2018 Handbook on HIV Drug Resistance Testing Availability, Accessibility and Capacity for Caribbean States

DISCLAIMER

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Arlene Darmanie (Chair, Caribbean Regional Reference Laboratory Group for HIV Laboratory Services, Caribbean Public Health Agency (CARPHA)) lead co-ordination of the core development team.

The following individuals also served as key consultants for the development of this handbook as part of the core development team: **Giselle Guevara** (CDC Caribbean Regional Office). **Valerie Wilson** (Caribbean Med Labs Foundation). **Tina Hylton-Kong** (University of the West Indies, Jamaica). **Nayra Rodriguez** (AIDS Research Program-Immunology Reference Laboratory, Puerto Rico). **Georges Dos Santos** (Service de Virologie Immunologie, CHUM, Martinique). **Indira Martin** (Ministry of Health, The Bahamas). **Michelle Hamilton** (National Public Health Laboratory, Jamaica). **Virginia Asin-Oostburg** (Caribbean Public Health Agency (CARPHA)). **Songee Beckles** (Best-Dos Santos Public Health Laboratory, Barbados).

PURPOSE

The purpose of this handbook is to promote and facilitate access to quality lab services for monitoring and surveillance of HIV drug resistance (HIV DR) in the Caribbean.

The primary audience of this handbook includes HIV National Program managers and officers, managers and technicians from public health and molecular biology labs, as well as service providers involved in HIV care and treatment.

MAIN OBJECTIVES

Provide guidance to Caribbean countries on how to access HIV genotyping services. The handbook includes:

- A general overview and basic concepts of HIV drug resistance (HIV DR) and HIV DR testing.
- Information on the HIV DR test service providers available for Caribbean countries.
- Guidelines and documents for preparation and shipping of specimens and samples for HIV DR testing by chosen service provider, including estimations of the cost and turnaround times of HIV DR testing from service providers.

INTRODUCTION

The objective of antiretroviral therapy (ART) is to suppress HIV replication so that viral load is maintained at undetectable levels by standard laboratory detection methods. In turn, this allows the immune system (typically measured via CD4+ T cell count) to reconstitute, halting disease progression to acquired immunodeficiency syndrome (AIDS).

In the case of suboptimal ART concentration environments, virus with mutations that reduce their susceptibility to treatment and allow viral replication in the presence of

antiretrovirals (ARVs) may be preferentially selected, eventually leading to detectable virological rebound and treatment failure. In addition, the presence of pre-therapy HIV DR can be detrimental for virological suppression in persons that re-start ART after treatment interruption, especially with non-nucleoside reverse transcriptase inhibitor (NNRTI)-based treatment regimens (Hamers et al., 2012, Johnson et al., 2008).

Several studies have shown that patients, whose HIV treatment and care providers use genotypic HIV DR data to manage their disease, could have better therapy outcomes than those without (Durant et al., 1999, Baxter et al., 2000, Falloon, 1999).

On the other hand, current approaches to resistance testing remain too costly and complex for routine use as part of a public health approach, especially in limited resource settings (WHO, 2016, WHO, 2017b), and the World Health Organization (WHO) does not currently recommend routine HIV DR testing to guide ART regimen selection. Nevertheless, middle-income countries increasingly use HIV DR testing to inform treatment decisions, and WHO recognizes the value of resistance testing for individual patients in such situations, provided that adequate treatment options are available and in-country expertise exists to properly interpret results. To inform population-level decision-making, the WHO recommends routine surveillance for HIV DR in populations initiating ART and in populations on ART for 12 months and more than 48 months. The results of these surveys support the choice of recommended first- and second-line ART, pre- and post-exposure prophylaxis (WHO, 2016).

There is currently limited information on HIV DR levels in the Caribbean. A meta-analysis, published in 2016, comparing pre-treatment drug resistance between the years 2000-2005 and 2006-2015 showed statistically significant increases in resistance to all major ARV drug classes used for 1st and 2nd line treatment in the Caribbean (RTIs and protease inhibitors (PI) respectively), the most significant increase being NNRTI resistance (Santiago Avila-Rios, 2016). It is important to note the under-representation of Caribbean states in this study as HIV DR data were only available from Cuba, the Dominican Republic and Jamaica. Therefore, although currently anecdotal for the Caribbean

because of limited HIV DR surveillance data, this has been a documented trend worldwide (WHO, 2017c). Even though it is not far-fetched to think that these findings could be applicable to the Caribbean, HIV DR surveillance initiatives need to be expanded and accelerated in order to generate local evidence for programmatic decision-making and public health actions to optimize treatment and improve outcomes.

Caribbean countries have committed to the 90-90-90 targets and a vision of ending AIDS by 2030, but while national programs are scaling-up ART, HIV DR, particularly NNRTI resistance, is slowly increasing and may become a threat to achieving these goals.

The WHO has developed new guidelines to address emerging drug resistance from a public health perspective (WHO, 2017c) and there is a renewed commitment to strengthen HIV DR surveillance at national level. In addition, support from the international community of technical cooperation partners has been called upon in the new WHO Global Plan of Action to prevent and address HIV DR (WHO, 2017a). Therefore, Caribbean countries should make sure that HIV DR surveillance strategies are in place, based on WHO guidance and with HIV genotyping performed by WHO designated laboratories. Countries that are adopting HIV genotyping as a monitoring tool for clinical decision making, along with the WHO empiric approach to ART switch at time of failure, should also make sure to access quality laboratory services for HIV genotyping.

HIV DR Basic Concepts

All mutations involved in conferring HIV drug resistance are not equal. Some single mutations confer enough resistance to render an ARV or multiple ARVs in a drug class ineffective. For example the mutation I84A/C in HIV protease, which represents the amino acid isoleucine (I) at position 84 of HIV protease mutated to alanine (A) or cytosine (C), is a more uncommon mutation, but when present, confers high level resistance to all protease inhibitors (PIs) currently used in ART regimens (Liu and Shafer).

Some mutations have little effect on drug susceptibility until they co-exist with other mutations, which is when they confer drug resistance. The thymidine analogue mutation (TAM) M41L in HIV reverse transcriptase confers low-level resistance to AZT, however when present with T215Y, together they confer significant resistance to multiple nucleoside reverse transcriptase inhibitors (NRTIs) (Liu and Shafer).

Some mutations simultaneously confer significant resistance to one drug while improving susceptibility to another. M184V/I in HIV reverse transcriptase confers significant resistance to NRTIs, 3TC and FTC, less significant resistance to ABC and ddI, while simultaneously improving susceptibility and slowing down the development of TAMs within the NRTI drug class (Liu and Shafer).

Most DRMs cause the mutated virus to replicate less efficiently compared to the un-mutated virus (termed wildtype virus), but there are additional mutations that can restore the replication capacity of the drug resistant virus. For example HIV protease mutations V82T, M36I and I54V together confer significant resistance to some PIs and the replication capacity of this virus can be less than 50% of the wildtype virus (Nijhuis et al.). But when the compensatory mutations and A71V is also present, the replication capacity of the drug resistant virus is restored to 100% (Nijhuis et al.).

Sub-optimal ART as a result of non-adherence to HIV treatment regimens remains the major route to HIV DR development and treatment failure. Drug resistant HIV strains may be transmitted, establish HIV infection in new individuals and affect future treatment

effectiveness and outcomes (WHO/HIVResNet, 2017). Pre-treatment resistance from previous exposure to ARV (e.g. ARV regimens for prevention of mother-to-child transmission of HIV like single-dose NVP) may also cause treatment failure, but this has been largely diminished because of updated HIV prophylaxis and treatment guidelines based on the findings of extensive surveillance studies on the topic.

The evolutionary biology of HIV can also influence DRV susceptibility. For example, HIV type 1 (HIV-1) is innately susceptible to non-nucleoside reverse transcriptase inhibitors (NNRTIs), while type 2 (HIV-2) is innately resistant to NNRTIs because HIV-2 does not contain region that NNRTIs need to bind to inhibit viral replication. In addition, the HIV-1 subtype can affect drug susceptibility. The best example of this is the increased prevalence of K65R selection in the reverse transcriptase gene of HIV-1 subtype C because of a viral mechanism that favors this mutation, particularly in this subtype (Coutsinos et al., 2011). The K65R mutation confers intermediate- or high- level resistance to all NRTIs except zidovudine (AZT).

