Phase I Placebo-Controlled Study of the Infectivity, Safety and Immunogenicity of a Single Dose of a Recombinant Live-Attenuated Respiratory Syncytial Virus Vaccine, LID ΔM2-2 1030s, Lot RSV#010A, Delivered as Nose Drops to RSV-Seronegative Infants 6 to 24 Months of Age

A Study of the International Maternal Pediatric Adolescent AIDS Clinical Trials Network

Sponsored by:
Office of Clinical Research Policy and Regulatory Operations (OCRPRO)
Division of Clinical Research (DCR)
National Institute of Allergy and Infectious Diseases (NIAID)
National Institutes of Health (NIH)

DAIDS ES # 30072
IND # 16990 Held by OCRPRO/NIAID/NIH
## Appendix II: Schedule of Events: Screening, Acute Phase, and Post-Acute Phase

<table>
<thead>
<tr>
<th></th>
<th>ACUTE PHASE</th>
<th>POST-ACTIVE PHASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>X</td>
<td></td>
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<tr>
<td>Day 2</td>
<td>X</td>
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<tr>
<td>Day 3</td>
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<tr>
<td>Day 4</td>
<td>X</td>
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<td>Day 5</td>
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<tr>
<td>Day 6</td>
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<tr>
<td>Day 7</td>
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<td>Day 8</td>
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<td>Day 9</td>
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<td>Day 10</td>
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<td>Day 11</td>
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<td>Day 12</td>
<td>X</td>
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<tr>
<td>Day 13</td>
<td>X</td>
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<tr>
<td>Day 14</td>
<td>X</td>
<td></td>
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<tr>
<td>Day 15</td>
<td>X</td>
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<tr>
<td>Day 16</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 17</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 18-27 (visit)</td>
<td>Per 6.5</td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 29</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Illness Visit</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Day 30-55</td>
<td>Per 6.5</td>
<td></td>
</tr>
<tr>
<td>Day 56</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Early DC</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

### In person visit
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Non-visit contact
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Informed consent
- Day 0: X

### History
- Day 0: X

### Interim History
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Physical exam (full)
- Day 0: X

### Clinical assessment (focused PE)
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Administer study product
- Day 0: X

### Blood for: immunologic assays
- Day 0: 5mL
- Day 1: 5mL
- Day 2: 5mL
- Day 3: 5mL
- Day 4: 5mL
- Day 5: 5mL
- Day 6: 5mL
- Day 7: 5mL
- Day 8: 5mL
- Day 9: 5mL
- Day 10: 5mL
- Day 11: 5mL
- Day 12: 5mL
- Day 13: 5mL
- Day 14: 5mL
- Day 15: 5mL
- Day 16: 5mL
- Day 17: 5mL
- Day 18-27: Per 6.5
- Day 28: 5mL
- Day 29: 5mL
- Day 30-55: 5mL
- Day 56: 5mL
- Early DC: 5mL

### Blood for: cellular immune assays (viable PBMCs)
- Day 0: 3mL
- Day 1: 3mL
- Day 2: 3mL
- Day 3: 3mL
- Day 4: 3mL
- Day 5: 3mL
- Day 6: 3mL
- Day 7: 3mL
- Day 8: 3mL
- Day 9: 3mL
- Day 10: 3mL
- Day 11: 3mL
- Day 12: 3mL
- Day 13: 3mL
- Day 14: 3mL
- Day 15: 3mL
- Day 16: 3mL
- Day 17: 3mL
- Day 18-27: Per 6.5
- Day 28: 3mL
- Day 29: 3mL
- Day 30-55: 3mL
- Day 56: 3mL
- Early DC: 3mL

### Nasal wash for: RSV antibody
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Nasal wash for: viral detection & quantification
- Day 0: X
- Day 1: X
- Day 2: X
- Day 3: X
- Day 4: X
- Day 5: X
- Day 6: X
- Day 7: X
- Day 8: X
- Day 9: X
- Day 10: X
- Day 11: X
- Day 12: X
- Day 13: X
- Day 14: X
- Day 15: X
- Day 16: X
- Day 17: X
- Day 18-27: Per 6.5
- Day 28: X
- Day 29: X
- Day 30-55: Per 6.5
- Day 56: X
- Early DC: X

