Current Status: *Pending*		**PolicyStat ID:** *8801081*
	Origination:	*N/A*
	Effective:	*Upon Approval*
	Final Approved:	*N/A*
	Last Revised:	*N/A*
Valley Laboratories	**Next Review:**	*2 years after approval*
	Owner:	*Lindsey Westerbeck: Dir, Lab*
	Policy Area:	*Lab - Microbiology*
	References:	
	Applicability:	*Valley Laboratories*

Performing an ID NOW Influenza A&B 2 Assay

PURPOSE

ID NOW Influenza A&B 2 assay performed on the ID NOW Instrument is a rapid (13 minutes or less) molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology intended for the qualitative detection and discrimination of influenza A and B viral RNA in direct nasal or nasopharyngeal swabs and nasal or nasopharyngeal swabs eluted in viral transport media from patients with signs and symptoms of respiratory infection. It is intended for use as an aid in the differential diagnosis of influenza A and B viral infections in humans 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 diagnosis, treatment or other patient management decisions.

POLICY

- Order Code: **FLUNAA**(*Influenza A/B Rapid NAAT)*
- Testing is to be performed upon receipt by trained CLS and / or MLT staff on all shifts.

EQUIPMENT, REAGENTS AND SUPPLIES

- ID NOW Analyzer
- **ID NOW Influenza A&B 2 Test Kit** - Store kit at 2-30°C and use until expiration date marked on the outer packaging and containers
  - Test Bases
  - Sample Receivers
  - Transfer Cartridges
  - Patient Swabs
  - Positive and Negative Control Swab
- PPE - To include mask, eye protection, lab coat and gloves
- One of the following acceptable cleaning agents:
  - 70% ethanol - available in commercial wipes or on a damp, lint free cloth
  - 70% isopropanol - available in commercial wipes or on a damp, lint free cloth
  - 10% bleach - on a damp, lint free cloth only

SPECIMEN REQUIREMENTS

- Use freshly collected specimens for optimal test performance.
- Inadequate specimen collection or improper sample handling/storage/transport may yield erroneous results.

Specimen Type	Acceptable Swab Type
Nasal **NOTE**: Perform sample collection from **one** nostril.	For optimal test performance, use the swabs provided in the test kit. • **Alternative swabs acceptable**: Rayon, foam, HydraFlock® Flocked swab (standard tip), HydraFlock® Flocked swab (mini tip), Copan Mini Tip Flocked Swab, or Copan Standard Flocked swabs • **NOT acceptable**: Puritan PurFlock Standard Tip Ultra Flocked Swabs, Puritan PurFlock Mini Tip Ultra Flocked Swabs and Copan Standard Rayon Tip Swabs
Nasopharyngeal	Sterile rayon, foam, polyester or flocked flexible-shaft NP swabs
Transport Media	Amie's Media, Dulbecco's Modified Eagles' Medium (D-MEM), Hank's Balanced Salt Solution, M4 Media, M4-RT Media, M5 Media, M6 Media, Phosphate Buffered Saline, Saline, Stuart's Media, Universal Transport Media, or Starplex Multitrans • **NOT acceptable**: Tryptose Phosphate Broth, Brain Heart Infusion Broth, Veal Infusion Broth, and Wako's E-MEM transport media

Specimen Type	Specimen Storage and Stability
Direct Nasal / NP Swabs	Direct nasal or nasopharyngeal swabs should be tested as soon as possible after collection. If immediate testing is not possible, the swab can be held in its original package at: • Room Temperature, 15-30°C, for up to 2 hours post collection • Refrigerated, 2-8°C, for up to 24 hours post collection
Transport Media	Elute swab in 0.5 to 3.0 mL of saline or viral transport media by rotating the swab in the liquid for 10 seconds, within 1 hour of sample collection. Remove the swab and discard. If immediate testing is not possible, eluted swab samples can be held at: • Room Temperature, 15-30°C, for up to 8 hours prior to testing • Refrigerated, 2-8°C, for up to 72 hours post collection Swirl eluted swab samples in transport media gently to mix before testing. • *Note: Minimal dilution of the sample is recommended as dilution may result in decreased test sensitivity.*

