



Kingdom of Cambodia
Nation Religion King

**SEROLOGIC ASSAYS FOR HUMAN
IMMUNODEFICIENCY VIRUS ANTIBODY
IN DRIED-BLOOD SPECIMENS COLLECTED
ON FILTER PAPER**

Prepared by

Surveillance Unit
National Center for HIV/AIDS and STD

Collaborate with

Laboratory Technical Unit, National Institute of Public Health

October, 2006

**SEROLOGIC ASSAYS FOR HUMAN
IMMUNODEFICIENCY VIRUS ANTIBODY
IN DRIED-BLOOD SPECIMENS COLLECTED
ON FILTER PAPER**

CONTENTS

| | Page |
|--|------|
| PREFACE | 2 |
| HOW TO USE THIS MANUAL | 3 |
| SAFETY | 3 |
| Guidelines to Prevent Transmission of HIV in the Laboratory | 3 |
| In Case of a Spill | 5 |
| SPECIMEN COLLECTION AND STORAGE | 6 |
| Sample Collection | 6 |
| Collection from finger sticks:..... | 6 |
| Collection from vacutainer of blood..... | 7 |
| Packaging Blood Spots..... | 7 |
| Storage of Specimens | 8 |
| Shipping Stored Specimens to Another Site | 8 |
| Logging and Tracking DBS Specimens..... | 8 |
| Punching Disks From Specimens for Testing | 8 |
| | |
| QUALITY CONTROL OF DBS SPECIMENS FOR ENZYME IMMUNOASSAY AND IMMUNOBLOT ASSAY..... | 10 |
| Quality Control for Elution Procedure | 11 |
| Quality Control of Enzyme Immunoassays for DBS Specimens | 11 |
| Quality Control for the Western Blot Assay..... | 11 |
| Interpretation of Quality Control Results | 12 |
| | |
| QUALITY CONTROL (QC) CRITERIA FOR A VALID RUN: | 13 |
| | |
| Figure 1. Dried-Blood-Spot Quality Control | |
| | |
| Appendix A..... | 14 |
| Reference..... | 17 |

PREFACE

Antibodies to the human immunodeficiency virus (HIV) can be detected in whole blood that has been collected and dried in filter paper. The dry blood spots (DBS) can be used to study the prevalence of HIV infection in populations and can be used to screen and confirm the HIV status of individuals.

Collection of blood samples on absorbent paper has unique advantages for large-scale screening programs and epidemiologic surveys. Sufficient blood to saturate the collection paper can be obtained easily by sticking the heel, finger, or ear, thereby eliminating the need for venipuncture. The DBS do not require refrigeration, and they can be mailed conveniently and inexpensively to a central laboratory for analysis. Specially formulated absorbent papers for blood collection (Schleicher & Schuell 903 or Whatman BFC 180) are commercially available and are registered as in vitro medical devices subject to Federal Food and Drug Administration regulations.

This manual was prepared to guide laboratorians in screening whole blood specimens collected on filter paper (to be referred to as blood spots) for HIV antibody using commercially available enzyme immunoassay (EIA) and enzyme-linked immunosorbent blot technique procedures. Since these procedures were developed for clear, non-hemolyzed serum or plasma samples, testing the hemolyzed specimens obtained by eluting requires considerable modification of existing assay procedures. Methods for eluting the blood from the paper and for testing microvolumes of serum by EIA and Western Blot are described in this manual. In addition, a quality assurance program to ensure reliable results when testing this type of specimen is described.

.....12th October, 2006



DR. MEAN CHHI VUN

HOW TO USE THIS MANUAL

This manual provides the laboratorian with information necessary to test DBS specimens for HIV antibody by enzyme immunoassay (EIA) and Western Blot assay.

Read all sections of the manual before performing any of the laboratory procedures. **Pay special attention to the sections on specimen storage, specimen processing, and quality control, since these procedures differ from those recommended by the manufacturers for the routine analysis of serum and plasma specimens.**

This manual complements the instructions provided by manufacturers of HIV-EIA antibody kits. The standard assays for serum and plasma specimens were changed as little as possible, and these changes are noted.

*Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health and Human Services

SAFETY

Working with DBS for human immunodeficiency virus (HIV) testing introduces no new biohazards to laboratorians working in either neonatal screening or HIV testing laboratories. In fact, the viability of HIV seems to be reduced in the dried state (1,2). Despite these findings, universal blood and body fluid precautions should be observed for ALL blood-spot specimens. These precautions are described in the August 21, 1987, supplement to the Morbidity and Mortality Weekly Report (3) and amplified in the April 1, 1988, supplement and June 24, 1988, issue of the same publication (4,5), and updated in the July 12, 1991 recommendations (6). Every laboratory should have a copy of these guidelines and observe the recommendations.

Guidelines to Prevent Transmission of HIV in the Laboratory (7)

1. Employ appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any person is anticipated:
 - Wear gloves when performing venipuncture and other vascular access procedures.
 - Use gloves for performing finger sticks on teens and adults and/or heel sticks on infants and children (5).
 - Change gloves and wash hands after contact with each patient.
 - Place all specimens of blood and body fluids in containers that will prevent leakage during transport. Avoid contaminating the outside of the container and the laboratory form accompanying the specimen.

NOTE: Whole blood dried on filter paper has not been shown to present a hazard

when mailed in paper envelopes. See the Specimen Collection and Storage section for more information.

- Wear gloves when processing blood and body fluid specimens. Remove gloves and wash hands with soap and water upon completion of specimen processing.
2. If hands or other skin surfaces become contaminated with blood or other body fluids, wash them immediately and thoroughly with soap and water.
 3. Employ a biological safety cabinet for procedures that have a high potential for generating droplets (blending, sonicating, vigorous mixing).
 4. Use mechanical pipetting devices to manipulate all liquids in the laboratory. **DO NOT PIPETTE BY MOUTH.**
 5. Take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments.
 - Do not recap needles, bend or break needles by hand, or remove needles from disposable syringes.
 - Discard all sharp instruments in puncture-resistant containers located close to work area.
 - Limit use of needles and syringes to situations in which there is no alternative.
 6. Decontaminate laboratory work surfaces at least daily with a freshly prepared chemical germicide such as a 1:10 dilution of household bleach (this dilution has a final concentration of 0.5% sodium hypochlorite). If bleach is to be used dilutions should be mixed daily as bleach loses its effectiveness within 24 hours. Other commercially available disinfectants can also be used (dilute as indicated by manufacturer).
 7. To decontaminate equipment that may come in contact with blood or body fluids:
 - Disinfect refrigerators by cleaning thoroughly and then by wiping with 1:10 dilution of household bleach.
 - Disinfect centrifuge components by swabbing head, bowl trunion, and carriers with 80% ethanol.
 - Autoclave or soak specimen racks in 1:10 dilution of household bleach for 5 minutes and then rinse thoroughly with water.
 - Discard as hazardous waste any disposable components of instrument systems that come in contact with patient specimens. Clean non-disposable components with 80% ethanol.
 - Allow disinfectant to remain in contact with surfaces for at least 5 minutes at ambient temperature for optimal effectiveness against dried blood or serum.
 - If equipment needs maintenance, clean and decontaminate it in the laboratory before transporting it to the manufacturer for repair.
 8. Use special precautions in handling microbiology laboratory waste, pathology waste, and blood specimens or blood products.

- Incinerate or autoclave all waste before disposal in a sanitary landfill. Solutions containing bleach may corrode the autoclave; therefore, these solutions may be poured down a drain connected to a sanitary sewer.
 - After decontaminating, carefully pour down a drain connected to a sanitary sewer bulk blood, suctioned fluids, excretions, and secretions.
9. Wash hands thoroughly after completing laboratory activities. Remove protective clothing before leaving the laboratory.

In Case of a Spill

Decontaminate spills of blood and body fluids:

- Wear disposable gloves.
- Cover visible blood or body fluids with paper towels and soak with a 1:10 dilution of household bleach. Allow to stand for at least 5 minutes.
- Discard contaminated towels in infective waste containers.
- Wipe down the area with clean towels soaked in a 1:10 dilution of household bleach.

SPECIMEN COLLECTION AND STORAGE

The accuracy of any laboratory procedure used to detect HIV antibody in DBS on filter paper depends on the manner in which the specimen is obtained and handled. DBS specimens have been used to screen newborns for congenital metabolic diseases for many years, and have been successfully used to assess the prevalence of HIV infection in childbearing women and other adult populations . The following recommendations reflect completed studies on stability and optimal storage conditions.

Best test results are obtained when the DBS have not been stored for prolonged periods at room temperature or exposed to conditions of high humidity or high temperatures.