### Request adventitious agent assay
- Day 0: X

### Total blood volume
- Day 0: 8mL
- Day 1: --
- Day 2: --
- Day 3: --
- Day 4: --
- Day 5: --
- Day 6: --
- Day 7: --
- Day 8: --
- Day 9: --
- Day 10: --
- Day 11: --
- Day 12: --
- Day 13: --
- Day 14: --
- Day 15: --
- Day 16: --
- Day 17: --
- Day 18-27: Per 6.5
- Day 28: --
- Day 29: --
- Day 30-55: --
- Day 56: --
- Early DC: --
- Total blood volume: 8mL

### Priority for blood samples:
1. 5 ml red top or SST serum tube for immunology assays
2. 3 ml EDTA blood for cellular immune assays (Viable PBMC)
Appendix III: Schedule of Events: RSV Pre-season Sampling, seasonal surveillance, and Post-season Sampling

<table>
<thead>
<tr>
<th>Visit Period</th>
<th>Pre-RSV season</th>
<th>Weekly contact</th>
<th>Post-RSV season</th>
<th>Illness Visit</th>
<th>Early DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct 1st to Oct 31st</td>
<td></td>
<td>Nov 1st to Mar 31st</td>
<td>Apr 1st to Apr 30th</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical assessment (focused PE)</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Interim history</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**LABORATORY EVALUATIONS**

<table>
<thead>
<tr>
<th></th>
<th>Pre-RSV season</th>
<th>Weekly contact</th>
<th>Post-RSV season</th>
<th>Illness Visit</th>
<th>Early DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood for:</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td>immunologic assays</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cellular immune</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>assay (viable PBMC)</td>
<td>3 mL</td>
<td>3 mL</td>
<td>3 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal wash for:</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>viral detection &amp;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quantification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Request adventitious</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>agent assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL BLOOD VOLUME</td>
<td>8 mL</td>
<td>--</td>
<td>8 mL</td>
<td>--</td>
<td>8 mL</td>
</tr>
</tbody>
</table>
### Protocol-Required Non-Standard Reagents and Supplies

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Reagent or Supply</th>
<th>Manufacturer part #</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snap Freezing either workstation can be used, one hold 30 vials the other 24 vials</td>
<td>CoolBox XT System</td>
<td>BCS-575</td>
<td>Sigma-Aldrich, Worldwide Life Sciences Division, Genesse Scientific, or Thomas Scientific <a href="http://www.biocision.com/products/CoolBox-CFT30-System/">Link</a> or Corning [Link](<a href="http://catalog2.corning.com/LifeSciences/en-US/Shopping/ProductDetails.aspx?categoryname=&amp;productid=432020(Lifesciences)">http://catalog2.corning.com/LifeSciences/en-US/Shopping/ProductDetails.aspx?categoryname=&amp;productid=432020(Lifesciences)</a></td>
</tr>
<tr>
<td></td>
<td>CoolBox 30 System</td>
<td>BCS-166</td>
<td>Corning 432020</td>
</tr>
<tr>
<td>Snap Freezing for Nasal Wash for RSV Viral Detection and Quantification</td>
<td>Sarstedt 2ml Cryovials</td>
<td>72.694.006</td>
<td>(Sarstedt or Fisher Scientific)</td>
</tr>
<tr>
<td>Snap Freezing for Nasal Wash (NW) for RSV Antibody</td>
<td>Sarstedt 2ml Cryovials with attached screw cap</td>
<td>72.694.106</td>
<td>(Sarstedt or Fisher Scientific)</td>
</tr>
<tr>
<td>Nasal Wash</td>
<td>Viral Transport Media (VTM)</td>
<td>N/A</td>
<td>JHU see mop for ordering instructions</td>
</tr>
</tbody>
</table>

