Recommended Sample Collection Procedures

Contents                                      Page
Purpose ................................................................. 2
Scope ................................................................. 2
Responsibilities .................................................. 2
Definitions ......................................................... 2
Principle ............................................................ 3
Policies ............................................................. 4
Serum Samples .................................................... 4
Virology Swab Samples ......................................... 6
Biopsy & Necropsy Tissue Samples ..................... 7
Cerebral Spinal Fluid (CSF) Samples ..................... 9
Whole Blood Samples for PCR ...............................10
Tissue Samples for Virus Re-Activation ...............12
Forms ............................................................... 12
References ......................................................... 12
PURPOSE

The purpose of this protocol is to describe the recommended types and procedures for collecting and transporting specimens to The National B Virus Resource Laboratory for B virus testing. These samples usually include serum, plasma, cerebral spinal fluid, whole blood, tissue, and/or virological swabs. These samples are obtained from human and non-human primate (NHP), as well as from fomite sources.

SCOPE

This protocol is written and intended for diagnostic and research laboratory personnel in The National B Virus Resource Laboratory, Department of Biology, Viral Immunology Center, Georgia State University, Atlanta, Georgia.

RESPONSIBILITIES

It is the responsibility of National B Virus Resource Laboratory personnel to advise clients who are collecting and shipping samples as to these recommended procedures. Any deviations from this protocol must be approved by the Laboratory Director prior to client advisement.

DEFINITIONS


B. **Cercopithecine herpesvirus 1, Herpesvirus simiae, herpes B virus, B virus, BV**: An alpha herpesvirus endemic to Old World non-human primates of the *Macaca* genus. If transmitted to a human host, this virus can be lethal if the host is not properly tested, the infection not identified, and proper medical treatment not begun immediately.

C. **Herpes simplex virus type 1, HSV-1**: An alpha herpesvirus endemic to humans. Due to its characteristics, antibody to HSV-1 is cross-reactive with B virus antigens when tested by current immunological assays. Because of this cross-reactivity, human serology samples may be tested on competition ELISA to confirm western blot data and distinguish antibody reactions.

D. **Herpes simplex virus type 2, HSV-2**: An alpha herpesvirus endemic to humans. Due to its characteristics, antibody to HSV-2 is cross-reactive with B virus antigens when tested by current
immunological assays. Because of this cross-reactivity, human serology samples may be tested on competition ELISA to confirm western blot data and distinguish antibody reactions.

E. **Simian agent 8, SA8**: An alpha herpesvirus endemic to some Old World monkey genus, other than macaques (i.e., African Green monkeys). Diagnostic testing for analysis of antibody to SA8 viral antigens may be preferred to B virus if an exposure/possible exposure occurs with one of these species. Although to date, there has been no confirmed case of transmission of SA8 infection to humans, the possibility of infection, and subsequent disease must be considered when such an exposure/possible exposure occurs.

F. **Herpesvirus papio 2, HVP-2**: An alpha herpesvirus endemic to some Old World monkey genus, other than macaques (i.e., baboons). Diagnostic testing for analysis of antibody to HVP-2 viral antigens may be preferred to B virus if an exposure/possible exposure occurs with one of these species. To date, there is one suggested case of HVP-2 being transmitted to a human host (see Palmer, 1987). The possibility of infection, and subsequent disease must be considered when such an exposure/possible exposure occurs.

G. **Herpesvirus langur, HVL**: An alpha herpesvirus endemic to some Old World monkey genus, other than macaques (i.e., langurs). Diagnostic testing for analysis of antibody to HVL viral antigens may be preferred to B virus if an exposure/possible exposure occurs with one of these species. Although to date, there has been no confirmed case of transmission of HVL infection to humans, the possibility of infection, and subsequent disease must be considered when such an exposure/possible exposure occurs.

H. **Herpesvirus cerocebus, HVC**: An alpha herpesvirus endemic to some Old World monkey genus, other than macaques (i.e., mangabeys). Diagnostic testing for analysis of antibody to HVC viral antigens may be preferred to B virus if an exposure/possible exposure occurs with one of these species. Although to date, there has been no confirmed case of transmission of HVC infection to humans, the possibility of infection, and subsequent disease must be considered when such an exposure/possible exposure occurs.

