# *Simplexa™* RSV & Influenza A/B Direct Procedure

**PURPOSE**

* This procedure provides instructions for preparing samples, setting up the PCR reaction and running the *Simplexa™* RSV & Influenza A/B Direct for the detection of RSV & influenza A/B from nasal specimens

#### POLICY STATEMENT

* PCR testing is performed daily, 0700 –1530

**ABBREVIATIONS**

|  |  |
| --- | --- |
| * ABC : Analyzer Before Computer * BSC: BioSafety Cabinet * BSL: BioSafety level * CBA: Computer Before Analyzer * Ct : crossing threshold * DAD : Direct Amplification Disc * F/T : freeze/thaw * IC : internal control * LIS: laboratory information system * MM : master mix * NA : Nucleic Acid * NEGC : negative control | * NP: nasopharyngeal swab * NW: nasal wash specimen * PCR: polymerase chain reaction * POSC: positive control * PPE: personal protective equipment * RIP: Simplexa RSV & Influenza A/B PCR * UNAC: Specimen unacceptable, please recollect * UTM: universal viral transport media   Area/Room 1: Clean room  Area/Room 2: Processing room  Area/Room 3: Amplification room |

## DOCUMENTATION/RECORDS

* Simplexa Flu A/B & RSV Direct Segment Report - run-specific
* LIS Incomplete and worksheets
* Pending Log
* Daily Maintenance Log

## SAFETY CONSIDERATIONS

* Standard precautions for infectious agents. Refer to MB 2.02, Biohazard containment
* Use of engineering controls: Refer to MB 3.01 Engineering Controls to Prevent Nucleic Acid Contamination
* General Safety: MB 2.01 Safe Work Practices
* *Caution:* PPE including protective eyewear must be worn when working with concentrated Extran

#### MATERIALS REQUIRED

|  |  |  |
| --- | --- | --- |
| **Equipment** | **Reagents** | **Supplies** |
| Room 1: Clean room   * -10 to -30° C freezer * Laminar flow Hood   Room 2: Processing   * Refrigerator 2 – 8° C * BSC BSL-2 * -70⁰ C freezer * 100 or 200 µl pipette   Room 3: Amplification   * Liaison MDX | Simplexa Flu A/B & RSV Direct kit MOL2651   * Reaction Mix (24) 50 µl | 2.0 mL cryovials |
| Simplexa Flu A/B & RSV Control Pack MOL1455   * 10 tubes, 100 µl | Nitrile gloves (powder-free) |
| Negative control – UTM | Filtered pipette tips, 100 or 200 µl |
| Sani-Cloth Bleach wipes | Gripper rack |
| 70% alcohol | Cryovial storage box |
| 5% Extran | Sharps disposal container |
| Universal viral transport media (UTM) | Replacement Foil wedge |

## QUALITY CONTROL

1. Assay Controls; refer to MB 9.03
   1. POSC and NEGC: run every 30 days and with new lots/shipment, performed on all instruments on a rotating basis
2. POSC – Simplexa Flu A/B & RSV Positive Control Pack
3. NEGC – UTM
   1. An IC is incorporated into each reaction mixture
4. QC Monitors:

|  |  |
| --- | --- |
| **Control** | **Control Monitor** |
| Positive Control (POSC) | Reagent failure and primer-probe integrity |
| Negative Control (NEGC) | Reagent and/or environmental contamination, cumulative effect |
| Internal Control (IC) | PCR inhibition in specimen, reagent failure or process error |

1. Before reporting patient results, all controls must yield valid results. Refer to MB 9.05, Procedures G, Evaluating and Interpreting Results.

