SIGNALCHEM
DIAGNOSTICS

User Manual

SignalChem Diagnostics

Canine/Feline Diarrhea Toxin 3-Plex Panel One-Pot Detection Kit

To enable point-of-care testing for the immediate detection of Clostridium perfringens in dog and cat samples

Catalog # PM001-K398 (Starter Kit)
Catalog # PM002-K398 (Standard Kit)

Diagnostic Reagents For Accurate And Reproducible Results

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Introduction

Clostridium perfringens is a major cause of acute hemorrhagic gastroenteritis (AHGE), necrotizing enteritis, and diarrhea in dogs and cats. Because clinical symptoms often overlap across different toxigenic strains, simultaneous identification of key toxin genes is critical for precise diagnosis and management.

The Canine/Feline Diarrhea Toxin 3-Plex Panel One-Pot Detection Kit provides simultaneous qualitative detection and profiling of key *C. perfringens* toxin genes alongside an internal Positive Control (PC) in a single test run. Each sample is evaluated across 4 dedicated reaction tubes:

Each kit includes 4-tube master mix strips, where each 4-tube strip contains pre-dispensed, lyophilized reagents for one sample:

- Tube 1 (cpa): Alpha-toxin gene
- Tube 2 (cpe): Enterotoxin gene
- Tube 3 (netF): NetF toxin gene
- Tube 4 (PC): Internal Positive Control (Assay run validation)

Clinical Cutoff Annotations: Tubes 1 (cpa) and 2 (cpe) are interpreted as clinically positive >300 copies/mg to differentiate pathogenic levels from normal commensal flora. Tube 3 (netF) is qualitative, where any detectable signal indicates a positive result due to its high virulence association.

Up to two 4-tube strips (2 samples / 8 tubes total) can be loaded into the CRISPOT® Detection Device for simultaneous testing in a single run.

This manual applies to both the Starter Kit (Cat# PM001-K398) and Standard Kit (Cat# PM002-K398). The kit is engineered for use with the CRISPOT® Detection Device (Cat# CSP01-V19A, SOLD SEPARATELY).

Disclosure

This kit is intended for use by veterinary professionals, veterinary clinic staff, and trained pet care personnel. Results should always be interpreted in conjunction with clinical signs and other diagnostic findings. Sensitivity may be affected by sample quality and collection timing. Pet owners and non-clinical users are strongly encouraged to consult a licensed veterinarian before making any treatment decisions based on test results.

Support

For inquires and assistance, please contact us at:
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Version History

Version 2.0
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Kit Components

Starter Kit-Only Components

#	Component	Amount
1	Tube Rack	1
2	Adjustable Pipette	1

Not Provided

Gloves
Timer

Standard Kit Components

#	Component	Quantity
3	Pipette Tips	10
4	Screw-Cap Collection Tubes (2 mL/tube)	4 tubes
5	Reaction Buffer ① — Blue-Strip Tubes	4 tubes
6	Reaction Buffer ② — Yellow-Cap Tubes	4 tubes
7	PCR Tube Strips With Lyophilized Master Mix (*cpa*, *cpe*, *netF*, PC)	4 strips (16 tubes total)
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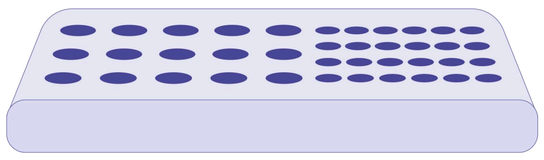
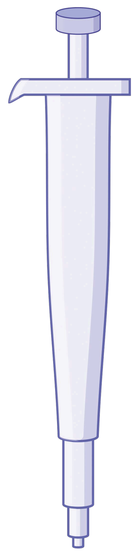
Kit Components: Visual Guide