### Section 3 (SCREENING, ACUTE, AND POST-ACUTE): Specimen Processing & Shipping Instructions

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Tube Type</th>
<th>Special Collection Notes</th>
<th>CRF # DMC Test Code</th>
<th>Processing</th>
<th>Shipping</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC and Plasma Storage</td>
<td>EDTA</td>
<td>Invert tubes 8-10 times to ensure mixing of anticoagulant and Blood</td>
<td>F3008</td>
<td><strong>Plasma:</strong> Centrifuge blood at 400xg for 10 minutes. Transfer plasma to a new centrifuge tube and centrifuge @ 800xg for 10 minutes. Freeze 3 x 0.50 mL aliquots. Store at -80°C or lower. LDMS Code BLD/EDT/PL2. <strong>PBMCs:</strong> See Cross-Network PBMC Processing SOP, lab must be IQA certified for PBMC cryopreservation. Store viable PBMCs in at least 2 aliquots of 3-5 x 10^6 per vial. Store all PBMC. Record cell # in LDMS. Store at -150°C or in LIQ N2 vapor phase. LDMS Code: BLD/EDT/CEL/DMS.</td>
<td>Send to local processing lab. All samples will be stored and batch shipped. See detailed shipping instructions at the end of the LPC.</td>
</tr>
</tbody>
</table>
### Section 3 (SCREENING, ACUTE, AND POST-ACUTE): Specimen Processing & Shipping Instructions

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Tube Type</th>
<th>Special Collection Notes</th>
<th>CRF # DMC Test Code</th>
<th>Processing</th>
<th>Shipping</th>
</tr>
</thead>
</table>
| Serum storage for Immunologic Assays (Serum RSV Ab) | NON or SST | OBTAIN AT LEAST 4 ML of BLOOD for the serum storage for immunology assays before drawing for PBMC/PLASMA storage | CRF: F3008  
DMC Test Code: ABRSVQT     | Allow to clot for at least 30 mins. Spin blood at 1000xg for 10 mins; remove serum and save at least 3 X 0.50 mL aliquots and store at -80°C. | Screen-  
Ship ONE aliquot real time Mon-Thurs. to JHU. Retain the remaining aliquots on site. They will be batched shipped at the end of study or when requested by team.  
For Day 56 samples-  
Ship ONE aliquot per participant to JHU, Batch ship multiple participants within 2 weeks of Day 56. Retain the remaining aliquots on site. They will be batched shipped at the end of study or when requested by team.  
Ship samples on dry ice priority overnight, Mon-Thurs for screening samples only |
| Nasal Wash (NW) for RSV Antibody | Nasal Wash | After collection, keep Nasal wash on wet ice until processed and frozen.  
See 2011 MOP for detailed NW collection instructions  
Nasal Washes for RSV Antibody should be snap frozen within 30 minutes of collection and transferred to a -80°C freezer. | CRF: F3008  
DMC Test Code: ABRSVQT | Measure and save 6 mL NW from total sample to be used for Viral Detection and Quantification (see below).  
Aliquot all remaining NW in three equal (1.5 mL) aliquots for RSV Ab assay. Use only sarstedt 72.694.106 2.0ml hinged cryovials for Ab storage.  
If volume exceeds 5 mL, prepare additional aliquots.  
Be sure cap is on tight, and then snap freeze in Biocision Coolbox, for a minimum of 15 min. Transfer frozen aliquots to -80°C freezer within 4 hours.  
The cool-box systems are capable of maintaining proper temperatures for 4 hours with 200ml dry ice.  
See performance data from the following link for your particular system http://www.biocision.com/products/coolbox-ice-free-cooling/  
Nasal wash aliquots for RSV Antibody do not contain VTM. | Day 0  
At the End of acute phase (Day 0-28): batched shipment(s) of TWO aliquots per participant. Batch ship the remaining aliquots when directed by team or at the end of the study  
Day 56  
Ship TWO aliquots per participant to JHU, multiple patient samples should be batched and shipped within 2 weeks of DAY 56, unless directed to ship sooner by team.  
Retain the remaining aliquots on site. They will be batched shipped at the end of study or when requested by team.  
Ship all samples on dry ice priority overnight, M-W only |
## Section 3 (SCREENING, ACUTE, AND POST-ACUTE): Specimen Processing & Shipping Instructions

