Laboratory Technical Procedure

# Rapid Diagnostic Testing for Influenza (RIDT)

This test determines whether viral influenza antigens are present in the patient's specimen sample. It requires testing a select few outpatients who have been showing symptoms of acute respiratory illnesses comparable to that of Influenza for the testing facility to determine the presence of influenza in the specified outpatient population, contributing to the efficient diagnosis and effective clinical judgment among health-care providers.

To detect the presence of Influenza A and/or Influenza B viral antigens in respiratory specimens (e.g., nasopharyngeal swab), the test is used for the rapid diagnosis of influenza in patients presenting with flu-like symptoms, such as fever, cough, and sore throat.

## Principle

The Rapid Influenza Diagnostic Test (RIDT) is an immunoassay that detects influenza viral nucleoprotein antigens. When the patient's specimen (typically a nasopharyngeal swab) is added to the test, viral antigens (if present) bind to influenza-specific antibodies embedded on a test strip. The antigen-antibody complex produces a visible colored line on the test, indicating a positive result for influenza A and/or B.

## Specimen Requirements

a) Specimen Collection

   RIDTs are approved for specific kinds of respiratory specimens. Specimen collection is to be done as immediately as possible, preferably less than four days after the patient starts showing symptoms. However, for younger patients, influenza viruses can still be present for longer periods, so samples from such can still be accepted.

b) Specimen Type

   Nasopharyngeal swab (other specimens such as nasal swabs, throat swabs, or aspirates may be acceptable depending on the kit).

c) Transport

   Place the swab in a transport medium or the extraction buffer provided in the test kit. Process the sample as soon as possible, or refrigerate if delayed (usually within 4 hours).

## Reagents or Media

1. Extraction Buffer (specific to the RIDT kit being used)
    - This buffer is required to extract the viral antigens from the patient specimen. It is usually supplied with the RIDT kit.
2. Control Samples (optional but recommended for quality control)
    - Positive Control: A sample containing known influenza antigens (Influenza A and/or B) to verify that the test detects the virus properly.
    - Negative Control: A sample free from influenza antigens to ensure no false-positive results occur.

## Supplies

1. Nasopharyngeal Swabs
    - For collecting respiratory specimens from the patient's nasopharynx. These are often provided with the test kit, but if not, swabs made from materials such as nylon or rayon are recommended for the best sample recovery.
2. Test Cassettes
    - The RIDT kit includes individual test cassettes, each designed to detect influenza antigens in the sample and display results visually (positive or negative).
3. Disposable Droppers
    - Transfer the specimen mixed with the extraction buffer into the sample well of the test cassette. These are typically included in the test kit.
4. Timer
    - Track the exact time required for the test to develop, usually 10-15 minutes, as specified by the manufacturer.
5. Disposable Gloves
    - For personal protection and to avoid contamination of the specimen or test components.
6. Biohazard Disposal Bags/Containers
    - For safe disposal of used swabs, test cassettes, and other materials that have come into contact with potentially infectious specimens.
7. Pipette or Dropper Tips (if not included in the kit)
    - To ensure precise handling of the extraction buffer and specimen mixture.
8. Labeling Materials
    - Permanent markers, labels, or pens for identifying patient samples and test cassettes.

## Preparation of Reagent

Extraction Buffer

- The extraction buffer is typically pre-prepared and supplied with the test kit, so no additional preparation is required. Ensure the buffer vial or tube is sealed and not expired before use.
- Open the buffer vial carefully, and use a sterile dropper or pipette to transfer the required amount of buffer into the specimen collection tube, following the manufacturer's instructions (usually 3-4 drops).
- After adding the patient specimen (swab) to the extraction buffer, ensure thorough mixing by rotating or gently swirling the swab in the buffer for the designated amount of time as per the manufacturer's guidelines (usually 10-15 seconds). Avoid vigorous shaking to prevent splashing.

## Specified Equipment Storage Requirements

1. For Test Cassettes
    - Store at room temperature (typically between 15°C and 30°C), as specified by the manufacturer.
    - Do not freeze the test cassettes, as freezing may degrade the components and lead to inaccurate results. Ensure they are stored in their sealed pouches until ready for use to protect them from moisture and contamination.
    - Test cassettes are typically marked with an expiration date. Do not use expired cassettes, as this can affect the test's reliability.
2. Extraction Buffer
    - Store at room temperature (typically between 15°C and 30°C), unless otherwise indicated by the manufacturer.
    - Once opened, the extraction buffer should be used within the time frame specified by the manufacturer (often within a single testing session or within a few hours). Always check for signs of contamination or degradation before use (e.g., cloudiness or discoloration).
3. Control Samples (Positive and Negative)
    - Store refrigerated at 2°C to 8°C unless otherwise specified by the manufacturer.
    - Control samples should be brought to room temperature before use. Always check the expiration date and discard any expired controls to maintain test accuracy.
4. Swabs
    - Store the sterile nasopharyngeal swabs in their original packaging at room temperature.

