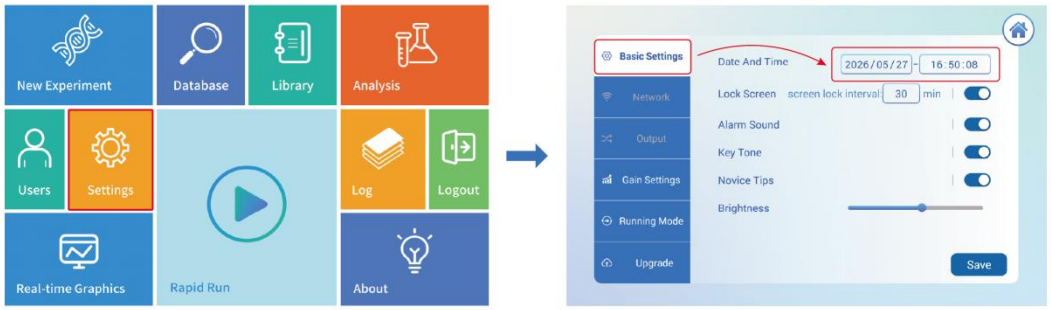

Figure 4. Setting Interface

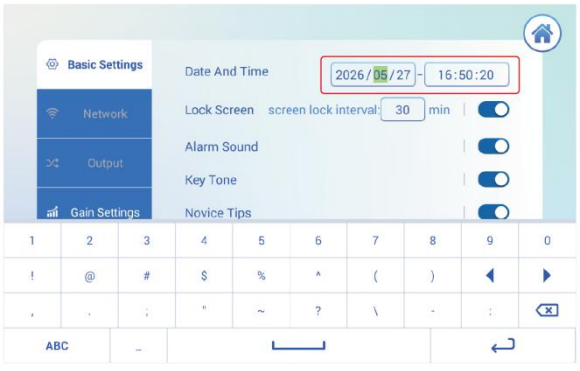

Figure 5. Time Setting

- Select '**New Experiment**' on the main interface, then select '**Scan QR code**' (Figure 6).

- The QR scanner laser will activate, and the scanning interface will be displayed on the screen with message 'Please aim the QR code/barcode at the center of the red scanning light!' (Figure 7).
- Align the center of the program QR code (Figure 8) with the scanner to load the program.
- Once the scanning is complete, the recognized program information will be displayed on the screen. The program name will be displayed in 'Cassette No.' and in the top-right corner of the interface (Figure 9).
- Select either the or to return to the main interface.
- Enter the '**Library**' to verify that the program has been successfully loaded (Figure 10).

