Attachment 4(c)

Adult and Pediatric HIV/AIDS Confidential Case Reports
for National HIV/AIDS Surveillance OMB No. 0920-0573

Supplemental Surveillance Activity 2: Variant, Atypical, and Resistant HIV Surveillance (VARHS) Technical Guidance

Technical Guidance for HIV/AIDS Surveillance Programs

Variant, Atypical, and Resistant HIV Surveillance (VARHS)

HIV Incidence and Case Surveillance Branch
Atlanta, Georgia

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Background

The Centers for Disease Control and Prevention (CDC) is responsible for maintaining a national surveillance system that provides data on the HIV/AIDS epidemic that can be used for national, state, and local public health HIV/AIDS prevention program planning and evaluation. Clinical and laboratory testing information is collected to characterize the epidemic and guide public health action. HIV-1 genetic sequence data based on the *pol* region (protease and reverse transcriptase genes) have been incorporated into the HIV/AIDS surveillance system to evaluate the distribution of HIV-1 mutations associated with HIV drug resistance and subtypes among persons with a new diagnosis of HIV infection.

In the late 1990s, several new nucleoside reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) were approved for treating HIV infection in the United States. These newer drugs, along with the NRTIs already available, provide clinicians with a variety of choices for initiating and changing antiretroviral treatment for patients infected with HIV-1. An international panel with expertise in antiretroviral research and HIV patient care, convened by the International AIDS Society–USA and an interagency work group of the Department of Health and Human Services (DHHS) and the Public Health Service, provide recommendations for prophylaxis or therapy that include all of the antiretroviral drugs approved by the Food and Drug Administration (FDA) and in use in the United States (1).

The therapeutic purposes of antiretroviral drugs include prophylaxis after known occupational exposure (postexposure prophylaxis), vertical transmission prophylaxis, treatment of primary infection (4 to 7 weeks after infection), initial treatment from early (little or no immunologic damage) to late (substantial immunologic damage) infection and changes in treatment regimens depending on virologic and immunologic response (1–5). Clinical trials are being conducted to evaluate the use of antiretroviral drugs for preexposure prophylaxis. Studies have demonstrated that the results (genotype and phenotype) of HIV drug-resistance testing can be used to predict clinical outcome and to guide drug treatment choices (5). Also, studies have shown that the results of *pol* region sequencing that indicate the presence of mutations associated with drug resistance predict phenotypic sensitivity to antiretroviral drugs and clinical response (1, 5). Studies have also demonstrated that the HIV-1 protease and reverse transcriptase genes of the *pol* region can be used to characterize HIV-1 subtypes and that a high prevalence of HIV-1 subtypes other than subtype B in a geographic area has implications for the appropriate selection of HIV diagnostic and clinical tests for populations and individuals (6).

Given the public health concerns about primary HIV drug resistance, the clinical impact on persons with a new diagnosis of HIV infection and the uncertain clinical significance of resistant HIV strains, CDC funded a project called Antiretroviral Drug Sentinel Surveillance to Examine Trends in Prevalence of Drug-Resistant Strains of HIV in 2002.

This demonstration project showed that it was feasible to use remnant sera for genotyping; in turn, this led to the inception of variant, atypical, and resistant HIV-1 surveillance (VARHS).

On June 25, 2004, VARHS received a non-research determination from the CDC Human Subjects Office (Appendix A). The document that serves as the basis for the federal regulations for protecting human research participants (Title 45 of the Code of Federal Regulations part 46, 45 CFR 46) is available at http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm). Subpart A, subsection 46.101c states that "the [federal] Department or Agency heads retain final judgment as to whether a particular activity is covered by this policy." The policy refers to the requirements for human subjects protection review for research protocols under the 45 CFR 46 regulations. *Disease surveillance* is one of the 4 major public health practice activities that usually involve data collection but are not, according to 45 CFR 46, research and thus do not need review by an institutional review board (IRB). Although CDC determined that VARHS is a disease surveillance activity by CDC (IRB review not required), this determination does not supersede state or local laws or regulations that may require IRB notification or review for public health surveillance activities.

On July 1, 2004, VARHS was incorporated nationally into routine HIV surveillance, and 22 surveillance areas were funded to participate in VARHS (Appendix B).

Objectives

The primary objectives of VARHS are to

1. Incorporate surveillance of transmitted strains of variant, atypical, and resistant HIV-1 into routine HIV surveillance activities by
    - amplifying and sequencing the *pol* region (protease and reverse transcriptase genes) from persons with a new diagnosis of HIV infection who have been reported to the HIV/AIDS Reporting System (HARS)
    - estimating the prevalence of mutations associated with HIV drug resistance, resistant patterns, and trends in dissemination of drug-resistant strains
    - determining the prevalence of genetic subtypes to gain insight into the genetic diversity of strains and distribution of B and non-B variants among populations
2. Provide data on HIV-1 drug resistance, subtypes, and factors associated with resistance to assist HIV treatment, prevention, and program planning and evaluation

Methods

VARHS is used to evaluate the prevalence of HIV-1 drug resistance and the distribution of HIV-1 subtypes among persons with a new diagnosis who have been reported to HARS by state, county, or city departments of health. Specimens from these persons should be included by using either of 2 methods.

First, if sufficient volume is available, aliquots of remnant diagnostic specimens should be set aside for HIV-1 drug-resistance testing from each blood specimen drawn for HIV testing from eligible persons in participating areas. For persons meeting VARHS criteria, HIV genetic sequencing (i.e., genotyping) will be performed on the protease and reverse transcriptase genes to detect the presence of mutations associated with HIV-1 drug resistance. HIV-1 subtypes will also be identified on the basis of these genes.

HIV-1 drug-resistance testing for VARHS is performed with standard tests, which are widely used clinically. Like drug-resistance testing in other infectious disease surveillance systems (e.g., tuberculosis, urinary tract infections, and sexually transmitted diseases), testing diagnostic specimens for HIV-1 drug resistance and HIV-1 subtype surveillance is not experimental and does not require informed consent (7–9). Areas may choose to include information in their HIV testing forms to inform persons that HIV drug-resistance testing is part of HIV diagnostic testing in participating areas. For persons with a new diagnosis who have been reported and whose specimens are successfully amplified and genotyped through the VARHS system, the health department will provide a hard copy of the results to the health care provider(s) designated by the persons tested.

Second, if private-sector laboratories performed genotyping on specimens from persons eligible for inclusion in VARHS, surveillance areas should request electronic sequence data from the laboratories.

Genotyping results and information from the HIV surveillance case report will be used to estimate the prevalence of HIV-1 drug resistance and the distribution of HIV-1 subtypes among persons with a new diagnosis who have been reported as having HIV infection. Prevalence will also be estimated for relevant demographic groups and HIV transmission categories. HIV sequence information may also be used to track the spread and clustering of atypical HIV strains that are of interest nationally.

Public Health Benefit

Although previous surveys have been based on convenience samples of specimens, VARHS is the first surveillance system in the United States designed to evaluate all persons who have been given a diagnosis of HIV infection and reported to HARS/eHARS from participating areas. VARHS will determine the distribution of drug-resistant strains and subtypes among persons with a new diagnosis who have been reported as having HIV-1, thus supporting efforts to characterize and track the HIV epidemic nationally. VARHS will also provide information on trends in the transmission of drug-resistant strains and the factors associated with resistance. Analyses will support evaluation of first-

line antiretroviral drugs and provide information that will be useful in developing new antiretroviral drugs. Strategies for using specific prophylactic drugs will be enhanced by information from the surveillance of HIV drug resistance.

VARHS will support evaluation of the usefulness of testing baseline clinical HIV-1 drug resistance in specific geographic areas. Current DHHS guidelines for the treatment of HIV-1 infection recommend baseline resistance testing for all HIV patients before the initiation of antiretroviral therapy. Even when therapy is deferred, the guidelines suggest that baseline testing should be considered: "In the absence of therapy, resistant viruses may decline over time to less than the detection limit of standard resistance tests but may still increase the risk of treatment failure when therapy is eventually initiated" (1). The DHHS guidelines cite a 2005 cost-effectiveness analysis that concluded, "A strategy of genotype-resistance testing at initial diagnosis of HIV infection increased per-person quality-adjusted life expectancy by 1.0 months, with an incremental cost-effectiveness ratio of $23,900 per quality-adjusted life-year gained, compared with no genotype testing. The cost-effectiveness ratio for resistance testing remained less than $50,000 per quality-adjusted life-year gained, unless the prevalence of resistance was ≤1%, a level lower than those reported in most regions of the United States and Europe…. Genotype-resistance testing of chronically HIV-infected, antiretroviral-naive patients is likely to improve clinical outcomes and is cost-effective…. Resistance testing at the time of diagnosis should be the standard of care" (10).

VARHS will also provide public health and clinical personnel with information on the distribution of HIV-1 subtypes in the United States over time. If the prevalence of HIV-1 non-B subtypes increases, this finding could have implications for vaccine studies. Also, should specific drug-resistant mutations be associated with some non-B subtypes, there could be implications for treatment guidelines.

In the surveillance of other organisms, such as *Mycobacterium tuberculosis*, molecular surveillance has allowed identification of atypical strains of special interest (9, 11). Although no such HIV strains have been identified, routine surveillance based on sequencing and phylogenetic analyses of the HIV *pol* region will allow such strains to be detected. Routine surveillance will also provide information on multidrug-resistant strains in various geographic areas and specific population subgroups. HIV-1 genetic sequencing surveillance data collected over time will also contribute to analyses of the evolution of HIV-1 in the United States.

Structural Requirements

Policies and Procedures

All persons with a new diagnosis of HIV infection after confidential testing should be reported to HARS in accordance with *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. I: Policies and Procedures* (12). VARHS is an extension of the national population-based HIV/AIDS case surveillance system, and state and local health

department partners and CDC will use the case surveillance infrastructure to collect the information necessary to estimate the prevalence of transmitted HIV-1 drug resistance and HIV-1 subtypes.