Starter Kit-Only

1 Tube Rack

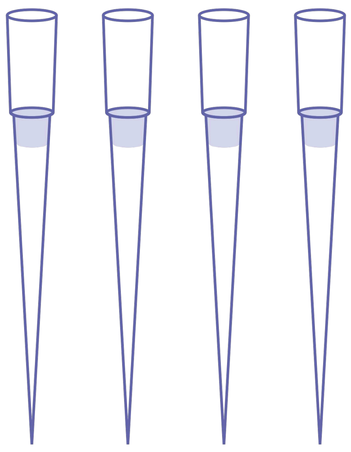


2 Adjustable Pipette

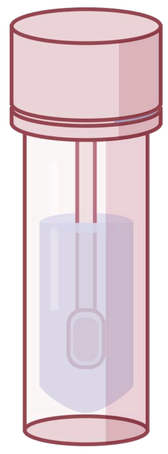


Standard Kit

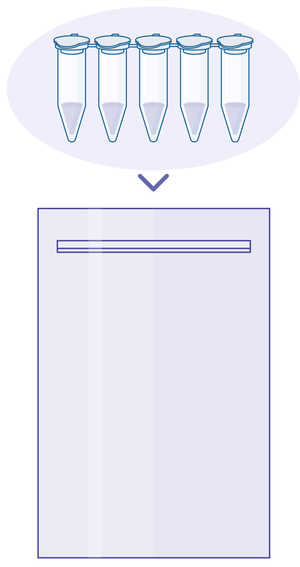
3 Pipette Tips



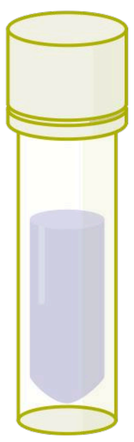
4 Screw-Cap Collection Tubes



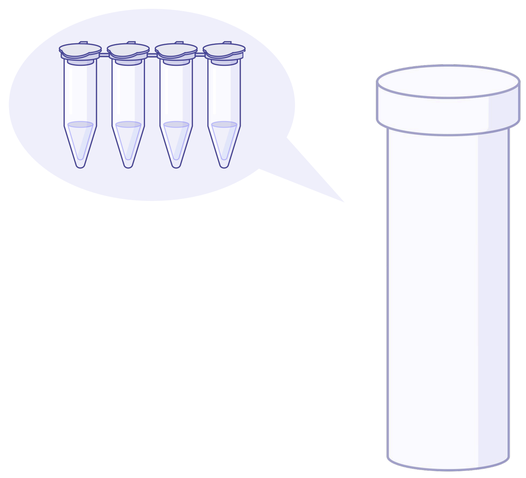
5 Reaction Buffer ① (Blue-Strip Tubes)



6 Reaction Buffer 2 (Yellow-Cap Tubes)



7 PCR Tube Strips With Master Mix in White Plastic Tube



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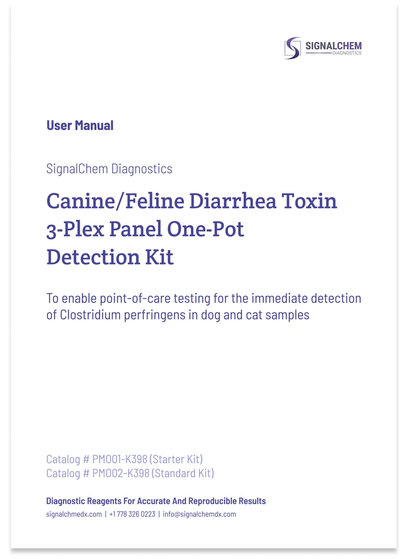

Storage and Precautions

Kit Storage

- Store all kit components at 2–25°C.
- Protect the Master Mix tubes from light. Keep the tube strips in their box until immediately before use. Exposure to light may cause reagent deterioration and lead to inaccurate results.
- Before use, allow all components to equilibrate at room temperature for at least 10 minutes to ensure optimal performance.
- Store all components in their original sealed packaging to protect from moisture or contamination.

Pipette Operation Guide

The pipette is preset to 10 µL for sample preparation steps. Adjust to 30 µL for the master mix loading step (Test Procedure Step 2).

1. Attach a sterile tip securely to the pipette.
2. Depress the plunger to the first stop before immersing the tip into the liquid.
3. Slowly release the plunger to aspirate the sample.
4. Transfer the sample to the destination tube and fully depress the plunger to dispense.
5. Keep the plunger depressed while withdrawing the tip from the tube.
6. Discard used tips immediately into an appropriate waste container.

Precautions

- Strictly follow the instructions in this manual to ensure accurate results.
- Always use a new pipette tip for each sample transfer to prevent cross-contamination.
- Perform all procedures in a clean environment.
- Wear gloves and appropriate personal protective equipment (PPE) when handling samples.
- Use reagents only within their expiration date and under recommended storage conditions.
- Do not alter reagent volumes or modify any procedure steps, as this may compromise test performance.
- **Do not open reaction tubes after amplification has begun**, to prevent contamination.
- Dispose of all used consumables in sealed waste bags in accordance with local biosafety guidelines.

Sample Collection and Preparation

Note: Each sample requires one 4-tube strip (cpa, cpe, netF, PC). You may run 1 strip (1 sample) or 2 strips (2 samples) per CRISPOT® batch run.

Note: Label lids of the tubes with the corresponding sample ID.

IMPORTANT: Ensure all liquid and pellets in tubes are at the bottom of the tubes before opening.

Step 1. Sample Collection

Preferred specimen: Freshly collected fecal sample gathered during suspected active enteric infection.

1. Unscrew the cap with the integrated scoop from the collection tube 4 .
2. Use the built-in scoop to collect a small portion of fresh feces (approximately the size of a pea, ~100–200 mg). Avoid collecting ground litter, soil, or straw.
3. Insert the scoop back into the collection tube and screw the cap on tightly to fully submerge the sample into the 2.0 mL of sample collection medium.

Homogenization:

4. Mix thoroughly by vortexing or vigorous shaking for 15–20 seconds to completely disperse and suspend the sample into the sample collection medium.

Settling:

5. Allow the tube to stand upright at room temperature for 1–3 minutes to let dense particulate matter settle to the bottom.

Step 2. Sample Processing

Note: Always use a new pipette tip for each sample to avoid cross-contamination.

6. For each sample, take out one tube of Reaction Buffer ① 5 and Reaction buffer ② 6 . Flick the tube downward sharply or tap it on the bench to ensure liquid settles completely at the bottom.
7. Using the adjustable pipette 2 set to 10 µL, transfer 10 µL of the non-viscous supernatant into a clearly labeled Reaction Buffer ① tube (blue strip tubes) 5 .
8. Mix thoroughly by pipetting up and down 5 times.
9. Transfer 10 µL of the processed sample from the Reaction Buffer ① 5 tube into a clearly labeled Reaction buffer ② tube (yellow cap) 6 .
10. Immediately cap the Reaction buffer ② 6 tube and mix by vortexing or vigorous agitation for 10–15 seconds.
11. Use the resulting sample for testing.