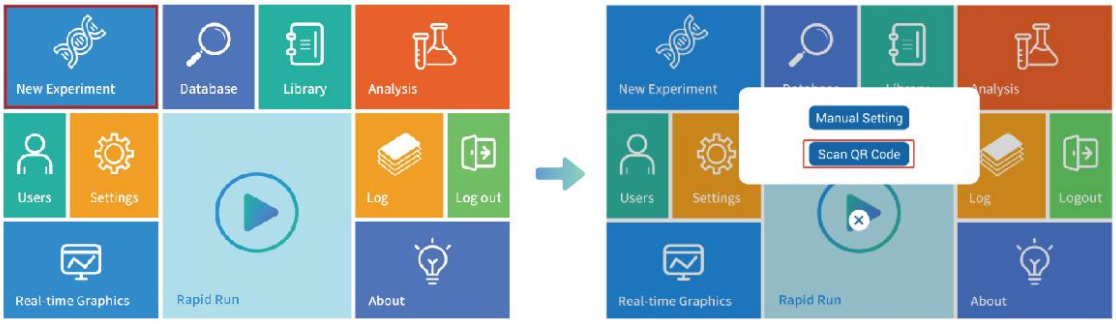

Figure 6. New Experiment Interface

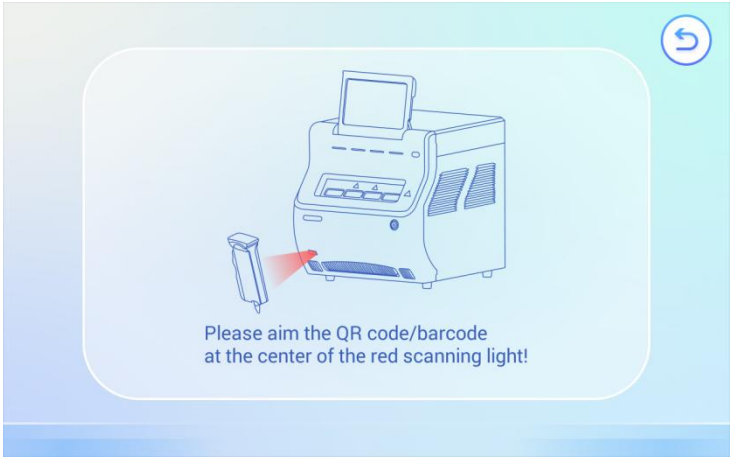

Figure 7. Scan QR Code Interface

Figure 8. Program QR Code

Figure 9. Program Code Installed

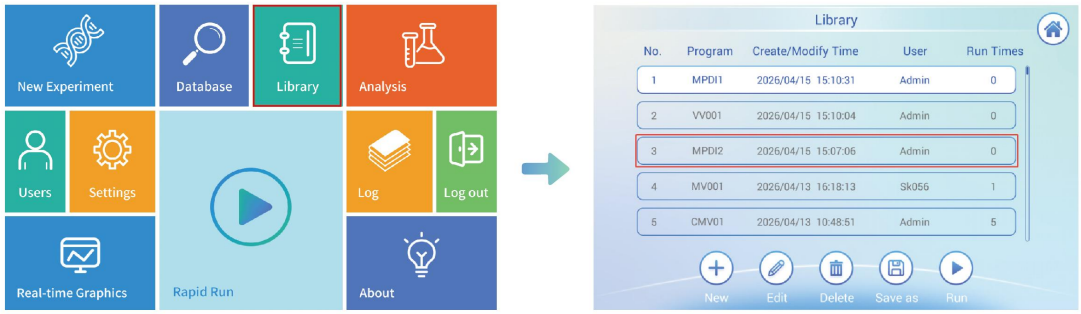

Figure 10. Library Interface

11. Test Procedure

11.1 Test Preparation

11.1.1 Power on a heat block to 25 ℃, 55 ℃ or 72 ℃, 95 ℃ according to the sample type (please refer to 11.2.2).

11.1.2 Set the centrifuge temperature to 2-8 ℃ before use.

11.1.3 Equilibrate the Magnetic Particles (NND065) at room temperature for 10 minutes and vortex briefly before use.

11.1.4 Dilute the MP Internal Control (NNA067) 10× with IC Buffer (NND069) before use.

11.2 Sample Preparation

11.2.1 Control Samples

- Negative control samples (NCS): 1 mL of Sample Buffer (NND070).
- Positive control samples (PCS): add 10 μL of MP Positive Control (NNA068) to 1 mL of Sample Buffer (NND070).

11.2.2 Test Samples

The recommended maximum sample volume is **1 mL**. Please follow the procedures below when handling samples with different total cell counts.

- **Samples (*Total cell counts no more than 10^6*)**

1）Matrix Substitution (optional)**:**

Centrifuge the sample in refrigerated centrifuge at 18,000×*g* for 10 minutes. Remove the supernatant and retain approximately 100 μL of the sample. Adjust the sample volume up to 1 mL using Sample Buffer (NND070).

Note 1*: If the sample contains human serum albumin (HSA) at a concentration higher than 10% (w/v), or other components that may inhibit efficient nucleic acid extraction (e.g., high concentrations of plasmid or virus), perform matrix substitution may eliminate the inhibition and improve the detection rate. A second round of substitution can be conducted if necessary.*

Note 2*:Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation. Before each centrifugation step, verify the tubes are intact and in good condition.*

2) Pre-digestion Preparation

Add 100 μL of Digestion Buffer (NND068) and 50 μL of Saline Buffer (NND067) to the sample. If the total volume is less than 1 mL, adjust to 1 mL using Sample Buffer (NND070).

