ThermoFisher SCIENTIFIC

INSTRUCTIONS FOR USE

Accula™ Flu A/Flu B Test

Catalog Number FAB1100CW

Pub. No. LBL-60009 Rev. C

For use with Accula™ Dock or Silaris™ Dock.

CLIA WAIVED: For use with nasal swabs.

CLIA COMPLEXITY: WAIVED.

A Certificate of Waiver is required to perform this test in a CLIA Waived environment. To obtain CLIA waiver information and a Certificate of Waiver, contact your state health department. Additional information is available at www.cms.hhs.gov/CLIA.

CAUTION! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Intended use

The Accula™ Flu A/Flu B Test performed on the Accula™ Dock or Silaris™ Dock is a molecular in vitro diagnostic test utilizing polymerase chain reaction (PCR) and lateral flow technologies for the qualitative, visual detection and differentiation of influenza A and influenza B viral RNA. The Accula™ Flu A/Flu B Test uses a nasal swab specimen collected from patients with signs and symptoms of respiratory infection. The Accula™ Flu A/Flu B Test assay is intended as an aid in the diagnosis of influenza infections in conjunction with clinical and epidemiological risk factors. The assay is not intended to detect the presence of influenza C virus.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established during the 2016-2017 influenza season. When other novel influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL-3+ facility is available to receive and culture specimens.

IVD Rx only

For In Vitro Diagnostic Use.

Summary and explanation

Along with the common cold, influenza is one of the most common acute respiratory infections. It produces symptoms such as headache, chills, dry cough, body aches and fever. It affects 10% to 20% of the United States population annually, resulting in more than 110,000 hospitalizations and 10,000 to 40,000 deaths (Cheung and Lieberman, 2002).

The influenza A virus is typically more prevalent and is associated with more serious influenza epidemics (Iha et al, 2016). Influenza B infections usually present with milder symptoms (Kim et al, 1979). Diagnosis of influenza and differentiation from other respiratory infections is difficult because the initial symptoms can be similar. Since the influenza virus is highly contagious, accurate diagnosis and prompt treatment of patients can have a positive effect on public health. Rapid diagnosis of viral infection can also help reduce the inappropriate use of antibiotics. Initiation of antiviral therapy for influenza within 48 hours of symptom onset is recommended for quick improvement of symptoms and reduction in viral shedding (Montalto and Byrd, 2003). The Accula™ Flu A/Flu B Test is a Nucleic Acid Amplification Test (NAAT). It is designed to be run at point of care locations and rapidly report detection of influenza viruses A and/or B from patients.

Principle of the test

The Accula™ Flu A/Flu B Test is a point of care Nucleic Acid Amplification Test (NAAT) for detection of influenza A virus and/or influenza B virus in patients with flu-like symptoms in approximately 30 minutes. Nasal swab specimens are added to the Accula™ Nasal Swab Buffer to solubilize the sample. An aliquot of the buffer is then dispensed into an Accula™ Flu A/Flu B Test Cassette.The cassette contains internal process positive and negative controls, enzymes, OscAR™ reagents, and a detection strip necessary for the 4 steps in the assay. These 4 steps are lysis of the virus, reverse transcription of viral RNA to cDNA, nucleic acid amplification, and detection. The Accula™ Dock controls reaction temperatures, timing, and fluid movements within the cassette resulting in a fast and automated Flu A/Flu B assay. After approximately 30 minutes the test results are interpreted by the visualization of blue test lines on the detection strip in the cassette. A blue process control line at the control (C) area is used to ensure proper reagent and Accula™ Dock function and to confirm a valid negative test result.

Reagents and materials

Materials provided

The Accula™ Flu A/Flu B Test kit contains materials sufficient for 25 tests.

Table 1 Accula™ Flu A/Flu B Test kit contents (Cat. No. FAB1100CW)

Component	Contents		Quantity	Storage
Sterile collection swabs	Sterile swab for nasal sample collection.		25 swabs	15°C to 30°C, 59°F to 86°F
Accula™ Nasal Swab Buffer	Single-use vial of solution to accept patient sample. Contains buffer with dimethyl sulfoxide and <0.01% sodium azide.		25 vials	
Accula™ Transfer Pipette	Single-use, fixed volume pipette used to transfer sample from the nasal swab buffer tube into the test cassette (located within the foil pouch of the test cassette).		25 pipettes[1]	
Accula™ Flu A/Flu B Test Cassette	Single-use, foil-pouched test cassette containing lyophilized reagents for the targeted amplification and detection of influenza A and B viral RNA. Includes desiccant pouch.		25 cassettes	
Accula™ Flu A Positive/Flu B Negative Swab (control swab; yellow shaft)	Positive for Influenza A and negative for Influenza B. Contains inactivated influenza A virus dried onto a swab.		1 swab	
Accula™ Flu B Positive/Flu A Negative Swab (control swab; blue shaft)	Positive for Influenza B and negative for Influenza A. Contains inactivated influenza B virus dried onto a swab.		1 swab	

[1] For your convenience, extra pipettes are provided in a separate plastic pouch within the kit.

Materials provided separately

Unless otherwise indicated, all materials are available through thermofisher.com.

Item	Source
Accula™ Dock	D2000 D2002 (Contact your sales representative for information on ordering this item.)
Accula™ Flu A/Flu B Control Kit (additional control swabs)	FAB1100CW-C

Storage and handling

- Store reagents at room temperature (15°C to 30°C, 59°F to 86°F). Do not refrigerate or freeze.
- Do not reuse kit contents: collection swabs, cassettes, pipettes, control swabs, or nasal swab buffer.
- Do not remove the cassette from the foil pouch until immediately before use.
- Do not use kit or reagents past the expiration date.

