**TITLE: Alere™ i RSV**

**Purpose:** This policy and procedure establishes guidelines for staff at Rush Copley that perform rapid RSV testing using the **Alere™ i** instrument RSV assay.

**CLIA COMPLEXITY: WAIVED – For Nasopharyngeal Swabs (Tested Directly or after Elution in Viral Transport Media)**

1. **Intended Use**

The **Alere™ i** RSV assay performed on the **Alere™ i** Instrument is a rapid molecular *in vitro* diagnostic test utilizing an isothermal nucleic acid amplification technology for the qualitative detection of respiratory syncytial virus (RSV) viral RNA in direct nasopharyngeal swabs and 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 diagnosis of RSV in children <18 years and adults ≥60 years in conjunction with clinical and epidemiological risk factors.

1. **Summary and Explanation of the Test**

Respiratory Syncytial Virus (RSV) is the single most important cause of severe respiratory illness in infants and young children and the major cause of infantile bronchiolitis. It is the most frequent cause of hospitalization of infants and young children in industrialized countries. In the USA alone, 85,000 to 144,000 infants with RSV infections are hospitalized annually, resulting in 20% to 25% of pneumonia cases and up to 70% of bronchiolitis cases in the hospital. Global RSV disease burden is estimated at 64 million cases and 160,000 deaths every year.1

RSV disease includes a wide array of symptoms, from rhinitis and otitis media to pneumonia and bronchiolitis. Spread of the virus from contaminated nasal secretions occurs via large respiratory droplets, and close contact with an infected individual or contaminated surface is required for transmission.

RSV is also a significant problem in the elderly, in persons with cardiopulmonary diseases and in immunocompromised individuals. Rates of RSV infection in nursing homes in the USA are approximately 5% to 10% per year with a 2% to 8% case fatality rate, amounting to approximately 10,000 deaths per year among persons >64 years of age.1

Rapid diagnostics with increased sensitivity are essential for the reliable detection of RSV, allowing immediate, effective patient management. Rapid accurate diagnosis of RSV can lead to reduced hospital stays and costs, reduction in antimicrobial use, reduced secondary complications and effective implementation of infection control measures.2

**Alere™ i** RSV is a rapid (less than 15 minutes), instrument-based isothermal test for the qualitative detection of RSV A and RSV B from nasopharyngeal swabs and nasopharyngeal swabs eluted in viral transport media. The **Alere™ i** Instrument has a small footprint and easy to use graphical user interface for convenience within a busy hospital or point-of-care environment. The **Alere™ i** RSV kit contains all components required to carry out an assay for RSV on the **Alere™ i** Instrument.

1. **Test Principle**

**Alere™ i** RSV utilizes isothermal nucleic acid amplification technology for the qualitative detection of RSV A and RSV B viral nucleic acids. It is comprised of a Sample Receiver, containing elution buffer, a Test Base, comprising two sealed reaction tubes, each containing a lyophilized pellet, a Transfer Cartridge for transfer of the eluted sample to the Test Base, and the **Alere™ i** Instrument.

The reaction tubes in the Test Base contain the reagents required for amplification of RSV A and RSV B, respectively, as well as an internal control. The templates (similar to primers) designed to target RSV A RNA amplify a unique region of the nonstructural gene NS2 while the templates designed to amplify RSV B RNA target the nucleocapsid gene N. Fluorescently-labeled molecular beacons are used to specifically identify each of the amplified RNA targets.

To perform the assay, the Sample Receiver and Test Base are inserted into the **Alere™ i** Instrument. The sample is added to the Sample Receiver and transferred via the Transfer Cartridge to the Test Base, initiating target amplification. Heating, mixing and detection are provided by the instrument, with results automatically reported as RSV positive, negative or invalid.

1. **Specimen Collection and Handling**

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

 **Nasopharyngeal Swab**

For optimal performance, use the swab provided in the test kit. Alternatively, sterile rayon, foam, or flocked flexible-shaft NP swabs can be used to collect nasopharyngeal samples.

Calcium alginate and Puritan Purflock® Ultra flocked swabs are not suitable for use in this assay.