- **Samples (*Total cell counts between 10^6 to 10^8*)**

1) Cell Lysis

Add Lysis Buffer (NND066) to the sample at a 1:5 volume ratio, vortex thoroughly to mix, and incubate at 25 ℃ for 5 minutes.

2) Removal of Cell Debris

Centrifuge the tubes at 7,250 ×*g* for 10 seconds to pellet the cell debris, then transfer the supernatant to a new microcentrifuge tube.

3) Sample Concentration

Centrifuge the supernatant in refrigerated centrifuge at 18,000 ×*g* for 10 minutes. Remove the supernatant and retain approximately 200 μL of the sample. Adjust the sample volume up to 1 mL using Sample Buffer (NND070).

4) Matrix Substitution (optional)：

Centrifuge the sample in refrigerated centrifuge at 18,000 ×*g* for 10 minutes. Remove the supernatant and leave the remaining volume at approximately 100 μL. Adjust the sample volume up to 1 mL with Sample Buffer (NND070).

***Note**: If the sample contains human serum albumin (HSA) at a concentration higher than 10% (w/v), or total cell count above 10^7, other components that may inhibit efficient nucleic acid extraction (e.g., high concentrations of plasmid or virus), perform matrix substitution may eliminate the inhibition and improve the detection rate. A second round of substitution can be conducted if necessary.*

5) Sample Digestion

Add 100 μL Digestion Buffer (NND068) and 50 μL Saline Buffer (NND067) to each tube, vortex to mix well, and spin in a microcentrifuge for 3 seconds. Incubate the mixture at 72℃ for 15 minutes, except for human serum albumin samples with the corresponding control samples at 55°C for 30 min. To ensure complete digestion, vortex the samples 2-3 times during incubation.

6) Heat Treatment (optional)

If the initial total cell counts of the sample higher than 10^7, after complete digestion, a following optional incubation at 95 °C for 10 minutes may be performed. Then cool the sample to room temperature.

Note: *For other complex samples or technical testing issues, please contact technical support team (info@shentekbio.com) for assistance.*