**PRINCIPLE**

The National B Virus Resource Laboratory receives samples from institutions worldwide for diagnostic testing to identify B virus and/or antibody to B virus (and sometimes other alpha herpesviruses). This protocol is utilized as a reference when advising clients in the proper procedures for the collection, storage and shipment of samples. Proper sample collection and sample handling until delivery to the laboratory is crucial to optimize test assay results and proper patient care. Recommendations for collecting, storing, and shipping serum samples are based upon data that serum antibody is stable at 2 – 6.0° C or if frozen at ≤ -15.0° C. Recommendations for collecting, storing and shipping virology samples are based upon data that alpha herpesviruses are stable at 2 - 6.0° C or if frozen at ≤ -60.0° C. These recommendations should provide optimum samples upon delivery for antibody detection and/or virus recovery.
POLICIES

A. All clients will be furnished with a written protocol for patient specimen collection, storage and shipping (Instruction Sheet) and test request forms (Submission Form).

B. Specimens that deviate from the written protocol and collection procedures may result in incomplete or sub-optimal results. Any and all deviations will be conveyed to the client in writing on the Case Evaluation form.

C. Requests are received from health care institutions, research facilities, private industry, and individuals.

D. All requests must be in writing.

E. All records will be retained for a minimum of 7 years.

F. The client is responsible for all supplies and materials as well as shipping costs. The laboratory will recommend possible supply sources and catalog numbers.

G. The client is responsible for ensuring that samples are shipped in accordance to mandated shipping regulations for Dangerous Goods and dry ice (42 CFR 72: Interstate Shipment of Etiologic Agents).

H. Instructions, forms and possible supply sources will be provided on The National B Virus Resource Laboratory web page at http://www.gsu.edu/bvirus. It is the responsibility of the Laboratory Supervisor to ensure that the information provided on the web page is current.

Sample Collection: Supplies, Collection Procedures, Storage & Shipment

I. Serum Samples

A. Supplies
   1. 5 to 7 ml red top or serum separator (SST) blood collection tubes.
   2. Venipuncture supplies.
   3. 2 ml non-glass serum storage tube.

B. Collection Procedures
   1. Use of Universal Precautions is recommended when collecting any biological specimen.
   2. Properly label a blood collection tube with patient or animal ID and collection date.
   3. Using acceptable venipuncture technique, collect 5 to 7 ml whole blood.
   4. Allow a minimum of 15 minutes to allow clot to form.
   5. Centrifuge sample to separate serum from clot. This can also be accomplished by storing the whole blood sample, in an upright position, overnight in the refrigerator (2 – 6.0° C).
   6. Properly label a 2 ml plastic storage tube with:
a) complete patient or animal ID;  
b) serum collection date.

7. Transfer 1.0 – 2.0 ml of serum to the storage tube.

C. Storage before shipment
   1. Serum samples can be stored in the refrigerator (2 – 6.0° C) for up to one week.
   2. Serum samples can be stored frozen (≤ -15.0 ° C) indefinitely.

D. Sample Packing and Shipment
   1. Diagnostic samples are shipped as “Dangerous Goods”. They do not need to be shipped as “Infectious Agent”. Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.
   2. Refer to the following web pages for regulated shipping instructions:
      a) http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
      b) http://www.cdc.gov/od/ohs/biosfty/shipdir.htm
      c) http://www.cdc.gov/od/ohs/pdffiles/who97.pdf
   3. Before packing, ensure that the Submission Form:
      a) is filled out complete and legible;
      b) has a name and phone number(s) of an individual to be contacted in emergencies;
      c) has a purchase order number or other billing information;
      d) is identical to the sample tubes.
   4. Ship serum with a minimum of 5 pounds dry ice or with frozen cold packs.
   5. Use extra dry ice or extra frozen cold packs for Friday, weekend or holiday shipments.
   6. Pack carefully to avoid sample breakage and leaks.
   7. Keep paperwork dry and separate from specimens.
   8. Contact The National B Virus Resource Laboratory prior to shipping by:
      a) telephone: 404-651-0808
      b) fax: 404-651-0814
      c) email: bvirus@gsu.edu
      d) emergency pager: 1-888-500-3504
      e) emergency phone: 404-358-8168
   9. Ship samples ASAP (priority overnight delivery) via overnight courier to:
      The National B Virus Resource Laboratory
      Viral Immunology Center
      Georgia State University
      50 Decatur Street
      Atlanta, Georgia 30303
   10. Packages that are shipped on Friday must have both the package and the airbill annotated for “Saturday Delivery”. **Do not** select the “Next Day Delivery” option. This is considered the next business day and the package will not be delivered on Saturday. Federal Express is the recommended courier for weekend delivery. Be sure to contact the B Virus Lab offices prior to shipping on Friday to provide the airbill number for tracking missing packages on the weekend.

E. Please do not:
1. ship serum in glass;
2. freeze or ship frozen whole blood samples for serum antibody testing;
3. ship samples with incomplete labeling;
4. label tubes with unnecessary (and confusing) information such as investigator’s name, study numbers, cage numbers, etc;
5. allow samples to freeze-thaw before shipping.