Sample Collection

In the United States, the National Committee for Clinical Laboratory Standards (NCCLS) has published a standard for collecting samples entitled "Blood Collection on Filter Paper for Neonatal Screening Programs" (LA4-A; 1997). This document is currently being updated and should be available in the next couple of months. The NCCLS recommendations are followed when samples are collected for HIV screening program.

Use only No. 903, Schleicher and Schuell, or No. BFC 180, Whatman, cotton-fiber-based paper product designed and used nationally within the United States for the collection of body fluids. Validate with Schleicher and Schuell or Whatman that the lot number of the paper is that currently in use. See Appendix A for a list of supplies and manufacturers for blood spot collection and storage.

Collection from finger sticks:

Add identification information for the client/patient to each filter paper card.

Select one of the 2 middle fingers.

Thoroughly cleanse the finger with 70% isopropanol.

Allow to air dry a few seconds.

Use a sterile, disposable lancet to puncture the skin off to the side of the finger tip. We recommend a single-use lancet such as BD Genie Lancet. These are spring loaded and once they have been used the blade/needle retracts into a plastic housing to prevent reuse. BD lancets come in a variety of needle or blade widths and depths. (See Appendix A)

Wipe away the first small blood drop with a gauze pad.

Place the card close to the lanced area but do not touch it. Apply gentle pressure to the base of the finger and allow the second LARGE blood drop to fall from the tip of the finger onto surface of the filter paper.

The filter paper cards may come with printed circles, apply blood to the inside of the circles. Attempt to fill the circle completely with a single drop before moving to the next empty circle.

Apply blood to only one side of the filter paper (the side with printing).

When all circles are filled (or client no longer bleeds) apply cotton to the puncture site until blood

flow stops.

NOTE: Avoid using capillary tubes to collect blood specimens. Considerable danger of infection exists for lab workers from puncture wounds resulting from accidental breakage of the capillary tubes.

Collection from vacutainer of blood:

Write on the filter paper card identification information for the client/patient.

Mix blood completely.

Use a pipet (with disposable tip) to aspirate 110 μ l of whole blood and apply to the center of each pre-printed circle.

Avoid touching the part of the card with the blood spot. Dry all specimens at least 3 hours in a suspended horizontal position. Depending on the climate it might be necessary to allow spots to dry over night. Schleicher and Schuell sell cardboard drying racks which have slots to hold about 12 cards. (See Appendix A)

Packaging Blood Spots

Once DBS are completely dry, stack them between sheets of glassine paper so that blood spot cards from different patients are not touching each other. Pack 10-15 blood spot cards in Low Gas Permeable zip-lock bags. The following items should also be placed in this bag: 5-10 desiccant packs (this will remove any residual moisture from the cards), humidity indicator cards (this will tell you the relative humidity inside the bag) and QC material. Press as much air out of the bag as possible and seal it shut. The humidity indicator cards and desiccant packs have a color indicator which changes from blue to pink as humidity increases. All cards and packs should be replaced with fresh material before they have all changed to a pink color.

Moist humidity cards and desiccant packs can be re-used. Simply place in a 65°C oven over night until the color indicator returns to a blue color. Remove from the oven and store in a sealed bag until reuse.

NOTE: **Plastic bags used for storage must be gas- impermeable. Bags available from grocery stores are inadequate. Use bags such as Bitran Saranex Series S multipurpose bags available from VWR Scientific, Fisher Scientific, or S&S (See Appendix A.) These bags come in variety of sizes.**

Storage of Specimens

For short term storage, DBS should be kept in zip-lock bags with desiccant and stored at 4°C. DBS should only be taken out of cold storage when they are needed for testing.

For long term storage (over 90 days), keep specimens in a freezer (-20°C).

Specimens stored differently from those just described are considered compromised and should not be tested. It is better to reject such specimens than to include potentially unreliable results that might bias the survey data.

Shipping Stored Specimens to Another Site

Use special care when shipping to another location any DBS that have been stored at 4 °C or -20 °C. Remove the bagged samples from the refrigerator and allow them to reach room temperature before opening the bag. Once the sealed bag has equilibrated, open it and remove the old desiccants. Add fresh desiccants and reseal the bag. Ship the bag by the fastest means. If a cooler is available for transport this will protect samples from short periods of high temperature. Upon receiving such specimens, store them immediately in a refrigerator (4°C) or freezer (-20°C).