Precautions

- For in vitro diagnostic use.
- Federal Law restricts sale of this device to or on the order of a licensed practitioner.
- To be used in conjunction with the Accula™ Dock or Silaris™ Dock.
- Follow universal precautions when handling patient samples. All patient samples should be treated as if potentially infectious. Follow standard BSL-2 guidelines when working with patient samples. Wear appropriate personal protective equipment, for example, gloves, mask, face and/or eye shield, and gown.
- Change to a new pair of gloves when handling each new patient sample.
- Inactivated and lypohilized viruses are used to make the control swabs. However, control swabs, patient samples and used cassettes should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
- Dispose of kit reagents and patient samples according to all local, state and federal regulations.
- Do not use swabs or nasal swab buffer other than those provided with the Accula™ Flu A/Flu B Test kit.
- Test the sample immediately after collection. If immediate testing is not possible, the nasal swab sample must be added to the nasal swab buffer immediately after collection. The prepared sample may be stored at room temperature (15°C to 30°C, 59°F to 86°F) for up to 2 hours.
- Do not remove the foil tab from the cassette until immediately before use. Once the tab is removed, add sample promptly and start testing.
- Once sample is added and the dock lid is closed, the test has started. Do not move the dock, open the lid, or unplug the dock until the dock indicates the test is completed.
- Do not use any damaged kit contents.
- To obtain accurate results, follow all instructions and heed all precautions in this instructions for use.
- Inadequate or inappropriate sample collection, handling, processing, and/or storage can yield inaccurate results.
- Sample collection and handling procedures require specific training and guidance.
- To obtain accurate results, use only the fixed volume transfer pipette provided in the kit to transfer the patient sample from the Accula™ Nasal Swab Buffer tube into the cassette port. Do not pour the patient sample from the buffer tube into the cassette sample port.
- Do not use visually bloody or overly viscous samples.
- When transferring the prepared patient sample, avoid drawing up large particulates, which may clog the transfer pipette.
- Due to the high sensitivity of the Accula™ Flu A/Flu B Test, contamination of the work area with previous samples may cause false positive results. Clean the dock and surrounding surfaces as described in the procedure in *Accula™ Dock Operator's Guide* (see “Related documentation” on page 14) or the *Silaris™ Dock Operator's Guide*.
- Do not attempt to open a used cassette or a cassette with closed sample port.
- Do not touch the heads of the control swabs. Cross contamination may occur due to the high sensitivity of the test.
- If infection with a novel influenza A virus is suspected, samples should be collected with appropriate infection control precautions for novel virulent influenza viruses. Follow the current clinical and epidemiological screening criteria recommended by public health authorities on whether to send the sample to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL-3+ facility is available to receive and culture the samples.
- To interpret results accurately, see “Interpretation of results” on page 7.

Quality control

Process controls

Each Accula™ Flu A/Flu B Test Cassette contains two internal process controls: an internal positive control (labeled ‘C’ on the cassette) and negative control (labeled ‘NC’ on the cassette). The positive process control is a non-infectious RNA bacteriophage in the cassette and is used as the positive process control to verify that all assay steps (lysis of the virus, reverse transcription, amplification and detection) were executed properly. A non-influenza nucleic acid target is used as a negative control for false positive results due to nonspecific binding.

For information on interpreting the results for the process controls, see “Interpretation of results” on page 7.

External positive and negative controls

External controls may be used to show that the Accula™ Flu A/Flu B Test is working properly.

The Accula™ Flu A/Flu B Test contains two control swabs:

- 1 Accula™ Flu A Positive/Flu B Negative Swab (yellow shaft)
- 1 Accula™ Flu B Positive/Flu A Negative Swab (blue shaft)

We recommend that a Flu A positive/Flu B negative and a Flu B positive/Flu A negative control be run:

- Once for each new lot or shipment of kits received.
- Once for each new operator.
- As deemed necessary in order to conform with your internal quality control procedures, with local, state and/or federal regulations, or accrediting groups.

Run control swabs using the same procedure as for a patient specimen. If external QC testing fails, repeat the test using a new control swab, reagent and test cassette, or contact Technical Support before testing patient samples.

Specimen collection and handling

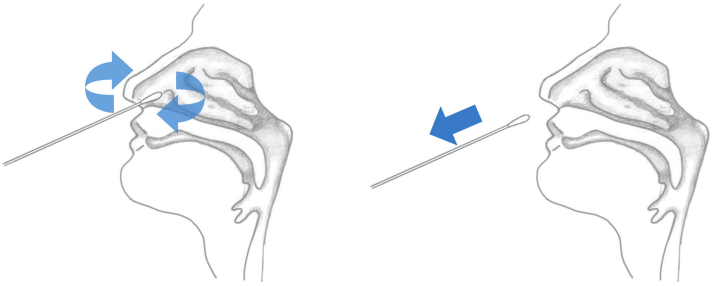
Proper sample collection is an important step for an accurate test result. Carefully follow the instructions for collection and handling.

1 Collect nasal swab sample

Note: Use only the collection swabs that are supplied with the kit.

1.1. Carefully insert the collection swab approximately 1 inch into the patient's nostril that exhibits the most secretions.

1.2. Gently rotate the swab several times against the nasal wall, then remove the swab .



2 Sample storage and sample extraction

For best results, nasal swabs must be eluted in Accula™ Nasal Swab Buffer immediately after collection.

IMPORTANT! Patient sample nasal swabs that have been previously stored in media other than Accula™ Nasal Swab Buffer are not recommended and may yield invalid results or false results.

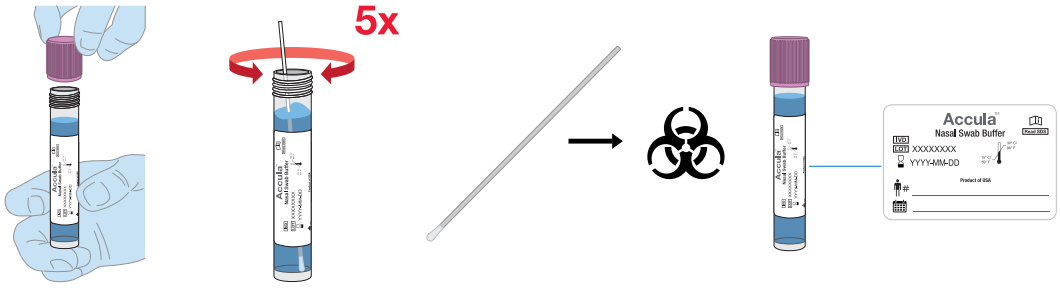
IMPORTANT! If immediate testing is not possible, the eluted sample may be stored at room temperature (15°C to 30°C, 59°F to 86°F) for up to 2 hours. Do not freeze the eluted sample before testing.

2.1. Remove the cap from the nasal swab buffer vial and set it aside.

2.2. Insert the nasal swab specimen into the nasal swab buffer and rotate it 5 times, rubbing it against the wall of the vial.