II. Virology Swab Samples:

A. Supplies
   1. Commercially prepared viral transport media (VTM) or laboratory prepared media. Note: Recipes for proper laboratory prepared media are provided on the Instruction Sheet included in the “Client Packet” or available on the web page.
   2. Sterile dacron or cotton tipped swabs with wooden or plastic shafts.

B. Collection Procedures
   1. Use of Universal Precautions is recommended when collecting any biological specimen.
   2. Properly label the viral transport sample tubes with complete patient or primate identification, the collection date and the swab site.
   3. Swab each collection site with a separate sterile swab.
   4. Place each swab into separate sample tubes containing 1 – 3.0 ml of viral transport media. If the shaft is longer than the tube, break it off by bending it against the side of the tube. Shafts that are too long will break through the cap, causing leaks and possible exposure to B virus.

C. Storage before shipment
   1. Swab samples can be stored in the refrigerator (2 – 6.0 °C) for up to one week.
   2. Swab samples can be stored frozen (≤-60.0 °C) indefinitely.

D. Sample Packing and Shipment
   1. Diagnostic samples are shipped as “Dangerous Goods”. They do not need to be shipped as “Infectious Agent”. Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.
   2. Refer to the following web pages for regulated shipping instructions:
      a) [http://www.cdc.gov/od/ohs/biosfty/shipregs.htm](http://www.cdc.gov/od/ohs/biosfty/shipregs.htm)
      b) [http://www.cdc.gov/od/ohs/biosfty/shipdir.htm](http://www.cdc.gov/od/ohs/biosfty/shipdir.htm)
   3. Before packing, ensure that the Submission Form:
      a) is filled out complete and legible;
      b) has a name and phone number(s) of an individual to be contacted in emergencies;
      c) has a purchase order number or other billing information;
      d) is identical to the sample tubes.
   4. Ship swab samples with a minimum of 5 pounds dry ice.
   5. Use extra dry ice for Friday, weekend or holiday shipments.
   6. Pack carefully to avoid sample breakage and leaks.
7. Keep paperwork dry and separate from specimens.
8. Contact the National B Virus Resource Laboratory prior to shipping by:
   a) telephone: 404-651-0808
   b) fax: 404-651-0814
   c) email: bvirus@gsu.edu
   d) emergency pager: 1-888-500-3504
   e) emergency phone: 404-358-8168
9. Ship samples ASAP (priority overnight delivery) via overnight courier to:
   The National B Virus Resource Laboratory
   Viral Immunology Center
   Georgia State University
   50 Decatur Street
   Atlanta, Georgia 30303
10. Packages that are shipped on Friday must have both the package and the airbill
    annotated for “Saturday Delivery”. **Do not** select the “Next Day Delivery” option.
    This is considered the next business day and the package will not be delivered on
    Saturday. Federal Express is the recommended courier for weekend delivery. Be sure
    to contact the B Virus Lab offices prior to shipping on Friday to provide the airbill
    number for tracking missing packages on the weekend.

E. Please do not:
   1. ship glass sample tubes;
   2. use bacterial or viral culturettes;
   3. use the same swab for more than one site;
   4. place more than one swab site in each tube;
   5. use less than 1.0 or more than 3.0 ml viral transport media;
   6. ship samples with incomplete labeling;
   7. label tubes with unnecessary (and confusing) information such as investigator’s name,
      study numbers, cage numbers, etc;
   8. allow samples to freeze-thaw before shipping.

III. Biopsy and Necropsy Tissue:
   A. Supplies
      1. Commercially prepared viral transport media (VTM) or laboratory prepared media.
         Note: Recipes for proper laboratory prepared media are provided on the Instruction
         Sheet included in the “Client Packet” or available on the web page.
      2. Sterile instruments for tissue collection.
   B. Collection Procedures
      1. Use of Universal Precautions is recommended when collecting any biological
         specimen.
      2. Properly label the viral transport sample tubes with complete patient or primate
         identification, the collection date and the tissue source.
      3. Collect a 3 x 5 mm (approximate) tissue sample.
      4. Place each tissue site into separate sample tubes containing 1 – 3.0 ml of viral
         transport media.
5. Recommended nerve tissue to collect at necropsy is right and left trigeminal ganglia and sacral dorsal root ganglia.

C. Storage before shipment
   1. Tissue samples can be stored in the refrigerator (2 – 6.0°C) for up to one week.
   2. Tissue samples can be stored frozen (≤ -60.0°C) indefinitely.

