RSV protocols - Laboratory Issues

- Viral Transport Medium (VTM)
  - Ordering and tracking stocks
- Cool-boxes
  - Ordering
  - Requirement
- Blood Draw prioritization
- Shipment, testing and labelling
  - Screen sera
  - Nasal wash
    - for RSV Viral Detection and Quantification
    - for RSV Antibody
  - Plasma and PBMC
Ordering VTM

- VTM will be supplied in plastic vials by JHU
  - 1.5 mL/vial of a 5x concentrate
  - Commercial VTM should not be used

- Initial shipment will be sufficient for 5 subjects
  - Order two weeks in advance of need
  - Storage of VTM (minus 20°C or minus 80°C)
  - Expiration date of VTM ~ 6 months if kept frozen
  - Thawed VTM is stable at 4°C for two weeks

- Please send request via email to Bhavin Thumar, Kim Wanionek and Jennifer Oliva at CIRLab@jhu.edu with shipping address
COOL BOXES

• ORDERING
  • Coolbox System
  • Corning-

• FREEZING
  • Maintains temperature for 4-5 hours
    • can be extended with larger cool-box or extension ring for home visits.

• REQUIRED
  • Provides long term temperature control
  • Provides uniform freezing of sample
Blood processing and labelling

• Collect blood samples as per site standard protocol.

• Process blood samples as per IMPAACT standard protocols

• Isolate PBMC according to HANC -Cross-Network PBMC Processing SOP.

• Label specimens as per MOP and Lab Processing Chart (LPC) protocol.
Blood Draw Prioritization at Screen

- SoE/LPC blood requirements at SCREEN
  - 5 ml NON (red top) or SST vacutainer
  - 3 ml spray dry EDTA

- Prioritization
  - Draw and obtain ~ 4ml for serum separation before drawing EDTA
  - Aliquot at 0.50 ml each
    - Minimum requirement of 2 aliquots
      - If insufficient number of aliquots are obtained additional blood should be obtained at ENTRY
    - Preferred 3+ aliquots
      - Screening serology, baseline and back-up
Screening sera: shipment and testing

• One aliquot of each screening specimen should be shipped on dry ice on Monday- Wednesday. Thursday optional if > 3 aliquots are obtained
  • Retain remaining aliquot(s).

• Please inform Bhavin Thumar and Kim Wanionek of plans for shipment of specimens no later than the preceding Friday at 2 PM Eastern Time by email CIRLab@jhu.edu

• Results will be emailed to the sites and the Clinical Trials Specialist (CTS) on Tuesday afternoons.

• In September and October, seroeligibility results will be emailed to the sites and the Clinical Trials Specialist (CTS) on Monday afternoons for samples shipped on Monday and Tuesday and Tuesday afternoons for sample shipped on Wednesday/Thursday.

• There should be no shipment to JHU on Fridays.
Post-inoculation Blood specimens

• Collect blood sample and separate serum as per site specific protocol.
• Day 56, Early DC and Pre/Post RSV Season
  • 5 ml NON (red top) or SST vacutainer
    • Obtain at least two serum aliquots of 0.50 ml
  • 3 ml spray dry EDTA
    • Process for Plasma and viable PBMC

• Label specimens as per LPC protocol.

• Ship samples according to detailed instructions in the LPC

• Notify Bhavin Thumar, Kim Wanioneck, and Jennifer Oliva at CIRLab@jhu.edu of any shipments
Nasal wash samples Shipping and Testing

• Collect nasal wash as outlined in the MOPS.
• Add 6 ml nasal wash to each plastic vial of VTM.
• MIX and aliquot 1 mL of nasal wash per vial in 7 vials.
• Label specimens as per LPC and MOP.
  • PID #
  • Protocol #: IMPAACT 2013
  • Specimen Date
  • Specimen type : NW/VTM
  • Vial #1, 2, 3, 4, 5, 6, 7
  • Visit day – (i.e., 3, 5, 7, 10, 12, sck, etc)

• Send 4 vials on dry ice and retain 3 vials for later shipment.
  • See detailed shipping instructions in LPC

• Email JHU with request for adventitious virus testing when subject is ill and for subjects who come for regular study visits but meet illness criteria.

• Notify Bhavin Thumar, Kim Wanionek and Jen Oliva at CIRLab@jhu.edu that specimens are being sent
Nasal wash for RSV Antibody Detection

- Collect nasal wash as outlined in the MOP.
- After removal of 6 ml of nasal wash for Viral detection.
- Aliquot the remaining nasal wash **WITHOUT** VTM into vials
  - 1.5ml per vial
  - 3-8 vials
  - Use hinged vials

- Label specimens as per LPC and MOP.
  - PID #
  - Protocol #: IMPAACT 2013
  - Specimen Date
  - Specimen type: NW/Ab
  - Vial #1, 2, 3, 4, 5, 6, 7, 8
  - Visit day – Day 0, Day 28, Day 56, Early DC, Pre/Post RSV season (Day XXX)
Plasma and PBMC collections

- Collect 3ml of blood in a Spray-dry EDTA vacutainer
- Store plasma in 0.50 ml aliquots at minus 70/80°C
- Process PBMC according to HANC - Cross-Network PBMC Processing SOP.
  - Site must be IQA certified for PBMC Cryopreservation
  - Store viable PBMCs in at least 2 aliquots of 3-5 x 10^6 per vial.
  - Store PBMC as viable at -150°C or vapor-phase LN2
- All Plasma and PBMC samples will be stored on-site until requested by the Team
Shipping

ALL Serum, Nasal Wash and Vaccine aliquot samples

- **NASAL WASHES FOR VIRAL DETECTION** MUST BE SHIPPED IN A STP-320 SHIPPING CONTAINER (or equivalent) WITH AT LEAST 10 kg DRY ICE. NO EXCEPTIONS.

- **SERUM OR VACCINE ALIQUOTS** may be shipped in a STP-308 shipping container (or equivalent) WITH AT LEAST 6 kg DRY ICE (no more than one 5”x5”x2” freezer box per shipping container)

- Refer to LPC for more detailed shipping instructions

- Please ship priority overnight

**STP-320 for NASAL WASHES**

**STP-308 (11”x10”x15”)**