2.3. Remove the patient nasal swab from the nasal swab buffer vial and discard it into a biohazardous waste container.

2.4. Replace the cap on the nasal swab buffer vial and write the patient identification (ID) information and testing date on the label in the area provided.



IMPORTANT! If immediate testing is not possible, recap the nasal swab buffer vial. The eluted sample may be stored at room temperature (15°C–30°C, 59°F–86°F) for up to 2 hours.

Test procedure

IMPORTANT! All clinical samples must be at room temperature before beginning the assay.

Check the expiration date on each individual test cassette foil pouch or outer box before using. Do not use any test after the expiration date on the label.

1 Connect the dock to a power source

1.1. Place the dock on a flat surface.

1.2. Connect the AC adapter to the power cord, then insert the round connector of the AC adapter into the dock.

1.3. Plug the AC end of the power cord into an electrical outlet.

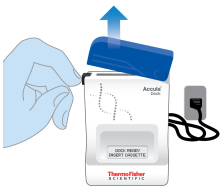
2 Open the dock and verify readiness

2.1. Open the dock by depressing the black button located on the top left side of the device.

2.2. Verify that the dock screen displays:

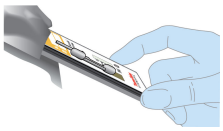
DOCK READY

INSERT CASSETTE



3 Remove the cassette and pipette from the package

3.1. Remove a test cassette and transfer pipette from the foil package (these items are packaged together).



IMPORTANT! Do not open the foil pouch until the sample is ready for testing. The test must be initiated within 30 minutes of opening the foil package.

Note: The foil pouch also contains a desiccant pack. This can be discarded with the foil pouch after the cassette and transfer pipette are removed.

3.2. Write the patient identification (ID) information and testing date onto the cassette label in the area provided.

Do not write on the cassette except in the indicated area on the cassette label for recording sample identification and test date.

4 Insert the cassette into the dock

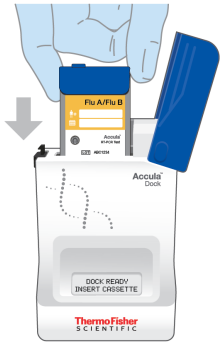
4.1. Insert the cassette into the dock, leaving the lid open. Press the cassette down firmly to seat it in the dock.

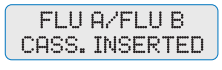
IMPORTANT! Do not remove the foil tab covering the sample port until immediately before testing.

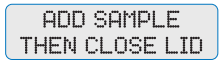
IMPORTANT! Once the test cassette is placed into the Dock, add the sample into the cassette within 5 minutes.

4.2. Verify that the dock screen first displays, **FLU A/FLU B CASS. INSERTED**, then displays **ADD SAMPLE THEN CLOSE LID**.

Note: Do not close the dock lid until the sample has been added to the cassette.

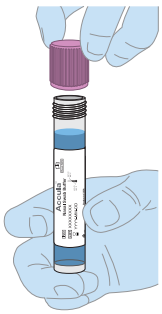






5 Withdraw sample from the sample tube

5.1. Invert the nasal swab buffer vial to mix, then remove the cap from the prepared patient sample in the nasal swab buffer and set it aside.



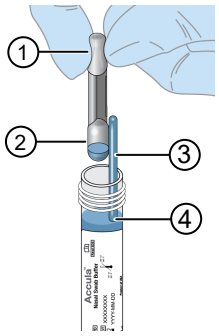
5 Withdraw sample from the sample tube *(continued)*

5.2. Firmly squeeze the top bulb of the pipette.

5.3. While continuing to squeeze the top bulb firmly, place the pipette tip well below the surface of the liquid in the nasal swab buffer vial.

5.4. Keep the pipette tip well below the surface of the liquid of the vial containing the prepared patient sample in nasal swab buffer. Slowly release the top bulb to completely fill the pipette stem with sample. Some liquid may also be in the overflow chamber.

Note: Although excess liquid will enter the pipette's overflow chamber, only the liquid in the pipette stem will be dispensed.



① Top bulb.
② Overflow chamber; do not squeeze.
③ Pipette stem.
④ Pipette tip.

6 Load the sample into the cassette

6.1. Completely remove and discard the foil tab covering the sample port on the cassette, then immediately proceed to step 6.2.

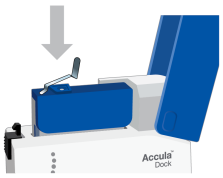
6.2. Insert the tip of the pipette all the way into the sample port of the cassette (until resistance is met), then firmly squeeze the top bulb of the pipette to dispense the sample into the cassette.

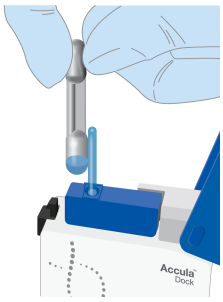
Note: A small amount of sample may remain in the overflow chamber (lower bulb). This is normal.

After the sample is loaded the dock screen displays:

SAMPLE LOADED

CLOSE LID





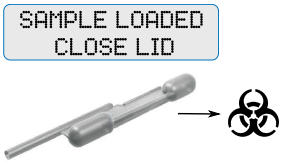
6.3. Dispose of the pipette in a biohazardous waste container.

6.4. Close the lid of the dock immediately, then verify that the dock screen displays:

SAMPLE LOADED

LID CLOSED

The test begins automatically after the lid of the dock is closed.

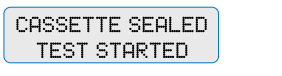


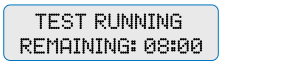
7 Run the test

7.1. Verify that the dock screen displays the following messages during the test:

CASSETTE SEALED
TEST STARTED

TEST RUNNING
REMAINING XX:XX





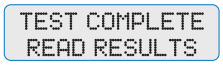
Note: The screen continues to display **TEST RUNNING** until complete. The dock beeps at the end of test processing.

IMPORTANT! Do not re-open the dock lid until the display indicates the test is complete. Opening the lid terminates the test. Do not move or unplug the dock while the test is processing.