A lack of bioavailability of ARVs is yet another factor that can influence the drug susceptibility of HIV. A prime example of reduced bioavailability due to drug-drug interaction that can cause suboptimal ART and consequent selection of HIV DR is the anti-tuberculosis drug, rifampicin, which reduces plasma concentrations of the NNRTI, namely efavirenz (EFV) and nevirapine (NVP); as much as ~30% and ~60% reduced plasma concentrations respectively (Maartens et al.).

HIV DR Genotypic Testing Approaches

HIV DR genotyping is a complex technology. All WHO HIVResNet Laboratory Network laboratories should have detailed and approved laboratory protocols for procedures to allow the collection of comparable genotyping information. If laboratory capacity is not available in-country and planners wish to develop such capacity, a WHO-designated laboratory can assist with protocol development (Appendix B).

GENOTYPIC TESTING

Genotypic HIV DR testing involves sequencing of key regions of the HIV genome and analysis of these sequences. Single amino acid changes (point mutations) in antiviral targets of viral proteins are the most common form of HIV DR. These point mutations disallow ARVs from binding to their targets in viral proteins. Multiple point mutations that can confer resistance to multiple drug classes cumulatively reduce HIV's susceptibility to cART. Such mutations can be determined genotypically and phenotypically. In clinical practice, genotypic and phenotypic HIV DR testing characterize antiviral activity and/or resistance profiles of the drug. HIV DR conferring point mutations can be identified by DNA sequence analysis of the part of the viral genome that encodes the relevant proteins.

HIV DR TEST CHARACTERISTICS

Commercially available assays that are routinely used for HIV DR have established performance characteristics that can validate their use. Commercial HIV DR tests are available on the market and validated "in-house" / "homebrew" methods are also used; none are considered the gold standard. The WHO recommends (WHO/HIVResNet, 2017):

1. **ViroSeq TM HIV Genotyping Kit**

   Vendor: Abbott Molecular

   Content: Protocols and reagents for sample extraction, amplification and sequencing of the regions of the HIV genome that encode full-length protease (amino acids 1-99) and most of reverse transcriptase (amino acids 1-320).

   Website: https://www.abbottmolecular.com/us/products/viroseq-hiv-1-genotyping-system.html

2. **GeneThink TM HIV-1 Genotyping Kit**

   Vendor: Research Think Tank

Content: Protocols, controls, reagents, sequencing instrument, product support and a fully integrated reporting software for amplification and sequencing of the regions of the HIV genome that encode the clinically relevant portions of protease (amino acids 10-99) and reverse transcriptase (amino acids 41-237).

Website: http://www.researchthinktank.com/

3. US CDC HIV-1 Pan Group-M Drug Resistance Genotyping Assay

Vendor: Thermo Fisher

Content: Protocols and reagents for amplification and sequencing of the HIV DR relevant regions of the HIV genome that protease (amino acids 13-99) and reverse transcriptase (amino acids 1-251). It can detect HIV DR in plasma or dry blood spots derived from multiple HIV-1 subtypes and circulating recombinant forms with a viral load ≥1000 copies/ml.

Website: http://www.thermofisher.com/

4. "Home-brew" or “in-house” Methods

“In-house” methods (also known as “home-brew” methods) commonly use reagents that are not marketed as HIV DR genotyping kit. Reagents and procedures can be less standardized than commercial kits. Advantages of these methods are: processing price per sample is significantly less than commercial kits; home-brew methods can also be more adaptable than kit-based methods.

As the Caribbean continues to develop HIV DR testing and surveillance capacity, the type and the extent of validating performance characteristics can be further discussed and developed in order to create standardized HIV DR testing characteristics guidelines.

OTHER RESOURCES AND ANALYSES

The WHO Global HIV Resistance Network (HIVResNet) Laboratory Strategy is an important resource "to support national, regional, and global HIV DR surveillance and monitoring by the timely provision of accurate genotyping results in a standardized format that meets the WHO specifications." Strategy and concept notes can be found here:

1. Strategy: http://www.who.int/hiv/pub/drugresistance/hiv_reslab_strategy.pdf
2. Concept Notes: http://www.who.int/hiv/topics/drugresistance/protocols/en/

Drug resistance testing and analysis can be complex and there is a lack of standardization of the processes involved. The key quality assurance processes being developed by WHO/HIVResNet involve the use of:

- RECall: HIVResNet tool developed for quality assurance of HIV sequences
- Stanford HIV Database: Stanford HIV Drug Resistance Database to detect the presence of HIV DRMs in HIV sequences
- REGA 3.0: Stanford University Tool to determine the subtype of HIV sequences
- MEGA 7.0: to determine the diversity of HIV sequences

The websites at which these tools are available can be found in Appendix C.

HIV DR Test Service Providers Available to Caribbean States

Table 2. HIV DR test service providers available to Caribbean states. Appendix A provides further details of processes and protocols required for each service provider.

WHO/HIVResNet Designated – WHO designated laboratories that are part of the HIV Resistance Network are fully functional and currently accepting specimens for HIV DR detection and analyses.

FDA Approved Testing – Institutions that provide FDA approved HIV DR detection services are currently accepting specimens for HIV DR detection.

CDC Supported – The CDC supported laboratories in Jamaica and Barbados are currently developing or validating HIV DR testing capacity. These laboratories anticipate accepting specimens for HIV DR testing within the next 12 months.

Table 2. HIV DR service providers.

Service Provider	Country	Designation	Key Contact	Telephone Email	Page#
BC Centre for Excellence in HIV and AIDS	Canada	WHO / HIVResNet designated	Dr. Richard Harrigan	+1-604-806-8775 prharrigan@cfenet.ubc.ca	14-15
Service de Virologie Centre Hospitalier et Universitaire de Martinique	Martinique	WHO / HIVResNet designated	Dr Georges Dos Santos	+ 596 696 31 81 31 Georges.dos-santos@chu-martinique.fr	16-18
Ponce School of Medicine, Immunology Reference Laboratory	Puerto Rico	WHO / HIVResNet designated	Lcda. Nayra Rodriguez-Hornedo	+1-787-841-5150 nrodriguez@psm.edu	19-32
Molecular Pathology Laboratory at New York-Presbyterian/Weill Cornell Medical Center	USA	FDA approved testing	Phyllis Ruggiero	+1-212-746-2994 pcr9004@nyp.org	34-36
Quest Diagnostics	USA	FDA approved testing	Meghan Starolis	+1-703-802-7049 meghan.w.starolis@questdiagnostics.com	37-38
Best-Dos Santos Public Health Laboratory	Barbados	CDC supported	Songee Beckles	+1-246-266-0823 slb5@yahoo.com songee.beckles@health.gov.bb	40-41
National Public Health Laboratory	Jamaica	CDC supported	Dr. Michelle Hamilton	+1-876-317-8583 Hamiltonm@moh.gov.jm	42-44
Reference Lab, Ministry of Health	The Bahamas	CDC supported	Dr. Indira Martin	+1-242-432-9754 indiramartin333@gmail.com	45-47

APPENDIX A

WHO/ HIVRESNET DESIGNATED HIV DR LABORATORIES

PONCE SCHOOL OF MEDICINE, IMMUNOLOGY REFERENCE LABORATORY

Street Address: 395 Industrial Reparada #2, Ponce, Puerto Rico 00716-2348

Director of Department: Vanessa Rivera-Amill, PhD

Email: vrivera@psm.edu

Laboratory Director & Contact for HIV DR: Lcda. Nayra Rodriguez-Hornedo

Email: nrodriguez@psm.edu

Position of Contact Person for HIV DR: Laboratory Director

Phone Number: +1-787-841-5150

Mobile phone: +1-787-317-2411

Fax Number: +1-787-841-5150

Email: nrodriguez@psm.edu

Date this data was collected: 7-Apr-2017

Footnote: This is WHO assigned laboratory for HIV Drug Resistance Testing and part of the WHO HIV Resistance Network.

HIV DR Detection: Methodology Overview

- PI and RTI DRM detection: A single amplicon of full-length Protease and Reverse Transcriptase PR.