**PROCEDURE A:** Follow the steps in the table below to prepare specimens for testing

Testing Preparation

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| RIP | 1 | Call worksheet **RIP**; use this worksheet for sample identification throughout testing. | MB 1.01 Specimen Management |
| **Sample Order**  **Room 2** | 2 | Position samples and controls (when applicable) in first disc as follows:   |  |  | | --- | --- | | Sample | Position | | POSC | Position 1 | | NEGC | Position 2 | | Patient samples | 3 – nn | | MB 3.01 Engineering Controls  MB 2.01  Safe Work Practices |
| **Organizing run**  **Room 2** | 3 | Using the RIP worksheet as a layout, organize patient specimens and labels   |  |  | | --- | --- | | Step | Action | | a | Color code worksheets and labels per run | | b | Number patients on worksheet in consecutive order | | c | Number corresponding patient labels according to assigned numbers on worksheet, color coded by run | | d | Number each primary patient specimen according to worksheet | |  |
| **Transfer NP swabs, nasal washes/aspirates** | 4 | Number and label a 2.0 ml cryovial for each nasal wash/aspirate and NP swab specimen to be tested   |  |  | | --- | --- | | Step | Action | | a | Number cap of each cryovial according to assigned number on worksheet | | b | Properly label the cryovial with patient bar-coded label matching the number on the cap to the number on the label | | c | Vortex specimen in original container until well mixed | | d | Verify number on primary and secondary container before transfer | | e | Transfer specimen into cryovial with corresponding number on cap   * Only one tube can be open at a time | |  |
| **Change gloves** | 5 | Change gloves |  |

**PROCEDURE B:** Follow the steps in the table below for setting up the computer

Computer set-up

|  |  |  |  |
| --- | --- | --- | --- |
| **Activity** | Step | **Action** | **Related Doc** |
| **Computer Set-up**  **Room 3** | 1 | Set up Liaison; take run specific patient labels into room 3   |  |  |  | | --- | --- | --- | | Step | Prompt | Action/Entry | | a | ------ | Turn on the Liaison MDX (ABC) | | b | ------ | Turn on the Liaison computer | | c | ------ | Log on computer | | d | User name | administrator | | e | Password | focusIC#1 | | f | ----- | Double-click on Integrated Cycler DX icon to open program | | g | User name | Enter personal user code | | h | Password | Enter personal password code | | i | Assay definition | From the main screen, scan the reagent lot barcode, small data matrix located on the lower left corner of the REF card | | j | Disc ID | Scan the disc barcode on the DAD to show disc layout   * Used wedges are shown in black and unavailable for use * Available wedges are shown in gray Fig. 1   Figure 1 | | k | ----- | Number available wedges according to worksheet layout | | l | ----- | Enter sample IDs: scan barcode ID from each label consecutively   * **Type** drop down box: **:** select **Unknown** (default) | | m | ----- | Enter controls according to layout   * POSC – scan the barcode provided on the positive control vial label * NOTE: the positive QC vial label is to be placed on   the back of the RIP reagent lot barcode card after use of the first vial. If the QC barcode is unavailable type in the lot number.   * NEGC – select **NTC** from the Type drop down box | | n | ----- | Load DAD into instrument | | o | ----- | Select the instrument from the drop down box (lower right)   * NOTE: Rotate Simplexa used daily so that controls are performed on a regular rotating basis. | | p | ----- | Click **Run** to begin processing the disc  **!** Once run is started, it cannot be cancelled; canceling will require  reloading new samples into unused wedges.  **!** Users cannot be changed while running | | q | ----- | Recycle labels when run is complete; do not take back to room 2 | |  |
| **New user** | 2 | To switch users: Select **File: Switch Users**  *Note*: Change users before starting instrument(s) |  |
| **Change PPE** | 3 | Remove lab coat and change gloves before leaving area |  |