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Tube Type</th>
<th>Special Collection Notes</th>
<th>CRF # DMC Test Code</th>
<th>Processing</th>
<th>Shipping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Wash for RSV Viral Detection and Quantification (and/or rtPCR for adventitious agents)</td>
<td>Nasal Wash</td>
<td>After collection, keep Nasal wash on wet ice until processed and frozen. See 2011 MOP for detailed NW collection instructions. Nasal wash for RSV viral detection must be mixed with Viral Transport Media (VTM), aliquoted and snap frozen within 30 min of collection.</td>
<td>CRF: F3008 DMC Test Code: CXRSVQT and/or ADVENTTS</td>
<td>Sterilely Transfer 6mL of Nasal Wash into vial containing 1.5 mL cold (2-8°C) VTM. Gently mix to assure even distribution of specimen in VTM. Aliquot into 7 X 1.0mL aliquots (Sarstedt 2ml cryovials 72.694.006) Be sure cap is on tight, and then snap freeze in BioCision Coolbox, for a minimum of 15 min. Transfer frozen aliquots to -80° freezer within 4 hours. The cool-box systems are capable of maintaining proper temperatures for 4 hours with 200ml dry ice. See performance data from the following link for your particular system <a href="http://www.biocision.com/products/coolbox-ice-free-cooling/">http://www.biocision.com/products/coolbox-ice-free-cooling/</a> See specific freezing instructions in MOP.</td>
<td>Shipping: ACUTE Phase Ship FOUR aliquots to JHU at the end of the acute phase, on dry ice priority overnight, M-W only. Retain 3 aliquots on site until requested by team. Multiple patient samples can be batched and shipped within 2 weeks of the time points, unless directed to ship sooner by team. Shipping: Illness Visits Complete Adventitious Agent Assay request for rRT/PCR on nasal wash for adventitious agents. Ship these samples with the next batch to JHU. Include a copy of F3008. However, if illnesses meet criteria for Pausing and Stopping Rules (protocol section 8.1.3), specimens should be shipped REAL-TIME to JHU. Please notify JHU of any changes made to LDMS or the F3008 form after the sample has been shipped. NOTE: Lab must enter comment in LDMS for all aliquots that have a different time on label than in the LDMS (for example: “LDMS time is actual sample time as noted on CRF. Time on label is not correct.”)</td>
</tr>
<tr>
<td><strong>LDMS ENTRY- Illness Visit</strong> Add “Adventitious agent assay requested” in the comments field of the LDMS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CRF:** F3008 **DMC Test Code:** CXRSVQT and/or ADVENTTS **Shipping:** ACUTE Phase Ship FOUR aliquots to JHU at the end of the acute phase, on dry ice priority overnight, M-W only. Retain 3 aliquots on site until requested by team. Multiple patient samples can be batched and shipped within 2 weeks of the time points, unless directed to ship sooner by team. **Shipping:** Illness Visits Complete Adventitious Agent Assay request for rRT/PCR on nasal wash for adventitious agents. Ship these samples with the next batch to JHU. Include a copy of F3008. However, if illnesses meet criteria for Pausing and Stopping Rules (protocol section 8.1.3), specimens should be shipped REAL-TIME to JHU. Please notify JHU of any changes made to LDMS or the F3008 form after the sample has been shipped. NOTE: Lab must enter comment in LDMS for all aliquots that have a different time on label than in the LDMS (for example: “LDMS time is actual sample time as noted on CRF. Time on label is not correct.”)
### Section 4 APPENDIX II (SCREENING, ACUTE AND POST-ACUTE PHASE):