Requirements for Sample Preparation and HIV DR Test Request

- At least 700µL of plasma (from whole blood collected in EDTA).
- Each vial containing plasma in the package that is shipped to the Immunology Reference Laboratory for HIV DR testing must be labelled with its Sample ID and correlate to the itemized list of samples you must send with the samples (page 20), or else it will be discarded.
- The completed sample list must be sent with the samples and ideally, a copy of this should be emailed to nrodriguez@psm.edu
- Samples must be shipped to Ponce School of Medicine according to instructions (pages 21-32)

List of Samples For HIV Drug Resistance Testing Shipped To The Immunology Reference Laboratory at Ponce School of Medicine

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	700ul plasma (Yes/No)	Comments
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			

This form must accompany samples shipped to the Immunology Reference Laboratory for HIVDR testing
For assistance, contact Nayra Rodriguez-Hornedo: 1-787-841-5150 or nrodriguez@psm.edu

TRLv1.0

Sample Shipping Instructions for Ponce School of Medicine, Immunology Reference Laboratory

SOP # 2-1
Ponce Health Sciences University
Research Institute
Immunology Reference Laboratory
Laboratory Standard Operating Procedure
Transportation of Biological Specimens

Prepared by: _____ Date: 07-2016
Nayra Rodríguez Hornedo, Lcda.

Reviewed by: _____ Date: 03-2017
Omayra De Jesús Matos, MT ASCP

_____ Date: 03-2017
Nayra Rodríguez Hornedo, Lcda.

Approved by: _____ Date: 03-2017
Vanessa Rivera-Amill, PhD

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1. INTRODUCTION

The purpose of developing this Standard Operating Procedure is as a reference for our laboratory. This is to ensure that the biological specimens are packaged and handled in a suitable manner to safeguard the health, safety and welfare of employees handling the pathological specimens and also to ensure that the specimens are packaged in suitable receptacles and maintained under suitable environmental conditions for transport.
It is the sender responsibility to ensure compliance with all packaging and transport regulations.

2. OBJECTIVE

This procedure is to ensure the proper and safe transportation of all biological materials. This procedure also ensures that the integrity of the specimens is preserved for accurate analysis by the receiving laboratory.

3. SCOPE

This procedure will be used for packing and transporting biological samples in a safe environment. This procedure helps us to preserve the integrity of the specimens.

4. DEFINITIONS

a. **Patients' specimens:** Those collected directly from humans or animals, including, but not limited to, excreta (feces & urine), secretions (body fluids), blood and its components, tissue (including fresh tissue, preserved tissue, paraffin blocks and glass slides), swabs, and body parts being transported for purposes such as diagnosis, research, investigational activities, disease treatment and prevention.

b. **Dangerous Goods:** Articles or substances which represent a risk to health, safety, property or the environment and which are classified in the IATA Dangerous Goods Regulations. The Dangerous Goods should meet the criteria of one or more of the nine UN hazards classes.

c. **Infectious substances:** Substances which are known or are reasonably expected to contain pathogens. Pathogens are defined as microorganisms (including bacteria, viruses, rickettsia, parasites, fungi and other agents such as prions) which can cause disease in humans or animals.

d. **Primary container:** A container or receptacle in contact with the biological or environmental material to be transported.

e. **Secondary packaging:** Provides additional protection for the primary container is leak-proof and may include absorbent material.

f. **Outer container:** A sturdy, leak-proof container, for example, a box, flask, Styrofoam box, chiller box that is used to contain the secondary container.

g. **Referral Laboratory or Institution: (PHSU)** Laboratory which receives specimens from another facility for investigation.

h. **Referring laboratory or Program:** A laboratory that sends biological substance or environmental sample to a referral laboratory for further investigations.

5. PROCEDURES FOR LAND TRANSPORT

5.1 General Requirements

The packaging shall be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport. This includes transshipment between transport units and laboratories as well as removal from an overpack for subsequent manual handling.

The packaging shall consist of three components:

a. A primary receptacle: Examples of a primary receptacle are: a urine container, a screw capped container or a blood tube. The primary container must be labeled with the name of patient, identification card or hospital registration number, and test request. Primary receptacles shall be packed in secondary packaging in such a way that under normal conditions of transport, they cannot break, be punctured or leak their contents. If multiple primary receptacles are placed in a single secondary packaging, they shall be secured together, individually wrapped or separated to prevent contact between them.

b. A leak-proof secondary packaging: Examples of a secondary packaging are: a snap lock plastic bag and an empty clean screw cap jar. Secondary packaging shall be secured in an outer packaging with suitable cushioning material. Any leakage of the contents shall not compromise the integrity of the cushioning material or of the outer packaging.

c. Outer packaging: The outer packaging shall be a solid strong and durable container fitted with a secure closure to prevent loss of contents under normal transport conditions. Place primary receptacle into the secondary packaging. Each primary receptacle may be individually wrapped or separated with absorbent material or bubble wrap. If multiple primary receptacles are placed in a single secondary packaging, a rubber band may be used to secure all inner receptacles.

5.2 Labelling, Marking and Documentation

Packaging of Category B biological materials for surface transport should be labeled clearly with the following information on the outer packaging

- Contact name and organization address of both referral and referring laboratories including 24 hours emergency contact number of the referring laboratory.

- If refrigerants are used, their presence is indicated.
- Documents identifying the contents of the primary receptacle or request forms should be outside the secondary package.
- Any documents required by a transporter shall be accessible without opening the package.

5.3 Refrigerants

- Mark the outer packaging to indicate which refrigerant is being used. This is important because some of the refrigerants pose some hazards.
- Use a freezer brick or gel pack or within the outer packaging or overpack. All the primary receptacles should not be in direct contact with the refrigerants
- **If using Dry ice:** design and construct the outer packaging so that the release of carbon dioxide gas is permitted to prevent a build-up of pressure that could rupture the packaging. Mark the outer packaging "Dry ice".
- Shipper shall ensure adequate and appropriate refrigerants being used in order to maintain required temperature upon arrival at the referral laboratories .This is important to ensure good quality specimens.

6 SPECIMEN AIR TRANSPORT

6.1 General Requirement

- Separate plasma from whole blood within 6 hours of collection by centrifugation at 800-1600 x g for 20 minutes at room temperature.
- Transfer plasma to a sterile polypropylene tube.
- Frozen at -20°C to -80°C.

7 BIOLOGICAL SPECIMENS, CATEGORY B

An infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances transported for diagnostic or investigational purposes. A Category B infectious substance must be described as ``Biological substance, Category B" and assigned identification number UN 3373. This does not include regulated medical waste, which must be assigned identification number UN 3291. [DOT 49CFR173.134(a)(1)(ii), 72 FR 55692, Oct. 1, 2007]

7.1 General Packaging Instructions for Biological Specimens, Category B (i.e. UN 3373)

All Category B infectious substance must be packaged in a "Triple Packaging" consisting of a primary receptacle, a secondary packaging, and a rigid outer packaging (a.k.a. the tertiary container). These packaging requirements are set forth in the DOT regulations (49CFR173.199) which harmonize with the IATA Standards (Packing Instructions #650). The requirements are:

a. **Primary receptacles** (sealed test-tubes being the most common) must be leak-proof if shipping liquids (i.e. blood) or sift proof if shipping solids (i.e. swab). You

should always assure that any seals (i.e. rubber stoppers) are secure and if using a screw top, the screw top should be reinforced with tape.

b. Primary receptacles must be packed in **secondary packaging** (sealed plastic bags being the most common) in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Note that the secondary packaging must also be leak proof if shipping liquids (i.e. blood) or sift proof if shipping solids (i.e. swab).
   - When packaging liquids, absorbent material must be placed between the primary receptacle and secondary packaging. The absorbent material must be of sufficient quantity to absorb the entire contents of all of the primary receptacles and not compromise the integrity of the cushioning material or the outer packaging.
   - If several fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them. The wrapping or separation mechanism may also be the absorbent material required for liquids if it is capable of absorbing the entire contents of all of the primary receptacles as indicated above.
   - If residual liquid may be present in the primary receptacle during transportation OR if the solid material may become liquid during transportation (i.e. frozen specimens), the solid must be packaged as if it were a liquid.

c. Secondary packaging must be secured in **rigid outer packaging** (fiberboard boxes being the most common) with suitable cushioning material such that any leakage of the contents will not impair the protective properties of the cushioning material or the outer packaging.

d. The following mark must be displayed on the outer packaging on a background of contrasting color. The label may be on its point or askew.