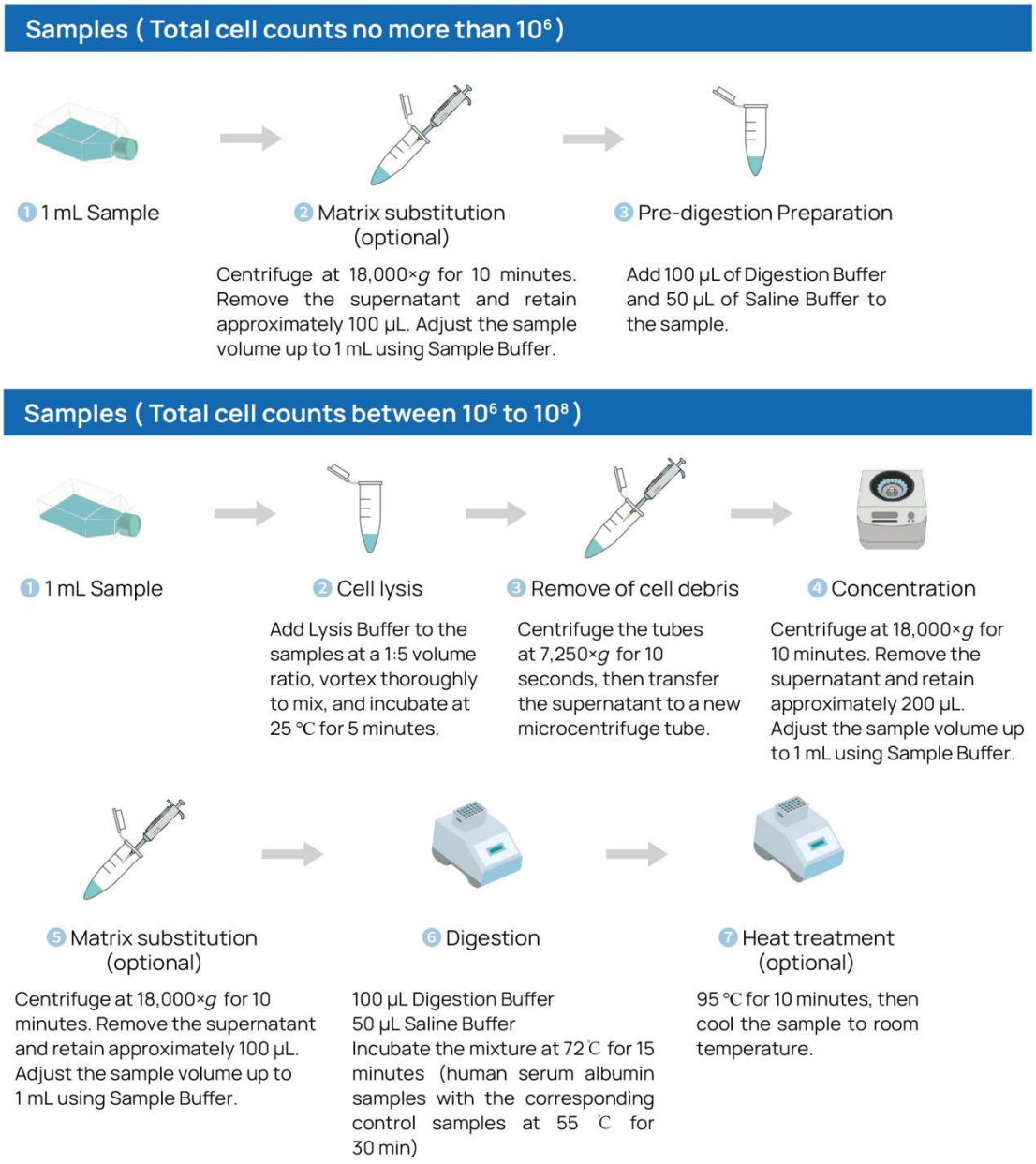

Figure 11. Sample Preparation Flowchart

11.3 Sample Loading

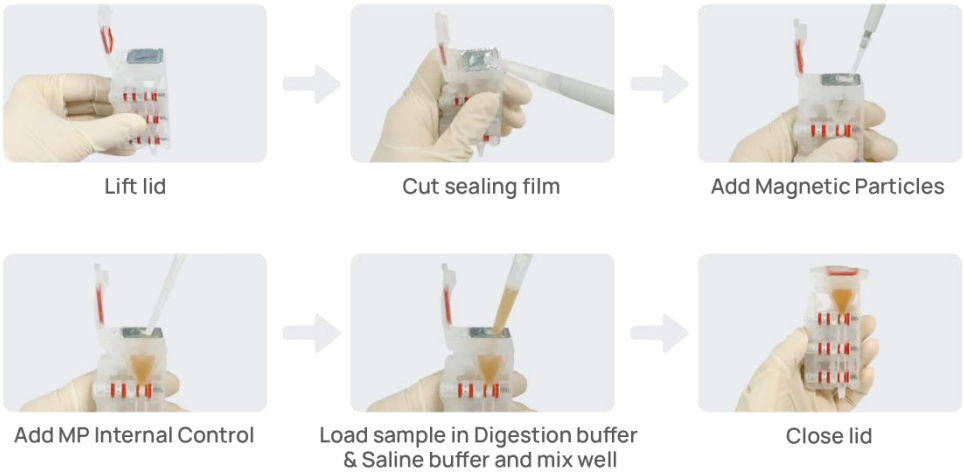

Figure 12. Flowchart of Sample Loading

11.3.1 Make an incision in the film cover: Lift the cassette lid, then use a pipette tip to make an incision in the film cover.

11.3.2 Add Magnetic Particles (NND065) and MP Internal Control (NNA067): Use a pipette to add 10 μL of well-mixed Magnetic Particles by placing the pipette tip below the liquid surface. Then add 10 μL of diluted MP Internal Control.

11.3.3 Add the prepared sample as described in Section 11.2 into the cassette, and securely close the lid.

11.4 Run Test

11.4.1 Select ‘Rapid Run’ on the main interface to display the sample information input screen (Figure 13). A date and time value is automatically set as the default test name. Tap the ‘Test Name’ field to edit it.