7.2. Verify that the dock screen displays the following before proceeding:

TEST COMPLETE

READ RESULTS

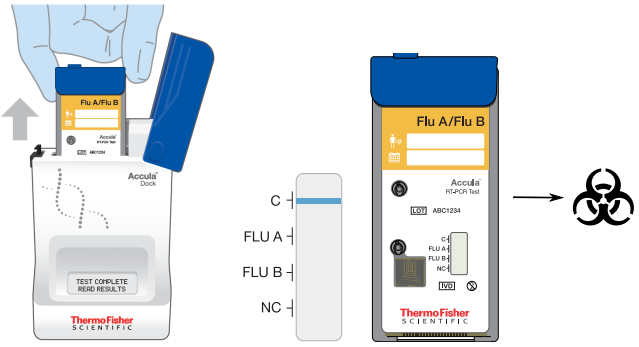


8 Remove the cassette, then read results

8.1. Open the lid of the dock, then remove the cassette.

8.2. Read and interpret results as described in “Interpretation of results” on page 7.

IMPORTANT! Results should be interpreted within 1 hour of test completion.



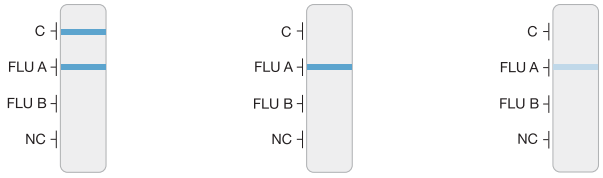
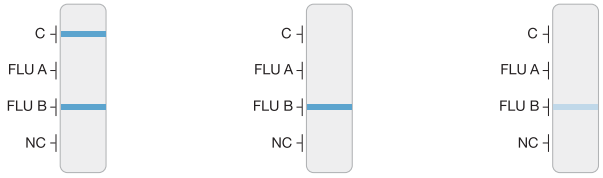
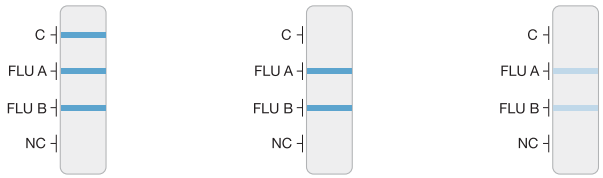
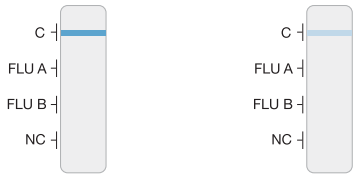
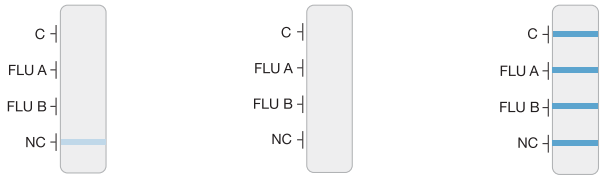
After reading and interpreting the results, dispose of the cassette in a biohazardous waste container.

Interpretation of results

The appearance of any shade of blue test line at the FLU A and/or FLU B positions is a valid result that is interpreted as positive for the influenza A and/or influenza B viral RNA. A negative result contains only a blue test line at position C.

Note: The absence of a blue test line at the C position in conjunction with a blue test line at the FLU A and/or FLU B positions means that Influenza A and/or Influenza B viral RNA was amplified and detected as a valid result. This can occur due to the overabundance of Influenza A and/or Influenza B RNA that competes with the positive control target.

Key: C = Internal Positive Process Control FLU A = Influenza A FLU B = Influenza B NC = Internal Negative Process Control

Window	Interpretation	Criteria
	Positive test for FLU A	A positive result for the presence of FLU A is indicated by: • ANY blue test line at the FLU A position (even if faint) • WITH or WITHOUT a blue process control line at the C position • AND the absence of a negative process control line at the NC position
	Positive test for FLU B	A positive result for the presence of Flu B is indicated by: • ANY blue test line at the FLU B position (even if faint) • WITH or WITHOUT a blue process control line at the C position • AND the absence of a negative process control line at the NC position
	Positive test for FLU A and FLU B	A positive result for the presence of both FLU A and FLU B is indicated by: • ANY blue test line at BOTH the FLU A and FLU B positions (even if faint) • WITH or WITHOUT a blue process control line at the C position • AND the absence of a negative process control line at the NC position
	Negative test for FLU A and FLU B	A negative result for the presence of both FLU A and FLU B is indicated by: • The absence of ANY blue test line at BOTH the FLU A and FLU B positions • AND a blue process control line at the C position • AND the absence of a negative process control line at the NC position
	Invalid result[1]	An invalid test result is indicated by: • The absence of any lines in the C, FLU A, FLU B positions, AND ANY process control line at the NC position (even if faint) • The absence of lines in the C, FLU A, FLU B, and NC positions • The presence of lines in both the C and NC positions, regardless of the presence or absence of lines in the FLU A or FLU B positions (the example shows lines at all four positions)

[1] If an invalid result is obtained, the sample may be re-run with a fresh cassette only if the eluted sample in the nasal swab buffer has been stored for less than 2 hours at room temperature (15°C to 30°C, 59°F to 86°F)

Dock cleaning

We recommend that the dock be cleaned each day that it is used. For more information, see *Accula™ Dock Operator's Guide* (see "Related documentation" on page 14) or the *Silaris™ Dock Operator's Guide*.

Limitations

- The performance of the Accula™ Flu A/Flu B Test was determined using the procedures provided in these instructions for use. Failure to follow these procedures may alter test performance.
- The Accula™ Flu A/Flu B Test is for use with nasal swab specimens only.
- Improper collection, storage or transport of specimens may lead to false negative results.
- Test results should be interpreted in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests performed.
- As with other tests, negative results do not rule out Flu A or Flu B infections and should not be used as the sole basis for patient management decisions.
- This is a qualitative test. Test line intensity is not indicative of the quantity of virus in the sample.
- Positive and negative predictive values are dependent upon prevalence. Test performance was established for the 2016-2017 influenza season. Performance may vary depending on the prevalence and population tested.
- False negative results may occur if viruses are present at levels below the test's limit of detection.
- False negative results may occur if mutations are present in the regions targeted by the test.
- Test performance has not been evaluated for patients without signs and symptoms of influenza infection.
- Cross-reactivity with respiratory tract organisms other than those listed in the analytical specificity study may lead to erroneous results. See "Analytical specificity (cross reactivity)" on page 10.
- Test performance has not been evaluated for the purpose of monitoring antiviral treatment.
- Test performance has not been evaluated in immunocompromised patients.
- Test performance has not been evaluated in patients who received inhaled influenza vaccine.
- This test cannot rule out diseases caused by other viral or bacterial pathogens.
- Analyte targets (viral nucleic acid) may persist in vivo, independent of virus viability. Detection of analyte targets does not imply that the corresponding viruses are infectious, or are the causative agents for clinical symptoms.
- The presence of inhibitors in the sample can lead to invalid results.
- Patient sample nasal swabs previously stored in media other than Accula™ Nasal Swab Buffer are not recommended and may yield invalid or false results.