VARHS will be implemented in areas that also conduct HIV incidence surveillance. CDC recommends that HIV incidence surveillance procedures be in place before VARHS implementation is planned. In some circumstances, however, the simultaneous implementation of both surveillance systems may be beneficial. Before implementing VARHS, surveillance areas must

- Consult their CDC HIV incidence surveillance epidemiologist and the CDC VARHS project officer or coordinator about the appropriate time to plan and implement VARHS
- Develop VARHS guidance outlining local policies and procedures, which must be reviewed and approved by CDC
- Host a site visit by CDC
- Document CDC approval before collecting specimens for VARHS

Because VARHS is an integrated component of HIV/AIDS surveillance, documentation of VARHS activities should be incorporated into locally tailored policy and procedures manuals developed for HIV/AIDS surveillance (12) to establish standardization, maintain continuity of meaning, document changes over time, and develop training programs. In addition to the information in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. I: Policies and Procedures* (12), VARHS-specific policies and procedures should include information related to

- Responsibilities of staff
- Training in VARHS procedures for health care providers, counselors, and other staff at participating testing sites and clinics
- Identification of participating laboratories
- Eligibility determination
- Specimen handling and transport procedures
- Collection of VARHS-specific specimen tracking and laboratory data elements
- Entering and importing data into the Incidence and Viral Resistance (IVR) database
- Data management practices
- Return of genotyping results
- Monthly data transmission to CDC
- Security and confidentiality
- Local analysis and dissemination plan
- Expansion plan (including a timeline for jurisdiction-wide implementation of VARHS)

At the end of some sections in this document, a box (similar to the one headed Sample VARHS Implementation Task) is used to present task-specific guidance that the surveillance area will need to address in order to implement VARHS. The local VARHS guidance should include a description of local plans and policies and procedures related to each task, as the completion of these tasks is key in the successful implementation of VARHS.

Sample VARHS Implementation Task

VARHS areas should complete each implementation task that appears at the end of selected sections in this document.

Laboratory Procedures

Specimen handling and transport procedures are stringent for VARHS, because proper specimen handling and transport contribute to the rate of successful amplification for genetic sequencing. Specimen handling and transport procedures should be decided by the public health laboratory and local sites. If necessary, procedures can be modified when new aspects of the surveillance system are instituted. At a minimum, surveillance areas planning to implement VARHS need to take the following laboratory procedures into consideration.

- If volume seems sufficient for the purposes of all basic laboratory tests and other local priorities, such as archiving and HIV incidence surveillance, a 1 mL (optimal) aliquot from all enzyme immunoassay (EIA)–reactive specimens should be frozen at –70ºC as soon as possible after the first reactive EIA.
- The following will maximize chances for successful amplification for genetic sequencing
  - Plasma and serum should be separated as quickly as possible, and neither should be refrigerated until after separation.
  - HIV diagnostic testing or other relevant clinical testing should begin immediately after separation of plasma or serum.
  - Sera or plasma should be kept on the bench at room temperature for as short a time as possible before being returned to the refrigerator or placed on ice.
  - Once frozen, specimens for genotyping **should not** be thawed until they reach the genotyping laboratory. A freeze-thaw cycle will reduce the chance of successful amplification for genotyping.
  - Specimens should be shipped on dry ice to the genotyping laboratory.
- A system must be in place to collect all required data elements for specimen tracking and to transmit them to CDC (see Appendix F).

Staffing Needs

Implementation of VARHS requires personnel with specific skills and dedicated time to effectively integrate VARHS into the core HIV/AIDS surveillance system. In general, personnel who work primarily on core HIV/AIDS surveillance or HIV incidence surveillance will develop work plans to integrate VARHS into HIV/AIDS surveillance.

VARHS staff should have

- An understanding of VARHS and the characteristics of the local HIV/AIDS surveillance system, including HIV incidence surveillance
- An understanding of how HIV diagnostic laboratories function and how they are related to the public health system
- Good communication skills
- Strong leadership skills
- An ability to work closely with CDC, other states, local HIV diagnostic and clinical sites, private providers, and laboratories

CDC recommends a full-time VARHS coordinator position dedicated to implementing and maintaining the system. The number of other personnel assigned to VARHS may vary, depending on the implementation phase, prevalence of HIV/AIDS, and available resources.

VARHS coordinator

- Provide overall management of VARHS
  - Develop local VARHS guidance (to be reviewed and approved by CDC)
  - Serve as the lead for area-specific implementation of the VARHS technical guidance
  - Serve as the primary CDC contact for VARHS
  - Manage employee or other service contracts related to VARHS
- Develop training materials and courses, including presentations for local clinical and diagnostic sites and laboratories to introduce VARHS
- Train providers, counselors, and other staff at participating clinical and diagnostic sites
- Oversee data collection processes
  - Directly manage or oversee the process for determining the eligibility of specimens for VARHS
  - Work with the genotyping laboratory to develop procedures for shipment of specimens and receipt of genotyping results
  - Develop and manage the system for returning genotyping results to designated providers
- Collaborate with other VARHS staff
- Participate in CDC site visits, trainings, and workshops

Epidemiologist

- Plan and implement the integration of VARHS activities into HIV core surveillance and HIV incidence surveillance activities
- Develop a VARHS analysis plan
- Develop systems to ensure data quality, analyze local VARHS data, and produce reports
- Participate in data dissemination activities
  - Collaborate with stakeholders to determine data needs and frequency of reporting
  - Identify results and surveillance issues for review and dissemination
  - Develop a data dissemination plan in collaboration with the VARHS coordinator and CDC
- Participate in CDC site visits, trainings, and workshops

Laboratory liaison

- Serve as the liaison between the public health department and laboratories from which specimens are being shipped
- Oversee transfer of specimen tracking data from laboratories to the IVR database at the health department
- In collaboration with liaisons based at participating laboratories, develop local procedures for processing and shipping specimens to the genotyping laboratory
- In collaboration with liaisons based at participating laboratories, develop quality control procedures for preparing specimens
- Develop and oversee procedures to maintain security and confidentiality of specimen identifiers and results
- Participate in CDC site visits, trainings, and workshops

Laboratory liaison based at a participating HIV diagnostic laboratory

- Work with the VARHS coordinator and the health department laboratory liaison to develop the laboratory-specific plan for processing, storing, determining eligibility, tracking, and shipping specimens to the genotyping laboratory
- Oversee preparation and shipping of VARHS specimens to the genotyping laboratory
- Monitor quality control procedures for preparing VARHS specimens
- Record specimen tracking data in the log or database

Data manager

- Assist the VARHS coordinator with daily management of VARHS data
- Serve as subject matter expert on VARHS data elements and data management programs

- Receive data from other health department entities and the genotyping laboratory, and incorporate those data into the IVR database and data sets for transfer to CDC
- Assess data quality
- Obtain a Secure Data Network (SDN) certificate and renew the certificate annually to allow for data transmission to CDC
- Identify at least 1 backup person to hold an SDN certificate and maintain the list of persons with certification, notifying CDC of changes
- Oversee VARHS data management
  - Assess data collection methods
  - Modify CDC's generic data management programs for use at the local level
  - Maintain the IVR database
  - Run SAS statistical software programs to produce monthly data sets
  - Develop and implement edit checks and edit data
  - Collaborate with the VARHS coordinator, epidemiologist, and other area surveillance and prevention staff on data editing, data entry, and data set preparation
  - Prepare monthly data set for transmission to CDC
  - Transfer data through SDN to CDC
  - Prepare data sets for local analysis
  - Collaborate with CDC on data set preparation for national resistance estimates
  - Prepare HIV resistance data reports for local use in collaboration with the VARHS coordinator, epidemiologist, and CDC
- Maintain security and confidentiality of data
- Participate in CDC site visits, trainings, and workshops

VARHS Implementation Task

Areas should identify the staff needed to implement VARHS and define staff roles, responsibilities, and the time each staff member should spend on VARHS.

Process Standards

VARHS involves the following processes:

- Determining eligibility
- Identifying laboratories and obtaining remnant specimens or sequence data
- Developing specimen handling and transport procedures
- Collecting required data elements

- Returning genotyping results
- Ensuring security and confidentiality
- Managing data
- Analyzing and disseminating data

Determining Eligibility

Specimens confirmed as HIV-1–positive by Western blot or other methods acceptable to the department of health are potentially eligible for VARHS. All specimens yielding indeterminate or negative Western blot results in the absence of other proof of HIV infection should be handled according to standard laboratory procedures for handling, storage, and discarding specimens.

The VARHS coordinator will identify the confirmed positive frozen aliquots that are eligible for VARHS by matching laboratory and HARS data (or data from an equivalent HIV/AIDS reporting system). The VARHS coordinator also will identify cases with electronic sequence data that are eligible for VARHS. Eligibility should be established according to the following inclusion and exclusion criteria at the case and the specimen level.

Inclusion criteria

- The specimen is the diagnostic specimen (the HIV-1–positive specimen that led to the case report to HARS).
- If the diagnostic specimen is unavailable, a later specimen is eligible provided that
  - the later specimen was drawn <3 months after the date of the diagnostic specimen **and**
  - the later specimen was drawn for an HIV-related test (viral load, polymerase chain reaction, CD4 count).
- Electronic sequence data are eligible if the specimen used for genotyping was drawn <3 months after the date of the diagnostic specimen.

Exclusion criteria

- The specimen is not from the diagnostic specimen that led to the report to HARS.
- The specimen is from an HIV-related test (viral load, polymerase chain reaction, CD4 count) or a genotyping test and was drawn >3 months after the date of the diagnostic specimen.
- The person had received antiretroviral drugs before the VARHS specimen was collected.
- There is no method to link the person to HARS (i.e., the person was tested anonymously).
- An earlier specimen had been sequenced and documented in VARHS.
- The person's diagnosis was HIV-2 infection.
- The specimen was collected before the surveillance area implemented VARHS.

How often HARS is checked for eligibility is left to the discretion of the surveillance area; however, at a minimum, HARS should be checked to determine VARHS eligibility

- before sending specimens for genotyping **and**
- before each monthly data transfer to CDC.