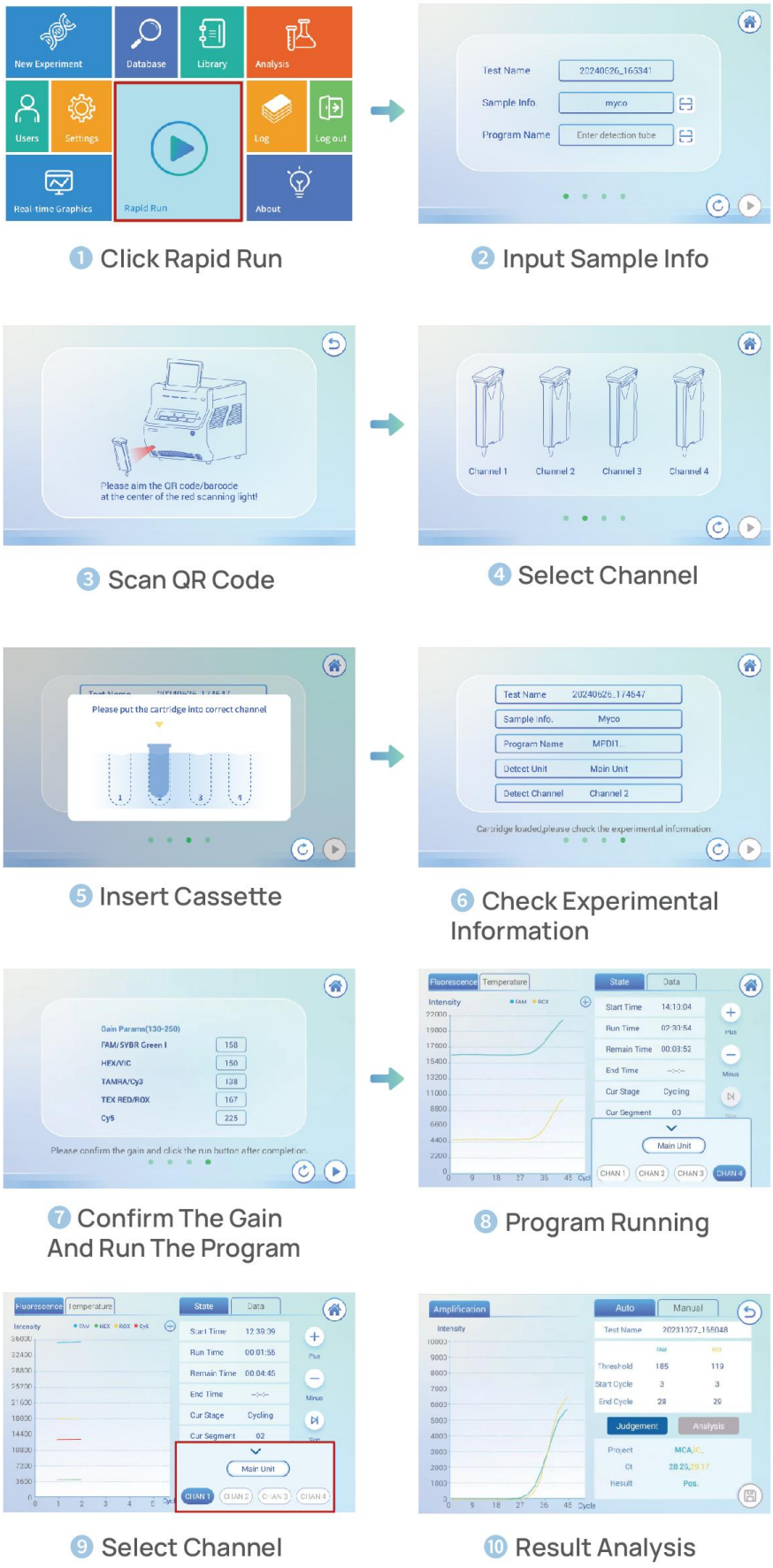

Figure 13. Program Running Graphic Scheme

11.4.2 Enter the sample name in the ‘Sample Info’ field or select the button next to the field to scan the sample code using the scanner on the front cover, if available..