Logging and Tracking DBS Specimens

DBS arriving from other locations should be examined for the quality of the spots and packaging:

All spots should be in appropriate low gas permeable zip-lock bags described elsewhere in this document.

Each blood card should be examined for collection quality and possible damage and a note should be made of any poor quality samples.

Samples should be separated by glassine paper.

NOTE: Desiccant packs and humidity indicator cards which have changed to a pink color should be replaced with fresh material.

All DBS specimens arrived in your lab should be logged into your existing specimen inventory system (whether it is a notebook or a computer software package).

Punching Disks From Specimens for Testing

Dried-bloodspot specimens or controls must be acclimated to room temperature before punching disks. If they have been stored in sealed bags with desiccant at 4 °C or -20 °C, allow the sealed bags to reach room temperature before opening them (a minimum of 30 minutes).

Examine DBS before testing. Do not test spots that:

- Are obviously contaminated by some foreign substance

- Contain blood clots
- Are not saturated with blood or appear spotted
- For any other reason appear to be of poor quality

In general, these guidelines for rejecting specimens are the same as those used in all newborn screening programs.

Punch the disk in an area that is saturated with blood. Do not take the punch from the edge of the circle where the degree of saturation may be uneven. Avoid blood clots.

Whether using an automated punching machine or a manual punch, remove paper fiber residue as it accumulates on the instruments. Although no cases of false-positive results have been associated with transfer of paper dust, periodic cleaning reduces this possibility.

QUALITY CONTROL OF DBS SPECIMENS FOR ENZYME IMMUNOASSAY AND IMMUNOBLOT ASSAY

Quality assurance is the dynamic and ongoing process of monitoring a system for reproducibility and reliability that permits corrective action when established criteria are not met. Maintaining these acceptable levels of performance is quality control. Laboratory aspects of quality assurance include the numerous steps from specimen collection through confirmation of positive specimens. Good laboratory practice for repeat and confirmatory testing requires that the original specimen, not an intermediate dilution, be used as the sample source.

The overall quality control scheme allows serum controls to monitor the effectiveness of each commercial kit's reagents throughout the analysis and permits linkage of the D controls throughout the system, thus controlling the effectiveness of each analytical system.

The criteria for accepting or rejecting an analysis are based on the performance of the quality control materials (serum and DBS) and must be predetermined as part of the laboratory protocol. Quality assurance and quality control steps must be outlined in writing in the laboratory operations manual. Quality control specimens must be handled in a manner identical to the manner in which unknown specimens are handled. All quality control events must be documented, from specimen processing to reporting. Good quality assurance for the HIV screening laboratory involves setting criteria of performance for all steps in the specimen logistics, from collecting specimens to reporting data.

Each enzyme immunoassay (EIA) plate is considered a separate run and must have its own serum and DBS controls. Data from EIA plates and Western blots are accepted or rejected on the basis of the performance of the serum controls supplied in the kits and on the DBS controls in an interactive process. The analytical system presented in Figure 1 illustrates one possible scheme for incorporating quality control material in a testing algorithm. This scenario uses an initial EIA, a repeat duplicate EIA, and a Western blot.

The serum kit controls (negative and positive) verify that the kit reagents are performing correctly and are used to calculate the positive and negative cutoff. The DBS controls (low- positive, high-positive, and negative) monitor the performance of the lab testing system. DBS quality controls incorporated into specimen sets during collection monitor the stability of the specimens throughout the collection and testing process.

Quality Control for Elution Procedure

Establish the placement of the DBSS disks in the elution plate so that the blank wells for the kit serum controls follow the configuration of the plate as directed by the manufacturer's protocol. DBS of all specimens and controls must be at room temperature before they are punched. Specimens or controls stored cold or frozen in sealed bags with desiccant must be allowed to equilibrate at room temperature before the bags are opened. See the chapter on specimen collection and storage for details. Duplicate DBSS control disks (low-positive, high-positive, and negative) are eluted along with the DBSS specimens.

Before performing the initial EIA, examine the eluted DBS to visually ensure complete elution. The filter paper punch should be white. If it is not, complete elution has not occurred. Continue incubation and do not analyze the specimen until elution is effective. If complete elution is not accomplished within 24 hours, discontinue the analysis of these specimens and document the observation. Several factors, such as specimen age and heat exposure, will affect the elution of blood dried on filter paper.