Test Procedure

Step 1. Prepare the Tube Strip

1. Take one 4-tube strip (7) per sample to be tested:
   - Tube 1: *cpa*
   - Tube 2: *cpe*
   - Tube 3: *netF*
   - Tube 4: PC
2. Label the strip with the corresponding Sample ID.

Step 2. Sample Loading

3. Set the pipette (2) to 30 µL.
4. Using a fresh pipette tip for each tube, transfer 30 µL of Processed Sample (from Reaction buffer ② yellow cap tube (6)) into each of the 4 tubes within its designated strip (7).
- **Change the pipette tip before dispensing into Tube 1, Tube 2, Tube 3, and Tube 4.**
5. If running additional samples in the same batch, repeat Step 2 with a new 4-tube strip, continuing to use a fresh pipette tip for every tube.

IMPORTANT: CONTAMINATION PREVENTION

- **Change pipette tips between every single tube**, even when dispensing the same sample into the 4 tubes of a strip, to prevent cross-contamination of liquid reagents between reaction tubes.
- Avoid direct contact between the pipette tip and the lyophilized pellet. When dispensing, gently touch the tip to the inner wall of the tube slightly above the reagent pellet.

Step 3. Mixing

6. Close the tube cap firmly to ensure a liquid-tight seal (7).
7. Gently tap the bottom of the tube approximately 5 times.
8. Flick the tube downward sharply or tap it on the bench to ensure liquid settles completely at the bottom, fully rehydrating the lyophilized pellet. Verify that no bubbles remain in the bottom of the tube.
- If a microcentrifuge is available, briefly centrifuge the tube to bring the entire volume to the bottom and eliminate any air bubbles.

CRITICAL: Check that all liquid is at the bottom of the tube and no large bubbles are present before proceeding to ensure accuracy.

Test Procedure

Step 4. Run the Test

9. Insert Strip: Place the loaded 4-tube strip into the CRISPOT® device reaction chamber. Press down gently to ensure the strip is fully and correctly seated.
10. Power On: Turn on the CRISPOT® device.
11. Select Program: Use the UP and DOWN buttons to select CUSTOM.

Note: The device should default to CUSTOM. Do not select any other program, as incorrect heating parameters will invalidate test results.

12. Verify Parameters: Confirm that the display shows the correct run settings: 63°C and 35 minutes.
13. Start Run: Press START to initiate the assay. The display will show "Testing...".

Note: The test will run for approximately 35 minutes. Do not open the lid or move the device during operation.

14. Completion: When the run finishes, the screen will display "Completed".

Note: Refer to the CRISPOT® Detection Device Instructions for Use for full device operating instructions.

Sample Collection and Preparation: Visual Guide

Note: Each sample requires one 4-tube strip (cpa, cpe, netF, PC). You may run 1 strip (1 sample) or 2 strips (2 samples) per CRISPOT® batch run.

Note: Label lids of the tubes with the corresponding sample ID.

IMPORTANT: Ensure all liquid and pellets in tubes are at the bottom of the tubes before opening.

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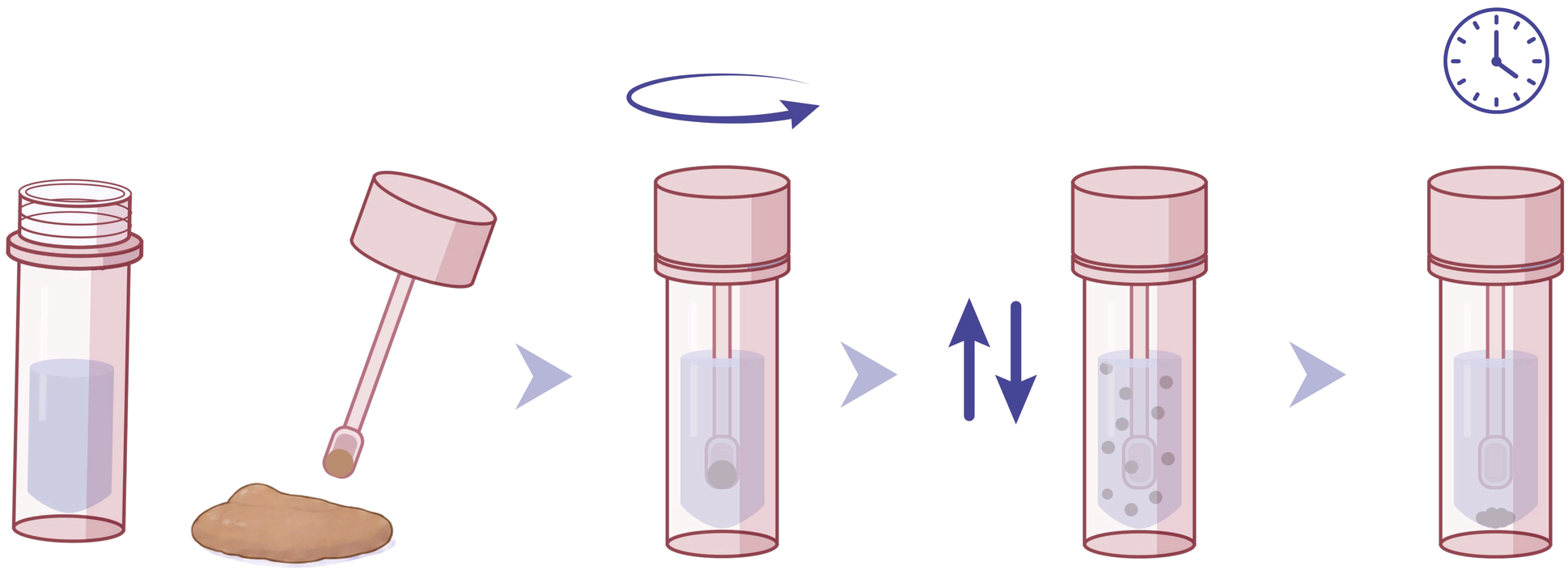
Settling:

5. Allow the tube to stand upright at room temperature for 1–3 minutes to let dense particulate matter settle to the bottom.