Next, select the button next to the ‘Cassette No.’ field to scan the cassette QR code. Align the center of the QR code with the scanner to load the program (Figure 13). The recognized information will then be automatically displayed in the ‘Cassette No.’ field.

11.4.3 Select the appropriate cassette channel on the screen (Figure 13). Press the ‘**Lid Door Switch**’ button on the instrument’s front panel (Figure 14) to open the lid door. Insert the cassette into the the corresponding channel, and press firmly to ensure secure placement. Then press the ‘**Lid Door Switch**’ to close the lid door.

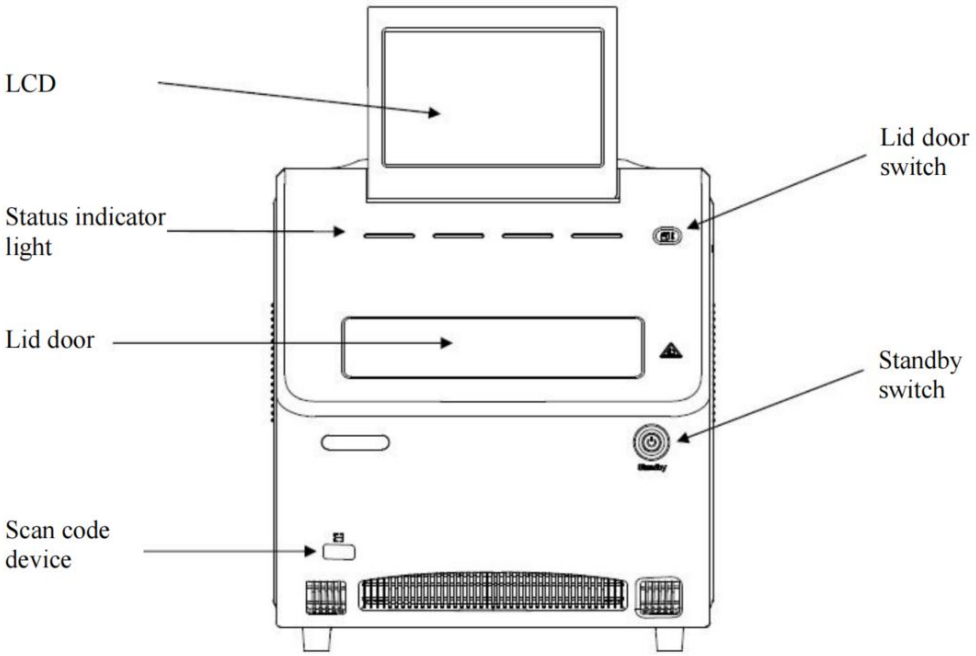

Figure 14. Program Running Graphic Scheme

Note: *Ensure the cassette orientation is correct with the QR code facing upward.*

11.4.4 Proceed with the preset the gain values (All parameters must be established through calibration and remain unchanged for testing.), then select the  button in the bottom right corner of the screen. The program will start and the program interface displayed on the screen (Figure 13).

11.4.6 Check the real-time graphics during testing, and review the results after the analysis is complete (Figure 13).

Note: *It is recommended to include NCS and PCS in each run:*

1. *IC: The amplification status of the IC is used to verify that the process was operating normally and to detect any sample inhibition.*
2. *PCS: A positive result confirms the successful amplification of the positive control (PC) and proper cassettes performance.*
3. *NCS: A negative result indicates the proper functioning of the negative control (NC) and cassettes.*

Contamination may cause unexpected positive results in negative controls. If such results occur, thoroughly clean and decontaminate the workspace to eliminate potential contaminants. Contact Customer Support for further assistance if the issue persists.

12. Result Analysis

Once the program is complete, select the 'Analysis' button and select the data set analyze. Then select the  button on the right side of each row shown in Figure 15. The Ct value interpretations of test samples are displayed. Guidance for result analysis is provided in Table 2.

