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Recommended Sample Collection, Storage, & Shipment

<u>Contents</u>	<u>Page</u>
Purpose	2
Scope	2
Responsibilities	2
Definitions	2
Principle	3
Policies	4
Serum Samples	5
Virology Swab Samples	7
Biopsy & Necropsy Tissue Samples	8
Cerebral Spinal Fluid (CSF) Samples	10
Whole Blood Samples for PCR	11
Tissue Samples for Virus Re-Activation	13
Recommended Materials	13
Forms	15
References	17

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

- A. **Macaque:** Old World monkeys of the *Macaca* genus, natural hosts to B virus. Members of this genus include, but are not limited to, *Macaca mulatta* (rhesus macaque), *Macaca fascicularis* (Cynomolgus monkey/Java macaque/Crab-eating macaque), *Macaca fuscata* (Japanese snow macaque), *Macaca silenus* (Lion-tailed macaque), *Macaca nemestrina* (Pig-tailed macaque), *Macaca nigra* (Celebes “ape”/Sulawesi-crested macaque/Sulawesi black “ape”), *Macaca sylvanus* (Barbary macaque or “ape”), *Macaca radiata* (Bonnet macaque), *Macaca arctoides* (Stump-tailed macaque/Red-faced macaque/Bear monkey), *Macaca thibetana* (Tibetan stump-tailed macaque), *Macaca maurus* (Moor macaque), *Macaca ochreata* (Sulawesi-booted macaque), *Macaca cyclopsis* (Taiwan macaque/Formosan rock macaque), *Macaca sinica* (Toque macaque) and *Macaca assamensis* (Assamese macaque).
- B. ***Macacine 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 2.0 – 6.0° C or if frozen at $\leq -15.0^{\circ}$ C. Recommendations for collecting, storing and shipping virology samples are based upon data that alpha herpesviruses are stable at 2.0 - 6.0° C or if frozen at $\leq -60.0^{\circ}$ 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 (*Recommended Sample Collection, Storage, & Shipment*) 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 known 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 (see page 13).
- G. Samples shipped to the B Virus Resource Laboratory for diagnosis are classified as “Clinical Samples, Biological Substance Category B (UN3373)”. These samples are not classified as “Infectious Agents”.
- H. Samples known to contain active B Virus must be shipped as a “Select Agent”. You must contact the B Virus Resource Laboratory prior to shipping these samples. The CDC must issue an approval before such a shipment can occur. More information can be found at <http://www.selectagents.gov/> (National Select Agents Registry).

I. Clients shipping from the 50 United States and/or Puerto Rico.

The client is responsible for ensuring that samples are shipped in accordance with mandated U.S. regulations governing the shipment of Diagnostic Samples, Dangerous Goods and dry ice (42 CFR 72: Interstate Shipment of Etiologic Agents). These regulations are mandated under the authority of the Center for Disease Control (CDC). These regulations are specified within this document and it is the policy of the B Virus Resource Laboratory that these requirements MUST be met in all cases.

J. Foreign clients.

1. Foreign clients are responsible for ensuring that samples are shipped in accordance with mandated shipping regulations (see I). Foreign clients are also responsible to ensure that samples are shipped in accordance with regulations that are mandated by the Centers for Disease Control (CDC), the United States Fish & Wildlife Service (USFWS), the Endangered Species Act (ESA) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Proper permits must be obtained before shipping samples collected from any non-human primate. It is the policy of the B Virus Resource Laboratory that these requirements MUST be met in all cases.
2. A CITES export permit will be necessary if you are shipping non-human primate samples. Please visit the CITES website <http://www.cites.org/eng/app/> where you will observe that

all non-human primates are listed under Appendix II of the Convention. As a result, you will be required to obtain a CITES Export permit from the Management Authority responsible for implementation of CITES in your country before shipment of any samples to the U.S. Under CITES, blanket permits for such exports are authorized and in some countries they are available. If they are not available, it will be necessary to obtain an individual export permit before shipment to the U.S. Failure to obtain the appropriate permit can cause serious problems in the U.S. Unless you are already familiar with the requirements, you should check with your local Management Authority to determine the appropriate requirements. If this CITES permit is not properly completed and attached to the exterior of the shipping container, it is possible that your shipment may be detained when it enters the U.S.

3. If the species is listed on Appendix I of CITES (i.e. *Macaca silenus*, a.k.a. lion tailed macaque), then please contact our laboratory. You will still be required to obtain a CITES Export permit, but in addition, we will be required to supply you with a copy of a CITES Import permit, which could take up to 6 months. Both the Export permit and a copy of the Import permit must be affixed to the exterior of the shipping container.
4. The word “CITES” should be the first word entered on the waybill in the Description Section. Please see the example at the end of this document under *Forms*. The example is a Federal Express form, but the “CITES” entry would apply for all courier services.
5. Please contact our laboratory with any inquiries you may have regarding procedures, regulations, and/or permits.