Figure 1: UN3373 Label

The width of the line must be at least 2 mm (0.08 inches) and the letters and numbers must be at least 6mm (0.24 inches) high. The size of the mark must be such that no side of the diamond is less than 50 mm (1.97 inches) in length. The proper shipping name ``Biological substances, Category B" must be marked on the outer packaging adjacent to the diamond-shaped mark in letters that are at least 6 mm (0.24 inches) high.

e. When packages are placed in an overpack (such as in placing a package into a fourth container or combining several packages into one box), all package markings required must be either clearly visible (i.e. through a clear plastic window) or reproduced on the outside of the overpack.

f. The name and telephone number of a person who is either knowledgeable about the material being shipped and has comprehensive emergency response and incident mitigation information for the material, or has immediate access to a person who possesses such knowledge and information, must be included on a written document (such as an air waybill or bill of lading) or on the outer packaging.

g. A packaging containing inner packagings of Category B infectious substances may not contain other hazardous materials except:

- Refrigerants, such as dry ice or liquid nitrogen, as authorized under paragraph (d) of this section;
- Anticoagulants used to stabilize blood or plasma; or
- Small quantities of Class 3, Class 8, Class 9, or other materials in Packing Groups II and III (as classified on the HazMat table) used to stabilize or prevent degradation of the sample (such as preservatives), provided the quantity of such materials does not exceed 30 mL (1 ounce) or 30 g (1 ounce) in each inner packaging.

h. For shipments by aircraft, there are some size and weight limitations
- For liquids, the maximum quantity contained in each primary receptacle, including any material used to stabilize or prevent degradation of the sample, may not exceed 1 L (34 ounces), and the maximum quantity contained in each outer packaging, including any material used to stabilize or prevent degradation of the samples, may not exceed 4 L (1 gallon). The outer packaging limitation does not include ice, dry ice, or liquid nitrogen when used to maintain the integrity of the material.

i. Packaging's must be filled and closed in accordance with the information provided by the packaging manufacturer or subsequent distributor.

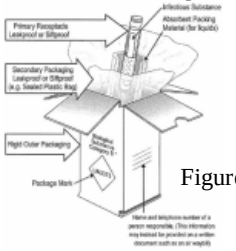


Figure 2: A properly packaged package

While most organizations who ship do not manufacture the materials used to ship, it is important to note that you cannot use just any materials. Manufacturers must pass certain quality control parameters in their products as defined in 49CFR178.609 entitled "Test requirements for packagings for infectious substances". This section requires exposing the packaging to things such as extremes in temperature, "drop tests" by dropping the boxes from heights at least 1.2 meters and pressure tests producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi) to assure that the primary receptacles remain intact and not separated from the absorbent material. Additionally, there are certain size restrictions (such as at least one surface of the outer packaging must have a minimum dimension of 100 mm by 100 mm or 3.9 inches). While most individuals rely on the quality control of the manufacturer to assure their materials meet these specifications, it is important to note that if your packaging materials seem damaged in any way; this may have compromised the system so that it will not meet the specifications. As the shipper, not the manufacturer, is ultimately responsible to assure the integrity of the system, you should not use any packaging materials that seem damaged or compromised in any way.

7.2 General Packaging Instructions for Carbon Dioxide, Solid (i.e. UN 1845)

As samples often need to be shipped frozen, "dry ice" is commonly used as a refrigerant and also contained in the packaging. Due to the risks of dry ice (formally known as "Carbon Dioxide, Solid" or UN1845 in the HazMat table) its packaging and shipment is also regulated and has certain packaging and labeling requirements. These requirements are set forth in the DOT regulations (49CFR173.217) which harmonize with the IATA Standards (Packing Instructions #904). The requirements for shippers are:

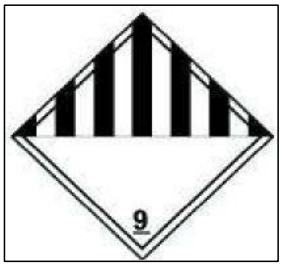
a. Carbon dioxide, solid (dry ice), when offered for transportation or transported by aircraft or water, must be packed in packaging's designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packaging. Fiberboard boxes and styrofoam chests suffice for this provided they are not subsequently sealed "airtight".

b. Dry ice is placed between the secondary receptacle and the rigid outer packaging. It is not placed within the primary container or the secondary receptacle as the dissipation of the dry ice will build pressure In these sealed containers potentially causing rupture in these leak-proof or sift proof protective containers.

c. When offered or transported by aircraft, in quantities not exceeding 2.3 kg (5 pounds) per package and used as a refrigerant for the contents of the package, the package must be marked ``Carbon dioxide, solid" or ``Dry ice", marked with the name of the contents being cooled (such as your UN3373 label) and marked with the net weight of the dry ice or an indication the net weight is 2.3 kg (5 pounds) or less (See attachment A)

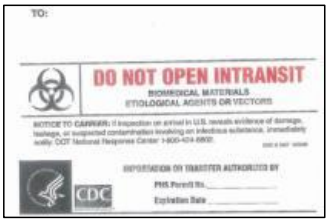
8 SAMPLING AND SHIPPING SPECIFICATIONS

Attachment A

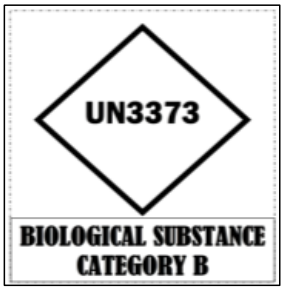


Dry Ice Sticker to be placed when shipping with dry ice as a refrigerant.

biohazard Sticker to be placed in the outer box.



Labels for the box



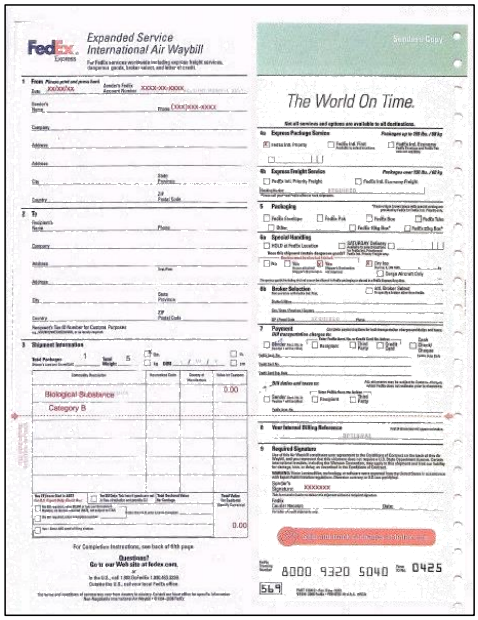
Biological substance Sticker to be placed in the outer box.

Referral Laboratory or Institution: (PHSU)

Lcda. Nayra Rodriguez-Hornedo
787-317-2411 (24 hrs)

Referring Laboratory or Program: _____
_____
787-_____

Contact information Sticker to be placed in the outer box.



International Air Waybill to be filled.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE

Centers for Disease Control and Prevention
Office of Health and Safety, MS A-46
Atlanta, Georgia 30333
TEL: 404-718-2077; FAX: 404-718-2093; Email: importpermit@cdc.gov

SAFER • HEALTHIER • PEOPLE

Permit to Import Infectious Biological Agents, Infectious Substances, and Vectors

In accordance with 42 CFR Section 71.54 of the Public Health Service Foreign Quarantine Regulators, cited on the bottom of this permit, permission is granted the permittee to import into any port under control of the United States, or to receive by transfer within the United States, the material described in Item 1 below.