To collect a nasopharyngeal swab sample, carefully insert the swab into the nostril exhibiting the most visible drainage, or the nostril that is most congested if drainage is not visible. Pass the swab directly backwards without tipping the swab head up or down. The nasal passage runs parallel to the floor, not parallel to the bridge of the nose. Using gentle rotation, insert the swab into the anterior nare parallel to the palate advancing the swab into the nasopharynx, leave in place for a few seconds, and then slowly rotate the swab as it is being withdrawn.

To ensure proper collection, the swab should be passed a distance that is halfway of that from the nose to the tip of the ear. This is about half the length of the swab. **DO NOT USE FORCE** while inserting the swab. The swab should travel smoothly with minimal resistance; if resistance is encountered, withdraw the swab a little bit without taking it out of the nostril. Then elevate the back of the swab and move it forward into the nasopharynx.

**Specimen Transport and Storage**

Nasopharyngeal swab in viral transport media(VTM) is the specimen of choice as the same sample can be used to perform Alere I Influenza testing as well.

The transport media listed below were tested and are acceptable for use in **Alere™ i** RSV. Elute the swab into 0.5 to 3.0 mL of viral transport media by rotating the swab head in the liquid for 10 - 20 seconds, within 1 hour of sample collection. If immediate testing is not possible, eluted swab samples can be held at room temperature (15-30°C) for up to eight (8) hours prior to testing. If the eluted swab sample will be held longer than eight (8) hours, it must be refrigerated at 2-8°C and tested within 24 hours from the time of sample collection. If refrigerated, samples must be warmed to room temperature before testing with **Alere™ i** RSV.

**Transport Media:**

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| Amie’s Media |
| Dulbecco’s Modified Eagles Medium (DMEM)  |
| M4 Media |
| M4-RT Media |
| M5 Media |
| M6 Media |
| Phosphate Buffered Saline |
| Saline |
| Tryptose Phosphate Broth |
| Veal Infusion Broth |
| Universal Transport Media |
| Starplex Multitrans Media |
| Vircell Media |

1. **Reagents and Materials**
2. **Materials Provided**

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| **Component** | **Content** |
| **Test Bases** | Orange plastic components containing two reaction tubes of lyophilized reagents for the targeted amplification of RSV A and RSV B viral RNA. |
| **Sample Receivers** | Blue plastic components containing 2.5 mL of elution buffer. |
| **Transfer Cartridges** | White plastic components used to transfer 2 x 100 µL of sample extract from the Sample Receiver to the Test Base. |
| **Nasopharyngeal Swabs** | Sterile swabs for use with the **Alere™ i** RSV Test. |
| **Positive Control Swabs** | The positive control swab is coated with inactivated RSV A and B viruses. |
| **Negative Control Swabs** | The negative control swab is coated with inactivated Group C *Streptococcus.* |
| **Plastic disposable pipettes capable of delivering 200µL VTM sample** |
| **Package Insert** |
| **Quick Reference Instructions** |

1. **Materials Required but not Provided**

**Alere™ i** Instrument

1. **Storage and Stability**

Store kit at 2-30˚C. The **Alere™ i** RSV kit is stable until the expiration date marked on the outer packaging and containers. Ensure all test components are at room temperature before use.

1. **Quality Control**

**Alere™ i** RSV has built-in procedural controls. 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.

**Procedural Controls:**

**Alere™ i** RSV contains an internal control that has been designed to control for functionality of the amplification/detection process and reagents. 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 low frequency, clinical samples can contain inhibitors that may generate invalid results.

Procedural Control Valid displayed on the instrument screen indicates that the assay reagents maintained their functional integrity and the sample did not significantly inhibit assay performance.

**External Positive and Negative Controls:**

New lot/shipment positive and negative controls ensure that test reagents are working and that the test is correctly performed. **Alere™ i** RSV kits contain Positive and Negative Control Swabs. These swabs will monitor the entire assay. Test these swabs once with each new lot/shipment.

**Control Swab Procedure**

External Positive and Negative Control swabs are provided and should be tested following the Run QC Test instructions on the **Alere™ i** Instrument. Refer to Quality Control Swab Test Procedure or Instrument User Manual for further details.

*Note: The* ***Alere™ i*** *Instrument reports QC results as Pass or Fail. RSV Positive QC pass indicates a positive result for both RSV A and RSV B.*

If the correct control results are not obtained, do not perform patient tests or report patient results. Contact Technical Support before testing patient specimens.