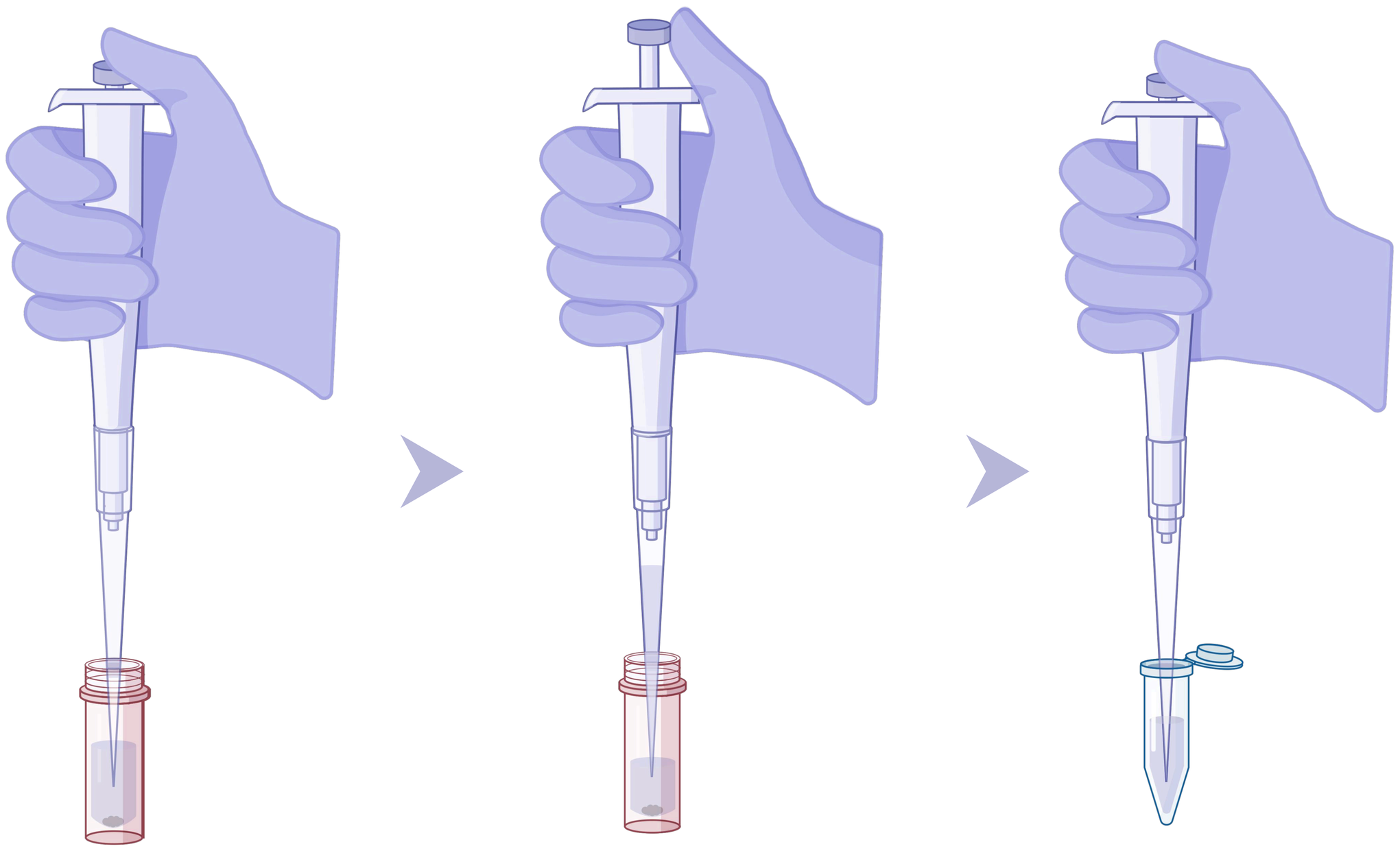


Sample Collection and Preparation:Visual Guide

Step 2. Sample Processing

Note: Always use a new pipette tip for each sample to avoid cross-contamination.