PHS PERMIT NO.	**2017-05-030**	
DATES	**ISSUED: Monday, May 08, 2017**	**EXPIRES: Tuesday, May 08, 2018**
1. DESCRIPTION OF MATERIAL	**HUMAN BLOOD, BLOOD PRODUCTS OR OTHER BODY FLUIDS WHICH MAY CONTAIN HUMAN IMMUNODEFICIENCY VIRUS.**	
2. PERMITTEE (NAME, ORGANIZATION, ADDRESS AND CONTACT INFORMATION)	**NAYRA RODRIGUEZ PONCE HEALTH SCIENCE UNIVERSITY 395 ZONA INDUSTRIAL REPARADA-2 PONCE, PR 00716-2348**	**TEL: 787-841-5150 FAX: 787-841-5150**
2a. OTHER AUTHORIZED PERMIT USERS	**PABLO LOPEZ PONCE HEALTH SCIENCE UNIVERSITY 395 ZONA INDUSTRIAL REPARADA-2 PONCE, PR 00716-2348**	**TEL: 787-841-5150 FAX: 787-841-5150**
	OMAYRA DE JESUS PONCE HEALTH SCIENCE UNIVERSITY 395 ZONA INDUSTRIAL REPARADA-2 PONCE, PR 00716-2348	**TEL: 787-841-5150 FAX: 787-841-5150**
3. SOURCE OF MATERIAL (NAME, ORGANIZATION, ADDRESS, COUNTRY)	**WORLDWIDE**	
4. TYPE OF PERMIT AND INSTRUCTIONS FOR USE	As the permittee, your facility will be subject to inspection at some time in the future to confirm that the importer's biosafety measures are commensurate with the hazard posed by the items to be imported and the level of risk given its intended use. ☐ Single Importation into the U.S. ☑ Single Transfer Within the U.S. ☑ Multiple Importation into the U.S. ☐ Multiple Transfer Within the U.S. A. Record of each importation shall be maintained on permanent file by permittee. B. Enclosed label(s) must be forwarded to the shipper(s). C. One label shall be affixed to shipping container. Enclosed labels may be photocopied.	
5. CONDITIONS OF ISSUANCE ITEMS APPLICABLE WHEN CHECKED	☐ A. Subsequent distribution, within the U.S., of the material described in this permit is prohibited without prior authorization by the Public Health Service. ☑ B. All material is for laboratory use only - Not for use in the production of biologics for humans or animals. ☑ C. All material is free of tissues, serum and plasma of domestic and wild ruminants, swine and equines. ☐ D. Additional Requirements: ☐ IATA Packaged to preclude escape. ☐ USDA permit may be required (Telephone: 301-851-3300). ☑ E. Work with the agent(s) described shall be restricted to areas and conditions meeting requirements in the CDC/NIH publication "Biosafety in Microbiological and Biomedical Laboratories." ☑ F. Packaging must conform to 49 CFR Sections 171-180.	
	6. Signature of Issuing officer Samuel S. Edwin Samuel S. Edwin, Ph.D. Director, Division of Select Agents and Toxins	

CDC 0728 (F 13.40) REV. 4-13

42 CFR 71.54. Permit to Import Biological Agents, Infections Substances, and Vectors
A person may not import into the United States any infectious biological agent, infectious substance, or vector unless: It is accompanied by a permit issued by the Centers for Disease Control and Prevention (CDC). The possession of a permit issued by the CDC does not satisfy permitting requirements placed on materials by the U.S. Department of Agriculture that may pose hazards to agriculture or agricultural production in addition to hazards to human health.

Attachment C

Sampling and Shipping Specifications

Test Type	Collection Tubes	Comments
Elisa HIV I/II	1 plain tube- (red top) (Plasma) or 1 EDTA tube (lavender top) (plasma)	Spin and separate serum or plasma
HIV-I Confirmatory test	1 plain tube- (red top) (Plasma) or 1 EDTA tube (lavender top) (plasma)	Spin and separate serum or plasma
HIV Viral Load	2 EDTA tubes (lavender top) (Plasma)	Spin and separate plasma in a period no longer than 6 hours after sample collection.
HIV RNA-Tropism (In-House 50 clones)	1 EDTA tube (lavender top) (Plasma)	Spin and separate plasma in a period no longer than 5 hours
HIV DNA-Tropism (In-House 50 clones)	1 EDTA tube (lavender top)	Whole blood is needed
Immunoprofile(CD3/CD4,CD3/CD8, Ratio, CBC)	1 EDTA tube (lavender top)	Whole blood is needed
HIV Genotype: In-House Protocol (Drug Resistance)	1 EDTA tube (lavender top) (Plasma)	Spin and separate plasma in a period no longer than 5 hours
HCV Viral Load	2 EDTA tube (lavender top)	Spin and separate plasma in a period no longer than 5 hours
HCV Genotype	1 EDTA tube (lavender top)	Spin and separate plasma in a period no longer than 5 hours

Mix well, if EDTA tube

BC CENTRE FOR EXCELLENCE IN HIV/AIDS

Street Address: 604-1081 Burrard St, Vancouver, Canada BC V6Z 1Y6

Director of Department or Institution: Dr. Julio Montaner

Email: jmontaner@cfenet.ubc.ca

Laboratory Director & Contact for HIV DR: Dr. Richard Harrigan

Email: prharrigan@cfenet.ubc.ca

Position of Contact Person for HIV DR: HIV DR Laboratory Director

Phone Number: +1-604-806-8775

Fax Number: +1-604-806-9463

Email Address: prharrigan@cfenet.ubc.ca

Date this data was collected: 7-Apr-2017

HIV DR Detection: Methodology Overview

- PI and RTI DRM detection: a single amplicon of full-length protease and full-length Reverse Transcriptase / RNaseH.
- INSTI DRM detection: a single amplicon of full-length Integrase

Other Tests Offered Using the Same Samples

- HLA-B*5701 for ABC hypersensivity
- CCR5 Tropism for CCR5 antagonist susceptibility

Requirements for Sample Preparation and HIV DR Test Request

- At least 1.2mL plasma (from whole blood collected in EDTA) and/or 3mL whole blood collected in EDTA.
- The sample request form explains all aspects of sample preparation for the tests provided.
- EACH SAMPLE MUST BE ACCOMPANIED BY A COMPLETED SAMPLE REQUEST FORM.

BC Centre for Excellence in HIV/AIDS
604-1081 Burrard St, Vancouver, BC V6Z 1Y6
Dr. Richard Harrigan BCCfE Research Laboratory
Phone 604 806-8775 FAX 604 806-9463

LABORATORY REQUISITION FORM OUTSIDE BRITISH COLUMBIA

Patient Information

Patient ID _____

Patient Name _____
Last

_____
First

Date of Birth _____/_____/_____
dd mmm yyyy

Physician Information

Physician _____

Address _____

_____

Telephone/Fax _____/_____

Patient's HIV Viral Load _____ **Patient's CD4 Count** _____

A. HLA-B*5701 for Abacavir Hypersensitivity ☐

The HLA genotype of a patient does not change and needs to be tested only once.
This test requires whole blood. * see whole blood collection below

B. HIV Drug Resistance Testing (Genotype)

These drug resistance tests require plasma with HIV viral loads **> 250** copies/mL. ** see plasma collection below

☐ **Standard** HIV Protease-RT Drug Resistance
For susceptibility to:
nRTI's e.g. lamivudine, tenofovir, abacavir, zidovudine
NNRTIs e.g. efavirenz, nevirapine, etravirine
Protease Inhibitors e.g. atazanavir, lopinavir, darunavir

Investigational HIV Drug Resistance Tests
Patient must be currently or previously treated with drug(s) in the following classes to qualify for testing.

☐ **Integrase Inhibitors**
For susceptibility to integrase inhibitors e.g. raltegravir

☐ **Fusion inhibitors (gp41 test)**
For susceptibility to fusion inhibitors e.g. enfuvirtide

C. HIV CCR5 Tropism Testing (V3) Investigational

These tests are used to determine HIV susceptibility to CCR5 antagonists (e.g. maraviroc). Tropism can change over time. Testing should be done just prior to starting a CCR5 antagonist.

Choose ONE of the tropism tests below, based on the patient's viral load.

☐ Plasma HIV V3 Genotyping (Tropism)
This test requires plasma.** see below
HIV viral load **must be > 500** copies/mL.

OR

☐ Proviral HIV DNA V3 Genotyping (Tropism)
This test requires whole blood.* see below
HIV viral load **must be ≤ 500** copies/mL or undetectable.

Collection of PLASMA ** (as appropriate for test ordered)

Collect 1 X 7 mL EDTA (lavender top) tube.
Centrifuge the EDTA tube for 15 min. at 800-1600 g.
Transfer **at least 1.2 mL** of plasma to a 2 mL screw cap cryovial.

Collection Date _____/_____/_____
dd mmm yyyy

Collection of WHOLE BLOOD * (as appropriate for test ordered)

Collect 1 X 3 mL EDTA (lavender top) tube.
Do **NOT** centrifuge.
Transfer whole blood to a 2 mL screw cap cryovial.

Collection Date _____/_____/_____
dd mmm yyyy

Shipping of Samples to be Tested

Store samples frozen at -15° to -80° C until ready to ship.
Ship cryovials frozen on dry ice by overnight courier.
Ship Monday – Wednesday only.
Ship according to IATA and TGD dangerous goods regulations.
Notify laboratory by fax.