All specimens that are not eligible for VARHS should be handled according to local standard laboratory procedures.

Identifying Laboratories and Obtaining Remnant Specimens or Sequence Data

In each surveillance area, laboratories that could participate in VARHS will be identified from a review of local HIV surveillance data and laboratory licensing records. In practice, VARHS will usually begin in public health laboratories, which collaborate closely with the health department.

The preferred default practice for VARHS is to have eligible specimens shipped by the central public health laboratory to the CDC-contracted genotyping laboratory for VARHS testing. Deviations should be discussed with CDC. Shipping costs associated with sending specimens from state public health laboratories to the genotyping laboratory should be paid from the funds provided through CDC's cooperative agreement with each participating area. Surveillance areas are responsible for including estimated shipping costs in their request for funds and for budgeting accordingly.

To minimize the possibility that a person with a new diagnosis might begin taking antiretroviral drugs before a specimen is collected, HIV genetic sequencing for VARHS must be performed on an HIV-1–positive specimen collected at the time of HIV diagnosis or at the time of a specimen collected at a follow-up visit no more than 3 months after diagnosis. If volume and logistics permit, a remnant specimen for VARHS will be obtained from all eligible, confirmed HIV-positive diagnostic specimens. Specimen types suitable for VARHS are serum, plasma, and whole blood.

The use of dried fluid spots for VARHS is being evaluated as an alternative type of specimen collection but has not been approved for use in VARHS.

National commercial reference laboratories and private laboratories where HIV diagnostic or clinical testing is performed can also participate in VARHS, if resources are available, to allow remnant specimens to be processed within the time frames described in the following sections or to allow time for the receipt of genotyping results (i.e., sequence data). Reimbursements to local commercial and private laboratories will be determined, on the basis of local policies, by each surveillance area.

When sequence data are available from commercial or private laboratories for cases that are eligible for VARHS, the following should be done:

- Secure the fasta files (standard text-based format for representing nucleic acid sequences) containing the nucleotide sequence.
- Match the sequence data with case information in the IVR database.
- Indicate in the appropriate field in the IVR database that the specimen was handled through a commercial or private laboratory.

VARHS Implementation Task

Identify laboratories that are required to report HIV-positive test results, the total number of new diagnoses made annually in each laboratory, and the number of HIV-positive specimens suitable for VARHS (plasma, serum, or whole blood).

Specimen Handling and Transport Procedures

Specimen handling and transport procedures should be decided by the public health laboratory and local sites. If necessary, procedures can be modified when new aspects of the surveillance system are instituted. The following sections describe tube types, transport times, and processing times that are optimal for VARHS. Before implementing VARHS, surveillance areas should take these factors into consideration when developing local procedures.

In general, 1 or more central public health laboratories will process and ship VARHS specimens to the genotyping laboratory. (See Appendix E for detailed shipping procedures for serum and plasma specimens.)

Tube type for HIV diagnostic specimens

The decision on the type of tube to use for HIV diagnostic and follow-up blood draws should be made on the basis of local diagnostic and surveillance needs and available resources. If changes in practice are being considered, note that the HIV diagnostic or the follow-up blood draw should be 8 to 10 mL to ensure sufficient volume for additional HIV surveillance uses, including VARHS.

Specimen handling

After the blood draw and before serum or plasma separation, the tube should be stored at room temperature. The blood draw and specimen transport should be timed so that specimens arrive at the HIV testing laboratory and are processed (separated, aliquoted, and frozen) within 96 hours or as soon as possible after the blood draw. The likelihood of amplification may be reduced when specimen processing takes longer.

Serum and plasma processing

For HIV testing and VARHS, it is recommended that specimens be centrifuged to remove red blood cells and prevent hemolysis. Successful HIV amplification for sequencing is less likely with hemolyzed specimens. After separation of the serum or plasma, specimens should be maintained in a refrigerator at 4ºC or kept on ice until the specimens are EIA tested and eligible aliquots are frozen for potential use in VARHS.

Cryovials and aliquots

Cryovials for sera or plasma aliquoted for VARHS should be 2 mL, polypropylene, with screw caps and external threads. If labels are not used, the tube should have a writing area.

After the VARHS identification number (VARHS ID) is generated locally, each specimen should be labeled with the appropriate VARHS ID, by either writing the ID number on the tube with a permanent marker or attaching a label to the tube. Labels that adhere to frozen tubes can be supplied at no cost by CDC upon request.

Ideally, an aliquot of 1 mL per specimen should be prepared for HIV genetic sequencing and sent to the genotyping laboratory. When volume is limited, priority is given to (1) the laboratory's standard operating procedures for HIV diagnostic and clinical testing and serum archiving, (2) HIV incidence surveillance activities, and (3) VARHS. If less than 1 mL is available for genetic sequencing when all other needs have been met, the specimen may still be sent for sequencing after consultation with the genotyping laboratory.

Diagnostic and clinical testing and freezing of serum or plasma aliquots

The following will maximize chances for successful amplification for genetic sequencing.

- HIV diagnostic testing or other relevant clinical testing should begin immediately after separation of plasma or serum.
- Sera or plasma should be kept on the bench at room temperature for as short a time as possible before being returned to the refrigerator or placed on ice.
- If volume appears sufficient for the purposes of all basic laboratory tests and other local priorities, such as archiving and HIV incidence surveillance, a 1 mL aliquot from all EIA-reactive specimens should be frozen at –70ºC as soon as possible after the first reactive EIA.

  Note: Optimally, the specimen should be frozen at –70ºC within 96 hours after the blood draw. After 96 hours, the likelihood of amplification may be reduced, but the specimens should still be sent for genetic sequencing provided that amplification results are followed up carefully.

- Once frozen, specimens for genotyping should not be thawed until they reach the genotyping laboratory. A freeze-thaw cycle will reduce the chance of successful amplification for genotyping.

After the first reactive EIA, the aliquot is still considered an HIV diagnostic specimen; it will not be defined as a VARHS specimen until it has been confirmed as HIV-positive and until it is clear that specimen needs for all higher priority laboratory tests, including diagnostic HIV testing and HIV incidence testing, have been fulfilled. Aliquots frozen as potential VARHS specimens can be thawed at any time if they are needed for higher priority tests. The freeze-thaw cycle will not affect the HIV diagnostic test results or STARHS (serologic testing algorithm for recent HIV seroconversion) results for HIV incidence testing.

Specimen tracking data elements to be recorded in VARHS specimen log or database

VARHS data are considered part of routine HIV surveillance data and should be held to the standards of security and confidentiality for HIV/AIDS surveillance outlined in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13). The diagnostic testing laboratory should maintain a VARHS specimen tracking log or database to provide information for evaluation if problems with amplification, specimen mixup, or specimen contamination occur. (See Table 2, Appendix F, for a list of the minimum required data elements for specimen tracking.) The specimen tracking data elements listed in Table 3 are optional unless the amplification rates for a particular surveillance area or local site are low (<90%). If the rates are low, all of the specimen tracking data elements listed in Table 3, in addition to those in Table 2, will be **required**. A Microsoft Access database for storage of the required specimen tracking and laboratory data is supplied by CDC.

Specimen tracking and laboratory data may be recorded first on person laboratory slips or specimen labels. Batch slips may be used for the initial recording of information applicable to batches of specimens, such as site of the blood draw, lists of patient identification numbers for specimens transported in a batch, date/time of receipt in the diagnostic laboratory, range of laboratory accession numbers for a batch, date/time of centrifugation, date/time of separation, date/time of first positive EIA result, date/time of aliquoting, and date/time aliquots were frozen. All or some of this information may also be captured from in-house laboratory databases. Information will be transferred to the IVR database for each specimen that is confirmed as HIV-positive and **not** identified as **ineligible**. Laboratory or health department personnel may work with CDC to develop methods of information transfer to minimize duplicate recording of information.

Shipment to the VARHS genotyping laboratory

Shipments should be made to the genotyping laboratory at least monthly. The frequency of shipments per month should be determined locally: the goal is to return resistance test results to designated health care providers as soon as possible. (For handling, packaging, and shipping of specimens, see Appendix E.)

Surveillance staff will compile a list of VARHS–eligible specimens to be packaged and shipped by the state or local public health laboratory to the genotyping laboratory. VARHS ID numbers should be recorded on the specimen manifest included in the shipment. Two additional copies of the specimen manifest should be made: 1 should be sent to sent to CDC by the US Postal Service or through SDN; the other should be filed with the VARHS coordinator (see Appendix E).

VARHS Implementation Task

VARHS areas should identify all laboratories that will transport specimens to a central public health laboratory, which will ship specimens to the genotyping laboratory. The local version of this guidance should include descriptions of the procedures by which the VARHS coordinator will receive monthly information about specimen tracking, receive shipping manifests whenever shipments are made, and communicate at least quarterly with laboratories making the shipments.

VARHS areas should include detailed laboratory procedures in the local version of this guidance (e.g., information on obtaining and processing specimens, determining eligibility, recording specimen tracking data for local use, storing specimens, and shipping specimens). All procedures should be developed by the VARHS coordinator in consultation with participating laboratories.

Required Data Elements

VARHS data are considered part of routine HIV surveillance data and should be held to the security and confidentiality standards for HIV/AIDS surveillance outlined in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13).

Data used to determine VARHS eligibility

HARS or equivalent HIV/AIDS reporting systems will serve as the primary means of determining eligibility by using the date of the first positive HIV test result. Information on the prior use of antiretroviral drugs may be obtained from the incidence surveillance Testing and Treatment History Data or other sources, provided that information is collected on whether antiretroviral drugs have **ever** been used (i.e., no time limit should be placed on the history of antiretroviral drug use).

The elements of specimen tracking and laboratory data are linked with the HIV surveillance database to determine whether the person was reported to HARS more than 3 months before the relevant blood draw. Given the commitment to make reports available to providers in "real time," the shipment of specimens for VARHS should not be delayed more than 30 days for the purposes of such evaluation (if possible, specimens should be shipped within 6 weeks after the HIV diagnostic test or other blood draw).