QUALITY CONTROL - EXTERNAL

- Positive Control Swab and Sterile Swab (Negative Control), which are included in the ID NOW Influenza A&B 2 kit, should be tested once with each new lot/shipment to ensure that test reagents are working and that the test is correctly performed.
  - External QC will be rotated on ID-NOW instruments with each lot/shipment
- Control swabs should also be tested once for each untrained operator.
- The QC test is run in the same manner as outlined in the ***"TEST PROCEDURE***" for direct nasal and NP swabs.
  - Refer to subsection in procedure for step by step instructions to process control swab for testing.
  - To program QC testing to run, refer to instructions below.

Step	Action
1	Touch 'Run QC Test' on home screen.
2	Touch 'Influenza A&B'
3	Select the QC Test to be Run
4	Confirm Test • Confirm the test type to match the QC sample intended for testing by touching 'OK' and following the on screen prompts to complete testing.
5	The ID NOW Instrument will report QC results as **Pass** or **Fail** once testing is complete.
6	If QC passes, lot/shipment is acceptable to use for patient testing.
7	If QC fails, **do not** perform patient tests or report patient results. • Contact Technical Support (855-731-2288) during normal business hours before testing patient specimens on instrument. • If Technical Support is not available, repeat QC using control swabs from a different kit from same lot/shipment. ◦ If QC passes upon testing with new control swabs, quarantine kit that did not pass and do not use for patient testing. ◦ If QC fails again, do not use any kits from shipment until contacting technical support for further troubleshooting. • Notify supervisor immediately of any QC failures and actions taken.
8	Document all external QC results and actions taken on *Form A: ID NOW Influenza A&B 2 External QC* log

QUALITY CONTROL - INTERNAL

- ID NOW Influenza A&B has built-in procedural controls run with each test performed.
  - Contains an internal control that has been designed to control for sample inhibition and assay reagent function.
  - In positive samples where target amplification is strong, the internal control is ignored and the target amplification serves as the '**control'** to confirm that the clinical sample was not inhibitory and that assay reagent performance was robust.
    - At a very low frequency, clinical samples can contain inhibitors that may generate invalid results.
- The result of the Procedural Control is displayed on the screen and is automatically stored in the instrument with each test result. This can be reviewed later by selecting Review Memory on the instrument.

PRECAUTIONS

- For *in vitro* diagnostic use.
- Federal Law restricts this device to sale by or on the order of a licensed practitioner (US only).
- To be used in conjunction with the ID NOW Instrument.
- Performance characteristics of this test have been established with the specimen type listed in the Intended Use section only. The performance of this assay with other specimen types or samples has not been validated.
- Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Leave test pieces sealed in their foil pouches until just before use.
- Do not tamper with test pieces prior to or after use.
- Do not use kit past its expiration date.
- Do not mix components from different kit lots or from other ID NOW assays.
- Solutions used to make the control swabs are inactivated using standard methods. However, patient samples, controls, and test pieces should be handled as though they could transmit disease. Observe established precautions against microbial hazards during use and disposal.
- If any assay components are dropped, cracked, found to be damaged or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur.
- Do not open the Sample Receiver before placing in the instrument. It will prohibit the Elution Buffer from reaching temperature and may impact test performance.
- If the Sample Receiver is spilled while opening, clean the instrument per instructions provided in the instrument User Manual and cancel test. Repeat test with a new Sample Receiver.
- All test pieces must be removed from the instrument according to removal instructions displayed on the instrument, and disposed of according to country and local requirements. Pieces must not be separated once they are assembled.
- 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 departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
- All test pieces are single use items. Do not use with multiple specimens.
- Performance characteristics for influenza A were established when influenza A/H3 and A/H1N1 pandemic were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
- Once reacted, the Test Base contains large amounts of amplified target (Amplicon). Do not disassemble the Test Base and Transfer Cartridge. In the case of a positive sample, this could lead to amplicon leakage and potential ID NOW Influenza A & B 2 false positive test results.
- At a low frequency, clinical samples can contain inhibitors that may generate invalid results. Site to site invalid rates may vary.
- Due to the high sensitivity of the assays run on the instrument, contamination of the work area with previous positive samples may cause false positive results. Handle samples according to standard laboratory practices. Clean instruments and surrounding surfaces according to instructions provided in the cleaning section of the instrument User Manual. Refer to Section 1.6, Maintenance & Cleaning, for further information.
- Visibly bloody samples must not be used with ID NOW Influenza A & B 2.
- Do not touch the heads of the Control Swabs. Cross contamination with the Positive Control Swabs may occur due to the high sensitivity of the assays run on the instrument.