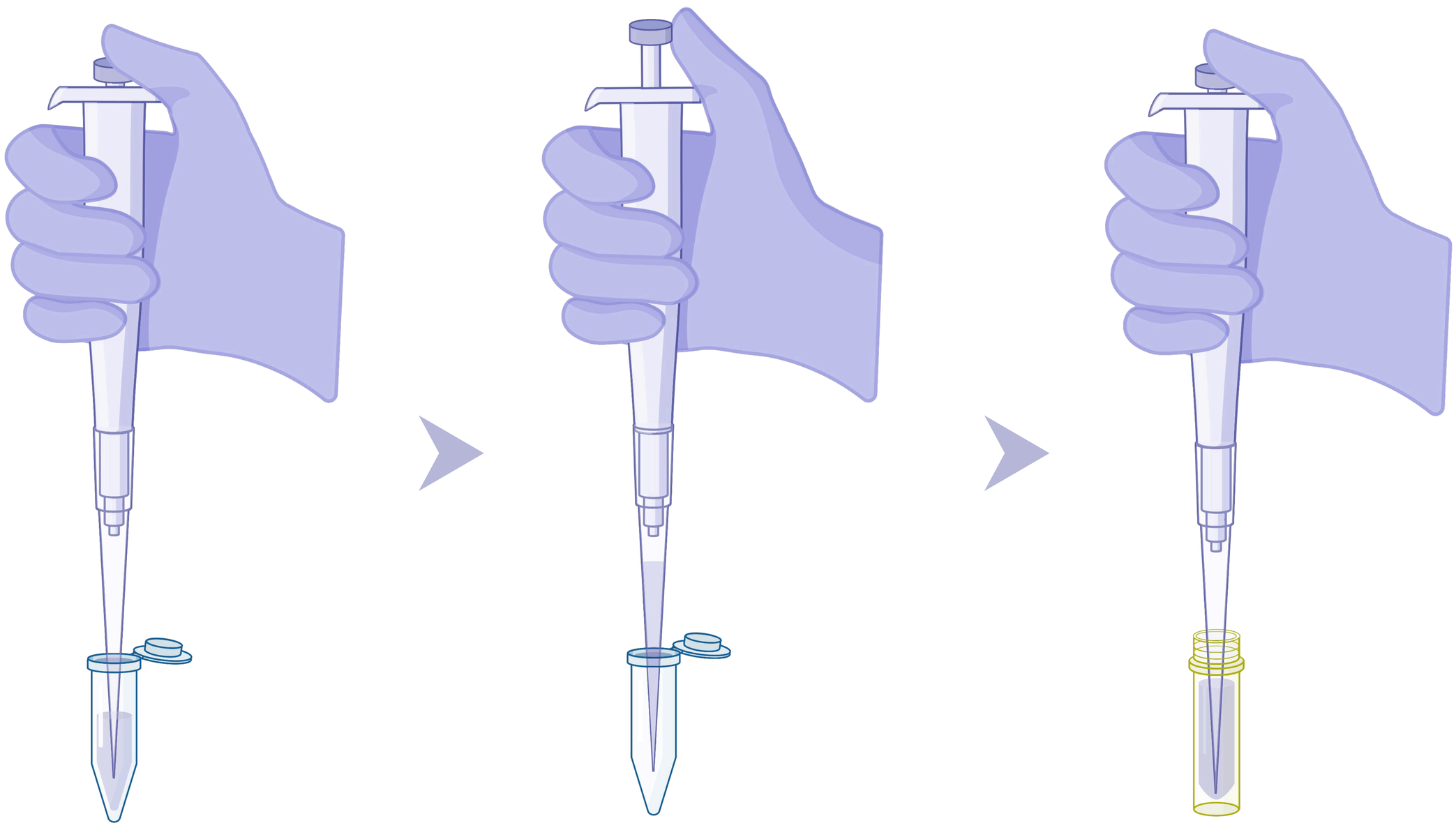
6. For each sample, take out one tube of Reaction Buffer ① 5 and Reaction buffer ② 6 Flick the tube downward sharply or tap it on the bench to ensure liquid settles completely at the bottom.
7. Using the adjustable pipette 2 set to 10 µL, transfer 10 µL of the non-viscous supernatant into a clearly labeled Reaction Buffer ① tube (blue strip tubes) 5
8. Mix thoroughly by pipetting up and down 5 times.



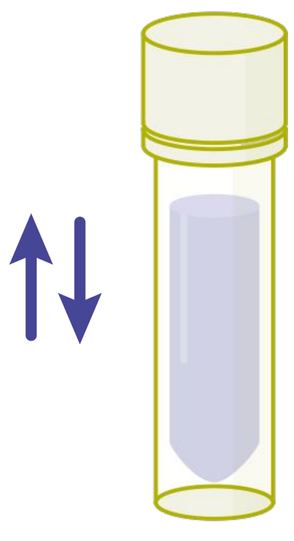
Sample Collection and Preparation:Visual Guide

Step 2. Sample Processing

9. Transfer 10 µL of the processed sample from the Reaction Buffer ① **5** tube into a clearly labeled Reaction buffer ② tube (yellow cap) **6**



10. Immediately cap the Reaction buffer ② **6** tube and mix by vortexing or vigorous agitation for 10–15 seconds.
11. Use the resulting sample for testing.



Test Procedure: Visual Guide

Step 1. Prepare the Tube Strip

1. Take one 4-tube strip (7) per sample to be tested:
   - Tube 1: *cpa*
   - Tube 2: *cpe*
   - Tube 3: *netF*
   - Tube 4: PC
2. Label the strip with the corresponding Sample ID.