Quality Control of Enzyme Immunoassays for DBS Specimens

Examine the results of serum controls as described in the manufacturer's product insert. Accept or reject assay results on the basis of the performance criteria for the serum controls as described by each manufacturer. Reject any analytical run (EIA plate) that violates these standards, regardless of results obtained for other control materials.

If the serum controls are within specifications, examine the blood-spot controls. Accept the assay results if all blood-spot controls are correctly classified as positive or negative. If any blood-spot control is incorrectly classified (positive / negative) reject the entire plate and repeat the EIA on the original eluates produced for this EIA plate or create fresh elutes from blood-spot controls and specimens. If blood-spot controls are still incorrectly classified, troubleshoot the method before proceeding.

Record in the quality control log the absorbance values of the DBSS controls, along with the calculated serum kit cutoff for each run. Plot these values as quality control charts, to identify trends in data and single control values that differ significantly from previous observations. See QC criteria for a valid run below.

Quality Control for the Western Blot Assay

The Western blot assay employs both serum and DBS control material. The serum controls should consist of a high-positive serum, displaying all of the significant viral bands; a low-positive serum of defined reactivity; and a negative serum.

If particular bands are missing from the high-positive DBS control, a stability or elution problem must be considered in the interpretation of the specimens.

Interpretation of Quality Control Results

Examine the serum controls first. The high-positive serum control must consistently demonstrate all significant viral specific protein bands at an intensity equal to that usually observed in the test system. These bands are identified, numbered, and used as a reference for identifying bands present in the unknown specimens. If bands usually present in the high-positive control are absent in a particular run, the run must be rejected. The system should be examined to determine the source of the performance problem before repeating the assay.

The low-positive serum control is used as a control for assay sensitivity. The serum selection for this purpose should have all the viral specific protein bands critical in identifying a positive specimen. The intensity of these bands should make them definitely visible but weak in comparison to the high-positive control. If a serum having these properties is not available, a serum containing only key viral protein bands (p24, gp41) can be substituted. Again, the bands present in a particular run must always be of the intensity usually observed for that control specimen. To compensate for run-to-run variations in the assay conditions, the low-positive serum control is used to adjust the substrate incubation period to ensure a specified reactivity for each run. The test is incubated until a selected band (or bands) present in the low-positive control reach an expected intensity, based on previous experience. If the expected intensity is not achieved within a 30-minute incubation with substrate, the run should be rejected and the method should be examined for the source of the malfunction.

The negative serum control should not demonstrate any viral protein bands.

QUALITY CONTROL (QC) CRITERIA FOR A VALID RUN: HIV Antibodies in DBS

A. Monitoring Control Values with QC Charts

QC charts must be made using the OD values for negative control (NC), low positive control (LP) and high positive control (HP). QC limits (either $\pm 95\%$ and 99% confidence intervals or ± 2 or 3 standard deviations) should be calculated using data from the first 10 analytical runs. These limits should be recalculated and held after 20 runs. The mean values from each run, the overall mean of the 10 (or 20) mean values, and upper and lower 95 and 99% confidence intervals or 2 and 3 standard deviations must be plotted on one chart. For each subsequent analytical run, the mean OD for the control materials must fall within the defined control limits for each material.

An analytical run is considered out of control if any of the following events occur:

1. The mean OD for NC, LP or HP falls outside the upper or lower 99% control limits (or 3 SD). The 99% control limits (or ± 3 SD) are considered action limits. The run must be repeated if these limits are exceeded.
2. Two successive mean ODs for NC, LP or HP fall outside of the upper or lower 95% control limits (or ± 2 SD). The second run must be repeated. The 95% control limits (or ± 2 SD) are considered warning limits.
3. Eight successive mean ODs (for NC, LP or HP) are above or below the mean value line. The eighth run must be repeated.

B. Troubleshooting Guidelines

The list below should be used to troubleshoot the assay if control values fall outside of the established ranges.

1. Check to be sure all pipettes or instruments used for making dilutions are calibrated and capable of making accurate dilutions.
2. Check all instrumentation used to wash and read plates.
3. Review kit lot information to be sure kits are not outdated. Reviewing kit lot information may also reveal a kit lot bias. Perform 5 runs and recalculate control limits for new kit lots.
4. Check storage and handling conditions of control materials.
5. Check quality of substrate solution. Substrate solution that has been reconstituted and stored for a period of time may increase background.