**PROCEDURE C:** Follow the steps in the table below for reagent handling

Reagent Handling

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
| **Room 1** | 1 | Remove one MM tube for each sample to be tested from - 20⁰ C freezer and thaw at room temperature   * *Use MM within 30 min* | MB 9.04  Reagent and Control Prep |
|  | 2 | Remove lab coat; move the MM tubes to room 2 |
|  | 3 | Remove POSC from -70⁰ C freezer to thaw at room temperature |
| **Room 2** | 4 | When thawed, gently flick POSC and MM tubes to mix; briefly centrifuge   * Do not vortex * Do not refreeze | MB 9.03 Storage and Stability |
|  | 5 | Proceed to PCR set-up |  |

**PROCEDURE D:** Follow the steps in the table below for PCR set-up and amplification

**PCR set-up and amplification**

| **Activity** | Step | **Action** | | **Related Doc** |
| --- | --- | --- | --- | --- |
| **Vortex samples**  **Room 2** | 1 | Vortex specimen tubes prior to set-up if they have been sitting for more than 30 min | |  |
|  | 2 | Remove DAD from package and set on disc cold block | |  |
|  | 3 | Number wedges according to worksheet layout | |  |
| **Load MM**  **Room 2** | 4 | Peel back the foil cover, one at a time, to expose the SAMPLE and Reaction (R) wells.  **!** Do not touch underside of foil to prevent cross contamination | | [Simplexa Operator's Manual IVD](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212355.pdf) |
|  |  | Figure 2: | Figure 3:  SAMPLE well  Reagent (R) well |  |
|  | 5 | Pipette 50 µl of MM into the Reaction (R) well first before sample   |  |  | | --- | --- | | ***Tip*** |  | | * To prevent aerosols and possible contamination, hold the pipette at a 30-degree angle inserting the tip under the roof of the well to reduce possible contamination      * *Caution:* Avoid placing pipette tip at the bottom of the well to prevent possible punctures in the foil that may lead to instrument contamination | | |  |
| **Load samples** | 6 | Pipette 50 µl of sample/control into the SAMPLE well   * *Caution:* Pipette leakage outside of well may lead to external disc contamination when resealing wedge | |  |
| **Seal disc remove tabs** | 7 | Seal the foil wedge before opening the next foil cover | |  |
| 8 | After all wedges are filled, carefully remove the perforated foil tab   * If foil is torn, it must be replaced with a replacement foil wedge to prevent carryover contamination | |  |
|  | 9 | Use the disc applicator to seal the foil firmly on all wedges | |  |
| **Change gloves** | 10 | Remove lab coat and change gloves | |  |
|  | 11 | Move to room 3 | |  |
| **Room 3** | 12 | Place disc into the instrument; close lid | |  |
| **Start Run** | 13 | Select test instrument from drop down box | |  |
|  | 14 | Start run | |  |
| **Change PPE** | 15 | Remove lab coat | |  |
|  | 16 | Change gloves before leaving room 3 | |  |
| **Run time** | 17 | Approximate run time: 1 h 15 min | |  |
|  | 18 | On the screen, a progress bar indicates time to completion; refer to Fig. 4 | |  |
|  | 19 | When run is complete, remove disc from instrument; *check well volumes* | |  |
| **Run completion** | 20 | Place in bio-bag | |  |
|  | 21 | If disc is completely used, discard in red biohazard container | |  |
|  | 22 | If there are unused wedges, retain disc in a sealed bio-bag in room 2 | |  |