Step 2. Sample Loading

3. Set the pipette (2) to 30 µL.
4. Using a fresh pipette tip for each tube, transfer 30 µL of Processed Sample (from Reaction buffer ② yellow cap tube (6)) into each of the 4 tubes within its designated strip (7).
- **Change the pipette tip before dispensing into Tube 1, Tube 2, Tube 3, and Tube 4.**
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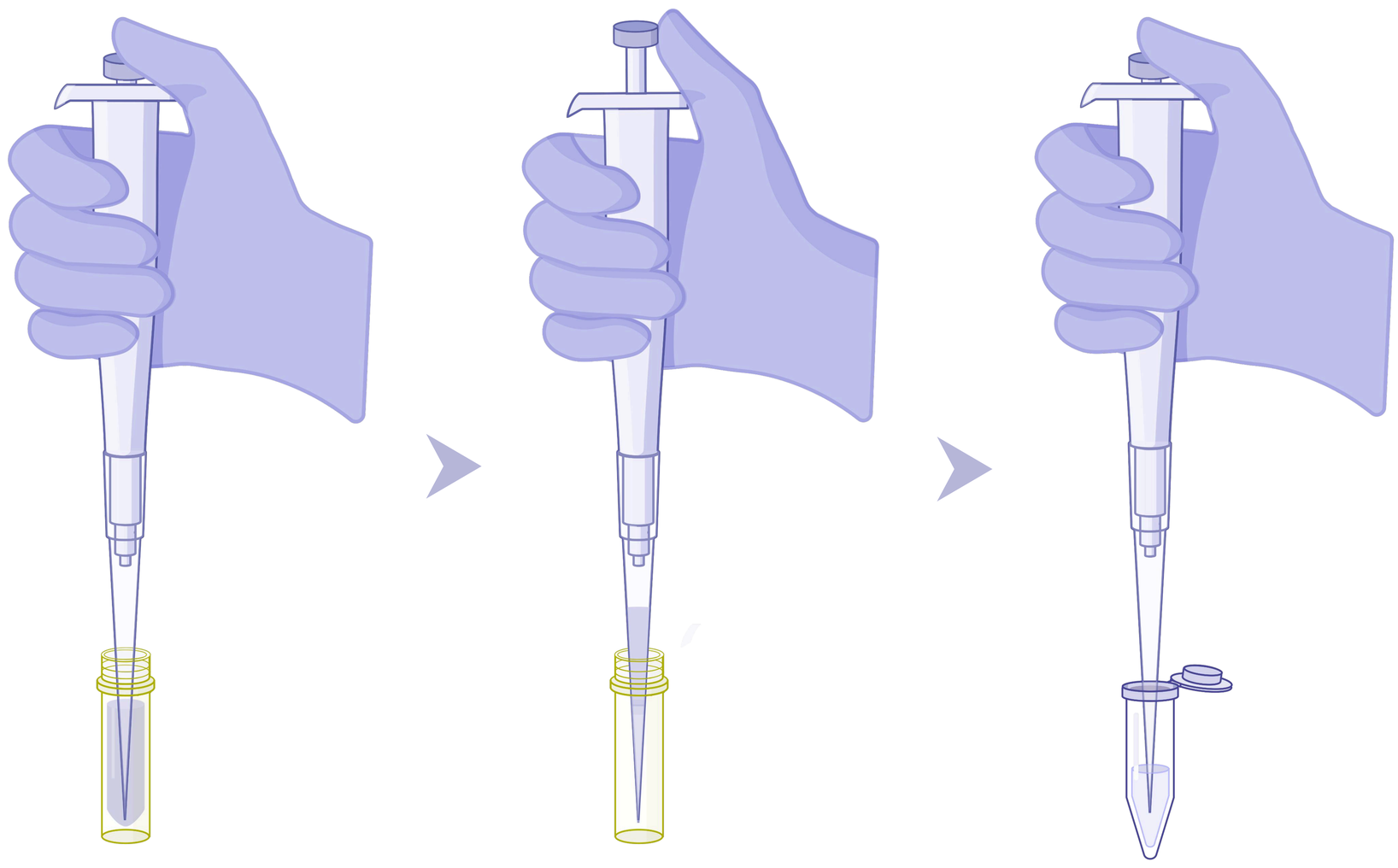