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

Sample Collection: Supplies, Collection Procedures, Storage & Shipment*

***All samples must be collected by appropriately trained individuals.**

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 Standard 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 (2.0 – 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 (2.0 – 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 “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). 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) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, ([World Health Organization](http://www.who.int))
 - b) The IATA Dangerous Goods Regulations, (International Air Transport Association) <http://www.iata.org/ps/publications/dgr/Pages/index.aspx>
 - c) Title 49 Code of Federal Regulations, Parts 100-185. Hazardous Materials regulations (Department of Transportation) <http://www.phmsa.dot.gov/hazmat/regs>
 - d) Title 42 Code of Federal Regulations, Part 72. Interstate shipment of etiologic agents (Department of Health and Human Services) <http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
 - e) Title 42 Code of Federal Regulations, Part 72.6. Additional requirements for facilities transferring or receiving select agents (Department of Health and Human Services) <http://www.selectagents.gov/>
 - f) Biosafety in Microbiological and Biological and Biomedical Laboratories (CDC/NIH) <http://www.cdc.gov/od/ohs/biosfty/bmb1/bmb1-1.htm>
3. Foreign clients must ship samples in accordance to CDC, USFWS, and CITES regulations. See part J in the Policies section.
4. 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.
5. Ship serum with a minimum of 5 pounds dry ice or with frozen cold packs.
6. Use extra dry ice or extra frozen cold packs for Friday, weekend or holiday shipments.
7. Pack carefully to avoid sample breakage and leaks.
8. Keep paperwork dry and separate from specimens.
9. Contact The National B Virus Resource Laboratory prior to shipping by:
 - a) telephone: 404-413-6550
 - b) fax: 404-413-6556
 - c) email: bvirus@gsu.edu
 - d) emergency phone: 404-358-8168
10. Ship samples ASAP (priority overnight delivery) via overnight courier to:

The National B Virus Resource Laboratory
Viral Immunology Center
Georgia State University
161 Jesse Hill Jr. Drive
Atlanta, Georgia 30303

11. 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 within the *Recommended Materials* section of this document.
2. Sterile dacron or cotton tipped swabs with wooden or plastic shafts.

B. Collection Procedures

1. Use of Standard 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 1.0 – 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 (2.0 – 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 “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). 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) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, ([World Health Organization](#))
 - b) The IATA Dangerous Goods Regulations, (International Air Transport Association)
<http://www.iata.org/ps/publications/dgr/Pages/index.aspx><http://www.cdc.gov/od/ohs/biosfty/shipdir.htm>
 - c) Title 49 Code of Federal Regulations, Parts 100-185. Hazardous Materials regulations (Department of Transportation)
<http://www.phmsa.dot.gov/hazmat/regs>
 - d) Title 42 Code of Federal Regulations, Part 72. Interstate shipment of etiologic agents (Department of Health and Human Services)
<http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
 - e) Title 42 Code of Federal Regulations, Part 72.6. Additional requirements for facilities transferring or receiving select agents (Department of Health and Human Services) <http://www.selectagents.gov/>
 - f) Biosafety in Microbiological and Biological and Biomedical Laboratories (CDC/NIH) <http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl-1.htm>
3. Foreign clients must ship samples in accordance to CDC, USFWS, and CITES regulations. See part J in the Policies section.
4. 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.
5. Ship swab samples with a minimum of 5 pounds dry ice.
6. Use extra dry ice for Friday, weekend or holiday shipments.
7. Pack carefully to avoid sample breakage and leaks.
8. Keep paperwork dry and separate from specimens.
9. Contact the National B Virus Resource Laboratory prior to shipping by:
 - a) telephone: 404-413-6550
 - b) fax: 404-413-6556
 - c) email: bvirus@gsu.edu
 - d) emergency phone: 404-358-8168
10. Ship samples ASAP (priority overnight delivery) via overnight courier to:

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11. 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 within the *Recommended Materials* section of this document.
2. Sterile instruments for tissue collection.