Ship to:
Dr. Richard Harrigan
BC Centre for Excellence in HIV/AIDS Research Laboratory
604—1081 Burrard St., St. Paul's Hospital
Vancouver, BC V6Z 1Y6
Tel: 604 806-8775 FAX: 604 806-9463

FCD-0023 v10

SERVICE DE VIROLOGIE CENTRE HOSPITALIER ET UNIVERSITAIRE DE MARTINIQUE (CHUM)

Street Address: CHUM de Martinique Nouveau Plateau Technique, Niveau-3, 97261 Fort de France, Martinique

Director of Institution: Mr N. Estienne

Email Address: N/A

Laboratory Director: Prof Raymond Cesaire

Email Address: raymond.cesaire@chu-martinique.fr

Contact Person for HIV DR: Georges Dos Santos, PhD

Position of Contact Person for HIV DR: Head, CHUM

Phone Number: + 596 696 31 81 31

Fax Number: N/A

Email Address: **georges.dos-santos@chu-martinique.fr**

Footnote: This is WHO assigned laboratory for HIV Drug Resistance Testing and part of the WHO HIV Resistance Network. This laboratory is ISO 15189 accredited.

Date this data was collected: 11/16/2017

HIV DR Detection: Methodology Overview

- PI and RTI DRM detection: A single amplicon of full-length Protease and Reverse Transcriptase (aa 1-260).
- INSTI DRM detection: a single amplicon of full-length Integrase.
- Both are In House protocol (ANRS protocol; http://www.hivfrenchresistance.org/ANRS-procedures.pdf)
- These tests are validated for both blood plasma and DBS. Viral load thresholds are:
    a. Plasma: PI and RTI > 50 copies/ml
    b. Plasma: INSTI > 100 copies/ml
    c. DBS > 500 copies/ml;

Other Tests that can be offered

- HLA-B*5701 for ABC hypersensitivity (CHUM laboratory is European Federation for Immunogenetics (EFI) Accredited)
- CCR5 Tropism for CCR5 antagonist susceptibility

Before sending samples to CHUM a contract must be established, in order to clearly define the pre-analytical requirements (ISO15189).

Requirements for Sample Preparation and HIV DR Test Request

- At least 1200-2000 µl of plasma (whole blood collected in EDTA; plasma separated from whole blood within 6 hours). DO NOT collect whole blood in heparin tubes. DO NOT freeze plasma in plasma preparation tubes (PPT).
- Store plasma in two 2ml screw-cap cryovials. Label vials appropriately. Store plasma between -20ºC and -80°C.

Plasma Preparation

- Centrifuge whole blood collected in a sterile EDTA tubes (lavender) at 1200 x g at room temperature for 20 minutes within 2-6 hours of collection.
- Transfer plasma to 1.5 - 2.0 mL polypropylene screw-cap tubes.
- Plasma may be stored at 2-8°C for up to 24 hours or frozen at - 20°C to -80°C for up to six months before testing and not freeze-thawed more than 2 times.

Dried Blood Spot (DBS) Preparation

- Dispense 80-100 µl of anti-coagulated EDTA venous blood onto a Whatman filter paper as soon as possible and within 24 hours of collection.
- Obtain at least 4 saturated circles for each specimen.
- Package dry filter cards in a single gas-impermeable, sealable zip-lock bag containing 2-3 desiccant packs to remove residual moisture along with one humidity indicator card.
- Desiccant packs must remain dry during storage.
- Keep zip-lock bags in the dark since UV light can damage DBS.
- If processing specimens within 14 days, store at ambient temperature.
- If processing specimens for longer than 14 days DBS may or store at -20°C or colder.

Before shipping samples to Service de Virologie CHUM, email georges.dos-santos@chu-martinique.fr to obtain the CHUM specimen submission form(s). Email the completed CHUM specimen submission form(s) before ship

- Ship samples on dry ice if unavailable use coolers in order to keep samples as cold as possible.
- Sample packages should be made according to IATA regulation and declared as Biological Samples Category B (UN3373) and carbon dioxide (UN 1845) if appropriate.
- Shipment should only be shipped on a Monday or Tuesday to ensure the samples arrive frozen at Service de Virologie Centre. Hospitalier Universitaire de Martinique

FDA APPROVED HIV DR TESTING PROVISION

MOLECULAR PATHOLOGY LABORATORY AT NEW YORK-PRESBYTERIAN / WEILL CORNELL MEDICAL CENTER

Street Address: 525 East 68th Street, New York USA, 10065

Director of Department or Institution: Dr. Jacob Rand

Email Address: N/A

Laboratory Director: Hanna Rennert

Email Address: har2006@med.cornell.edu

Contact Person for HIV DR: Phyllis Ruggiero

Position of Contact Person for HIV DR: Supervisor Molecular Pathology Laboratory

Phone Number: +1-212-746-2994

Fax Number: +1-212-745-4546

Email Address: pcr9004@nyp.org

Date this data was collected: 4-Apr-2017

HIV DR Detection: Methodology Overview

- ViroSeq HIV-1 Genotyping System v.2.0 (Celera, Alameda, CA, USA; Abbott Molecular).
- Approved for clinical use for HIV DR detection of HIV-1, Subtype B strains only and viral loads >2000 copies RNA per mL blood.
- PI and RTI DRM detection: A single amplicon containing full-length Protease and full-length Reverse Transcriptase

Requirements for Sample Preparation and HIV DR Test Request

- At least 1200µL of plasma (whole blood collected in EDTA and plasma separated from whole blood with 6 hours). DO NOT collect whole blood in heparin tubes.
- Each vial of plasma that is shipped to Weil Cornell Medical Centre for HIV DR testing must be labelled with its Sample ID and correlate to the samples list sent (page 35), or else it will be discarded.
- The completed sample list must be sent with the samples and ideally, a copy of this should be emailed to pcr9004@nyp.org

List of Samples For HIV Drug Resistance Testing Shipped To the Molecular Pathology Laboratory at NewYork-Presbyterian (WCMC)

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	1.2ml plasma (Yes/No)	Comments
1			
2			
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This form must accompany Samples Shipped to the Molecular Pathology Laboratory at NewYork-Presbyterian/Weill Cornell Medical Center for HIVDR Testing

For assistance, contact Phyllis Ruggiero: 212-746-2994 or pcr9004@nyp.org

TRLv1.0

Printed 04/04/17
Test information subject to change

NewYork-Presbyterian

Human Immunodeficiency Virus 1 Genotyping

COLLECTION

Collect:

Routine Venipuncture. Deliver to laboratory immediately after collection

Unacceptable Conditions:

Plasma not separated (decanted) from whole blood within 6 hours of blood draw. Specimen contaminated with Heparin. A minimum HIV viral concentration of 2000 copies/mL HIV RNA, is required for this test.

Storage/Transport Temperature:

Whole blood must be transported at 2-25ºC. Plasma should be separated from cells within 6 hours of collection, and may be stored at 2-8ºC f to 72 hours or frozen at -20ºC or colder (-70ºC).

Performed:

Central Laboratory, Payson 8.

Remarks:

Deliver to laboratory as soon as possible after collection. Specimen must be received by the laboratory within 6 hours of collection.
It is highly recommended that the patient be referred to the K-09 Patient Service Center located in the lobby of the C.V. Starr building for colle
Alternatively: Centrifuge specimen after collection, aliquot plasma into a plastic transport tube and freeze. Label aliquot with patient's name, I and "FROZEN PLASMA". Place specimen and completed requisition into a plastic transport bag. Label the outside of the transport bag with "FROZEN PRIORITY HANDLING" label. Call Courier Service for a "FROZEN SPECIMEN" pick-up.

Phone #:

For test or section specific questions, call Molecular Pathology at (212)746-2431 (Mon-Fri 8AM-5PM)

Availability:

Batched once/week

Specimen:

Blood 5 mL (Min: 2 mL)

Container:

1 White Top Plasma Preparation Tube (PPT)

ORDERING

Ordering Recommendations:

The test may be used to detect HIV genomic mutations that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring a treating HIV infection. The assay detects the most prevalent HIV subtype (B) found in the United States. HIV genotyping should be performed HIV infected individuals upon initial presentation before initial drug therapy. HIV genotyping should also be performed in response to drug the failure as reflected by increased viral load, before switching to new therapy.

Performed:

Central Laboratory, Payson 8.

Methodology:

ViroSeq HIV-1 Genotyping System V.2.0 (Celera Diagnostics/Abbott Molecular). Test includes NRTI, NNRTI and PI mutations as well as NO\ Protease and Reverse Transcriptase mutations for predicting HIV-1 Subtype B resistance to Protease and Reverse Transcriptase inhibitors a retroviral drugs.