**Figure 4**: Progress in Real-Time

Progress bar shows estimated end time



**PROCEDURE E:** Follow the steps in the table below for reviewing data and sample failures

Reviewing and printing Completed Runs

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Analysis Complete** | 1 | When the run is complete, the results will display on the screen; positive results appear red **Figure 5:** Analysis Complete |  |
| **Print**  **Review amplification curves** | 2 | Click the Print button to print a full report of the results, Fig. 5  |  |  | | --- | --- | | Step | Action | | a | √ Include Ct values | | b | √ Include graphs | | c | Scroll through the report , reviewing comments, failures and amplification curves | | d | A valid curve shows a smooth, exponential increase, Fig. 6 | | E | Invalid curve may be linear or a curve with data “spikes” where the curve crosses the threshold | | f | Click **Print** | | g | Export results to LIS; refer to procedure F |   **Figure 6:** Valid and invalid amplification curves  **Valid Valid Invalid** |  |
| **Detailed analysis** | 3 | For a detailed analysis of the completed run, click the Details button to open the Analysis Window |  |
| **Analyzing Runs**  ***Detail tab*** | 4 | Click on the run Details tab to display a summary of the run, fluid checks, Ct values and any sample failures that are highlighted in yellow **Figure 7**: Details Screen |  |
| **Analyzing Runs**  ***Data tab***  To view graphs by dye, click on the dye checkbox  Data / Detail tabs | 5 | Click Data tab to *Select* or *Deselect* samples to be exported to LISSelect or deselect samples to view graphs (optional); reviewed in Fig. 5Select or deselect samples to export to LIS  * Export results to LIS; refer to procedure F   **Figure 8: Data Screen**  Export drop down  Select and Deselect buttons | Refer to procedure G for evaluating QC and patient results  Refer to procedure F for Exporting results to LIS |

**PROCEDURE F:** Follow the steps in the table below for exporting data to LIS from the analysis screen

Exporting Data to LIS

| **Activity** | Step | **Action** | **Related Doc** |
| --- | --- | --- | --- |
|  | 1 | Confirm daily POSC and NEGC are valid before reporting patient results. Record valid control results on the appropriate worksheet. | MB 9.09.F5 Simplexa Influenza A,B and RSV 30 Day QC Worksheet |
|  | 2 | Positive patient results: Confirm name, accession number and disc location of primary sample before releasing results |  |
| **Select data** | 3 | If all test results were valid upon review, select **√** results to be exported onthe **Data** tab, refer to Fig.8 | MB 9.07  Reporting and Archiving Results |
|  | 4 | *Do not* send invalid patient results or POSC and NEGC. Deselect by clicking on individual box(es) |  |
| **Export** | 5 | From the Export drop down box, select **LIS** and then **LIS folder;** click **OK**  **Figure 9: Export to LIS** |  |
|  | 6 | A message that the run exported successfully will appear. Click **OK** |  |