B. Collection Procedures

1. Use of Standard 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 1.0 – 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 (2.0 – 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 “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). 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) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, ([World Health Organization](http://www.who.int/publications/m/item/guidelines-for-the-safe-transport-of-infectious-substances-and-diagnostic-specimens))
 - b) The IATA Dangerous Goods Regulations, (International Air Transport Association)
<http://www.iata.org/ps/publications/dgr/Pages/index.aspx><http://www.cdc.gov/od/ohs/biosfty/shipdir.htm>
 - c) Title 49 Code of Federal Regulations, Parts 100-185. Hazardous Materials regulations (Department of Transportation)
<http://www.phmsa.dot.gov/hazmat/regs>

- d) Title 42 Code of Federal Regulations, Part 72. Interstate shipment of etiologic agents (Department of Health and Human Services) <http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
 - e) Title 42 Code of Federal Regulations, Part 72.6. Additional requirements for facilities transferring or receiving select agents (Department of Health and Human Services) <http://www.selectagents.gov/>
 - f) Biosafety in Microbiological and Biological and Biomedical Laboratories (CDC/NIH) <http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl-1.htm>
3. Foreign clients must ship samples in accordance to CDC, USFWS, and CITES regulations. See part J in the Policies section.
 4. 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.
 5. Ship tissue samples with a minimum of 5 pounds dry ice.
 6. Use extra dry ice for Friday, weekend or holiday shipments.
 7. Pack carefully to avoid sample breakage and leaks.
 8. Keep paperwork dry and separate from specimens.
 9. Contact the National B Virus Resource Laboratory prior to shipping by:
 - a) telephone: 404-413-6550
 - b) fax: 404-413-6556
 - c) email: bvirus@gsu.edu
 - d) emergency phone: 404-358-8168
 10. Ship samples ASAP (priority overnight delivery) via overnight courier to:
The National B Virus Resource Laboratory
Viral Immunology Center
Georgia State University
161 Jesse Hill Jr. Drive
Atlanta, Georgia 30303
 11. 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 Standard 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 3.0 – 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 3.0 – 4.0 ml of clean CSF to the storage tube.

C. Storage before shipment

1. CSF samples can be stored in the refrigerator (2.0 – 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 “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). 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) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, ([World Health Organization](http://www.who.int))
 - b) The IATA Dangerous Goods Regulations, (International Air Transport Association)
<http://www.iata.org/ps/publications/dgr/Pages/index.aspx><http://www.cdc.gov/od/ohs/biosfty/shipdir.htm>
 - c) Title 49 Code of Federal Regulations, Parts 100-185. Hazardous Materials regulations (Department of Transportation)
<http://www.phmsa.dot.gov/hazmat/regs>
 - d) Title 42 Code of Federal Regulations, Part 72. Interstate shipment of etiologic agents (Department of Health and Human Services)
<http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
 - e) Title 42 Code of Federal Regulations, Part 72.6. Additional requirements for facilities transferring or receiving select agents (Department of Health and Human Services) <http://www.selectagents.gov/>
 - f) Biosafety in Microbiological and Biological and Biomedical Laboratories (CDC/NIH) <http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl-1.htm>
3. Foreign clients must ship samples in accordance to CDC, USFWS, and CITES regulations. See part J in the Policies section.
4. 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.

5. Ship CSF samples with a minimum of 5 pounds dry ice.
6. Use extra dry ice for Friday, weekend or holiday shipments.
7. Pack carefully to avoid sample breakage and leaks.
8. Keep paperwork dry and separate from specimens.
9. Contact the National B Virus Resource Laboratory prior to shipping by:
 - a) telephone: 404-413-6550
 - b) fax: 404-413-6556
 - c) email: bvirus@gsu.edu
 - d) emergency phone: 404-358-8168
10. Ship samples ASAP (priority overnight delivery) via overnight courier to:
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11. 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.

B. Collection Procedures

1. Use of Standard 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.

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 “Clinical Samples, Biological Substance Category B (UN3373)”. They DO NOT need to be shipped as “Infectious Agent”. Proper labeling includes the “Biological Substance – Category B” label (replaces Diagnostic Sample label), the UN 3373 label, and proper dry ice labeling (UN 1845). 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) Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens, ([World Health Organization](http://www.who.int))
 - b) The IATA Dangerous Goods Regulations, (International Air Transport Association)
<http://www.iata.org/ps/publications/dgr/Pages/index.aspx><http://www.cdc.gov/od/ohs/biosfty/shipdir.htm>
 - c) Title 49 Code of Federal Regulations, Parts 100-185. Hazardous Materials regulations (Department of Transportation)
<http://www.phmsa.dot.gov/hazmat/regs>
 - d) Title 42 Code of Federal Regulations, Part 72. Interstate shipment of etiologic agents (Department of Health and Human Services)
<http://www.cdc.gov/od/ohs/biosfty/shipregs.htm>
 - e) Title 42 Code of Federal Regulations, Part 72.6. Additional requirements for facilities transferring or receiving select agents (Department of Health and Human Services) <http://www.selectagents.gov/>
 - f) Biosafety in Microbiological and Biological and Biomedical Laboratories (CDC/NIH) <http://www.cdc.gov/od/ohs/biosfty/bmbl/bmbl-1.htm>
3. Foreign clients must ship samples in accordance to CDC, USFWS, and CITES regulations. See part J in the Policies section.
4. 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.
5. Ship whole blood samples with ample cold packs to assure delivery in a cool condition.
6. Use extra cold packs for Friday, weekend or holiday shipments.
7. Pack carefully to avoid sample breakage and leaks.
8. Keep paperwork dry and separate from specimens.
9. Contact The National B Virus Resource Laboratory prior to shipping by:
 - a) telephone: 404-413-6550
 - b) fax: 404-413-6556
 - c) email: bvirus@gsu.edu
 - d) emergency phone: 404-358-8168
10. Ship samples ASAP (priority overnight delivery) via overnight courier to:

The National B Virus Resource Laboratory
Viral Immunology Center
Georgia State University
161 Jesse Hill Jr. Drive
Atlanta, Georgia 30303
11. 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. Julia Hilliard. Dr. Hilliard will advise the client as to proper sample collection, storage and shipment.

Recommended Materials

I. Serology

- A. Use red top or serum separator vacutainer tubes for blood collection.
- B. Transfer sera to 2 ml storage tube for shipment.
- C. Possible sources and catalog numbers for blood collection and shipment supplies:
 1. **Fisher**
www.new.fishersci.com
1-800-766-7000
Red top: 02-683-94
Serum separator: 02-683-97
Storage tube: 03-341-18A
 2. **VWR**
www.vwrsp.com
1-800-932-5000
Red top: BD367815
Serum separator: BD367986
Storage tube: 66021-974

II. Virology

- A. Our laboratory suggests the use of commercially prepared viral transport media (VTM).
- B. Possible sources and catalog numbers for swab/tissue collection and shipment supplies:

1. Bartel's (Division of MarDx)

<http://www.trinitybiotech.com/Pages/home.aspx>

or

http://www.trinitybiotech.com/InfectiousDisease/Pages/ProductDetails.aspx?ProductNumber=B1029-35C&Filter=*B1029-35C*

or

http://www.trinitybiotech.com/InfectiousDisease/Pages/ProductDetails.aspx?ProductNumber=B1029-90D&Filter=*B1029-90D*

1-800-331-2291 or 1-800-325-3424

Item #: B1029-35C (must be stored at 2° to 6° C prior to use)

Item #: B1029-90D (can be stored at room temperature prior to use)

Email: info@trinitybiotech.com or customerservice@trinityusa.com

2. Micro Test (Division of Remel)

<http://www.remel.com/Clinical/Virology/MicroTest.aspx>

1-800-255-6730

M4 Kit (must be stored at 2° to 6° C prior to use): Item #r12550

M4RT Kit (can be stored at room temperature prior to use) Item #r12552

Email: remel@remel.com or csemail@thermofisher.com

3. Meridian Bioscience, Inc

<http://www.meridianbioscience.com/diagnostic-products/viral-dfa/meridian/meridian-viral-transport.aspx>

1-800-543-1980

Meridian Catalog #505021 (must be stored at 2° to 8° C prior to use)

C. The following media may also be used with the addition of 200 U/ml Pen-Strep-Fungizone or 50 µg/ml Gentamycin with 0.50 µg/ml Fungizone.

- A. Tryptose phosphate broth
- B. Earle's balanced salt solution (Sigma #E6267)
- C. Hank's balanced salt solution (Sigma #H9394)
- D. Normal saline
- E. Phosphate buffered saline
- F. Any sterile, osmotic, buffered solution, pH 7.0

D. The addition of a cryopreservative such as 1% calf serum or 1% fetal bovine serum is recommended for the above media other than tryptose phosphate broth.

FORMS

A. *Recommendations for Sample Collection, Storage, & Shipment*, 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

- A. HHS. 42 CFR Part 493. Clinical Laboratory Improvement Amendments of 1988; Final Rule. Federal Register 57: 7137-7186, 1992. (493.1101-3, 1105)
- B. DHR. Chapter 290-5-29. Rules and Regulations for Licensure of Clinical Laboratories. Official Code of Georgia Annotated 715-744.10, 1995.
- C. Recommendations for Prevention of and Therapy for Exposure to B Virus (*Cercopithecine herpesvirus 1*). Clinical Infectious Diseases 2002; 35.

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Laboratory Director

Date