TEST PROCEDURE

Please refer to the ID NOW™ Instrument User Manual for full instructions.

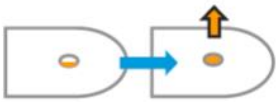
- Before testing with ID NOW™ Influenza A&B 2:
  - Allow all samples to reach room temperature.
  - Allow all test pieces to reach room temperature.
  - Check that a reagent pellet is visible at the bottom of each of the reaction tubes prior to inserting the Test Base in the ID NOW Instrument.
    - Do **not** use the Test Base if a pellet is not visible at the bottom of each reaction tube.

IMPORTANT: Work surfaces should be cleaned and decontaminated before and after processing samples to prevent cross-contamination.

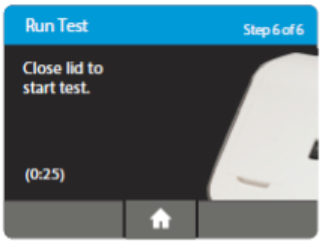
- Use Standard Precautions when handling samples and used test pieces, as they are capable of transmitting infectious agents.
- Change gloves between samples.
- Change or remove gloves before using computer.

Step	Action

1	Turn on the ID NOW Instrument - press the power button on the side of the instrument. • **NOTE**: If the unit is unattended for one hour, the instrument will go to a black screen power save mode. Touch the screen to return the unit to active display operation.
2	Enter User ID Press '✓' after entry.
3	Touch 'Run Test' • This will begin the test process.
4	Touch 'Influenza A&B Test' • This starts a Influenza A&B test.
5	Select Sample Type
6	Scan Sunquest barcode label affixed to patient sample for FLUNAA test at "Enter Patient ID" prompt using barcode scanner. • Touch '✓'. • Verify that the ID was entered correctly, then touch '✓' to confirm entry.
7	Open the Lid and insert Orange Test Base into Orange Test Base holder. • **CAUTION**: Do not apply excessive force. Excessive force could damage the instrument.
8	Confirm that the correct test is displayed on the screen. Touch 'OK' to proceed. • **CAUTION**: Once the Test Base has been placed in the holder, the user will have **10 minutes** to confirm the test. If the test is not confirmed within 10 minutes, the instrument will time out and the Test Base must be removed and discarded. ◦ If the incorrect Test Base has been inserted, remove and dispose of the incorrect Test Base. Close the lid. The instrument will then run a self-test before proceeding to the Home screen. Press Run Test and restart the test using the correct Test Base.
9	Insert Blue Sample Receiver into the Blue Sample Receiver holder. • **CAUTION**: Do not apply excessive force. Excessive force could damage the instrument. • **CAUTION**: Once the Sample Receiver has been placed in the holder, the user will have **10 minutes** to start the test. If the test is not started within 10 minutes, the instrument will time out and all test pieces (Test Base and Sample Receiver) must be removed and discarded. The instrument will proceed to the Home screen. Press Run Test and restart the test using a new Test Base and Sample Receiver.
10	Wait for the Sample Receiver to Warm Up. Do not remove the Sample Receiver from the instrument once the Warm Up begins. • **CAUTION**: DO NOT REMOVE THE FOIL SEAL UNTIL PROMPTED BY THE INSTRUMENT. DO NOT close the lid or insert the sample until prompted by the instrument
11	When prompted, remove the foil seal from Sample Receiver. • **CAUTION**: To ensure that the Sample Receiver remains in the instrument while removing the foil seal, place two fingers along the outer edge of the Sample Receiver to hold it in place. If the Sample Receiver spills after warm up, cancel the test by pressing the Home button. Remove and discard the test pieces (Sample Receiver and Test Base) and clean the instrument. Press Run Test to start a new test using a new Test Base and Sample Receiver.
12	**If** / **Then** Direct nasal or NP swab → • Place the patient swab to be tested into the Sample Receiver. • Vigorously mix the swab in the liquid for 10 seconds. ◦ Press the swab head against the side of the Sample Receiver as you mix it. This helps remove the sample from the swab. • Carefully remove and discard swab. Nasal or NP swab eluted in transport media → • Add 0.2ml of sample to the Sample Receiver using the disposable pipettes provided in the kit. • Vigorously mix the sample in the liquid for 10 seconds. ◦ Use the pipette tip to swirl the liquid. • Carefully remove and discard pipette.
13	Touch 'OK' to proceed.
14	Press the White Transfer Cartridge into the Blue Sample Receiver