1. **Precautions**
2. Federal Law restricts this device to sale by or on the order of a licensed practitioner.
3. To be used in conjunction with the **Alere™ i** Instrument.
4. 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.
5. Treat all specimens as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
6. Proper sample collection, storage and transport are essential for correct results.
7. Leave test pieces sealed in their foil pouches until just before use. Storage of unpouched test components at temperatures greater than 30°C or at high relative humidity prior to use may result in Invalid or false results.
8. Do not tamper with test pieces prior to or after use.
9. Do not use kit past its expiration date.
10. Do not mix components from different kit lots.
11. 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.
12. **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.**

12. 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.

13. 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.

14. 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.**

15. All test pieces are single use items. Do not use with multiple specimens.

16. 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 **Alere™ i** RSVfalse positive test results.

17. 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.

18. 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.

1. **Test Procedure**

Before testing with **Alere™ i** RSV:

* 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 **Alere™ i** Instrument.
* Do not use the Test Base if a pellet is not visible at the bottom of each reaction tube.

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| **To Perform a Test:****Step 1**Turn on the **Alere™ i** 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.* | C:\Users\darcy.whitlock\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\Alere i Loading Application.jpg |
| **Enter User ID** Press ‘√’ after entry. | C:\Users\darcy.whitlock\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\Alere i_Enter User ID_Screen CMYK 2015_02.jpg |
| **Touch ‘Run Test’**This will begin the test process. | C:\Users\darcy.whitlock\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\Alere i_Home_Screen CMYK.JPG |
| **Touch ‘RSV’**This starts an RSV test. |  |
| **Select Sample Type (if prompted)**If the sample type has already been specified by the Admin, the instrument will automatically advance to the next step. |  |
| **Enter Patient ID** using on screen keyboard or barcode scanner.Touch ‘√’Verify that the ID was entered correctly, then touch ‘√’ to confirm entry. | C:\Users\darcy.whitlock\AppData\Local\Microsoft\Windows\Temporary Internet Files\Content.Word\Alere i_EnterOrScan Patient ID_Screen CMYK 2015_02.jpg |
| **Step 2****Open the Lid and Insert Orange Test Base into the Orange Test Base holder****Caution: Do not apply excessive force. Excessive force could damage the instrument.** |  |
| **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. |
| **Step 3****Insert Blue Sample Receiver into the Blue Sample Receiver holder****Caution: Do not apply excessive force. Excessive force could damage the instrument.****Caution: Confirm that the foil seal on the Sample Receiver indicates that it is for use with Alere™ i RSV. If not, then remove the Sample Receiver and replace it with a new Sample Receiver for Alere™ i RSV.****Caution: Once the Sample Receiver has been placed in the holder, the user will have 10 minutes to start the test (Steps 3 through 5). 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.** | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_RunTest_PlaceSR_ScreenCMYK.jpg |
| **Wait for the Sample Receiver to Warm Up.****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. |  |
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| **Step 4****Nasopharyngeal Swab Eluted In Viral Transport Media Test Procedure****Before testing vortex or vigorously mix the sample for 10 seconds.****When prompted, remove the foil seal and 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 to swirl the liquid. Once the sample is mixed and the pipette is removed, **immediately press ‘OK’ to proceed.** Continue to Step 5a.**Caution: To ensure 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.** |  |
| **Step 5a****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.** | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_RunTest_PlaceTR_intoSR_ScreenCMYK.jpgC:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_OrangeIndicatorUp.jpg |
| **Step 5b****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.** | **C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_OrangeIndicatorDown.jpg** |
| **Step 6****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.* **Caution: 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. The instrument will proceed to the Home screen. Collect a new sample from the patient. Press Run Test and restart the test using a new Test Base and Sample Receiver.** | *C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_RunTest_CloseLid_ScreenCMYK.jpg*C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_RunTest_Testing_ScreenCMYK.jpg |
| **Caution: DO NOT OPEN THE LID. The test will be cancelled and 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.** |
| 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.** | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere i_Run Test Saving CMYK 2015_02.jpg |
| 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 the Interpretation of Results. **Press Print to print test results, press New Test to run another test, Press Home to return to the Home screen.** |  |
| After printing, or if New Test or Home is selected, the instrument will prompt to open the lid and discard the used test pieces. |  |
| 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.**All test pieces will be connected and can now be removed from the instrument and disposed of according to federal, state and local regulations.**Caution: DO NOT disassemble the Transfer Cartridge and the Test Base before disposal.** | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere_i_DiscardPieces_AttachTestBaseScreenCMYK.jpg |
| 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. |  |
| **Quality Control Swab Test Procedure**For QC testing, select Run QC Test on the Home screen, and follow the displayed instructions. Refer to Running a QC Test in the **Alere™ i** Instrument User Manual for further details. |
| 1. **Touch ‘Run QC Test’**
 | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere i_RunQC_Screen CMYK_en.jpg |
| 1. **Touch ‘RSV’**
 |  |
| 1. **Select the QC Test to be Run**
 | C:\Users\darcy.whitlock\Documents\_Files\Alere i\Screen Shots\Alere i_Run QC Test_Screen CMYK.jpg |
| 1. **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.*Note: The QC test is run in the same manner as a Direct Nasopharyngeal Swab. See the* ***To Perform a Test*** *section above for step by step instructions for direct nasopharyngeal swab samples.* |  |