#### SCREEN: (no more than 42 days prior to enrollment-Day 0).

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Specimen</th>
<th>CRF</th>
<th>Aliquots</th>
<th>LDMS Code</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum storage for Immunologic Assays (Serum RSV AB)</td>
<td>5 mL NON or SST Blood</td>
<td>F3008</td>
<td>Save all SER in at least 3 X 0.50 ml aliquots.</td>
<td>BLD/NON/SER OR BLD/SST/SER</td>
<td>NOTE: One screening serum aliquot must be shipped to JHU real time. Ship ONE aliquot real time Mon-Thurs. to JHU.</td>
</tr>
<tr>
<td>Blood for: cellular immune assay (viable PBMC) and Plasma</td>
<td>3 ml EDTA</td>
<td>F3008</td>
<td>PBMC: Save at least 2 aliquots of 3-5 million viable PBMC. Store all PBMC.</td>
<td>BLD/EDT/CEL/DMS</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma: Save 3 x 0.50 ml aliquots</td>
<td>BLD/EDT/PL2</td>
<td></td>
</tr>
</tbody>
</table>

#### ENTRY DAY 0: Day 0 Must be no more than 42 days from screening and within 3 days of randomization, section 6.2

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Specimen</th>
<th>CRF</th>
<th>Aliquots</th>
<th>LDMS Code</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Wash for RSV Antibody</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Prepare at least three 1.5mL aliquots. Save all nasal wash. See MOP for additional instructions.</td>
<td>NPW/RLS/RLS</td>
<td>Nasal Washes for RSV Antibody should be snap frozen within 30 minutes of collection Use hinged vials <strong>72.694.106</strong> for Ab storage NOTE: Nasal wash for antibody does not have the addition of VTM.</td>
</tr>
<tr>
<td>Nasal Wash for RSV Viral Detection and Quantification (and/or rtPCR for adventitious agents)</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Divide into 7x1.0mL aliquots, snap freeze</td>
<td>NPW/RLS/RLS/VTM</td>
<td>Nasal Washes for RSV viral detection must be processed and snap frozen within 30 min of collection. If sample cannot be received and processed in lab within this time, must be processed at the collection site.</td>
</tr>
</tbody>
</table>

#### DAY 3, 5, 7, 10, 12, 14, 17, or 28 (±1 days)

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Specimen</th>
<th>CRF</th>
<th>Aliquots</th>
<th>LDMS Code</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal Wash for RSV Viral Detection and Quantification (and/or rtPCR for adventitious agents)</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Divide into 7x1.0mL aliquots, snap freeze</td>
<td>NPW/RLS/RLS/VTM</td>
<td>Nasal Washes for RSV viral detection must be processed and snap frozen within 30 min of collection If sample cannot be received and processed in lab within this time, must be processed at the collection site. For Illness visits: Add the following statement “Adventitious agent assay requested” in the comments field of the LDMS</td>
</tr>
</tbody>
</table>
## DAY 56 (+ 7 days) POST-ACUTE PHASE

### Visit Code: Day 56

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Specimen</th>
<th>CRF</th>
<th>Aliquots</th>
<th>LDMS Code</th>
<th>Special Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum storage for Immunologic Assays (Serum RSV AB)</td>
<td>5 mL NON or SST Blood</td>
<td>F3008</td>
<td>Save all SER in at least 3 X 0.50 ml aliquots</td>
<td>BLD/NON/SER OR BLD/SST/SER</td>
<td>Nasal Washes for RSV Antibody should be snap frozen within 30 minutes of collection</td>
</tr>
<tr>
<td>Nasal Wash for RSV Antibody</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Store at least three 1.5 ml aliquots. Save all nasal wash.</td>
<td>NPW/RLS/NPW</td>
<td>Use hinged vials 72.694.106 for Ab storage</td>
</tr>
<tr>
<td>Blood for: cellular immune assay (viable PBMC) and Plasma</td>
<td>3 ml EDTA</td>
<td>F3008</td>
<td>PBMC: Save at least 2 aliquots of 3-5 million (viable PBMC). Save all PBMC. Plasma: 3 x 0.50 ml</td>
<td>BLD/EDT/CEL/DMS</td>
<td>NOTE: Nasal wash for antibody does not have the addition of VTM.</td>
</tr>
</tbody>
</table>