D. Sample Packing and Shipment
   1. Diagnostic samples are shipped as “Dangerous Goods”. They do not need to be shipped as “Infectious Agent”. Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.
   2. Refer to the following web pages for regulated shipping instructions:
      a) http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
      b) http://www.cdc.gov/od/ohs/biosfty/shipdir.htm
   3. Before packing, ensure that the Submission Form:
      a) is filled out complete and legible;
      b) has a name and phone number(s) of an individual to be contacted in emergencies;
      c) has a purchase order number or other billing information;
      d) is identical to the sample tubes.
   4. Ship tissue samples with a minimum of 5 pounds dry ice.
   5. Use extra dry ice for Friday, weekend or holiday shipments.
   6. Pack carefully to avoid sample breakage and leaks.
   7. Keep paperwork dry and separate from specimens.
   8. Contact the National B Virus Resource Laboratory prior to shipping by:
      a) telephone: 404-651-0808
      b) fax: 404-651-0814
      c) email: bvirus@gsu.edu
      d) emergency pager: 1-888-500-3504
      e) emergency phone: 404-358-8168
   9. Ship samples ASAP (priority overnight delivery) via overnight courier to:
      The National B Virus Resource Laboratory
      Viral Immunology Center
      Georgia State University
      50 Decatur Street
      Atlanta, Georgia 30303
   10. Packages that are shipped on Friday must have both the package and the airbill annotated for “Saturday Delivery”. **Do not** select the “Next Day Delivery” option. This is considered the next business day and the package will not be delivered on Saturday. Federal Express is the recommended courier for weekend delivery. Be sure to contact the B Virus Lab offices prior to shipping on Friday to provide the airbill number for tracking missing packages on the weekend.

E. Please do not:
   1. ship glass sample tubes;
   2. place more than one tissue site in each tube;
3. ship tissues dry;
4. use less than 1.0 or more than 3.0 ml viral transport media;
5. ship samples with incomplete labeling;
6. label tubes with unnecessary (and confusing) information such as investigator’s name, study numbers, cage numbers, etc;
7. allow samples to freeze-thaw before shipping.

IV. Cerebral Spinal Fluid (CSF):

A. Supplies
   1. A sterile CSF storage and transport tube.
   2. Lumbar puncture supplies.

B. Collection Procedures
   1. Use of Universal Precautions is recommended when collecting any biological specimen.
   2. Properly label the CSF transport sample tube(s) with complete patient or primate identification and the collection date.
   3. Using acceptable lumbar puncture techniques, collect a 3 – 4.0 ml CSF sample. The CSF sample must be clean. A bloody sample may compromise results.
      Safety precaution: CSF should never be centrifuged prior to shipping.
   4. Transfer 3 – 4.0 ml of clean CSF to the storage tube.

C. Storage before shipment
   1. CSF samples can be stored in the refrigerator (2 – 6.0° C) for up to one week.
   2. CSF samples can be stored frozen (≤ -60.0 ° C) indefinitely.

D. Sample Packing and Shipment
   1. Diagnostic samples are shipped as “Dangerous Goods”. They do not need to be shipped as “Infectious Agent”. Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.
   2. Refer to the following web pages for regulated shipping instructions:
      a) http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
      b) http://www.cdc.gov/od/ohs/biosfty/shipdir.htm
      c) http://www.cdc.gov/od/ohs/pdffiles/who97.pdf
   3. Before packing, ensure that the Submission Form:
      a) is filled out complete and legible;
      b) has a name and phone number(s) of an individual to be contacted in emergencies;
      c) has a purchase order number or other billing information;
      d) is identical to the sample tubes.
   4. Ship CSF samples with a minimum of 5 pounds dry ice.
   5. Use extra dry ice for Friday, weekend or holiday shipments.
   6. Pack carefully to avoid sample breakage and leaks.
   7. Keep paperwork dry and separate from specimens.
   8. Contact the National B Virus Resource Laboratory prior to shipping by:
      a) telephone: 404-651-0808
b) fax: 404-651-0814  
c) email: bvirus@gsu.edu  
d) emergency pager: 1-888-500-3504  
e) emergency phone: 404-358-8168

9. Ship samples ASAP (priority overnight delivery) via overnight courier to:  
The National B Virus Resource Laboratory  
Viral Immunology Center  
Georgia State University  
50 Decatur Street  
Atlanta, Georgia 30303

10. Packages that are shipped on Friday must have both the package and the airbill annotated for “Saturday Delivery”. Do not select the “Next Day Delivery” option. This is considered the next business day and the package will not be delivered on Saturday. Federal Express is the recommended courier for weekend delivery. Be sure to contact the B Virus Lab offices prior to shipping on Friday to provide the airbill number for tracking missing packages on the weekend.