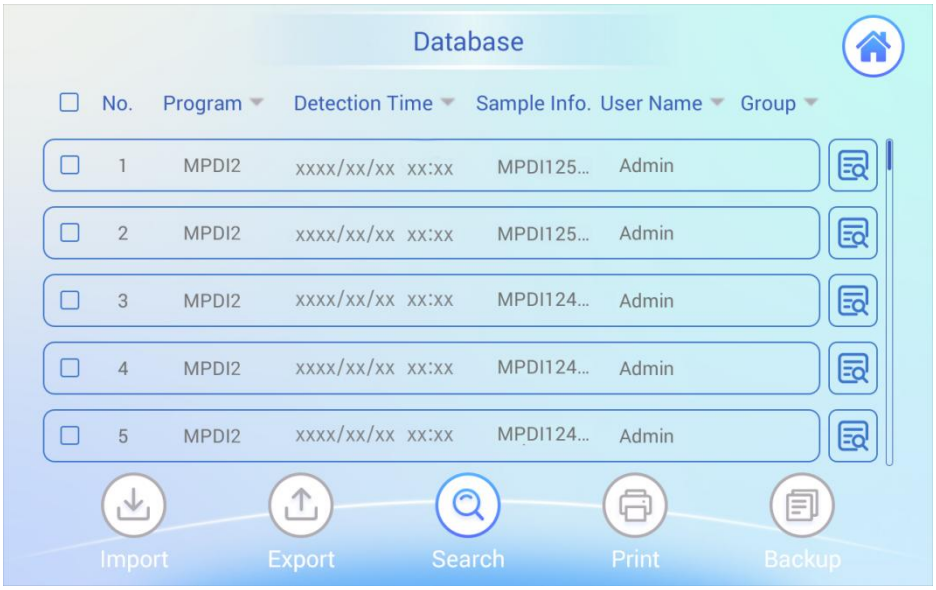

Figure 15. Database Interface

Table 2. Mycoplasma Detection Result Analysis

FAM (Target)	ROX (IC)	Result	Conclusion
Positive	Positive	Positive	Acceptable
Positive	Negative	Positive	Not Conclusive
Negative	Positive	Negative	Acceptable
Negative	Negative	Failure	Not Acceptable

13. Retests

If any of the following conditions occur, repeat the test using new cassettes.

- Abnormal IC gain: Low signal value or abnormal IC gain curve.
- No IC amplification signal: If IC was unintentionally omitted, repeat the test.

Note: *If amplification is abnormal, verify proper reagent dilution and ensure the reaction is proceeding as expected.*

- Abnormal sample amplification curve: Irregular curve.
- Other conditions: For example, liquid leakage or improper sealing of the cassette lid during operation.

14. Precautions

1. The performance of the AdvSHENTEK Mycoplasma DetectInnova Cassette has been validated exclusively on the AdvSHENTEK DetectInnova System.

2. The AdvSHENTEK Mycoplasma DetectInnova Cassette is a qualitative test and does not provide quantitative results.

3. False positive and false negative results can arise from various sources; therefore, results should be analyzed by qualified personnel.

4. Detection of mycoplasma nucleic acid depends on proper specimen collection, handling, transport, storage, and preparation. Failure to follow proper procedures in any of these steps may lead to atypical results. Improper sample collection, transport, or handling increases the risk of false positive or false negative outcomes.

5. Partial PCR inhibition may occur due to high cell densities and potential inhibitory factors.

6. The environment must be kept clean before and after the experimental tests to prevent mycoplasma contamination.

7. There is a risk of false positive results due to contamination of organisms, including nucleic acids, vaccine material, amplified product or non-specific signals in the assay.

8. It is recommended to designate separate areas for negative, positive, and amplification zones with clear markings. Equip each area with independent equipment, reagents, and consumables to avoid cross-contamination. This minimizes unnecessary movement and contamination risks.

9. Centrifuge the tube before opening to reduce the risk of contaminating gloves or pipettes with reagents. Used tips and liquid waste must be disinfected, then discarded in a designated container, and，if necessary, shipped off-site.

10. Remove the cassette immediately after the test is completed, seal it in a designated plastic bag, and place it in the appropriate disposal container. Do not open the cover of the cassette.

11. Do not use if the cassette is damp or the lid seal is damaged.

12. Each cassette is for single use only. Do not reuse.

For further inquiries, please contact us for technical support.

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Support & Contact

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