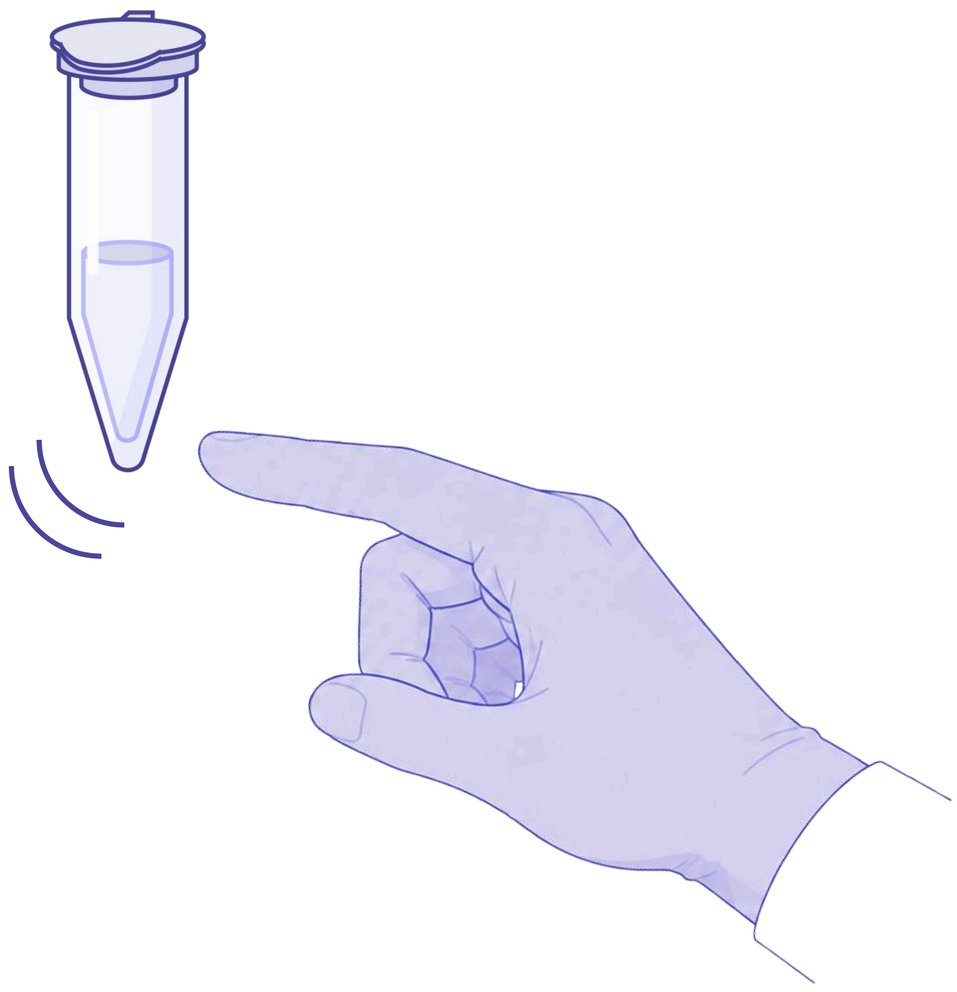
Test Procedure: Visual Guide

Step 3. Mixing

6. Close the tube cap firmly to ensure a liquid-tight seal (7).
7. Gently tap the bottom of the tube approximately 5 times.
8. Flick the tube downward sharply or tap it on the bench to ensure liquid settles completely at the bottom, fully rehydrating the lyophilized pellet. Verify that no bubbles remain in the bottom of the tube.

- If a microcentrifuge is available, briefly centrifuge the tube to bring the entire volume to the bottom and eliminate any air bubbles.

CRITICAL: Check that all liquid is at the bottom of the tube and no large bubbles are present before proceeding to ensure accuracy.



Test Procedure: Visual Guide

Step 4. Run the Test

9. Insert Strip: Place the loaded 4-tube strip into the CRISPOT® device reaction chamber. Press down gently to ensure the strip is fully and correctly seated.
10. Power On: Turn on the CRISPOT® device.
11. Select Program: Use the UP and DOWN buttons to select CUSTOM.

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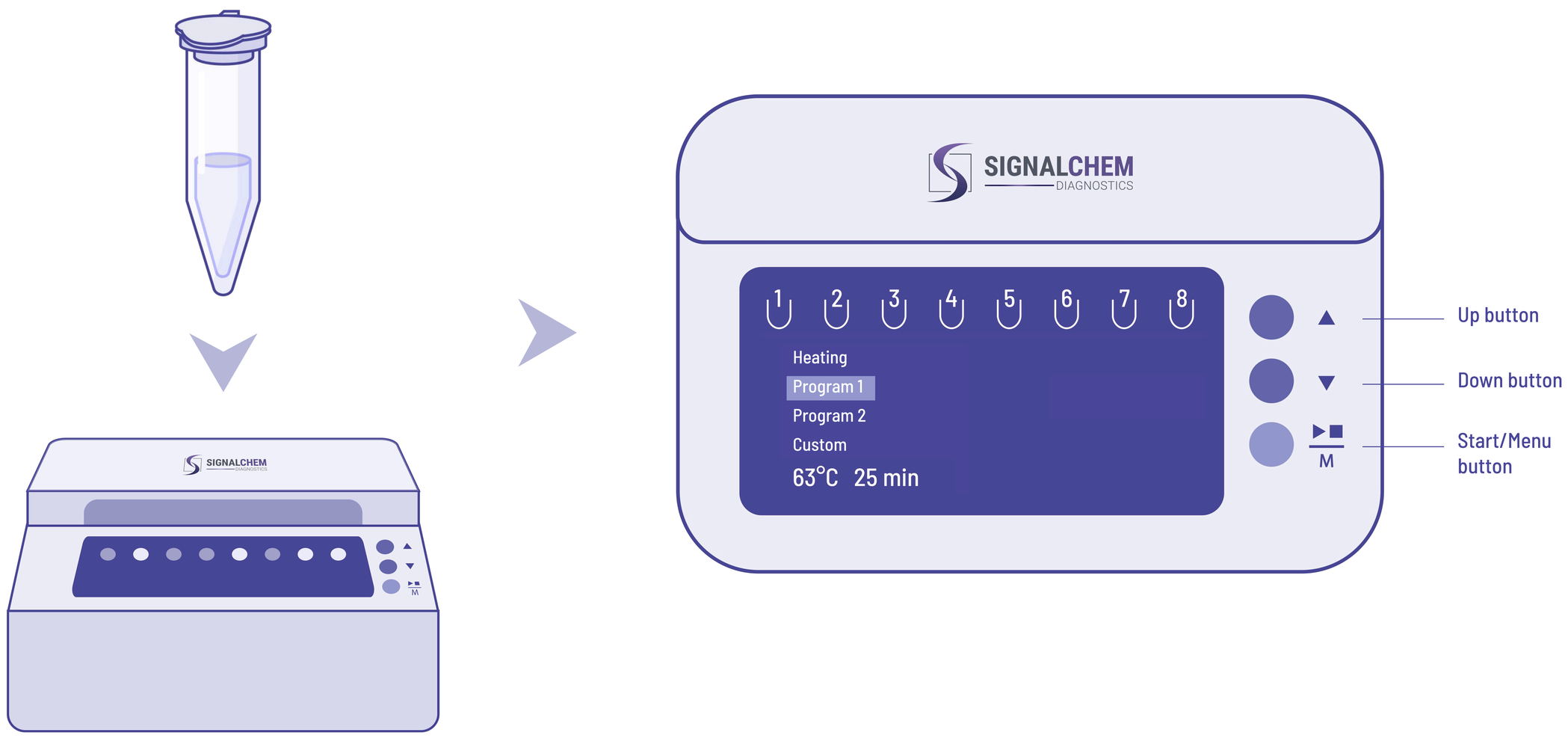
Note: Refer to the CRISPOT® Detection Device Instructions for Use for full device operating instructions.