    - Ensure swabs remain sealed in their sterile packaging until ready to collect the specimen to avoid contamination.
5. Test Kit
    - Store complete test kits (including all components such as test cassettes, buffers, and controls) at room temperature or according to the manufacturer's specified conditions (usually 15°C to 30°C).
    - Ensure the test kit is within its expiration date. Discard the kit if expired.

## General Storage Requirements

- Store test components in a dry, cool environment away from direct sunlight and humidity, as these factors can degrade the reagents.
- Do not freeze the reagents or test kits unless explicitly indicated by the manufacturer, as freezing can damage the test materials and invalidate results.
- If a refrigerator is used to store controls or other components, ensure it is monitored regularly to maintain a stable temperature between 2°C and 8°C.

# Quality Control

- Perform quality control testing with both positive and negative control samples daily or as required by laboratory protocol.
- As prescribed in the instruction run-down below, the specimen must be stored accordingly so as to keep it unchanged by external and environmental factors.
- Standard PPE must be always be used when handling a sample.
- The control line (C) must appears in all tests. If the control line does not appear, the test is invalid and should be repeated.
- Record all quality control results in the official laboratory logbook.

## Step-by-step Instructions

**Step 1**: Prepare Work Area

- Ensure the workspace is clean and well-organized.
- Put on appropriate PPE (gloves, mask, face shield).
- Ensure that the test kit and all materials are at room temperature.

**Step 2**: Specimen Collection

- Using a nasopharyngeal swab, collect the specimen by inserting the swab into the patient's nostril, reaching the nasopharynx, and gently rotating it.
- Remove the swab and immediately place it into the extraction buffer or transport medium.

**Step 3**: Test Setup

- Open the RIDT test cassette pouch just before use.
- Add the required number of drops of the extraction buffer to the sample well on the test cassette (usually 3-4 drops, depending on the manufacturer's instructions).

**Step 4**: Add Specimen

- Insert the collected swab into the buffer solution, ensuring that the swab is thoroughly mixed in the buffer for proper antigen extraction.
- Remove the swab, and using the provided dropper, add the required number of drops of the mixed specimen into the designated sample well of the test cassette.

**Step 5**: Run Test

- Set a timer according to the test manufacturer's instructions (typically 10-15 minutes).
- Place the test cassette on a flat surface and wait for the test to develop.

**Step 6**: Interpret Results

- After the indicated time (usually 10-15 minutes), read the test results.
    - Positive Result: A visible line appears in the control line (C) region and one or both test line regions (A and/or B). A line in the A region indicates Influenza A; a line in the B region indicates Influenza B.
    - Negative Result: A line appears only in the control (C) region, with no line in either test (A or B) regions.
    - Invalid Result: If no control line appears, or only a test line without a control line, the result is invalid, and the test must be repeated with a new cassette.

## Reporting Results

**Reference Ranges/Interpretation**

- Positive for Influenza A
    - The presence of a line in the "A" test line region indicates the detection of Influenza A antigens.
- Positive for Influenza B
    - The presence of a line in the "B" test line region indicates the detection of Influenza B antigens.
- Negative
    - The absence of a test line in either the "A" or "B" regions with the presence of a control line (C) indicates no detection of Influenza A or B.

- Invalid Result
    - No control line (C) indicates an invalid test.

## Procedure Notes

1. Handle all specimens as if potentially infectious. Wear appropriate PPE (gloves, mask, face shield).
2. Dispose of all biological waste (swabs, test cassettes) in a biohazard container.
3. Disinfect the workbench and equipment after testing.
4. Follow your laboratory's standard safety protocols for handling infectious material.

### Sources of Error

- False-negative results may occur if the specimen is not collected properly or if viral antigen levels are below the detection limit.
- False-positive results can occur in patients recently vaccinated with the live attenuated influenza vaccine (LAIV).
- Negative results do not rule out influenza infection. Further testing with more sensitive methods (e.g., PCR) may be required in cases of suspected influenza with a negative RIDT.
- Other respiratory pathogens may cause similar symptoms but will not be detected by the RIDT

## Troubleshooting

1. If No Control Line (C) Appears on the Test Cassette
    - The test cassette or reagents may be defective, or the procedure was not followed correctly.
    - Troubleshooting Steps:
        - Ensure that the correct amount of extraction buffer and specimen was added to the sample well.
        - Verify that the test cassette was stored properly and has not expired.
        - Check for visible damage to the test cassette or any contamination.
        - Repeat the test with a new cassette and fresh reagents, ensuring that the instructions are followed carefully.

If multiple test cassettes fail to show a control line, switch to a different lot of test kits, or use an alternative test method, such as a molecular test (e.g., PCR), if available.