	• Listen for a click. When the Transfer Cartridge is properly attached to the Sample Receiver, the orange indicator on the Transfer Cartridge will rise. If the orange indicator does not rise, continue pushing onto the Sample Receiver until it does. • **CAUTION**: The orange indicator should be observed closely. If the orange indicator does not fully rise, the Transfer Cartridge may not collect enough sample. 
15	Lift and then connect the Transfer Cartridge to the Test Base • When the Transfer Cartridge is properly attached to the Test Base, the orange indicator on the Transfer Cartridge will descend. If the orange indicator does not descend, continue pushing onto the Test Base until it does. • **CAUTION**: If the orange indicator does not fully descend, not enough sample will be dispensed. This may potentially result in invalid or false negative results. 
16	Proceed to Test Run and Completion Procedure.

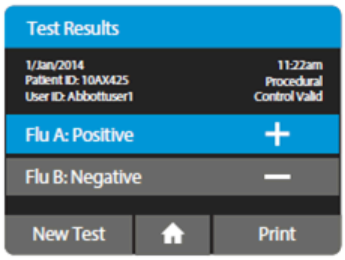
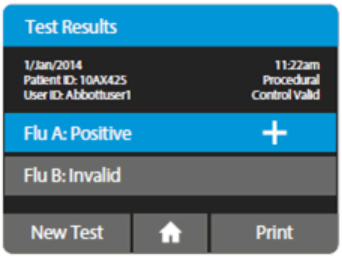
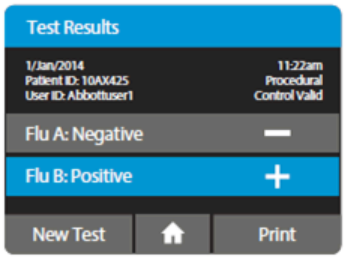
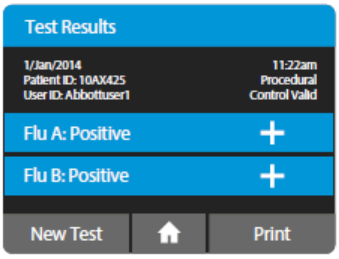
TEST RUN AND COMPLETION PROCEDURE

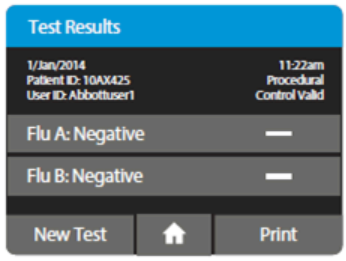
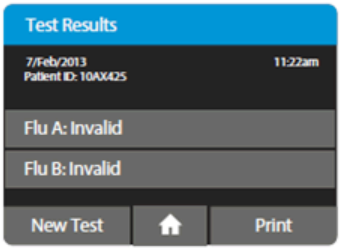
Step	Action
1	Close the Lid. **DO NOT OPEN THE LID** until the Test Complete message appears on the screen. • **NOTE**: The test will be cancelled if the lid is opened all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded. ◦ A test result will not be reported or saved in the instrument memory.
2	**IMPORTANT**: This screen will be displayed for up to 30 seconds once the Transfer Cartridge is detected. If the instrument does not detect that the lid has been closed by then, it will time out and all test pieces (Sample Receiver, Test Base, and Transfer Cartridge) must be removed and discarded.  • If it times out, the instrument will proceed to the Home screen a new specimen will be required. Press Run Test and restart the test using a new Test Base and Sample Receiver.
3	When amplification and detection is complete, the instrument will automatically save the data before advancing to the results screen • **CAUTION**: The test is not saved until the completed result is displayed. **DO NOT OPEN THE LID** until the results are displayed.
4	The Test Results screen displays either a Negative or Positive result for a successfully completed test. • If a test error occurs, the display will read 'Invalid'. Refer to the Result Interpretation Section for Interpretation of Results.
5	Press Print to print test results if they do not print automatically. • Press New Test to run another test. • Press Home to return to the Home screen.
6	After printing, or if New Test or Home are selected, the instrument will prompt to open the lid and discard the used test pieces.
7	Remove test pieces by lifting the Transfer Cartridge attached to the Test Base, and clicking it into the Sample Receiver, by pressing into the Sample Receiver.