1. **Result Interpretation**

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| When the test is complete, the results are clearly displayed on the instrument screen. **Instrument Display** | **Interpretation/Reporting of Results**  |
|  | **Positive for RSV viral RNA.**Select from keypad }PRSP ?Pos RSV PCR“Respiratory Syncytial Virus RNA DETECTED”?Internal QC OKCall result to caregiver and document in LIS. |
|  | **Negative for RSV viral RNA.**Select from keypad }NRSP ?Neg RSV PCR“Respiratory Syncytial Virus RNA NOT DETECTED”?Internal QC OK |
|  | **Invalid.**Immediately repeat test. If invalid again probable interfering substance present. Recollection required or RESPA can be performed on sample.Select from keypad }QCFR ?QC fail x2 recollectCall result to caregiver and document in LIS. |

1. **Limitations**
2. The performance of the **Alere™ i** RSV was evaluated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
3. **Alere™ i** RSVperformance 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.
4. 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, RSV viruses 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.
5. 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.
6. Mucin may interfere with RSV detection at levels greater than 0.0625% w/v.
7. This test is not intended to differentiate RSV subtypes. If differentiation of specific RSV subtypes is needed, additional testing, in consultation with state or local public health departments, is required.
8. Negative results do not preclude infection with RSV and should not be the sole basis of a patient treatment decision.
9. This test has not been evaluated for patients without signs and symptoms of respiratory infection.
10. Cross-reactivity with respiratory tract organisms other than those tested in the Analytical Specificity Study may lead to erroneous results.
11. This assay has not been evaluated for immunocompromised individuals.
12. The test is a qualitative test and does not provide the quantitative value of detected organism present.
13. Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2015 to 2016 respiratory season. The positive and negative predictive values may vary depending on the prevalence and population tested.
14. **Expected Values**

The prevalence of RSV varies from year to year; the rate of positivity found in RSV testing is dependent on many factors including the method of specimen collection, the test method used, time of year, age of the patient, and the disease prevalence in specific localities. In the **Alere™ i** RSV multi center prospective clinical study (described in the “Clinical Study” section below), a total of 506 nasopharyngeal swab specimens were determined to be evaluable. The number and percentage of RSV positive cases per specified age group, as determined by the **Alere™ i** RSV assay, are presented below:

**RSV Positives by the** **Alere™ i RSV Assay per Age Group**

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| --- | --- | --- | --- |
| **Age Group (Years)** | **Number of Nasopharyngeal Swab Specimens** | **Number of RSV Positives** | **RSV Positivity****Rate** |
| <1 | 122 | 58 | 48% |
| 1 to 5 | 243 | 82 | 34% |
| 6 to 10 | 58 | 0 | 0% |
| 11 to 18 | 41 | 1 | 2% |
| ≥60 | 42 | 5 | 12% |
| Total | 506 | 146 | 29% |

1. **References**
2. World Health Organization (WHO) Acute Respiratory Infections (Update September 2009). [Online] Available from: http://apps.who.int/vaccine\_research/diseases/ari/en/index2.html Accessed: 20 Nov 2015

2. Williams, KM, Jackson MA, Hamilton M. Rapid Diagnostic Testing for URIs in Children: Impact on Physician Decision Making and Cost. Infect. Med. 19(3): 109-111, 2002.