E. Please do not:
   1. ship glass sample tubes;  
   2. ship samples with incomplete labeling;  
   3. label tubes with unnecessary (and confusing) information such as investigator’s name, study numbers, cage numbers, etc;  
   4. allow samples to freeze-thaw before shipping.

V. Whole Blood for PCR

   Note: The National B Virus Resource Laboratory does not recommend performing PCR analysis on whole blood samples. B virus is not known to be a blood borne pathogen, and therefore viral DNA is not expected to be found in such samples. One exception has been noted in the literature, from an animal that appeared very ill and viremic. Whole blood PCR analysis might be considered for animals that appear in such an unhealthy condition.

   A. Supplies  
      1. 5 to 7 ml EDTA or ACD blood collection tubes.  
      2. Venipuncture supplies.  
      3. 2 ml non-glass serum storage tube.

   B. Collection Procedures  
      1. Use of Universal Precautions is recommended when collecting any biological specimen.  
      2. Properly label a blood collection tube with patient or animal ID and collection date.  
      3. Using acceptable venipuncture technique, collect 5 to 7 ml whole blood.  
      4. Properly label a 2 ml plastic storage tube with:  
         a) complete patient or animal ID;  
         b) whole blood collection date.  
      5. Thoroughly mix whole blood sample and transfer 1.0 – 2.0 ml of whole blood to the storage tube.
C. Storage before shipment
   1. Whole blood samples can be stored in the refrigerator (2 - 6.0°C) for up to one week.
   2. Do not freeze or ship frozen.

D. Sample Packing and Shipment
   1. Diagnostic samples are shipped as “Dangerous Goods”. They do not need to be shipped as “Infectious Agent”. Dangerous goods and dry ice shipping regulations must be followed for any diagnostic sample.
   2. Refer to the following web pages for regulated shipping instructions:
      a) http://www.cdc.gov/od/ohs/biosfty/shipregs.htm
      b) http://www.cdc.gov/od/ohs/biosfty/shipdir.htm
      c) http://www.cdc.gov/od/ohs/pdffiles/who97.pdf
   3. Before packing, ensure that the Submission Form:
      a) is filled out complete and legible;
      b) has a name and phone number(s) of an individual to be contacted in emergencies;
      c) has a purchase order number or other billing information;
      d) is identical to the sample tubes.
   4. Ship whole blood samples with ample cold packs to assure delivery in a cool condition.
   5. Use extra cold packs for Friday, weekend or holiday shipments.
   6. Pack carefully to avoid sample breakage and leaks.
   7. Keep paperwork dry and separate from specimens.
   8. Contact The National B Virus Resource Laboratory prior to shipping by:
      a) telephone: 404-651-0808
      b) fax: 404-651-0814
      c) email: bvirus@gsu.edu
      d) emergency pager: 1-888-500-3504
      e) emergency phone: 404-358-8168
   9. Ship samples ASAP (priority overnight delivery) via overnight courier to:
      The National B Virus Resource Laboratory
      Viral Immunology Center
      Georgia State University
      50 Decatur Street
      Atlanta, Georgia 30303
   10. Packages that are shipped on Friday must have both the package and the airbill annotated for “Saturday Delivery”. **Do not** select the “Next Day Delivery” option. This is considered the next business day and the package will not be delivered on Saturday. Federal Express is the recommended courier for weekend delivery. Be sure to contact the B Virus Lab offices prior to shipping on Friday to provide the airbill number for tracking missing packages on the weekend.

E. Please do not:
   1. ship whole blood in glass;
   2. freeze whole blood samples;
   3. ship samples with incomplete labeling;
4. label tubes with unnecessary (and confusing) information such as investigator’s name, study numbers, cage numbers, etc.

VI. Tissue Samples for Virus Re-Activation

Virus re-activation from tissue sources requires special procedures not explained in this protocol. If a client inquires about such procedures, refer the client to Dr. Peter Krug, Marty Wildes, or Dr. Julia Hilliard. One of them will advise the client as to proper sample collection, storage and shipment.

FORMS

A. Instruction Sheet, included in the “Client Packet” or available on the web page.

B. Mini-SOP, included in the “Client Packet” or available on the web page.

C. Submission Form, included in the “Client Packet” or available on the web page.

D. Case Evaluation, printed on the back of the test result page.

REFERENCES


C. Recommendations for Prevention of and Therapy for Exposure to B Virus (Cercopithecine Herpesvirus 1). Clinical Infectious Diseases 2002; 35.

_________________________________________  _______________________
Julia K. Hilliard, Ph.D.  Date
Laboratory Director