Synonyms:

- HIV GENO
- HIV-1 Genotyping

Printed 04/04/17
Test information subject to change

Turn Around Time:

3 to 10 days

RESULT INTERPRETATION

Reference Interval:

See interpretation on report

ADMINISTRATIVE

CPT Codes:

87901

QUEST DIAGNOSTICS

Street Address: 14225 Newbrook Dr, Chantilly, VA USA, 201551

Director of Department or Institution: Meghan Starolis

Email Address: meghan.w.starolis@questdiagnostics.com

Laboratory Director: Patrick Mason

Email Address: N/A

Contact Person for HIV DR: Meghan Starolis

Position of Contact Person for HIV DR: Science Director

Phone Number: 703-802-7049

Fax Number: 703-802-7153

Email Address: meghan.w.starolis@questdiagnostics.com

Date this data was collected: 6-Apr-2017

HIV DR Detection: Methodology Overview

- ViroSeq HIV-1 Genotyping System v.2.0 (Celera, Alameda, CA, USA; Abbott Molecular).
- Approved for clinical use for HIV DR detection of HIV-1, Subtype B strains only and viral loads >2000 copies RNA per mL blood.
- PI and RTI DRM detection: A single amplicon containing full-length Protease and full-length Reverse Transcriptase

Requirements for Sample Preparation and HIV DR Test Request

- At least 1200µL of plasma (whole blood collected in EDTA and plasma separated from whole blood with 6 hours). DO NOT collect whole blood in heparin tubes. DO NOT freeze plasma in plasma preparation tubes (PPT).
- Transfer plasma to a sterile, plastic, screw-capped vial, freeze at -80ºC, and ship on dry ice.
- Email the sample list (page 38) to meghan.w.starolis@questdiagnostics.com

List of Samples For HIV Drug Resistance Testing Shipped To Quest Diagnostics, Chantilly VA

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting Institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	1.2ml plasma (Yes/No)	Comments
1			
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This form must accompany samples shipped to the Quest Diagnostics, Chantilly VA for HIVDR testing
For assistance, contact Meghan Starolis: 1-703-802-7049 or meghan.w.starolis@questdiagnostics.com

TRLv1.0

CDC Supported Capacity Building for HIV DR Testing

(These laboratories are not yet accepting samples for HIV DR testing)

BEST-DOS SANTOS PUBLIC HEALTH LABORATORY, BARBADOS

Street Address: Enmore Complex, Martindale Road, St. Michael, Barbados

Director of Department or Institution: Dr. Anton Best

Email Address: anton.best@health.gov.bb

Laboratory Director: Clive Landis

Email Address: clive.landis@cavehill.uwi.edu

Contact Person for HIV DR: Songee Beckles

Position of Contact Person for HIV DR: Clinical Information Specialist

Phone Number: (246)266-0823

Fax Number: (246)437-8241

Email Address: slb5@yahoo.com or songee.beckles@health.gov.bb

Date this data was collected: 7/10/17

HIV DR Detection: Methodology Overview

- HIV-1 Genotyping for Drug Resistance Testing: In House Assay (CDC). Further references to be defined.
- Approved for HIV DR detection of HIV-1 Group M, multiple subtypes, viral loads and plasma or DBS. Viral load thresholds pending.
- PI and RTI DRM detection: A single amplicon containing the enzymatic regions of Protease and Reverse Transcriptase (*pol* codons 6-251)

Requirements for Sample Preparation and HIV DR Test Request

- **Plasma:** 140-1200 µL of plasma (whole blood collected in EDTA; plasma separated from whole blood within 6 hours). DO NOT collect whole blood in heparin tubes. DO NOT freeze plasma in plasma preparation tubes (PPT).
- Dried Blood Spot (DBS): Whole blood collected in K3-EDTA tubes. 100µL of EDTA-collected blood spotted on Whatman 903 paper and dried overnight at ambient temperature. Wrap each patient DBS sample is an air, water and grease resistant paper and then in a zip-lock bag with a humidity indicator. Store and ship at a temperature range of -20^{o}C to -70^{o}C (dry ice).
- Email the shipping sample list (page 41) to songee.beckles@health.gov.bb

List of Samples For HIV Drug Resistance Testing Shipped To Ladymeade Reference Unit Laboratory

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	1.2ml plasma (Yes/No)	Comments
1			
2			
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This form must accompany samples shipped to the Ladymeade Reference Unit Laboratory for HIVDR testing
For assistance, contact Songee Beckles: (246)266-0823 or slb5@yahoo.com or songee.beckles@health.gov.bb

NATIONAL PUBLIC HEALTH LABORATORY, JAMAICA

Street Address: 21 Slipe Pen Road, Kingston Jamaica, National Public Health Laboratory, Immunology Department PCR Lab

Director of Department or Institution: Dr. Michelle Hamilton

Email Address: Hamiltonm@moh.gov.jm

Laboratory Director: Prof John Lindo

Email Address: Lindoj@moh.gov.jm

Contact Person for HIV DR: Dr. Michelle Hamilton

Position of Contact Person for HIV DR: Director of Immunology

Phone Number: +1-876-317-8583

Fax Number: +1-876-967-0169

Email Address: Hamiltonm@moh.gov.jm

Date this data was collected: 30-Sept-2017

HIV DR Detection: Methodology Overview

- This test is validated for blood plasma or dry blood spot (DBS) or dry plasma spot (DPS) samples. Viral loads >1000 copies / ml.
- PI and RTI DRM detection: A single amplicon containing the enzymatic regions of Protease and Reverse Transcriptase (*pol* codons 6-251) (Buckton et al., Yang et al.)

Requirements for Sample Preparation and HIV DR Test Request

- Follow the instruction on page 43 for plasma, DBS and DPS preparations.
- Ship samples on dry ice
- Email a copy of the sample shipping list (page 44) to Hamiltonm@moh.gov.jm

Rejection Criteria

- Improper specimen collection. Packaging without humidity indicators and desiccants. Demonstrate any indication of humidity in zip lock bags. Insufficient volume for testing. Specimens with blood clots or clumps. Specimens with a halo around the blood spot indicating contamination (DBS specimens). Specimens with evidence of cross-contamination: congruency or commingling.

Plasma Preparation

- Centrifuge whole blood collected in a sterile EDTA tubes (lavender) at 1000 to 2000 x g at room temperature for 15 minutes within 2-6 hours of collection.
- Transfer plasma to 1.5 - 2.0 mL polypropylene screw-cap tubes.
- Plasma may be stored at 2-8°C for up to 24 hours or frozen at - 65° to -80°C for up to six months before testing and not freeze-thawed more than 2 times.

Dried Blood Spot (DBS) Preparation

- Dispense 100 µL of anti-coagulated EDTA venous blood onto a Whatman filter paper as soon as possible and within 24 hours of collection.
- Obtain at least 4 saturated circles for each specimen.
- Package dry filter cards in a single gas-impermeable, sealable zip-lock bag containing 2-3 desiccant packs to remove residual moisture along with one humidity indicator card.
- Desiccant packs must remain dry during storage.
- Keep zip-lock bags in the dark since UV light can damage DBS/DPS.
- If processing specimens within 14 days, store at ambient temperature.
- If processing specimens for longer than 14 days DBS/DPS may or store at -20°C or colder for up to 2 years or -70°C for up to 5 years.

Dried Plasma Spot (DPS) Preparation

- Centrifuge anti-coagulated EDTA venous blood within 2-6 hours after collection.
- Spot 50 µL of plasma onto a Whatman filter paper.
- Obtain at least 4 saturated circles for each specimen.
- Package dry filter cards in a single gas-impermeable, sealable zip-lock bag containing 2-3 desiccant packs to remove residual moisture along with one humidity indicator card.
- Desiccant packs must remain dry during storage.
- Keep zip-lock bags in the dark since UV light can damage DBS/DPS.
- If processing specimens within 14 days, store at ambient temperature.
- If processing specimens for longer than 14 days DBS/DPS may or store at -20°C or colder for up to 2 years or -70°C for up to 5 years.

List of Samples For HIV Drug Resistance Testing Shipped To National Public Laboratory, Jamaica

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	Sample Type (Plasma/DBS/DPS)	Comments
1			
2			
3			
4			
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This form must accompany Samples Shipped to the National Public Health Laboratory for HIVDR Testing

For assistance, contact Dr. Michelle Hamilton: +1 (876)317-8583 or Hamiltonm@moh.gov.jm

REFERENCE LAB, MINISTRY OF HEALTH, THE BAHAMAS

Street Address: Royal Victoria Gardens, Shirley Street

Director of Department or Institution: Dr Pearl McMillan

Email Address: pearlmcmillan@bahamas.gov.bs

Laboratory Director: Dr. Indira Martin

Email Address: indiramartin333@gmail.com

Contact Person for HIV DR: Dr. Indira Martin

Position of Contact Person for HIV DR: Laboratory Director

Phone Number: +1-242-432-9754

Fax Number: Not applicable

Email Address: indiramartin333@gmail.com

Date this data was collected: 07-Dec-2017

HIV DR Detection: Methodology Overview

- This test is validated for blood plasma or dry blood spot (DBS) or dry plasma spot (DPS) samples and viral loads >1000 copies/ml.
- PI and RTI DRM detection: A single amplicon containing the enzymatic regions of Protease and Reverse Transcriptase (*pol* codons 6-251) (Buckton et al., Yang et al.)