The dates of HIV diagnosis and the prescription of antiretroviral drugs may not be available in HIV/AIDS surveillance reports until after VARHS specimens have been shipped, tested, and entered in the IVR database. If additional data makes a case ineligible, the reason for ineligibility should be recorded in the database.

Demographic and clinical data

VARHS does not require the collection of additional demographic or clinical data. Instead, relevant data are supplied by HARS or the local equivalent. (See Table 1 of Appendix F for the minimum set of data elements required for determining HIV-1 drug resistance and HIV-1 subtype prevalence in the population and in population subgroups.) Demographic data used to estimate the prevalence of HIV-1 drug resistance may include age, sex, race/ethnicity, country of origin, and transmission categories associated with HIV infection. These elements will contribute to the calculation of prevalence estimates for national subgroups; local prevalence may be estimated in areas where the number of cases is sufficient.

Specimen tracking data

The required data elements for specimen tracking (i.e., must be collected for every specimen) are listed in Table 2 of Appendix F, and the optional data elements for specimen tracking are listed in Table 3 of Appendix F.

Note: CDC will analyze amplification rates stratified by surveillance area. If the amplification rates in a surveillance area are low (<90%), all the data elements for specimen tracking listed in Tables 2 and 3 will be **required**. These additional data must be collected until the amplification problem has been resolved or until CDC concludes that the problem is not associated with the procedures for handling or storing specimens.

Laboratory data

Data transmitted to CDC must not include personal identifiers and must be encrypted and password protected as specified in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13).

For specimens sent to the CDC contract and state laboratories, the genotyping laboratory will do the following:

- Transmit information on amplification to the health department and to CDC
- Transmit the complete sequence of the protease gene and at least the first 240 codons of the reverse transcriptase gene, both as nucleotides and amino acids
- Transmit a separate list of all mutations (including those associated with drug resistance and those not associated with drug resistance) of any strain that differs from the reference strain used at the laboratory
- Provide hard copy of laboratory reports (to the appropriate health department only)

VARHS Implementation Task

VARHS areas should review Appendix F and determine the completeness and quality of the data elements used for analysis in VARHS. Because all of the demographic and clinical data elements used are key elements for general HIV surveillance, areas may wish to explore methods for increasing the reporting of these elements (e.g., some areas have added specific elements, such as country of birth, to their counseling and testing laboratory request forms).

Returning Genotyping Results

HIV genetic sequencing is usually performed for clinical purposes for persons receiving treatment for HIV. Physicians who treat HIV-infected persons are familiar with the formats in which results are reported. The genotyping laboratory sends the hard copy of a report (similar to the reports of drug-resistance testing, with which physicians are familiar) to the health department no more than 30 days after the genotyping laboratory receives the specimen. Because the interpretation of these results requires familiarity and training, the health department should not return genotyping results directly to the patient, but to the patient's designated health care provider.

A person with HIV-1–positive results who returns for posttest counseling or follow-up should

- Receive a brief explanation of ancillary HIV diagnostic testing, including genotyping
- Receive instruction on how to designate a health care provider to receive, at that time or later, the genotyping result if the patient wishes to do so
- Receive a brief explanation on when the health department will send the results to the health care provider

The health department is responsible for

- Determining the method(s) by which the patient can designate a health care provider(s) who will receive the genotyping result
- Developing a method for the health care provider to obtain the genotyping results
- Sending the hard copy of reports to the health care provider of the patient's choosing
- Acting as a repository for the hard copy of reports for a locally specified time

If the blood draw that produced a VARHS specimen was performed at a clinical site to which a patient is returning for medical consultation or care, the health department may arrange for the genotyping report to be returned directly to the patient's provider at that clinical site.

VARHS Implementation Task

Before VARHS is implemented, staff should determine the method(s) by which the patient can designate a health care provider(s) who will receive the genotyping results. Methods should be put in place to allow the patient to designate a health care provider(s) at the time of the initial diagnosis or later.

Ensuring Security and Confidentiality

HIV testing is a medical procedure. Therefore, policies and procedures are in place to protect the confidentiality of tested persons and their medical records. VARHS will be performed only on specimens that have tested positive for HIV or specimens used for the follow-up of a confirmed HIV-positive result.

VARHS data are considered part of routine HIV surveillance data and should be held to the standards of security and confidentiality for HIV/AIDS surveillance outlined in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13). Current policies and procedures, based on these guidelines and local laws, are used by state and local health departments to secure hard copy and electronic information to protect the confidentiality of persons reported as having HIV infection. These measures must be extended to protect VARHS data held locally. Access by VARHS staff to information in HARS, HIV incidence surveillance data, and VARHS data is governed by the same security and confidentiality requirements.

All staff responsible for the transmission and receipt of specimen shipping manifests, VARHS sequence data, or reports of results must be trained in the security and confidentiality procedures for HIV/AIDS surveillance.

Information that could identify a person (e.g., name, Social Security number, full date of birth, address, ZIP code) will not be included in data sets transmitted from local surveillance areas to CDC. Data transmitted to CDC must be encrypted and password protected according to *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13).

VARHS Implementation Task

VARHS areas should review their security and confidentiality requirements and if necessary, draft specific procedures for handling VARHS data or add VARHS components to the local protocol. VARHS areas should also review their protocol and outline how VARHS data will be stored and secured. Personnel with access to the data should receive annual training in security and confidentiality procedures and should sign a confidentiality statement that outlines the procedures and the consequences of violating them.

Data Management

All data will be considered part of routine HIV surveillance data. Data entry and management will take place at state or local health departments by the use of HARS or eHARS and software developed by CDC or software that is compatible with CDC software.

CDC software

The IVR database supplied by CDC is a Microsoft Access database, an adjunct to HARS and eHARS, with tables for specimen tracking and HIV drug-resistance data. An additional table is provided for local data entry and for use by local HIV surveillance staff for the purposes of determining eligibility or for returning results.

CDC also provides a SAS program that facilitates the merging of specimen tracking and drug-resistance data (from the IVR database) with selected demographic and clinical data (from HARS or eHARS). The SAS program produces a data quality report along with a cumulative SAS data set to be submitted to CDC.

Identification of VARHS specimens

A VARHS ID will be assigned to each specimen sent for genotyping and used for importing HIV genetic sequencing information to the IVR database. The 14 digits of the VARHS ID are assigned as follows:

- Digits 1–4: These 4 digits represent the Federal Information Processing Standard (FIPS) code of the surveillance area.
- Digits 5–8: These 4 digits represent the site where the blood draw was performed.
- Digits 9–10: These 2 digits identify the last 2 digits of the year the blood was drawn.
- Digits 11–14: These 4 digits are a sequence assigned by the health department.

The IVR database includes functions to allow areas to enter their FIPS code only once and to create a menu of blood drawing sites and their codes. The VARHS ID will be created automatically in the IVR database when the 4 components listed above are entered for each specimen.

All data from the health department, local laboratories, genotyping laboratory, and HARS or an equivalent reporting system will be associated with either a VARHS ID number or a HARS State No. and then will be merged on the basis of these IDs. In addition to the VARHS ID, a specimen accession number may be entered in the database for local use.

Data encryption and transfer

Data transmitted to CDC must not include personal identifiers and must be encrypted and sent through SDN, as specified in *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13). Either SEAL or Pretty Good Privacy (PGP) encryption software must be used to encrypt all data being sent through SDN (13). SEAL software, for encrypting data sent to CDC, is provided by CDC at no cost. VARHS surveillance areas must purchase PGP software with at least 128-bit encryption to encrypt communications with genotyping laboratories. The PGP Personal Desktop edition is suitable; a perpetual license is recommended. All persons using PGP to encrypt and decrypt data must exchange PGP encryption keys. Any encryption software other than SEAL or PGP must be approved by CDC.

Electronic data sent from the genotyping laboratory to VARHS areas must be encrypted with PGP (or equivalent software). Please note that to receive genetic sequencing data from the CDC contract laboratory, VARHS areas must purchase PGP encryption software. The genotyping laboratory and the VARHS area must exchange PGP encryption keys.

CDC will use PGP software to encrypt quality assurance data and will send those data to VARHS areas through SDN (13).

Before the 15th day of each month, the complete VARHS data set from the preceding month should be transmitted to CDC.

VARHS Implementation Task

The local guidance should include the procedure for entering specimen tracking, genotyping, and local data into the VARHS database, exporting these data from the VARHS database, and using SAS to merge these data with selected demographic and clinical variables from HARS. Before transmitting the monthly VARHS data set to CDC, VARHS staff must have encryption software and a CDC-approved SDN certificate and must understand CDC's security and confidentiality guidelines (13). If PGP is used, all persons who encrypt and decrypt data must exchange encryption keys.

Data Analysis and Data Dissemination

To standardize the national resistance data set, CDC uses a regularly updated program to analyze resistance data transmitted from the genotyping laboratories. This program translates the nucleotide sequence and incorporates information on individual mutations of interest, the level of resistance to each antiretroviral drug in common use, and HIV-1 subtype.

The prevalence of resistance to at least 1 antiretroviral drug and the distribution of HIV-1 subtypes among persons with a new diagnosis of HIV infection should be reported annually at the national and local levels. The prevalence of resistance to individual antiretroviral drugs and categories of commonly used antiretroviral drugs (currently NRTIs, NNRTIs, and PIs) may also be reported. These data will be stratified by demographic factors or transmission category for subpopulation analyses at the national and local levels. If adequate case counts are available, stratification will allow comparisons of differing geographic areas and of differing transmission categories.

Aggregate data from the participating surveillance areas will be analyzed by CDC, and results will be disseminated. Each surveillance area will be responsible for conducting analyses at the local level.

As appropriate, results will be presented at conferences and published in peer-reviewed journals. The authors representing the surveillance areas and those representing CDC will be determined for each presentation or paper.

VARHS Implementation Task

VARHS areas should describe, in the local version of the VARHS guidance, the proposed plan for analyzing and disseminating VARHS data.