Expected values

The prevalence of influenza varies from year to year, with outbreaks occurring during the fall and winter months. The influenza positivity rate is dependent upon many factors, including specimen collection, test method, and geographic location. Prevalence varies throughout the flu season and from location to location.

The clinical study was conducted during the 2016-2017 influenza season. The following tables show the prevalence of influenza A and influenza B observed in three subject age categories in that clinical study.

Table 2 Prospective Clinical Study during the 2016/2017 Influenza Season - Influenza A

Age group	Number of nasal swab specimans	Number of Influenza A positives	Influenza A positivity rate
≤ 5 years of age	488	97	19.9%
6 to 21 years of age	601	172	28.6%
≥ 22 years of age	169	20	11.8%
Total	1258	289	23.0%

Table 3 Prospective Clinical Study during the 2016/2017 Influenza Season - Influenza B

Age group	Number of nasal swab specimans	Number of Influenza B positives	Influenza B positivity rate
≤ 5 years of age	488	27	5.5%
6 to 21 years of age	601	91	15.1%
≥ 22 years of age	169	8	4.7%
Total	1258	126	10.0%

Performance characteristics

Accula™ Flu A/Flu B Test vs. Molecular Influenza Assay: Prospective Clinical Study

Clinical performance characteristics of the Accula™ Flu A/Flu B Test were evaluated in a multi-site prospective study during the 2016-2017 flu season in the United States. A total of sixteen investigational sites throughout participated in the study. To be enrolled in the study, patients had to be presenting at the participating study centers with flu-like symptoms. Two nasal swabs were collected from one nostril from each subject using standard collection methods. One nasal swab was tested, following elution in 5-mL of Accula™ Nasal Swab Buffer, on the Accula™ Flu A/Flu B Test, according to product instructions. The other nasal swab was eluted in 3-mL of viral transport media (VTM) and transported to one of two central laboratories for testing using the comparator method, an FDA-cleared molecular influenza assay.

A total of 1331 subjects were enrolled in this study. Of those, 73 specimens are unevaluable (that is, failed to meet inclusion/exclusion criteria, were not transported to a Reference Laboratory per the conditions required by the clinical protocol, had invalid results for the comparator assay, or had two invalid results on the Accula™ Flu A/Flu B Test). A total of 1258 specimens were considered evaluable. The performance of the Accula™ Flu A/Flu B Test for influenza A and influenza B compared with the comparator method are presented in the following tables.

Table 4 Accula™ Flu A/Flu B Test Flu A performance against the Molecular Comparator Method

Accula™ Flu A/Flu B Test Flu A	Comparator		
	Positive	Negative	Total
Positive	289	60[1]	349
Negative	9[2]	900	909
Total	298	960	1258
Sensitivity:	97% (95% CI: 94.4% – 98.4%)		
Specificity:	94% (95% CI: 92.0% – 95.1%)		

[1] FLU A was detected in 47/60 false positive specimens using an alternative FDA-cleared molecular influenza assay.
[2] FLU A was not detected in 3/9 false negative specimens using an alternative FDA-cleared molecular influenza assay.

Table 5 Accula™ Flu A/Flu B Test Flu B performance against the Molecular Comparator Method

Accula™ Flu A/Flu B Test Flu B	Comparator		
	Positive	Negative	Total
Positive	126	14[1]	140
Negative	8[2]	1110	1118
Total	134	1124	1258
Sensitivity:	94% (95% CI: 88.7% – 97.0%)		
Specificity:	99% (95% CI: 97.9% – 99.3%)		

[1] FLU B was detected in 9/14 false positive specimens using an alternative FDA-cleared molecular influenza assay.
[2] FLU B was not detected in 5/8 false negative specimens using an alternative FDA-cleared molecular influenza assay.

Reproducibility studies

The reproducibility study was performed to demonstrate the reproducibility of the Accula™ Flu A/Flu B Test with contrived nasal swabs at three CLIA-waived sites and one moderately complex site based in the United States. The objective of this study was to test panels of contrived nasal swab samples with the Accula™ Flu A/Flu B Test to demonstrate reproducibility of the assay in the hands of multiple users at multiple sites over multiple non-consecutive days.

The test panel consisted of five samples at virus concentration near the respective LoD (that is, Flu A and B Negative, Flu A Low Positive, Flu A Moderate Positive, Flu B Low Positive and Flu B Moderate Positive). Each sample was prepared using the influenza A and B strains spiked into clinical matrix. The influenza A strain used in this study was Flu A/California/07/2009 and the influenza B strain used in this study was Flu B/Massachusetts/2/2012. The targeted concentrations for the Moderate Positive samples were approximately 3X the respective LoD, the targeted concentrations for the Low Positive samples were approximately 1X the respective LoD (C95 concentration), and the Negative samples contained no influenza virus.

Samples were provided to testing operators in panels of 5 samples (Flu A Low Positive and Moderate Positive, Flu B Low Positive and Moderate Positive, and Negative). Samples were blinded and randomized. Each operator tested one panel per day, testing a maximum of five samples at a time. Each sample was tested in triplicate (from separate swabs) (2 operators x 1 run x 3 swabs x 5 non-consecutive days = 30 observations for each site per sample type). Results are reported as percent agreement: actual result /expected result x 100. Results were evaluated by site, by operator and by day. Agreement was 100% across all sites, operators, and days. Two samples did not produce results because re-tests of invalid results were invalid on re-test. Results are shown below by site.

Agreement of actual results with expected results was 100%. There were no significant differences observed within run (replicates tested by one operator), between run (five different days), between sites (four sites), or between operators (eight operators).