## ILLNESS VISIT Section 6.10

### Visit Code: ____/SCK

<table>
<thead>
<tr>
<th>Evaluation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Nasal Wash for RSV Viral Detection and Quantification (and/or rtPCR for adventitious agents)</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Divide into 7x1.0mL aliquots, snap freeze and store at 80°C. Add the following statement “Adventitious agent assay requested” in the comments field of the LDMS</td>
<td>NPW/RLS/NPW/VTM</td>
<td>Nasal Washes for RSV viral detection must be processed and snap frozen within 30 min of collection. If sample cannot be received and processed in lab within this time, must be processed at the collection site. ALL Illness visit samples should be shipped with the next available batch. Include in the shipment a request for adventitious agent assay. Notify JHU via e-mail that Adventitious Virus Assay is to be done on the sample. Include copy of F3008 with shipped samples. Please notify JHU of any changes made to LDMS or the F3008 form after the sample has been shipped.</td>
</tr>
</tbody>
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### EARLY DC: ACUTE AND POST-ACUTE PHASE

<table>
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<tr>
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</tr>
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<td>Blood for: cellular immune assay (viable PBMC) and Plasma</td>
<td>EDTA</td>
<td>F3008</td>
<td>PBMC: Save at least 2 aliquots of 3-5 million viable PBMC. Store all PBMC.</td>
<td>BLD/EDT/CEL/DMS</td>
<td></td>
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<tr>
<td>Nasal Wash for RSV Antibody</td>
<td>Nasal Wash</td>
<td>F3008</td>
<td>Prepare at least three 1.5mL aliquots Save all nasal wash.</td>
<td>NPW/RLS/NPW</td>
<td>Nasal Washes for RSV Antibody should be snap frozen within 30 minutes of collection Use hinged vials 72.694.106 for Ab storage NOTE: Nasal wash for antibody does not have the addition of VTM.</td>
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### Section 4: APPENDIX III: SCHEDULE OF EVENTS: RSV PRE-SEASON SAMPLING, SEASONAL SURVEILLANCE, AND POST-SEASON SAMPLING

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<td>3 ml EDTA</td>
<td>F3008</td>
<td>PBMC: Save at least 2 aliquots of 3-5 million viable PBMC. Store all PBMC.</td>
<td>BLD/EDT/CEL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma: Save 3 x 0.50 ml</td>
<td>BLD/EDT/PL2</td>
<td></td>
</tr>
</tbody>
</table>

Visit Code: /Ext
## POST-RSV SEASON (APRIL 1 TO APRIL 30)

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## ILLNESS VISIT

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## EARLY DC: RSV Pre-season Sampling, seasonal surveillance, and Post-season Sampling

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<td>BLD/EDT/Cel/DMS</td>
<td></td>
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Visit Code: Day XX

Visit Code: ____/Sck

Visit Code: ____/Ext
## Section 5: Helpful Links and Shipping Addresses

ACTG/IMPAACT Laboratory Manual, Shipping Information and other useful information: [http://www.hanc.info/labs/labresources/Pages/informationActgimpaaclabs.aspx](http://www.hanc.info/labs/labresources/Pages/informationActgimpaaclabs.aspx)

### Detailed PBMC shipping Instructions summary

- Ship ALL PBMC when notified by the team
- All PBMC should be stored in LN2 vapor phase or -150°C freezer until shipped.

### Ship PBMC Priority Overnight in a dry shipper

- Include an LDMS shipping manifest in EXCEL format.
- Notify lab >48 hours in advance of pending shipment
- Notify Ulla Buchholz ([ubuchholz@niaid.nih.gov](mailto:ubuchholz@niaid.nih.gov)) and Cyril Le Nouen, ([lenouenc@niaid.nih.gov](mailto:lenouenc@niaid.nih.gov)) of shipment, include excel format of shipping manifest, Courier, and Tracking #
- **Ship on Monday or Tuesday ONLY**