Outcome Standards

Outcome standards described in the Introduction to Policies and Procedures and Data Quality sections of *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. I: Policies and Procedures* (12) should be applied to VARHS. Given the time-sensitive nature of VARHS data elements, meeting the core surveillance standards for case ascertainment and timeliness is essential to the success of VARHS. The quality of the HIV resistance estimate depends on the quality of data in the VARHS system. All outcome standards for VARHS relate only to cases in persons who resided in the surveillance area at the time of diagnosis.

- The minimum standard for collecting the required specimen tracking and laboratory data elements is 85% (goal, 100%).
- Each year, for at least 50% of the persons in a jurisdiction who have a new diagnosis and have been reported to HARS, a specimen should be sent for genotyping and documentation in the IVR database (50% of cases diagnosed in a year should have genotyping results 12 months after the diagnosis year).

References

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Notes

Appendix A

National Center for HIV, STD, and TB Prevention's Non-research Determination for HIV Variant, Atypical, and Resistant HIV-1 Surveillance

NCHSTP
Research/Non-research Determination
(Request to Classify Project as Not Involving Human Subjects or Research)

This form should be used to submit to NCHSTP ADS materials for projects involving CDC investigations that are not subject to human subjects regulations. Projects are eligible for this classification either as "non-research" projects (primary intent is not to generate generalizable knowledge) or as research projects that do not involve identifiable human subjects. Such projects do not require submission to the CDC Human Subjects Office for IRB review. Do **NOT** use this form for IRB "EXEMPT" research.

Project Title: Surveillance of variant, atypical, and resistant strains of HIV
(under PA 1194, 4017, 4118, and future program announcements supporting genotyping for this purpose)

Project Locations/Sites Current sites: Piloted for feasibility under Protocol 3575 (as research, because of pilot status, but consent was waived by all involved IRBs) in Colorado, Illinois, Maryland, and Seattle/King County.
We now propose to reclassify this activity from pilot status to routine HIV surveillance. Departments of Health in Chicago, Colorado, Florida, Illinois, Indiana, Louisiana, Massachusetts, Michigan, New York City, New York State, North Carolina, Pennsylvania, Puerto Rico, Seattle/King County, South Carolina, and Virginia have received funding under PA 4017 for this HIV surveillance activity.
Departments of Health in all other states performing HIV surveillance are eligible under a new program announcement (4118).

Project Officer(s) Diane Bennett Division: DHAP-SE Telephone: 404-639-5349

Proposed Project Dates: Proposed Start (as non-research routine surveillance): 7/1/2004 Ending: ongoing HIV surveillance activity

Categories of data collection that do not constitute human subjects research include are listed below. Please check appropriate category:

___I. **Activity is not research**. Primary intent is a public health practice disease control activity.
___A. Epidemic/endemic **disease control** activity; collected data directly relate to disease control needs.
XB. Routine **disease surveillance** activity; data used for disease control program or policy purposes.
___C. **Program evaluation** activity; data are used primarily for that purpose.
___D. **Post-marketing surveillance** of efficacy and/or adverse effects a new regimen, drug or device.

NCHSTP ADS Review **Date rec'd in NCHSTP ADS Office:**_____

✓ **Concur, project does not constitute human subjects research**

or

___**Project constitutes human subjects research**, submission for Human Subjects review required

Additional Comments:
1. This form cannot be used to document "IRB Exempt Research," which must instead be submitted to the CDC IRB.
2. Although CDC Human Subjects (IRB) review is not required in this instance, investigators/project officers are expected to adhere to ethical principles and standards by respecting and protecting to the maximum extent possible the privacy, confidentiality and autonomy of participants. All applicable State and Federal privacy laws must be followed.
3. Although this project does not constitute human subjects research, informed consent may be appropriate. Information disclosed in the consent process should address the eight standard consent elements.
4. Other:

Signed: _____ 6-25-04
Andrew Vernon, MD, MHS — Date
Associate Director for Science
National Center for HIV, STD, and TB Prevention

Appendix B

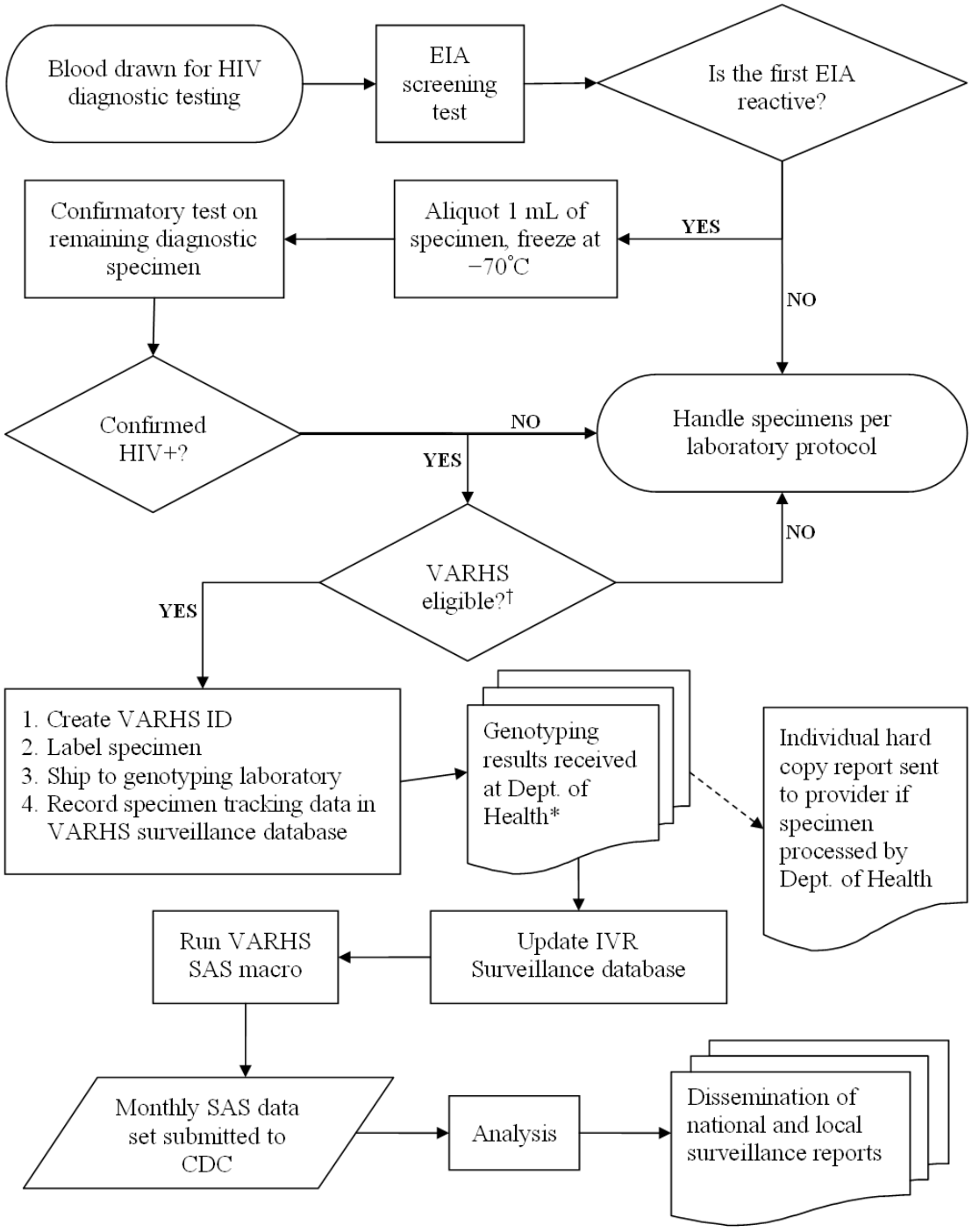
Participating Surveillance Areas

The following surveillance areas were funded to participate in VARHS (including sites that participated in the antiretroviral drug resistance testing [ARVDRT] evaluation project) as of 2004.

1. Chicago Department of Public Health
2. Colorado Department of Public Health and Environment
3. District of Columbia Department of Health
4. Florida Department of Health
5. Illinois Department of Public Health
6. Indiana State Department of Health
7. Louisiana Office of Public Health
8. Maryland Department of Health and Mental Hygiene
9. Massachusetts Department of Public Health
10. Michigan Department of Community Health
11. Mississippi State Department of Health
12. New Jersey Department of Health and Senior Services
13. New York City Department of Health and Mental Hygiene
14. New York State Department of Health
15. North Carolina Department of Health
16. Pennsylvania Department of Health
17. Puerto Rico Department of Health
18. Seattle and King County Public Health
19. South Carolina Department of Health
20. Texas Department of State Health Services
21. Virginia Department of Health
22. Washington State Department of Health