	• **CAUTION**: Do not try to remove the Sample Receiver by any other method as there is a risk of spilling the patient sample.
8	All test pieces will be connected and can now be removed from the instrument and disposed of in the biohazard waste. • **CAUTION**: DO NOT disassemble the Transfer Cartridge and the Test Base before disposal.
9	Close the lid. The instrument will then run a Self-Test before showing the Home screen or Enter Patient ID screen, depending on the previous selection

RESULT INTERPRETATION

Step	Action
1	When the test is complete, the results are clearly displayed on the instrument screen.

Instrument Display	Interpretation of Results and Follow-up Actions
	**Flu A Viral RNA Detected; Flu B Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu A Viral RNA Detected; The presence or absence of Flu B Viral RNA cannot be determined.** This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
	**Flu B Viral RNA Detected; Flu A Viral RNA Not Detected.** This result does not rule out co-infections with other pathogens or identify any specific influenza B virus lineage.
	**Flu A Viral RNA Detected; Flu B Viral RNA Detected.** Dual infections of Flu A and Flu B are rare. Repeat testing using new test components. Contact Technical Support during normal business hours if multiple samples provide this result. This result does not rule out co-infections with other pathogens or identify any specific influenza A or influenza B virus lineage.

		**Flu A Viral RNA Not Detected; Flu B Viral RNA Not Detected.**
		**The presence or absence of Flu A and Flu B Viral RNAs cannot be determined.** Repeat testing of the sample using new test components. If repeated Flu A and Flu B Invalid results are obtained, results should be confirmed by another method prior to reporting the results.
2	If an Invalid result is received, one additional test may be run using the same Sample Receiver. The instructions below should be followed: • Remove the connected Test Base and Transfer Cartridge from the instrument and connect the Test Base portion to an open, UNUSED Sample Receiver. ◦ The connected Test Base and Transfer Cartridge MUST be attached to a Sample Receiver prior to disposal. The Sample Receiver from a new Transfer Cartridge package may be used for this. • Remove the blue Sample Receiver separately and carefully from the instrument. ◦ The Sample Receiver should be retained and kept upright to avoid spilling the liquid contents. • From the Home Screen, start a new test. Follow the screen prompts; however, when asked to insert the Sample Receiver, reuse the Sample Receiver and **DO NOT** reuse/re-elute the swab.	
3	If instrument is not interfaced or interface is down, *Form B: ID NOW Test Result Log* can be used for result documentation and manually reporting of results.	

REPORTING RESULTS

If Result Is	Then Report
Negative for Flu A	• FLUAR**: NOTDET** (*Not Detected*)
Negative for Flu B	• FLUBR**: NOTDET**
Positive for Flu A	• FLUAR**: DET1** (*Detected*)
Positive for Flu B	• FLUBR**: DET1**
Invalid for Flu A *(after repeat testing)*	• FLUAR**: INDET2** *(Indeterminate)* • The following ETC will auto-append to result: ◦ **FLUAP:** *Presence or absence of Influenza A target RNA sequences cannot be determined.*
Invalid for Flu B *(after repeat testing)*	• FLUBR**: INDET2** • The following ETC will auto-append to result: ◦ **FLUBP:** *Presence or absence of Influenza B target RNA sequences cannot be determined.*
Flu A and/or B Positive	The following ETC will auto-append to result: • **MOLPOS:** *Positive results should be interpreted in conjunction with clinical evidence, since this assay does NOT distinguish between infectious and non-infectious (e.g. latent, treated) organisms.*
Flu A and B Negative	The following ETC will auto-append to result: • **MOLNEG**: *Negative results do not exclude the possibility of infection. Interpretation of all results must include*