APPENDIX A

SUPPLIES AND PREPARATION OF DRIED BLOOD SPOTS FROM BLOOD COLLECTED BY VARIOUS METHODS

1. Supplies

- a. FDA-approved Filter Paper Collection Device
1.73310--3" x 4.25" card with 5 circles/card.
Order # 10538414
Job # A01520
Schleicher & Schuell (S&S) 903™ paper
Keene, NH 03431
Contact: Judy Peter 800- 437-7003
2. Whatman BFC 180™ paper
Whatman, Inc.
6 Just Road
Fairfield, NJ 07004
Contact: Mark Fry 800-343-5853 ext 132
Fry@whatman.co
- b. Drying Racks
105-395-21, Dry Rak™
Schleicher & Schuell (S&S)
Keene, NH 03431
(800) 437-7003
- c. Double-sided Carpet Tape
ST501, DF Paper Tape ½" x 36 yds
Minimum order: 1 case of 72 rolls
Spectape of Atlanta
1661 Roadhaven Drive
Stone Mountain, GA 30083
(770) 934-4053
- d. Low-gas permeable plastic bags for card storage
VWR Scientific #11217-106
(800) 932-5000
Fisher Scientific #19240127
(800) 766-7000
7 x 8" bags are marketed by the above companies but manufactured by
Com-Pac International, (800) 824-0817, manufacturer #4743S)
S&S #79692
These are low gas perm. bags from S&S
- e. Desiccant Packs—1 gram desiccant packs with blue indicator that turns pink with high humidity

#02-00040-AG37 Minipax Indicating Silica Gel Tyvek Bag
Multisorb Technologies
325 Harlem Road, Buffalo, NY 14224
(800) 445-9890

f. Weigh Paper

#09-898-12C
Fisher Scientific
PO Box 829
Norcross, GA 30091
(800) 766-7000

g. Hole Puncher

1/4" stainless hole punch
Available from any office or school supply store

h. BD Genie Lancet (single use, permanently retractable lancet with 1.5 mm blade)

#02-683-105
Fisher Scientific
PO Box 829
Norcross, GA 30091
(800) 766-7000

i. 96-well microtiter plate, flat bottom, with lid

#07-200-90
Fisher Scientific
PO Box 829
Norcross, GA 30091
(800) 766-7000

j. PBS pH 7.4 with TWEEN 20 0.05% powder

#P3563
Sigma Aldrich
(800) 325-5052

The use of trade names is for identification only and does not imply endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

2. Description of collection device

DBS can be collected from a finger stick or venous blood and dried into FDA-approved filter paper. The paper is preprinted with circles that contain approximately 100 μ L blood when fully filled. Patient identifiers can be written in pen or pencil directly on the paper.

3. Collecting blood on filter paper

a. *From a finger stick:* Lightly touch the filter paper to a LARGE blood drop. Allow the blood to soak through and completely fill the circle with a single application of blood. To

enhance blood flow, gently apply intermittent pressure to the area surrounding the puncture site. Avoid “milking” the finger. Apply blood to only one side of the paper. Fill remaining circles in the same manner. Dry the filter paper horizontally for a minimum of 3 hours at room temperature (see “Drying dried blood spots” below)

b. *From blood collected into vacuum tubes:* Use blood from anticoagulant tubes (EDTA-lavender top** or Heparin-green top). Fill the blood collection tube to the recommended volume so anticoagulant is at proper dilution. Before making spots allow the blood to come to room temperature if it has been refrigerated. Gently and thoroughly mix the tubes to resuspend the red cells, either by inverting the tube by hand (25x) or by placing the tube on a mechanical hematology mixer for approximately 5 min. After the blood is mixed, remove 100 μ L aliquots of blood and apply the blood to the filter paper that has been secured (see “Drying dried blood spots” below).

c. *From blood collected from a finger stick into EDTA** or heparin capillary tubes:* Gently touch the capillary tube to the surface of filter paper that has been secured (see “Drying dried blood spots” below). The blood will flow from the tube into the filter paper. Do not scratch, poke, or otherwise score the filter paper with the capillary tube.

**EDTA can interfere with some analytical tests. Consult the testing protocol or package insert from a commercially available kit to determine which blood collection tubes to use.

4. Drying Dried Blood Spots

a. Use S&S #903™ Dry Rak™ to DBS cards.