Appendix C

Epidemiologic Flowchart for Variant, Atypical, and Resistant HIV-1 Surveillance

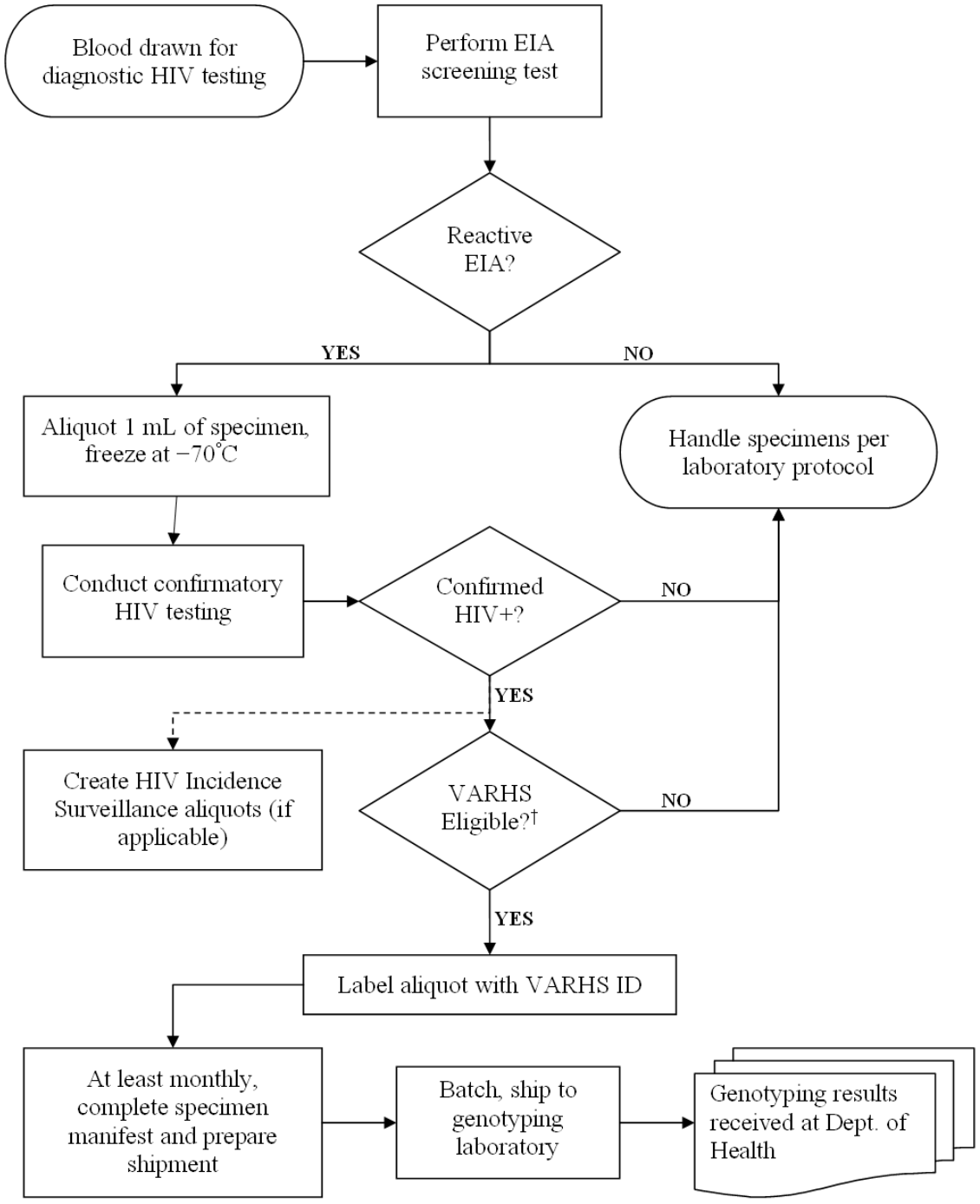


† See technical guidance section on determining eligibility.

* If sequences are received from commercial laboratories, Dept. of Health should determine eligibility.

Appendix D

Laboratory Flowchart for Variant, Atypical, and Resistant HIV-1 Surveillance



† See technical guidance section on determining eligibility.

Appendix E

Technical Guidance for the Processing, Storage, and Shipment of Variant, Atypical, and Resistant HIV-1 Surveillance (VARHS) Specimens to Genotyping Laboratories for Antiretroviral Drug-Resistance Testing

Purpose

This technical guidance describes the methods for the processing, storage, and shipment of VARHS serum and plasma specimens that will be tested for HIV antiretroviral drug resistance and HIV-1 subtyping.

Introduction

Serum or plasma from HIV diagnostic specimens should be collected and frozen at –70ºC. For the purpose of resistance testing, serum should ideally be separated within 48 hours after the blood draw and frozen within 96 hours after the blood draw. Frozen serum or plasma will be shipped to a CDC-designated testing laboratory for genotypic analysis.

Note: Surveillance areas may elect to store a backup aliquot for use in the event that something happens to the original aliquot sent to the laboratory or if a specimen needs to be retested for any reason.

Setting and Personnel Required for Specimen Processing

- Centrifugation, aliquoting, and shipping should be performed at or under the auspices of a laboratory that has been certified by CLIA (Clinical Laboratory Improvement Amendments of 1998) to handle HIV-positive specimens.

  Note: All personnel handling specimens should receive training in handling bloodborne pathogens. (See OSHA's [Occupational Safety and Health Administration's] standards regarding occupational exposure to bloodborne pathogens at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10051.)

- Personnel handling or processing specimens should receive training in the relevant laboratory techniques for handling HIV-positive specimens and for performing the specific tasks required.
- The setting in which centrifugation, aliquoting, and shipping are done should meet Biosafety Level 2 specifications, required by the US Department of Health and Human Services, for the handling of HIV specimens (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s2.htm).

Materials

The following materials are required for the collection and shipment of VARHS specimens:

- Cryogenic vials: 1.5 to 2 mL, with screw cap, external threads, O-ring, and made of polypropylene.

- Freezer labels that will remain on the tubes upon freezing. Many cryogenic vials bear a label area that is suitable for writing with a permanent marker. Writing on the printed area, instead of affixing a label, is acceptable.
- Cardboard storage boxes for cryogenic vials: 81 spaces per box.
- Low temperature freezer: –70ºC.
  - Keep a daily temperature log to ensure that the freezer is operating properly.
  - House the freezer in a location with proper ventilation to avoid overheating and freezer failure.
  - Be sure that the –70ºC freezer has enough space for the storage of VARHS specimens.
- A supply of dry ice in pellet form.
- Saf-T-Pak (http://www.saftpak.com) STP 320 insulated shipping containers certified for frozen diagnostic specimens (i.e., certified for HIV-positive specimens and dry ice).
- Courier's airbills.
- Materials for packing the shipping container (see 2.4).

Specimen Collection and Processing

1. All processing of specimens should be done by personnel qualified to handle HIV-positive specimens under the auspices of a laboratory equipped to handle HIV-positive specimens (http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s2.htm).

2. If the amount of serum seems to be sufficient for all diagnostic needs and HIV incidence testing, transfer an aliquot from the collection tube to the corresponding vial for HIV drug-resistance testing as soon as possible after separation (1 mL per cryogenic vial is optimal).

3. Ship the aliquot to the Stanford University laboratory or to the locally contracted genotyping laboratory. Surveillance areas may elect to store a backup aliquot for use in the event that something happens to the original aliquot sent to the laboratory or if a specimen needs to be retested for any reason.

4. Place the aliquots in cardboard boxes for cryogenic vials in a –70ºC freezer. Optimally, freeze the aliquot within 96 hours after the blood draw.

5. The aliquots frozen as potential specimens for HIV drug-resistance testing remain HIV diagnostic specimens until all HIV diagnostic tests and any other basic laboratory tests have been completed. If additional serum is needed for diagnostic testing or HIV incidence testing, the aliquot for potential HIV drug-resistance testing should be thawed and used for those purposes. If a backup specimen was stored, the backup specimen should be used first. If more volume is required, the specimen designated for the genotyping laboratory may also be used.

6. After a positive result from an HIV confirmatory test or at the time determined by standard laboratory procedures, label each cryogenic vial with the appropriate VARHS specimen ID. The VARHS ID is a 14-digit number that is assigned to each specimen sent for genotyping.

   The 14-digit VARHS specimen ID consists of the following:

   - The 4 digits that denote the **surveillance area** (digits 1–4), which correspond to the Federal Information Processing Standard (FIPS) code for the surveillance area (state or city)
   - The 4-digit **site number** (digits 5–8), which designates where the blood draw was performed
   - The last 2 digits of the **year of the blood draw** (digits 9–10)
   - The 4-digit **sequence number** (digits 11–14), which is assigned by the local health department

7. Every specimen that is eligible for VARHS should be entered into a specimen handling and processing log or a database. Each surveillance area is expected to develop its own log or database or to use the Microsoft Access database provided by CDC. In addition, each surveillance area is responsible for ensuring that all information for each specimen is logged before the specimen is shipped.

Shipping

1. Preliminary procedures for shipping to the Stanford University genotyping laboratory (CDC contract laboratory)

1.1. Specimens for genotypic resistance testing should be sent to the Stanford University laboratory. All specimens should be shipped as diagnostic specimens, according to International Air Transport Association (IATA) Packing Instruction 650. Dry ice should be included with each shipment, as specified in IATA Packing Instruction 904.

1.2. Because shipping specimens involves using dry ice, shipping personnel must be trained and certified to ship dangerous goods. (See Appendix E-1 for a list of companies that provide training.)

1.3. Establish contact with the point person at the Stanford University laboratory.

- Stanford Virology Main Lab: 650-498-5575
- Stanford HIV Genotyping Laboratory: 650-725-7165
- Christina Trevino: 650-725-7165
- Mary Arroyo: 650-725-4146

1.4. A "test" shipment must be sent before the first shipment of VARHS specimens to ensure that all procedures are in place. The test shipment should exactly duplicate a real shipment (i.e., ship frozen liquid in cryogenic vials on dry ice) and should be sent only once. (See the procedures outlined in 1.5–1.8.)

The purpose of the test shipment is to familiarize the sender with the processes for notifying the laboratory about the shipment and for packing and shipping the diagnostic specimens on dry ice. Shipping frozen water on dry ice without the infectious substance labels will accomplish the purpose of the test shipment.

1.5. Arrange for the preparation of the test shipment and the initial shipment to be overseen by laboratory staff experienced in the shipment of comparable specimens.

1.6. Notify the Stanford University laboratory that the test shipment is on the way by calling the main laboratory at 650-725-7165 or by e-mailing (do not e-mail or fax the shipping manifest).

In the call or e-mail, include the number of specimens being shipped and if applicable, the FedEx tracking number. Per CDC's *Technical Guidance for HIV/AIDS Surveillance Programs, Vol. III: Security and Confidentiality Guidelines* (13), do not e-mail or fax the shipping manifest (the manifest is included in the shipment).

1.7. Be sure to have an adequate number of STP 320 shipping containers or the equivalent are on hand. The Stanford laboratory will return these containers to the shipping laboratory (they are expensive and should be reused).

1.8. Be sure to have an adequate supply of the courier's airbills on hand.

2. Packing procedures for shipping to the Stanford University laboratory

2.1. Specimens must be shipped on the same day that they are packed. Plan to begin the packing process early so that the container(s) will be ready for the courier's last pick-up of the day. ***Pack and ship only Monday through Wednesday. Never pack and ship the week of Thanksgiving, Christmas, New Year's, or July Fourth***.