**PROCEDURE G:** Follow the activities below for evaluating QC and patient results

**QC and Patient Results**

| **Activity** | **Step** | **Action** | | | **Related doc** |
| --- | --- | --- | --- | --- | --- |
| LIS interps | 1 | Patient results will be translated in LIS as *Positive* or *Negative* for Flu A, Flu B and/or RSV. If the sample is interpreted as “Invalid” by Simplexa, results will need to entered manually as *Equivocal* or *Unresolved* after review | | | MB 9.07 Resulting and Archiving Results |
| Review | 2 | Review patient and QC amplification curves before releasing resultsCheck for exponential growth and data spikesCheck for possible inhibition or low target signalReview “QC statement/Note” on the Segment Report for failures and error messages | | |  |
| Simplexa software interps |  | QC and patient results are interpreted by the software | | |  |
|  | 3 | If | Then | |  |
|  |  | Detected; LIS positive | Flu A, Flu B and /or RSV are present in the sample | |  |
|  |  | Not Detected; LIS negative | Flu A, Flu B and /or RSV are absent in the sample | |  |
|  |  | Invalid result | Unable to determine the presence or absence of Flu A, Flu B and/ or RSV  * Possible IC failure * Insufficient sample volume  1. Retest sample with new mm from the same kit or from a new kit | |  |
|  |  | EC500 Data Quality Errors | Indicates a data quality error; weak or late amplification; Rerun sample directly and diluted 1:5 with UTM. | | Refer to Proc. I for EC500 errors |
| **QC conditions not met**  **Invalid assay** | 4 | Failure indications will be highlighted in yellow on the Details tab, Fig. 7  |  |  | | --- | --- | | Step | Action | | a | Click the Print Preview button to review the “Data Quality message” and error code on the Segment report under QC Notes, Fig. 7 | | b | Review sample graph for amplification and Ct values | | c | Refer to Troubleshooting Guide for additional information | | d | Click the **Print** button to generate a report; place in molecular office review box | | e | Record corrective action on QC and Equipment Failure Log | | f | Record number of failed samples on **Failed Run** log and a brief explanation |   **Figure 10: Review**  Review curves  QC Statements/Notes | | | [Simplexa Operator's Manual IVD](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212355.pdf) Appendix B: Troubleshooting  MB 9.06 Troubleshooting Guide |
|  |  | If | | Then |  |
|  | 5 | **Valid assay:** Controls as expected | | * Report patient results |  |
|  |  | **Invalid assay conditions:**  POSC/ NEGC failure | | * *Do not* report patient results; invalid run * Failure caused by reagent or system failure  1. Review graphs for amplification 2. Notify technical director or designee for review 3. Repeat testing | [Simplexa Operator's Manual IVD](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212355.pdf) Appendix B: Troubleshooting |
|  |  | NEGC positive | | * *Do not* report patient results; invalid run * Possible contamination of samples  1. Review graphs for amplification 2. Review the specimen handling/ preparation technique 3. Notify technical director or designee for review 4. Repeat testing | MB 9.06 Troubleshooting Guide  **Early IC Ct < 25:** |
|  |  | Internal Control failure | | * Possible sample inhibition  1. F/T sample; avoid pipetting mucus 2. Quick spin if large amount of mucus present 3. Repeat testing  * IC did not amplify due to pipetting error  1. MM and sample reversed; placed in wrong wells  * Early Ct < 25: Check DAD volumes | * Possible contamination * Bad DAD * Improper mixing * Reagent not completely thawed |
|  |  | Problem unresolved | | * Refer to Troubleshooting section of the Operator’s Manual and MB 9.06 Troubleshooting guide  1. Call DiaSorin technical service, **1-800-838-4548, option 3** 2. Notify section technical director or designee |  |
| **Problem Log** | 6 | Do not report patient results until problem is resolved | | |  |
|  | 7 | Record problem and corrective action in the ***QC and Equipment Failure* *Log*** | | |  |

**PROCEDURE H:** Follow the activities below for repeat testing

**Repeat Testing**

| **Activity** | **Step** | **Action** | **Related doc** |
| --- | --- | --- | --- |
| **Timeframe** | 1 | Perform repeat testing from original specimen aliquot |  |
|  | 2 | Repeat within 3 days if stored at 2 – 8⁰ C |  |
| **Vortex** | 3 | Vortex the specimen tubes prior to retesting |  |
| Type of Failure | 4 | Review type of failure and any error messages containing the cause of the problem and possible solutions; refer to available troubleshooting guides for additional information   |  |  | | --- | --- | | Failure | Action | | System error | * Read error dialog box containing software messages regarding the cause of the problem and possible solutions  1. Review amplification curves for exponential growth 2. Follow recommended actions 3. Repeat run including a POSC/NEGC 4. Contact technical service if problem does not resolve | | Reagent failure | 1. Review proper storage conditions 2. Use MM within 30 min after thaw 3. MM subjected to 1 F/T only 4. Repeat testing | | IC failure | * IC did not amplify due to sample inhibition  1. F/T sample; avoid pipetting mucus if present 2. Quick spin if large amount of mucus present 3. Repeat testing 4. If sample remains unresolved, call caregiver for new collection  * IC did not amplify due to pipetting error  1. MM and sample reversed; placed in wrong wells 2. Repeat testing  * Early Ct < 25: Check DAD volumes | | Insufficient volume | * Not enough sample reached the detection chamber for testing  1. Check sample for mucus 2. F/T or quick spin to remove mucus 3. Repeat testing | | POSC failure | * Target not detected  1. System/reagent failure 2. Repeat run including POSC and NEGC; vortex patient samples prior to testing 3. Flick POSC to mix before repeat testing 4. If POSC fails on repeat, thaw new POSC  * Target and IC not detected  1. Review pipetting, possible sample and MM reversed 2. Repeat run including POSC and NEGC | | NEGC | * NEGC contaminated  1. Repeat run including POSC and NEGC 2. Review patient graphs for low level contamination 3. Review specimen handling/processing technique  * IC not detected  1. System/reagent failure 2. Possible pipetting error, sample and MM reversed 3. Repeat run including POSC and NEGC | | Failure unresolved | 1. Call DiaSorin technical service 2. Notify section technical director or designee | | [Simplexa Operator's Manual IVD](http://khan.childrensmn.org/Manuals/Lab/SOP/MolBio/UserMan/212355.pdf) Appendix B: Troubleshooting  MB 9.06 Troubleshooting Guide  MB 9.03  Storage and Stability  DiaSorin technical service,  **1-800-838-4548, option 3**  **Early IC Ct < 25:**   * Possible contamination * Bad DAD * Improper mixing * Reagent not completely thawed |