2. If Test Line is Weak or Faint
    - THe probable cause my be the Low levels of viral antigen in the specimen, or the test was read too early.
    - Troubleshooting Steps:

        - Ensure that the specimen was properly collected and handled.
        - Recheck the timer to confirm the test was read at the correct time (typically after 10-15 minutes). Reading the result too soon or too late can cause misinterpretation.
        - If the test line is weak but visible, consider the result as positive, but recommend confirmatory testing (such as PCR) to verify the result.
        - For repeated faint lines, review the specimen collection technique to ensure the proper amount of sample was obtained.

Consider collecting a new specimen and repeating the test. If faint lines persist, use a more sensitive test (e.g., PCR).

3. If Invalid or Unexpected Reults are Yielded
    - The probable cause may be that the Quality control samples (positive or negative) produce invalid or unexpected results, indicating a potential issue with the test kit or reagent quality.
    - Troubleshooting Steps:
        - Check that the positive and negative controls were properly prepared and stored.
        - Ensure the controls are not expired and were handled according to the manufacturer's instructions.
        - Repeat the test using a new batch of controls and test materials.

If controls continue to fail, discontinue using the test kit and report the issue to the manufacturer. Switch to an alternative test method until the issue is resolved.

4. If Results are Invalid Due to Sample Quality
    - Improper specimen collection, contamination, or insufficient sample quantity may be the cause of thie issue.
    - Troubleshooting Steps:
        - Review the specimen collection protocol to ensure proper nasopharyngeal swabbing technique.
        - Ensure swabs are placed into the extraction buffer immediately after collection.
        - If the sample appears insufficient or contaminated, discard it and collect a new specimen.

If obtaining an adequate specimen remains a challenge, consider using alternative sampling methods (e.g., throat or nasal swabs) or refer the patient for a more sensitive molecular test.

5. If False Negative Result is Yielded
    - The test may yield false-negative results due to low viral load, improper storage or handling, or testing outside the detectable window (e.g., early or late in the infection cycle).
    - Troubleshooting Steps:
        - Check the specimen collection time relative to the onset of symptoms. Testing too early or too late may reduce the likelihood of detecting the virus.
        - Confirm that the test reagents and cassettes were stored and handled correctly, following manufacturer guidelines.
        - Recommend confirmatory testing with a molecular diagnostic test (e.g., RT-PCR) in cases where influenza is strongly suspected despite a negative RIDT result.

If repeat testing is not possible, or if the patient is critically ill, proceed with clinical management based on clinical judgment and epidemiological factors.

6. If False Positive Result is Yielded
    - Cross-reactivity with other respiratory pathogens or recent influenza vaccination (live attenuated influenza vaccine, LAIV) may lead to a false-positive result.
    - Troubleshooting Steps:
        - Review the patient's recent history, including vaccination status, and evaluate the possibility of cross-reactivity.
        - Use confirmatory testing (e.g., RT-PCR) to verify the result if the clinical picture does not match the test outcome.
        - Report any unusual positive results to the manufacturer or regulatory authorities if cross-reactivity with non-influenza pathogens is suspected.

Use alternative testing methods or clinical judgment to manage the patient's condition. For patients recently vaccinated with LAIV, a PCR test may be more accurate.

## Standard Laboratory Back-up Plan

1. Alternative Testing Methods
    - If troubleshooting fails or if test results are inconclusive, a more sensitive molecular test, such as reverse transcription polymerase chain reaction (RT-PCR), should be used to confirm influenza infection. PCR tests have higher sensitivity and specificity compared to RIDT.

- Although rarely used for acute diagnosis due to longer turnaround times, viral culture can serve as a backup option in cases where RIDT results are ambiguous and PCR is unavailable.

2. Re-test with New Specimen
    - If a test fails due to poor specimen quality, contamination, or an invalid result, recollect the specimen from the patient and repeat the RIDT using fresh reagents and materials.
3. Clinical Diagnosis
    - If testing is not possible or yields inconclusive results, clinicians may diagnose and manage influenza based on the patient's symptoms, history, and local epidemiological data (such as flu season prevalence).

## References


BD Veritor™ System for Rapid Detection of Flu A+B)

Centers for Disease Control and Prevention (CDC) guidelines for Influenza Testing

Clinical and Laboratory Standards Institute (CLSI) guidelines


### Sources


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*Rapid Influenza Antigen (Nasal or Throat Swab) - Health Encyclopedia - University of Rochester Medical Center*. (2020). Rochester.edu. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=rapid_influenza_antigen


*These Procedure Guidlines are effective starting this* ***eighth of October, 2024 (9October 8, 2024).***

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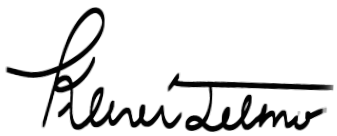

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