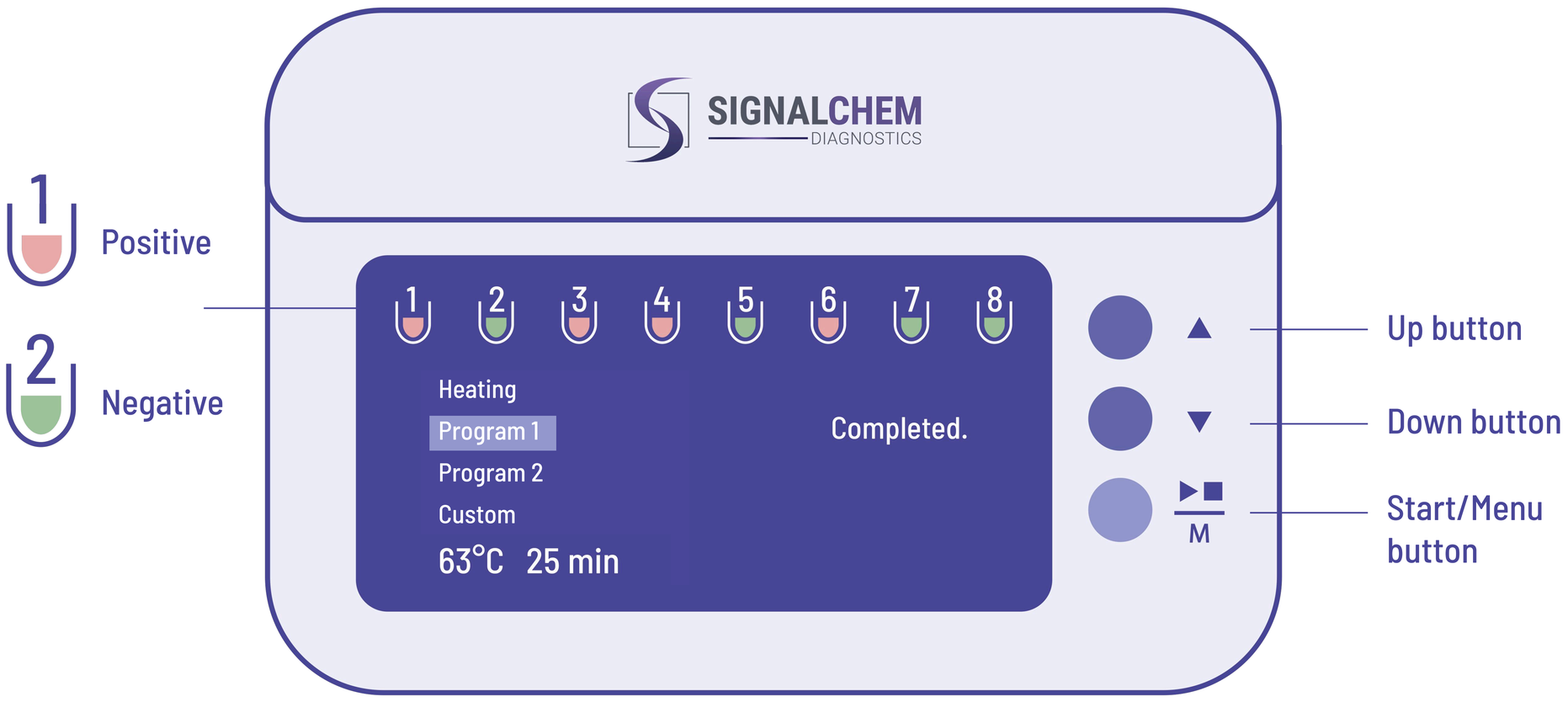
Interpretation of Results

A test run for a given sample is VALID only if its Positive Control (Tube 4 / PC) displays RED (Positive). If Tube 4 shows GREEN (Negative), the test result for that sample is invalid and must be retested.

After completion, the CRISPOT Detection Device automatically displays the results:

- Green well = Negative (target nucleic acid not detected, or well is unused)
- Red well = Positive (target nucleic acid detected)

Results for each well correspond directly to the pathogen toxins layout configured for the run.



Illustrated result: Wells 1, 3, and 4 tested positive for *Clostridium perfringens* (alpha-toxin, *cpa*, *netF* and Positive Control). All other wells were negative or contained no sample.

Index of Symbols

Do not reuse

Manufacturer

REF Catalog number

Temperature limit

LOT Batch code

Consult instructions for use

Caution

Use by

SIGNALCHEM DIAGNOSTICS

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