2.2. Personnel in the shipping laboratory should read and walk through all of these steps before starting to prepare the actual shipment in order to be familiar with the requirements.

2.3. Wear gloves and a lab coat.

2.4. Bring the STP 320 shipping container and other packing materials into the area where the shipment is being prepared. Check to be sure that the container includes the following items:

- 2 sheets of bubble wrap
- 2 STP 710 2-part secondary pressure vessels or equivalent certified containers
- Two 250 mL absorbent strips
- Class 9 label and label for the quantity of dry ice
- Other hazard and handling labels
- 1 instruction sheet

2.5. For a diagram of the contents of the shipping container, see the Saf-T-Pak catalog (http://www.saftpak.com). Of the listed contents, use only what is needed for each shipment. Save leftover supplies for future shipments.

2.6. If the STP 320 shipping container is being reused, the labels will already be in place on the outer cardboard container.

2.7. Be sure that adequate supplies of the other materials listed in 2.4 are on hand.

2.8. Prepare 3 copies of the shipping manifest (Appendix E-2). On the manifest, write the VARHS ID number on each vial to be shipped, along with the date the specimen was drawn and the date the specimen was frozen (freeze date).

Note: ***The freeze date entered here should be the last freeze date for the aliquot being shipped.***

Indicate (circle) whether the specimens are serum or plasma, and record the volume being shipped if it is less than 1 mL. Bring the copies of the manifest into the area where the shipment is being prepared.

- Copy 1 of the shipping manifest should be included in the shipment to the Stanford laboratory.
- Copy 2 of the shipping manifest should be sent to CDC (see 3.2). Send through SDN (preferred) or the US Postal Service.
- Copy 3 of the shipping manifest should be kept by the surveillance area's VARHS coordinator or the laboratory sending the specimens to the Stanford laboratory.

2.9. Prepare the courier's airbill (Appendix E-3); Stanford will use the airbill to return the shipping container for reuse. On the airbill, fill in the shipping laboratory's complete return address, Stanford University's address, and the billing number. Staple the airbill to the shipping box return form. Bring the copy of the airbill into the area in which the shipment is being prepared.

2.10. If dry ice is in another location, which requires leaving the area where the shipment is being prepared, put the dry ice needed for this shipment in a separate container and bring it to the shipping area.

2.11. Go to the freezer and remove the entire 2-inch freezer box containing the specimens to be sent.

2.12. Bring the specimens to the area where the shipment is being prepared. ***Specimens are to remain frozen at all times and therefore should not be removed from a –70°C environment for more than a few minutes.***

2.13. Recheck the screw-cap lids on the specimen vials and tighten if necessary.

2.14. Place the freezer box containing the specimens in the secondary pressure vessel (leak-proof) and make sure the specimens are surrounded by bubble wrap and absorbent strips. The vials should not move around or rattle inside the vessel.

2.15. Place the secondary pressure vessel in the inner box and place the inner box in the polystyrene cooler.

2.16. **Do not** put dry ice inside the inner box.

2.17. Pack pelleted dry ice in the shipping container and around the inner box. The STP 320 shipping container will hold ~8 kg of dry ice (~10 lbs); if the cooler is packed completely, this quantity of dry ice will keep the contents frozen for more than 80 hours.

2.18. Place the lid on the polystyrene cooler.

2.19. Fold 1 copy of the VARHS shipping manifest in half and place it on top of the shipping box return form with the airbill that Stanford laboratory will use to return the shipping container to the shipping laboratory (for reuse), and place on top of the polystyrene lid.

2.20. Fold over the top flaps of the outer box and seal with clear shipping tape.

2.21. Place the category B label ("UN 3373") over the 2½" × 2½" diamond-shaped outline on the outer box.

2.22. Write "Diagnostic Specimens" on the outer box, adjacent to the category B label.

2.23. Place the Class 9 hazard label over the 4" × 4" diamond-shaped outline.

2.24. Place the label for the net quantity of dry ice over the rectangular outline adjacent to the Class 9 hazard label. On the label, write the approximate amount (in kg) of dry ice used to pack the container.

Note: The labels (category B designation, Class 9 hazard, and quantity of dry ice) can be purchased.

2.25. Prepare the courier's paperwork as directed by the training and certification course and select the overnight shipping option.

2.26. If the preceding steps are not completed before the courier's last pickup of the day, unpack the specimens and put them back in the –70ºC freezer and begin the process again on the next appropriate day.

3. Procedures for shipping to the Stanford University laboratory

3.1. Ship only Monday through Wednesday. Never ship the week of Thanksgiving, Christmas, New Year's, July Fourth, or major local holidays.

3.2. Send the second copy of the shipping manifest to the CDC Laboratory Liaison, Richard Kline, through the SDN (preferred) or the US Postal Service. On the manifest, indicate the date that the specimens were shipped to the Stanford laboratory. ***Do not e-mail or fax the shipping manifest to CDC or to the Stanford laboratory.***

Mailing Address:

Attn: Richard Kline
Centers for Disease Control and Prevention
1600 Clifton Rd, NE, MS E-47
Atlanta, GA 30333
Phone: 404-639-4958

3.3. Keep the third copy of the shipping manifest.

3.4. Notify the Stanford laboratory that a shipment is on the way by calling the HIV genotyping laboratory at 650-725-7165 or by e-mailing (do not e-mail or fax the shipping manifest).

In the call or e-mail, include the number of specimens being shipped, the date the shipment will be sent, and if applicable, the FedEx tracking number. ***Do not e-mail or fax the shipping manifest (the manifest is included in the shipment).***

3.5. Track the shipment by using the FedEx tracking number (10–12 digits) on the airbill. Tracking can be done through the Web site (http://www.fedex.com) or by calling 1-800-GO-FEDEX.

If a problem is identified, please notify

Stanford HIV Genotyping Laboratory: 650-725-7165
and
Richard Kline (CDC): 404-639-4958

3.6. The Stanford laboratory will contact the shipping laboratory if expected shipments are not received.

4. Preliminary procedures for shipping to a locally contracted genotyping laboratory

Follow the same procedures, substituting the locally contracted laboratory's contact information for the Stanford laboratory's contact information (see 1.3 and 1.6).

5. Packing procedures for shipping to a locally contracted genotyping laboratory

Follow the procedures listed above, substituting local procedures as applicable and substituting the locally contracted laboratory for the Stanford laboratory.

6. Procedures for shipping from the processing laboratory to a locally contracted genotyping laboratory

Follow the procedures listed above, substituting local procedures as applicable and substituting the locally contracted laboratory for the Stanford laboratory.

7. Procedures for local transport from the processing laboratory to a locally contracted genotyping laboratory

7.1. Follow the procedures listed above or substitute local procedures as discussed with Richard Kline at CDC to ensure that specimens remain frozen during transport and until ready for genotyping at the genotyping laboratory. Always include a copy of the shipping manifest in the shipment.

7.2. Whenever specimens are transported, send a copy of the specimen manifest to Richard Kline at CDC through the SDN (preferred) or the US Postal Service. On the manifest, indicate the date that the specimens were shipped to the genotyping laboratory. ***Do not e-mail or fax the shipping manifest to CDC or the genotyping laboratory***.

Mailing Address:

Attn: Richard Kline
Centers for Disease Control and Prevention
1600 Clifton Rd, NE, MS E-47
Atlanta, GA 30333
Phone: 404-639-4958

7.3. Transport specimens only Monday through Thursday, unless local guidelines specify otherwise. If local HIV surveillance or laboratory staff are not personally handling the transport, do not transport specimens during the weeks of Thanksgiving, Christmas, New Year's, July Fourth, or major local holidays.

7.4. Track the shipment through FedEx's or another courier's tracking system.

If there are problems with the transfer, please notify

Richard Kline (CDC): 404-639-4958
and
the appropriate personnel at the genotyping laboratory

Develop an acknowledgment system so that the genotyping laboratory informs the shipping laboratory when the shipment has arrived.

8. Local transfer from the processing area to the genotyping area within one laboratory (i.e., the HIV diagnostic testing and genotyping for HIV drug resistance are performed within the same laboratory)

If HIV testing and genotyping are performed in the same laboratory, diagnostic testing is complete, and aliquots are identified as VARHS specimens, do the following:

8.1. Send a copy of the shipping manifest (Appendix E-2) to Richard Kline at CDC through the SDN (preferred) or the US Postal Service. On the manifest, indicate the date that the specimens were shipped to the genotyping laboratory. ***Do not e-mail or fax the shipping manifest to CDC or the genotyping laboratory.***

Mailing Address:

Attn: Richard Kline
Centers for Disease Control and Prevention
1600 Clifton Rd, NE, MS E-47
Atlanta, GA 30333
Phone: 404-639-4958

8.2. Ideally, the initial freezing of specimens should be done in a freezer convenient to the genotyping laboratory so that it will not be necessary to transfer frozen specimens.

8.3. If frozen specimens are to be transferred from a freezer in one area to a freezer in another area, a biohazard bag, cooler, and dry ice or cool packs should be ready before the specimens are removed from the freezer. Place the specimens (in their storage box) in the biohazard bag. Place the bag in the cooler with dry ice or cool packs and transport as quickly as possible to the other freezer.

8.4. If there are problems with the transfer, please notify

Richard Kline (CDC): 404-639-4958
and
the appropriate personnel at the genotyping laboratory

Appendix E-1. Training and Certification for Shipping Infectious Substances

The following are some of the companies that provide training in shipping dangerous goods. CDC does not endorse any particular company.