NON-LDMS LAB

**Ursula Buchholz, PhD**  
RNA Viruses Section  
Laboratory of Infectious Diseases  
NIAID, NIH  
Bldg. 50, Rm. 6503  
50 South Drive, MSC 8007  
Bethesda, MD 20892  
Phone: (301) 594-1533 Fax: (301) 480-1268  
email: [ubuchholz@niaid.nih.gov](mailto:ubuchholz@niaid.nih.gov)
Section 5: Helpful Links and Shipping Addresses

ACTG/IMPAACT Laboratory Manual, Shipping Information and other useful information: http://www.hanc.info/labs/labresources/Pages/informationActgImpaactLabs.aspx

Detailed shipping Instructions summary

**SERUM Samples**

- **Screening sample must be shipped real time,** Mon-Thurs to JHU. Ship ONE aliquot real time on dry ice priority overnight and retain the remaining aliquots on site until the end of study or requested by team.

- For Day 56 samples, Ship one aliquot within 2 weeks of that time point, multiple patient samples can be batched and shipped. Retain the remaining aliquots on site until requested by team.

- **EARLY DC,** Ship one aliquot within 2 weeks of that time point, multiple patient samples can be batched and shipped. Retain the remaining aliquots on site until requested by team.

- **PRE-RSV SEASON** (Oct 1-Oct 31), Ship one aliquot within 2 weeks of collection, multiple patient samples can be batched and shipped

- **POST RSV SEASON** (April 1-April 30), Ship one aliquot within 2 weeks of collection, multiple patient samples can be batched and shipped

**PLASMA samples**

- ALL plasma samples will be stored on site. The plasma samples will be batched shipped with remaining serum and nasal wash samples at the end of the study or when requested by the team.

**NASAL WASH for viral detection Samples**

Ship FOUR aliquot to JHU at the end of the acute phase (Day 0-28), on dry ice priority overnight, M-Wed only. Retain 3 aliquots on site until requested by team. Multiple patient samples can be batched and shipped within 2 weeks of the time points, unless directed to ship sooner by team

- **NASAL WASH: for adventitious agent assay.**
  - For Illness visits, request adventitious agent assay to be done. Ship these samples with the next batch to JHU. Include a copy of F3008.
  - However, if Illness visit meets Pausing and Stopping Rules (protocol section 8.1.3), Specimens should be shipped REAL-TIME to JHU.

  Ship FOUR aliquot to JHU on dry ice priority overnight, M-Wed- only. Retain 3 aliquots on site until requested by team

**NASAL WASH for Antibody Samples**

Ship two aliquots to JHU at the end of the post-acute phase (Day 56, on dry ice priority overnight, M-Wed only. Retain the remaining aliquots on site until requested by team. Multiple patient samples can be batched and shipped within 2 weeks of the time points, unless directed to ship sooner by team

Batch shipments can include serum, plasma and nasal washes

- Ship samples together when possible

SHIP TO (Plasma, Serum and Nasal wash) LDMS Lab code: 550

Bhavin Thumar/Kim Wanionek

Johns Hopkins Bloomberg School of Public Health
Room E5402, 615 N. Wolfe Street
Baltimore, MD 21205
Phone: (410) 955-7230 Fax: (443) 287-3167

NOTE: Notify Bhavin Thumar (bthumar@jhu.edu), Kim Wanionek (kwanion1@jhu.edu) and Jen Oliva (joliva2@jhu.edu) that a specimen is being sent. Include in your email if an adventitious agent assay is to be done on the sample Please notify JHU of any changes made to LDMS or the F3008 form after the sample has been shipped.
Revisions 14-JUL-2016
1) Shipping screening serum samples for RSV Ab has been revised. JHU will accept Thursday shipments for delivery on Friday by 10:30 am. Please refer to section 3.3 of the 2011 MOP for details.
2) Blood draw prioritization outlined
3) Included statement that labs should be IQA certified for PBMC cryopreservation.

Revisions 29-JUL-2016
1) Clarified tube type for immunology assays in blood sample priority listing (red top or SST serum tube)
2) Updated COOLBOX suppliers
3) Updated special collection notes for Serum storage for Immunologic Assays