Table 6 Site-to-Site Reproducibility: Percent Agreement and Total Counts (Observed/Expected)

Sample Category	Site								Overall % and 95% CI
	Site 1		Site 2		Site 3		Site 4		
	%	Count	%	Count	%	Count	%	Count	
Low Pos Flu A	100	30/30	100	30/30	100	29/29[1]	100	30/30	100% (119/119), (96.9%, 100%)
Mod Pos Flu A	100	29/29[1]	100	30/30	100	30/30	100	30/30	100% (119/119), (96.9%, 100%)
Low Pos Flu B	100	30/30	100	30/30	100	30/30	100	30/30	100% (120/120), (96.9%, 100%)
Mod Pos Flu B	100	30/30	100	30/30	100	30/30	100	30/30	100% (120/120), (96.9%, 100%)
True Neg	100	30/30	100	30/30	100	30/30	100	30/30	100% (120/120), (96.9%, 100%)

[1] Re-run of second test resulted in an invalid result.

Limit of detection

Multiple analyte levels were tested in 20 replicates until the LoD was determined (the level at which at least 19/20 results are positive). Four (4) influenza strains were run in replicates of twenty (20) for each concentration. The influenza strains selected for testing included a 2009-like seasonal H1N1 influenza A strain, an H3N2 influenza A strain, and two influenza B strains representing Victoria and Yamagata lineages. Virus was serially diluted into pooled negative clinical matrix. Only dilutions in clinical matrix were used for LoD determination.

The limit of detection (LoD) for the Accula Flu A/Flu B Test for both Influenza A and Influenza B were determined with ≥ 95% detection at:

- A/California/07/2009 (H1N1): 300 TCID50/mL
- A/Texas/50/2012 (H3N2): 1200 TCID50/mL
- B/Nevada/3/2011 (Victoria): 1350 CEID50/mL

- B/Massachusetts/2/2012 (Yamagata): 400 TCID50/mL

Table 7 Limit of Detection: Observed/Expected

Volume Spiked Swab	Final Concentration (TCID50/mL)	Observed/Expected Positives
10 µL	A/CA 300 TCID50/mL	20/20
10 µL	**A/CA 300 TCID50/mL**	**20/20**
10 µL	A Texas 2400 TCID50/mL	20/20
10 µL	**A Texas 1200 TCID50/mL**	**20/20**
10 µL	B Nevada 675 CEID50/mL	18/20
10 µL	**B Nevada 1350 CEID50/mL**	**20/20**
10 µL	B/MA 300 TCID50/mL	17/18
10 µL	**B/MA 400 TCID50/mL**	**20/20**

Analytical Reactivity

Inclusivity verification was evaluated for the Accula Flu A/Flu B Test. The panel consisted of 23 influenza strains. The chosen strains represented subtypes in the population, including A: H1N1, A: H3N2, A: H1N1 (2009), B Victoria lineage strains, and B Yamagata lineage strains. At least ten (10) Influenza A strains and five (5) Influenza B strains were included, and emphasis was placed on contemporary strains. Virus was diluted into a pooled clinical matrix and spiked onto a swab to create contrived swab samples. Each strain was tested in triplicate, at final concentration of about 2x LoD of each Influenza subtype. The following test results were obtained.

All twenty-three (23) strains of Influenza were detected with the Accula Flu A/Flu B Test at concentration of about 2X LoD.

Table 8 Inclusivity Verification: Influenza A and Influenza B Strains, Target Concentrations and Test Results

Influenza Strain	Subtype/Lineage	Viral Titer TCID50/mL[1]	Flu A Test Result (# of Flu A Positive/3)	Flu B Test Result (# of Flu B Positive/3)
A/Beijing/262/1995	H1N1	6.00E+02	3/3	0/3
A/Brisbane/59/2007	H1N1	6.00E+02	3/3	0/3
A/Brisbane/10/2007	H3N2	2.40E+03	3/3	0/3
A/England/42/1972	H3N2	2.40E+03	3/3	0/3
A/Fort Monmouth/1/1947	H1N1	6.00E+02	3/3	0/3
A/New Caledonia/20/1999	H1N1	6.00E+02	3/3	0/3
A/Perth/16/2009	H3N2-like	2.40E+03	3/3	0/3
A/Port Chalmers/1/1973	H3N2	2.40E+03	3/3	0/3
A/Puerto Rico/8/1934	H1N1	6.00E+02	3/3	0/3
A/Solomon Islands/3/2006	H1N1	6.00E+02	3/3	0/3
A/Switzerland/9715293/2013	H3N2-like	2.40E+03	3/3	0/3
A/Sydney/5/1997	H3N2	2.40E+03	3/3	0/3
A/Victoria/3/1975	H3N2	2.40E+03	3/3	0/3
A/Victoria/361/2011	H3N2	2.40E+03	3/3	0/3
A/Wisconsin/67/2005	H3N2-like	2.40E+03	3/3	0/3
B/Brisbane/60/2008	Victoria	2.70E+03	0/3	3/3
B/Florida/4/2006	Yamagata	8.00E+02	0/3	3/3
B/Lee/1940	Victoria	2.70E+03	0/3	3/3
B/Malaysia/2506/2004	Victoria	2.70E+03	0/3	3/3
B/Maryland/1/1959	Yamagata	8.00E+02	0/3	3/3
B/Phuket/3073/2013	Yamagata	8.00E+02	0/3	3/3
B/Russia/1969	Yamagata	8.00E+02	0/3	3/3
B/Wisconsin/01/2010	Yamagata	8.00E+02	0/3	3/3

[1] Concentration of contrived sample after 10 µL of virus dilution spiked onto swab and swirled in 5 mL assuming 100% viral elution recovery.

Analytical specificity (cross reactivity)

The analytical specificity was evaluated with a panel of common organisms when tested on the Accula™ Flu A/Flu B Test. Thirty-three (33) organisms were obtained from Zeptometrix Corporation except for *Chlamydia pneumonia* and *Corynebacterium glycinophilum* (were obtained

from ATCC). These potentially cross-reacting non-influenza organisms were tested in replicates of three (3) in this study. The organisms were diluted into a pooled negative nasal sample (PNNS) matrix to create samples at the concentration in the following table for testing.