- **FedEx**
  - 1-800-GO-FEDEX
  - 3-day IATA-based training
  - Covers all hazardous materials
  - Cost: ~$550

- **Saf-T-Pak**
  - 1-800-814-7484
  - Specifically for infectious and diagnostic substances and dry ice
  - 3 options: 1-day seminar, on-site program, or interactive CD (can be completed in 3–5 hours)
  - Certificate: valid for 2 years or until regulations change
  - Cost: ~$250

- **Viking Packing Specialist** (Oklahoma)
  - 1-800-788-8525; Contact: David Weilert
  - Monthly seminars in Tulsa
  - Covers all 9 classes of hazardous materials
  - Covers shipping according to IATA instructions
  - Certificate: good for 2 years
  - Cost: ~$300 per person
  - Group classes in local area: ~$3,000 plus travel costs

Appendix E-2. CDC VARHS Shipping Manifest

Stanford Hospital and Clinics
300 Pasteur Drive
Stanford, CA 94035

CDC VARHS Shipping Manifest

Clinical Virology Laboratory @ Hillview
(650) 498-5575
Directors: R. Sibley, MD / B. Patterson, MD

Date specimens sent to Stanford: _____

Link project number (VARHS ID number)	**Draw Date**	**Freeze Date**	**Serum or Plasma** (CIRCLE)	**Volume** (if < 1 mL)	**Stanford Laboratory number** (STANFORD USE)
			Serum or Plasma		
			Serum or Plasma		
			Serum or Plasma		
			Serum or Plasma		
			Serum or Plasma		
			Serum or Plasma		
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			Serum or Plasma		
			Serum or Plasma		

Shipping Instructions: 1) Call Stanford Virology Laboratory (650) 725-7165 when package is sent (keep a record of tracking number)
2) Complete this manifest form and include a copy with the specimens
3) Package and ship on Monday, Tuesday, or Wednesday only
4) Ship to: Attn: Virology Laboratory Specimen Processing
Stanford Clinical Lab at Hillview
3375 Hillview Ave
RM 2401
Palo Alto, CA 94304-1204

Mail hard copy of the report to: Attn: _____

Location _____

Street Address _____

City _____

State _____ ZIP code _____

Contact person name and phone number: _____; ( ) __ __ __ - __ __ __ __

Stanford Virology Test Code: AVRT

For office use only: **Billing Done**: _____

Original: **Box #**_____

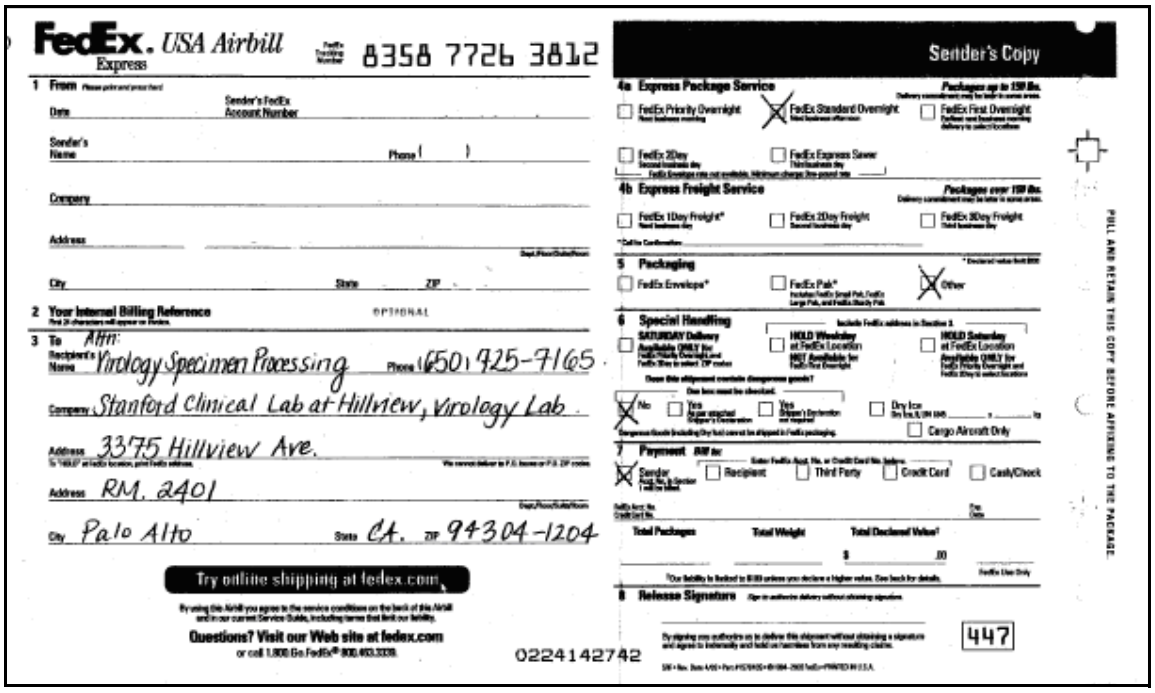
Extract:: **Box #**_____

Product: **Box #**_____

Appendix E-3. Stanford Laboratory's Return of Shipping Container

Sender: Attach return FedEx airbill. Complete all sections except the date, which will be completed at the Stanford laboratory. See example below.

Stanford Virology Receiving Desk: Give the empty shipping container, packing materials, and the return FedEx airbill to Hina, in Virology, for return to sender.



FedEx. USA Airbill Express

8358 7726 3812

Sender's Copy

1 From
Date
Sender's FedEx Account Number
Sender's Name
Phone ( )
Company
Address
City State ZIP

2 Your Internal Billing Reference OPTIONAL

3 To Attn:
Recipient's Name Virology Specimen Processing Phone (650) 725-7165
Company Stanford Clinical Lab at Hillview, Virology Lab
Address 3375 Hillview Ave.
Address RM. 2401
City Palo Alto State CA. ZIP 94304-1204

Try online shipping at fedex.com

Questions? Visit our Web site at fedex.com
or call 1.800.Go.FedEx® 800.463.3339.

0224142742

4a Express Package Service — Packages up to 150 lbs.
☐ FedEx Priority Overnight ☒ FedEx Standard Overnight ☐ FedEx First Overnight
☐ FedEx 2Day ☐ FedEx Express Saver

4b Express Freight Service — Packages over 150 lbs.
☐ FedEx 1Day Freight* ☐ FedEx 2Day Freight ☐ FedEx 3Day Freight

5 Packaging
☐ FedEx Envelope* ☐ FedEx Pak* ☒ Other

6 Special Handling
SATURDAY Delivery ☐
HOLD Weekday at FedEx Location ☐
HOLD Saturday at FedEx Location ☐
Does this shipment contain dangerous goods?
☒ No ☐ Yes ☐ Yes ☐ Dry Ice ☐ Cargo Aircraft Only

7 Payment Bill to:
☒ Sender ☐ Recipient ☐ Third Party ☐ Credit Card ☐ Cash/Check

Total Packages Total Weight Total Declared Value* $ .00

8 Release Signature

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PULL AND RETAIN THIS COPY BEFORE AFFIXING TO THE PACKAGE.

Appendix F

VARHS Data Elements

Table 1

The following data elements are used for estimations of the national prevalence and incidence of transmitted HIV-1 drug resistance and distribution of HIV-1 subtypes and for making estimates for major subpopulations.

Note: Most of the data elements are collected through routine HIV case surveillance.

Data Element	HIV-1 Drug Resistance Prevalence	HIV-1 Subtype Distributions
Demographic Data		
Age	X	X
Sex	X	X
Race/ethnicity	X	X
Transmission category for HIV infection	X	X
Country of origin	X	X
Current state of residence	X	X
State of residence at HIV diagnosis	X	X
Laboratory Data		
HIV pol gene nucleotide sequence	X	X
Mutation-specific assays	X	X
Previous HIV Testing Data		
Date of first HIV test	X	
Date of first positive HIV test	X	
Date of last negative HIV test	X	
Clinical Data		
Date of AIDS diagnosis	X	
CD4 counts	X	
Dates of CD4 counts	X	
Viral load	X	
Dates of viral loads	X	
Antiretroviral agents used	X	
Start/end dates of antiretroviral agent use	X	
Opportunistic infection(s) diagnosed	X	
Date(s) of opportunistic infection diagnosis	X	

Table 2. Required Data Elements for Specimen Tracking

The following required laboratory data elements are used for local tracking of specimens, evaluating problems with amplification of HIV for genotyping, supporting plans to optimize specimen handling processes for surveillance purposes, and evaluating problems with contamination.

Name	Label
SPECIMEN ID	VARHS ID number
DRAWDTTM	Date/time of blood draw
SPECSOURCE	Was specimen from a VARHS site or a commercial/private laboratory?
ACCESNUM	Laboratory accession number (if applicable)
TUBE TYPE	Blood collection method
BLDCOMP	Type of specimen sent from collection site
BLDLAB	Type of specimen sent for resistance test
VOLGENO	Volume collected for genotype aliquot
GFRZ1DTM	Date/time of first freeze of aliquot
NOT SENT	Was specimen sent for genotyping?
AMPLIFY	Was this specimen amplified?

Table 3. Optional Data Elements for Specimen Tracking

The following laboratory data elements are optional **unless** low amplification rates (<90%) are seen in a particular surveillance area. In this case, all of the specimen tracking data elements listed in Table 3, in addition to Table 2, will be required. The additional elements must be collected until the amplification problem has resolved or until CDC can conclude that the problem is not associated with specimen handling/storage procedures.

Name	Label
COURDTTM	Date/time of courier pick-up from collection site
GRESDATE	Date/time genotyping results received at DOH
DRECDTTM	Date/time of receipt in diagnostic laboratory
CENTDTTM	Date/time of centrifugation
SEPDTTM	Date/time of separation
ALIQDTTM	Date/time of aliquoting
VOLUME	Volume of serum/plasma after separation
COND	Specimen condition
SPECIMEN FROZEN	Was this specimen frozen prior to aliquoting?
GFRZ2+YN	Was the genotyping aliquot thawed or refrozen?
GTHW1DTM	Date/time of first thaw of genotyping aliquot
GFRZ2DTM	Date/time of second freeze of genotyping aliquot
GSHPDATE	Date of shipment to genotyping laboratory
BFRZ1DTM	Date/time of first freeze of local back-up aliquot
BTHW1DTM	Date/time of first thaw of local back-up aliquot
BFRZ2DTM	Date/time of second freeze of local back-up aliquot
VOLBACK	Volume of local back-up aliquot