If these are not available, follow procedure b.

b. Place double-sided carpet tape on two books, boards, boxes, or other suitable solid support to suspend the filter paper used to collect blood. Remove cover strip to expose sticky side of tape. Carefully place the paper containing DBS between the supports so as to avoid contact between the table and wet blood spots. Nothing should touch the wet paper. DBS should be dried at room temperature for a minimum of 3 hours. They may also be dried overnight at room temperature.

Note: Desiccant packs can be regenerated by heating overnight in a 60°C oven.

5. Storing Dried Blood Spots

After spots are dried, place DBS between 2 sheets of glassine paper (weighing paper), or wrap sheets of weighing paper around spots. Store spots at -20°C, in low gas permeable zip-closure bags with several desiccant packs. The desiccant packs should be checked often and changed when the indicator turns pink. Initially, desiccant packs may need to be changed frequently. After a few changes the cards will be drier and the desiccant packs will need less changing. The buildup of humidity can damage the quality of the sample.

6. Shipping

Transport low gas-permeable bags containing dried DBS by the fastest means possible. Enclose the bags inside a foam or plastic cooler for transport. This will provide a double-layer barrier that protects casual handlers from accidental exposure, and protects the specimens from the environment during transport.

References

1. Resnick L, Veren K, Salahuddin SZ, Tondreau S, Markham PD. Stability and inactivation of HTLV-III/LAV under clinical and laboratory environments. *JAMA* 1986;255:1887-91.
2. McDougal JS, Martin LS, Cort SP, Mozen M, Heldebrant CM, Evatt BL. Thermal inactivation of acquired immunodeficiency syndrome virus, human T-lymphotropic virus type-III/lymphadenopathy-associated virus, with special reference to antihemophilic factor. *J Clin Invest* 1985;76:875-7.
3. Centers for Disease Control. Recommendations for prevention of HIV transmission in health-care settings. *MMWR* 1987;36(suppl. no. 2S):3S-18S.
4. Centers for Disease Control. Agent summary statement for human immunodeficiency virus and report on laboratory-acquired infection with human immunodeficiency virus. *MMWR* 1988;37(suppl. no. S-4):1S-17S.
5. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other bloodborne pathogens in health-care settings. *MMWR* 1988;37:377-88.
6. Recommendations for Preventing Transmission of Human Immunodeficiency Virus and Hepatitis B Virus to Patients During Exposure-Prone Invasive Procedures. *MMWR* 1991;40:RR08;1-9.
7. American Public Health Association. Prevention of HIV transmission in laboratory settings. *Laboratory Section Newsletter*, October 1987:1-2.
8. National Committee for Clinical Laboratory Standards. NCCLS Approved Standard LA4_A3. Blood collection on filter paper for neonatal screening programs. Villanova, PA: National Committee for Laboratory Standards, 1997.
9. Knudson RC, Slazyk WE, Richmond JY, Hannon WH. 1993. Guidelines for the shipment of dried blood spot specimens. *Infant Screening*. Volume 16. Document can also be found at: <http://www.cdc.gov/od/ohs/biosfty/driblood.htm>

For more information please contact any of the following individuals:

Joanne V. Mei, Ph.D.

Lead Research Chemist
Newborn Screening Quality Assurance Laboratory
Division of Laboratory Science
National Center of Environmental Health
Centers for Disease Control and Prevention

Mail Stop F-19, 4770 Buford Hwy NE
Atlanta, GA 30341-3724
Phone: 770-488-7945
Fax: 770-488-7459
Email: jmei@cdc.gov

Timothy C. Granade

Division of AIDS, STD, and TB Laboratory Research(DASTLR)
NCSTP
Centers for Disease Control and Prevention
Mailstop A-25, 1600 Clifton Road
Atlanta, GA 30333
Phone: 404-639-3850
Fax: 404-639-2660
Email: txg1@cdc.gov

Kyle B. Bond

Global AIDS Program, Laboratory Support
NCHSTP/DASTLR
Centers for Disease Control and Prevention
Mailstop: A-12, 1600 Clifton Road
Atlanta, GA 30333
Phone: 404-639-2643
Fax: 404-639-2475
Email: kbb5@cdc.gov

Marie Downer, MD, MPH

Global AIDS Program, Laboratory Support
NCHSTP/DASTLR
Centers for Disease Control and Prevention
Mailstop: A-12, 1600 Clifton Road
Atlanta, GA 30333
Phone: 404-639-3050
Fax: 404-639-1286
Email: mld8@cdc.gov

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
Atlanta, Georgia 30333