Table 9 Analytical Exclusivity – Organisms Tested, Concentrations and Test Results

Organism key #	Organism name	Stock concentration	Test level	Flu A Test Result (# of Flu A Positive/3)	Flu B Test Result (# of Flu B Positive/3)
1	Adenovirus Type 1	1.02E+08 TCID50/mL	5.10E+05 TCID50/mL	0/3	0/3
2	Adenovirus Type 7	6.61E+06 TCID50/mL	3.31E+04 TCID50/mL	0/3	0/3
3	Human herpesvirus 5 (Cytomegalovirus)	2.19E+06 TCID50/mL	1.10E+04 TCID50/mL	0/3	0/3
4	Human coronavirus 229E	2.19E+06 TCID50/mL	1.10E+04 TCID50/mL	0/3	0/3
5	Human coronavirus OC43	5.89E+07 TCID50/mL	2.95E+05 TCID50/mL	0/3	0/3
6	Human Enterovirus 71 (HEV-71)	4.17E+05 TCID50/mL	1.04E+04 TCID50/mL	0/3	0/3
7	Epstein-Barr virus	7.95E+09 cp/mL	3.98E+07 cp/mL	0/3	0/3
8	Human parainfluenza virus 1	1.26E+06 TCID50/mL	1.26E+04 TCID50/mL	0/3	0/3
9	Human parainfluenza virus 2	2.19E+06 TCID50/mL	1.10E+04 TCID50/mL	0/3	0/3
10	Human parainfluenza virus 3	5.89E+05 TCID50/mL	1.18E+04 TCID50/mL	0/3	0/3
11	Measles virus	5.89E+07 TCID50/mL	2.95E+05 TCID50/mL	0/3	0/3
12	Human metapneumovirus	3.55E+05 TCID50/mL	1.01E+04 TCID50/mL	0/3	0/3
13	Mumps virus	1.95E+07 TCID50/mL	9.75E+04 TCID50/mL	0/3	0/3
14	Respiratory syncytial virus	3.16E+06 TCID50/mL	1.58E+04 TCID50/mL	0/3	0/3
15	Human rhinovirus 17	6.61E+06 TCID50/mL	3.31E+04 TCID50/mL	0/3	0/3
16	*Bordetella pertussis*	8.43E+08 cfu/mL	4.22E+06 cfu/mL	0/3	0/3
17	*Chlamydia pneumoniae*	≥ 5E+03 IFU/mL*	≥ 1.67E+04 IFU/mL	0/3	0/3
18	*Corynebacterium glycinophilum*	≥ 5.56E+07 IFU/mL**	≥ 1.59E+06 IFU/mL	0/3	0/3
19	*Escherichia coli*	3.83E+09 cfu/mL	1.92E+07 cfu/mL	0/3	0/3
20	*Haemophilus influenzae*	2.40E+08 cfu/mL	1.20E+06 cfu/mL	0/3	0/3
21	*Lactobacillus* sp.	6.00E+08 cfu/mL	3.00E+06 cfu/mL	0/3	0/3
22	*Legionella longbeachae*	1.93E+09 cfu/mL	9.65E+06 cfu/mL	0/3	0/3
23	*Moraxella catarrhalis*	3.97E+07 cfu/mL	1.99E+05 cfu/mL	0/3	0/3
24	*Mycobacterium tuberculosis*	7.23E+08 cfu/mL	3.62E+06 cfu/mL	0/3	0/3
25	*Mycoplasma pneumoniae*	5.62E+07 CCU/mL	2.81E+05 CCU/mL	0/3	0/3
26	*Neisseria meningitidis*	2.55E+08 cfu/mL	1.28E+06 cfu/mL	0/3	0/3
27	*Neisseria subflava*	1.46E+09 cfu/mL	7.30E+06 cfu/mL	0/3	0/3
28	*Pseudomonas aeruginosa*	1.21E+08 cfu/mL	6.05E+05 cfu/mL	0/3	0/3
29	*Staphylococcus aureus*	1.39E+10 cfu/mL	6.95E+07 cfu/mL	0/3	0/3
30	*Staphylococcus epidermidis*	6.47E+09 cfu/mL	3.24E+07 cfu/mL	0/3	0/3
31	*Streptococcus pneumonia*	4.17E+08 cfu/mL	2.09E+06 cfu/mL	0/3	0/3
32	*Streptococcus pyogenes*	5.43E+09 cfu/mL	2.72E+07 cfu/mL	0/3	0/3
33	*Streptococcus salivarius*	4.63E+08 cfu/mL	2.32E+06 cfu/mL	0/3	0/3

All 33 exclusivity organisms were negative at the concentrations tested. Exclusivity is verified for the strains tested.

Interfering substances

To assess substances with the potential to interfere with the performance of the Accula™ Flu A/Flu B Test, four (4) influenza strains were tested in replicates of three (3) with each interfering substance at the "worst case" concentration. The influenza strains selected for testing include a 2009 pandemic swine-like H1N1 influenza A strain, an H3N2 influenza A strain, and two influenza B strains representing Victoria and Yamagata lineages. Virus was serially diluted into a pooled clinical matrix to achieve a 1.5X LoD concentration.

Each influenza strain was tested with the "worst case" interferent concentration, representing the highest concentration likely to be found in a respiratory sample. Additionally, each strain was tested without the interfering substance as a control.

The results are shown in the following table. The Accula™ Flu A/Flu B Test performance is not negatively affected by the potentially interfering substances under "worst case" concentration conditions.

Table 10 Interfering Substances: Agreement of Observed/Expected

Interferent Description, Concentration	Target	% Agreement with Expected Results
Mucin, 20 µg Mucin/mL	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Blood (Human) 1% (v/v)	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Neo-Synephrine (phenylephrine nasal spray)	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Afrin (Oxymetazoline nasal spray)	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Nasacort (Triamcinolone, nasal corticosteroid	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
No Interferent	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Zicam (Nasal gel, homeopathic allergy relief medicine)	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Cepacol (throat lozenge)	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Zanamivir (anti-viral drug) 10mg/mL	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Mupirocin (antibiotic) 12 mg/mL	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)
Tobramycin (antibacterial) 2.43 mg /mL	Negative	100% (3/3)
	FluA/Cali	100% (3/3)
	FluA/Texas	100% (3/3)
	FluB/Nevada	100% (3/3)
	FluB/Mass	100% (3/3)

CLIA waiver studies

Clinical performance by intended users

The performance of the Accula™ Flu A/Flu B Test was evaluated at sixteen intended use sites by non-laboratory personnel in a prospective clinical study during the 2016-2017 flu season in the United States. Nasal swabs were collected from patients with flu-like symptoms and were tested with the Accula™ Flu A/Flu B Test and the comparator method, a FDA-cleared molecular influenza assay. All specimens generating discrepant results were investigated by testing using an alternative FDA-cleared molecular assay. The performance of the Accula™ Flu A/Flu B Test for influenza A and influenza B compared with the comparator method are presented in the following tables.