	clinical evaluation of the patient and other diagnostic procedures.
Indeterminate (Invalid)	The following ETC will auto-append to result: • **MOLIND**: *Indeterminate due to possible inhibiting or interfering substances. Recollect if clinically indicated.* Indeterminate results are called to the caregiver of the patient to notify of the need to recollect if clinically indicated.

TECHNICAL LIMITATIONS

- The performance of the ID NOW™ Influenza A & B 2 was evaluated using the procedures provided in this product insert only. Modifications to these procedures may alter the performance of the test.
- ID NOW™ Influenza A & B 2 performance depends on viral RNA load and may not correlate with cell culture performed on the same specimen. Viral nucleic acid may persist in vivo, independent of virus viability. Detection of analyte target(s) does not imply the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
- Performance of ID NOW™ Influenza A & B 2 has not been established for monitoring antiviral treatment of influenza.
- Although this test has been shown to detect A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for the A/H1N1 (pre-2009 pandemic), A/H7N9 (detected in China in 2013) and A/H3N2v viruses have not been established.
- There is a risk of false negative results due to the presence of sequence variants in the viral targets of the assay. If the virus mutates in the target regions, influenza viruses A or B may not be detected or may be detected less efficiently. Additionally, if the sequence variant occurs in the target sequence recognized by the fluorescently-labeled molecular beacon an invalid assay may result.
- False negative results may occur if a specimen is improperly collected, transported or handled. False negative results may occur if inadequate levels of viruses are present in the specimen.
- False negative results may occur if mucin concentrations of 1% (w/v) or greater are present in the specimen.
- False negative results may occur if Respiratory Syncytial Virus is present as a co-infecting organism.
- Potential interference effects from FluMist™ have not been evaluated. Individuals who have received nasally administered influenza vaccine may test positive in commercially available influenza rapid diagnostic tests for up to three days after vaccination.
- This test is not intended to differentiate Influenza A subtypes or Influenza B lineages. If differentiation of specific influenza subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- Negative results do not preclude infection with influenza virus and should not be the sole basis of a patient treatment decision. Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2016 to 2017 influenza seasons. The positive and negative predictive values may vary depending on the prevalence and population tested.
- This test has not been evaluated for patients without signs and symptoms of influenza infection.
- The test is a qualitative test and does not provide the quantitative value of detected organism present.
- Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
- This assay has not been evaluated for immunocompromised individuals.
- This test cannot rule out diseases caused by other bacterial or viral pathogens. The regions selected for amplification are conserved among all known Influenza A and Influenza B subtypes and strains (where sequence data is available from public databases). Laboratory testing has shown that ID NOW™ Influenza A & B 2 can readily amplify and detect H1N1 (pre-2009 pandemic), H3N2 (variant) and H7N9 (detected in China in 2013) influenza subtypes but the performance of the assay for detection of these subtypes in a clinical setting has not been established due to the lack of clinical samples.

REFERENCES

- ID NOW™ Influenza A&B 2 Product Insert, IN427000 Rev.7 2019/10/29.

All revision dates:

Attachments

Form A: ID NOW Influenza A&B 2 External QC
Form B: ID NOW Test Result Log

Approval Signatures

Step Description	Approver	Date
Lab Medical Directors	Kristen Vandewalker: MD	pending
Lab Medical Directors	Andrea Ong: MD	pending

Step Description	Approver	Date
Lab Medical Directors	Hannah Wong: MD	pending
Lab Medical Directors	Rowberry Ron: MD	pending
Lab Medical Directors	Jamie Cassity: MD	pending
Lab Medical Directors	Mary Keohane: MD	pending
Lab Medical Directors	Marian Butcher: MD	pending
	Lindsey Westerbeck: Dir, Lab	10/28/2020