**PROCEDURE I:** Follow the steps in the table below to Resolve EC500 Data Quality errors/unresolved results

EC500 Data Quality Errors

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **Dilute eluate** | 1 | Dilute 0.25 mL specimen in 1.0 mL of UTM to obtain a 1:5 dilution. |
|  | 2 | Retest according to the RIP protocol using 50 µl of the diluted eluate in UTM as well as repeating the direct specimen (undiluted). |
|  | 3 | |  |  | | --- | --- | | If | Then | | Error resolves with diluted sample | Report the result along with the comment code DILUT to indicate "Sample diluted due to inhibition.  Please consider submission of a new sample if clinical suspicion is high.” | | Error does not resolve with the diluted sample | Report as UNR and request new sample for testing. | |

**PROCEDURE J:** Follow the steps in the table below for Liaison MDX instrument shutdown in room 3

Computer and Instrument Shutdown

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **CBA** | 1 | **CBA**: Shut down computer and then the analyzers when all runs are completed (Computer before analyzer) |
|  | 2 | Click on the **Close** button or “X” out of the program |
| **Shutdown menu** | 3 | Click on the **Start** button (Windows icon) |
|  | 4 | Next to **Restart**, click on |
|  | 5 | Select **Shutdown** from the drop down menu |
| **CBA** | 6 | After the computer has shutdown, turn off the analyzers |
| **Clean** | 7 | Decontaminate work area; refer to MB 9.08 |

**PROCEDURE K:** Follow the steps in the table below for storing test specimens

Storage and Retention of test specimens

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **Storage** | 1 | Store test samples in -70⁰ C freezer, shelf 4, for 3 - 6 mo. |
|  | 2 | Write date range on cryo-storage box including month, day and year |
| **Disposal** | 3 | Discard samples after elapsed time in red biohazard container |

**PROCEDURE L:** Follow the steps in the table below for performing data backup monthly.

Monthly Data Backup

| **Activity** | **Step** | **Action** |
| --- | --- | --- |
| **Data Backup** | 1 | Insert USB Labeled “Simplexa Backup” into Simplexa computer |
|  | 2 | Go to Tools and then from the Database Tools menu choose Backup database. |
|  | 3 | Click “Create Backup” to save to the thumb drive. There is no need to change the file name. |
|  | 4 | Mark this activity as checked off on our monthly checklist so as to ensure it is completed on a monthly basis. |

**PROFICIENCY TESTING**

* CAP ID3, 3 shipments per year, 5 challenges each

#### METHOD PERFORMANCE

* Clinical Sensitivity/Specificity 2, 4:

1. Flu A: 97.1% / 97.9%
2. Flu B: 100% / 99.9%
3. RSV: 98.6% / 89.5%

* Analytical Sensitivity 2, 4:

1. Flu A: 0.1 – 0.05 TCID50/ml
2. Flu B: 1 – 20 TCID50/ml
3. RSV: 2 TCID50/ml

#### ALTERNATE METHOD

1. Viral Respiratory Culture
2. Sunquest Order code: VRSP
3. Specimen container

* 2 NP Swabs: BBL CultureSwab with Liquid Stuart’s
* Nasal wash/aspirate (0.5 – 1 ml): sterile screw top container

1. Logistics

* Transport at RT or refrigerated
* Laboratory: Transfer 1 ml of wash/aspirate into UTM or cut 2 NP swabs into UTM
* Analytic time: 1-3 days
* Testing : Daily

## LIMITATIONS

* Results must be considered in conjunction with the clinical history, epidemiology data and other information available to the clinician evaluating the patient.
* If a novel influenza A is suspected based on clinical and epidemiological data, specimens should be collected and sent to Minnesota Dept. of Health for testing.
* This assay does not differentiate between influenza A subtypes, H1, H3 and 2009 H1.
* This assay does not differentiate between types RSV A and RSV B.
* Negative results do not rule out influenza A, influenza B or RSV.
* PCR detection of influenza A, influenza B or RSV does not distinguish between viable and non-viable organism.
* Test performance is not established for monitoring treatment for influenza A, influenza B or RSV
* False-negative results can occur if the viruses are below the level of the analytical sensitivity and if performed very early in the course of the illness.
* False-negative results may occur if the virus has genomic mutations, insertions, deletions or rearrangements
* Specimen integrity is dependent on the proper collection, transport, handling and storage of the specimen. Failure to meet set criteria can result in falsely negative results.
* When very high levels of influenza A are present with very low levels of RSV and influenza B, the signal from the RSV and FluB may not be detected due to competitive interference.
* False positive results can occur if proper handling and processing protocols are not followed.

**REFERENCES**

1. Simplexa™ 3M™ Integrated Cycler Studio 5.0 , 3M™ Integrated Cycler Operator Manual Reference 34-8710-8239-1, PI.MOL1101.IVD\_REV. F for use with IVD assays, Focus Diagnostics 2009-2012, Focus Diagnostics, Inc. Cypress, CA
2. Simplexa™ *Flu A/B & RSV* Direct Circular PI.MOL2650.IVD, Rev. F, 18-September-2015, Focus Diagnostics, Cypress, CA 90630
3. Children’s Hospitals and Clinics of MN, Simplexa Flu A/B & RSV Direct Verification/Validation Study, 2016
4. 510(k) Substantial Equivalence Determination, Decision Summary, number K120413, July 13, 2012, Focus diagnostics, Inc., 11331 Valley view St, Cypress, CA, 90630
5. Mitchell W. Woodberry, Shankar R, Cent A, Jerome KR, Kuypers J, Comparison of the Simplexa Flu A/B & RSV Direct Assay and Laboratory-Developed Real-Time PCR Assays for Detection of Respiratory Virus, JCM 2013
6. Influenza viruses: http://www.cdc.gov/flu/about/viruses/index.htm
7. RSV: http://www.cdc.gov/rsv/about/index.html

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| --- | --- | --- | --- | --- |
| Historical Record | | | |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | 1 | P. Ackerman | 11.30.2016 | Initial Version |
|  | 2 | P. Ackerman | 03.30.17 | Added Proc. I *EC500 Data Quality Errors;* added early Ct < 25 to IC failures |
|  | 3 | M. Merryman/J. Laramie | 02.12.2018 | -Modified Procedure I: eliminated extraction and added dilution.  -Added Procedure L: Monthly data backup  -Added rotation of QC on instruments and scanning of pos control QC barcode |
|  | 4 | J. Laramie | 04.01.2018 | -Modified QC notes to reflect IQCP implementation . QC to be tested every 30 days, and upon arrival of new lots/shipments. |