Table 11 Accula Flu A/Flu B Test Flu A performance against the Molecular Comparator Method

Accula™ Flu A/Flu B Test Flu A	Comparator		
	Positive	Negative	Total
Positive	289	60[1]	349
Negative	9[2]	900	909
Total	298	960	1258
Sensitivity	97% (95% CI: 94.4% - 98.4%)		
Specificity	94% (95% CI: 92.0% - 95.1%)		

[1] FLU A was detected in 47/60 false positives specimens using an alternative FDA-cleared molecular influenza assay.
[2] FLU A was not detected in 3/9 false negative specimens using an alternative FDA-cleared molecular influenza assay.

Table 12 Accula Flu A/Flu B Test Flu B performance against the Molecular Comparator Method

Accula™ Flu A/Flu B Test Flu B	Comparator		
	Positive	Negative	Total
Positive	126	14[1]	140
Negative	8[2]	1110	1118
Total	134	1124	1258
Sensitivity	94% (95% CI: 88.7% - 97.0%)		
Specificity	99% (95% CI: 97.9% - 99.3%)		

[1] FLU B was detected in 9/14 false positive specimens using an alternative FDA-cleared molecular influenza assay
[2] FLU B was not detected in 5/8 false negative specimens using an alternative FDA-cleared molecular influenza assay

The study demonstrates the performance of the Accula™ Flu A/Flu B Test in a CLIA Waived clinical setting.

Performance near the cut-off

Three CLIA-waived sites that participated in the prospective clinical study participated in the near-cutoff study. The testing was performed by three (3) untrained intended operators at each of the sites. This study was conducted to demonstrate that untrained intended users could perform the Accula™ Flu A/Flu B Test and consistently detect low positive samples at the limit of detection.

The test panel consisted of three contrived samples: Flu A low positive, Flu B low positive, and a true negative. Each sample was prepared using Flu A and B strains spiked into clinical matrix. The Flu A strain used in this study was Flu A/California/07/2009 and the Flu B strain used in this study was Flu B/Massachusetts/2/2012. The targeted concentrations for the low positive samples were approximately 1X the respective LoD (C95 concentration), and the Flu A and B Negative samples contained no flu virus. Test samples of Influenza A or Influenza B were coded and blinded to the operators. Swab specimens were presented to the intended use operators throughout the course of a normal testing day and were masked as subject samples. Testing took place over the course of two weeks on non-consecutive days, while the clinical study was in progress. Each operator tested 5 samples each testing day. Each site ultimately tested a panel of 60 samples: 20 replicates of each sample. Testing was performed with one lot of Accula™ Flu A/Flu B Test cassettes.

Test results are shown in the following table. This study demonstrates untrained intended use operators are able to accurately perform and interpret the Accula™ Flu A/Flu B Test at the level of the LoD for both Influenza A and Influenza B.

Table 13 Near-Cutoff Study Test Results: Agreement of Observed/Expected

Site	Swab type		
	Low A Positive/Total	Low B Positive/Total	Negative/Total
ADP	19/20	19/20	19/19[1]
DCO	20/20	20/20	20/20
GVP	19/20	19/20	20/20
Total agreement	58/60 = 97%	58/60 = 97%	59/59 = 100%

[1] 1 negative result resulted in an unresolved Invalid (2 invalid results on the same sample)

Definition of symbols

Symbol	Description	Symbol	Description
	CONSULT INSTRUCTIONS FOR USE		MANUFACTURER
Read SDS	READ SAFETY DATA SHEET	REF	CATALOG NUMBER
	This symbol is used for both warnings and cautions. A warning indicates the risk of personal injury or loss of life if operating procedures and practices are not correctly followed. A caution indicates the possibility of loss of data or damage to, or destruction of, equipment if operating procedures and practices are not strictly observed.	LOT	BATCH CODE
	UPPER AND LOWER TEMPERATURE LIMIT		USE BY
 n	CONTAINS SUFFICIENT FOR <n> TESTS	CONTROL +	POSITIVE CONTROL
IVD	IN VITRO DIAGNOSTIC MEDICAL DEVICE	CONTROL −	NEGATIVE CONTROL
	PATIENT NUMBER		DATE
	DO NOT REUSE		BIOLOGICAL RISKS
Rx only	**CAUTION:** FEDERAL LAW RESTRICTS THIS DEVICE TO SALE BY OR ON ORDER OF A PHYSICIAN		

Related documentation

To obtain the documents in PDF format, go to **thermofisher.com**, search for the product catalog number, then click the **Documents** tab on the web page.

Document	Publication number
Accula™ Flu A/Flu B Test Quick Reference	LBL-60008
Accula™ Flu A/Flu B Control Kit Instructions for Use	LBL-60110
Accula™ Dock Operator's Guide	LBL-60058

Technical support

Email: **techsupport@thermofisher.com**

Telephone: 1-800-955-6288, option 2

For the latest service and support information at all locations, or to obtain Certificates of Analysis or Safety Data Sheets (SDSs; also known as MSDSs), go to **thermofisher.com/support**.

References

Cheung M, Lieberman JM (2002, Oct) Influenza: update on strategies for management. *Contemporary Pediatrics* 19:82.

Iha Y, Kinjo T, Parrott G, Higa F, Mori H, Fujita J (2016) Comparative epidemiology of influenza A and B viral infection in a subtropical region: a 7-year surveillance in Okinawa, Japan. *BMC Infect Dis* 16:650.

Kim HW, Brandt CD, Arrobio JO, Murphy B, Chanock RM, Parrott RH (1979, Apr) Influenza A and B virus infection in infants and young children during the years 1957-1976. *Am J Epidemiol* 109(4):464-79.

Montalto N, Byrd R. (2003, Jan) An Office-Based Approach to Influenza: Clinical Diagnosis and Laboratory Testing. *American Family Physician* 67:111-118.

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Revision	Date	Description
C	31 May 2022	First release of the document to customers, using the latest Thermo Fisher Scientific template, with associated changes to logos, trademarks, legal information, and format.