Requirements for Sample Preparation and HIV DR Test Request

- Follow the instruction on page 45 for plasma and DBS preparations.
- Ship samples according to instructions on page 45 and IATA shipping guidelines.
- Email a copy of the sample shipping list (page 44) to indiramartin333@gmail.com

Rejection Criteria

- Insufficient sample quantity; improper sample collection; inadequate documentation; leaked or otherwise visibly contaminated samples; samples that are too old

Plasma Preparation

- EDTA Plasma should be used. Heparinized plasma is a known PCR inhibitor and should not be used.
- Transport plasma at room temperature within 24 hours. If this is not possible, store at 2–8°C and transport within 5 days.
- If plasma has been frozen at –70°C, make sure it is transported at the same temperature on dry ice.

Dried Blood Spot (DBS) Preparation

- 100 uL blood should be applied inside each circle on Whatman 903 filter cards.
- Both sides of paper must be saturated beyond the delineated circle.
- Allow DBS to air dry at room temperature for at least 3 hours but no more than 24 hours.
- Store DBS samples in re-sealable bags with desiccant sachets and humidity indicator cards at room temperature for up to 1 month.

Shipping Samples To The Reference Laboratory

- All samples should be labelled with two unique identifiers and collection date and be accompanied by a completed correspondence form. Contact the laboratory director for these details: Dr. Indira Martin indiramartin333@gmail.com
- Samples shipped by air or sea should be packaged according to IATA regulations by appropriately trained shippers.

Other

- Turnover time to receive HIV DR results is 6-8 weeks when the sample was received at the Reference Laboratory.

List of Samples For HIV Drug Resistance Testing Shipped To The Reference Laboratory, The Bahamas

Date of Test Request:_____

Sent by (Institution):_____

Address of requesting Institution:
Name and position of key contact:
Telephone number: Fax number:
Email Address:

Number of Samples in Package:_____

Sample Number	Sample ID (Multiple Sample Numbers per Sample ID is permitted)	Sample Type (Plasma/DBS/DPS)	Comments
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This form must accompany samples shipped to the Reference Laboratory for HIVDR testing

For assistance, contact Dr. Indira Martin: +1-242-432-9754 or indiramartin333@gmail.com

TRLv1.0

Appendix B

List of World Health Organization Designated HIV DR Genotyping Laboratories (as of December 2017)

WHO Region	Type	Country	Laboratory Name/Institution	City	Contact Name	Contact Email	Assay
AFRO	National	Cameroun	IMPM-IRD/CREMER	Yaounde	Avelin Aghokeng	avelin.aghokeng@ird.fr	IH*
AFRO	National	Cote d'Ivoire	RETRO CI	Abidjan	Christiane Adje	cia9@cdc.gov	IH
AFRO	National	Ethiopia	HIV and Other Viral Disease Research, EHNRI	Addis Ababa	Dawit Assefa	dawitarm@gmail.com	IH
AFRO	National	Senegal	Bacteriology-Virology UTH A Le Dantec	Dakar	Coumba Toure Kane	ctourekane@yahoo.co.uk	VS
AFRO	National	Uganda	CFAR Molecular Biology Laboratory, Joint Clinical Research Centre	Kampala	Immaculate Nankya	inankya@hotmail.com	IH
AFRO	Regional	Kenya	KEMRI/CDC HIV Research Laboratory	Kisumu	Maxwell Majiwa	MMajiwa@kemricdc.org	VS, IH
AFRO	Regional	South Africa	AIDS VIRUS RESEARCH UNIT, National Institute for Communicable Diseases	Johannesburg	Gillian Hunt	GillianH@nicd.ac.za	IH
AFRO	Regional	South Africa	CLS Genotyping Laboratory, Johannesburg General Hospital	Johannesburg	Wendy Stevens	wendy.stevens@nhls.ac.za	VS
AFRO	Regional	Uganda	MRC/UVRI Basic Sciences Laboratory	Entebbe	Pontiano Kaleebu	Pontiano.Kaleebu@mrcuganda.org	IH
AMRO	National	Brazil	Laboratory of AIDS and Molecular Immunology, Oswaldo Cruz Foundation - FIOCRUZ	Rio de Janeiro	Jose Carlos Couto-Fernandez	coutofer@ioc.fiocruz.br	VS
AMRO	Regional	Brazil	Laboratorio de Virologia Molecular, Universidade Federal do Rio de Janeiro (LVM-UFRJ)	Rio de Janeiro	Amilcar Tanuri	atanuri@biologia.ufrj.br	IH

AMRO	**Regional**	**Martinique**	**Service de Virologie Immunologie Centre Hospitalier et Universitaire de Fort-de-France**	**Fort de France**	**Georges Dos Santos**	**gmeg@wanadoo.fr**	**TG, IH**
AMRO	Regional	Mexico	Centro de Investigación en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias (CIENI/INER)	Mexico City	Santiago Ávila Ríos	santiago.avila@cieni.org.mx	VS
AMRO	**Regional**	**Puerto Rico**	**AIDS Research Program-Immunology Reference Laboratory**	**Ponce**	**Nayra Rodriguez**	**nrodriguez@psm.edu**	**TG, IH**
AMRO	Specialised	Canada	National Laboratory for HIV Genetics, PHAC	Winnipeg	Paul Sandstrom	Paul.Sandstrom@phac-aspc.gc.ca	VS, IH
AMRO	Specialised	Canada	BC Centre for Excellence in HIV/AIDS (BCCfE)	Vancouver	Richard Harrigan	prharrigan@cfenet.ubc.ca	IH
AMRO	Specialised	United States	Drug Resistance Unit, International Laboratory Branch, DGHA, CGH, CDC	Atlanta	Artur Ramos	cer9@cdc.gov	VS, IH
EURO	Specialised	France	Laboratoire de Virologie, CHU	Bordeaux	Herve Fleury	herve.fleury@chu-bordeaux.fr	IH
EURO	Specialised	France	UMI 233, TransVIHMI, IRD and UM1	Montpellier	Martine Peeters	martine.peeters@mpl.ird.fr	IH
EURO	Specialised	Netherlands	Department of Virology, University Medical Center Utrecht	Utrecht	Rob Schuurman	rob.schuurman@umcutrecht.nl	VS
EURO	Specialised	United Kingdom	Public Health England, London	London	Tamyo Mbisa	tamyo.mbisa@phe.gov.uk	IH
SEARO	National	India	Department of Clinical Research Tuberculosis Research Centre (ICMR)	Chennai	Luke Hanna	hannatrc@yahoo.com	VS
SEARO	National	India	National AIDS Research Institute, Indian Council of Medical Research	Pune	Swarali Kurle	skurle@nariindia.org	VS
SEARO	National	Thailand	Dept Microbiology, Siriraj Hospital	Bangkok	Ruengpung Sutthent	sirst@mahidol.ac.th	VS

SEARO	National	Thailand	National Institute of Health, Department of Medical Sciences	Bangkok	Siriphan Saeng-aroon	siriphas@gmail.com	TG, IH
WPRO	National	China	Shanghai Municipal Center for Disease Control and Prevention	Shanghai	Ping Zhong	zhongp56@hotmail.com	IH
WPRO	National	China	Key Laboratory of Immunology of AIDS, Ministry of Health	Shenyang	Hong Shang	P3lab@yeah.net	IH
WPRO	National	Vietnam	Laboratory of Molecular Diagnostics, National Institute of Hygiene and Epidemiology	Hanoi	Lan Anh Nguyen	lananh_2003@yahoo.com	TG
WPRO	National	Vietnam	HIV/AIDS laboratory, Pasteur Institute	Ho Chi Minh City	Truong Thi Xuan Lien	truongxuanlien@gmail.com	IH
WPRO	Regional	Australia	NSW State Reference Laboratory for HIV and Molecular Diagnostic Medicine	Sydney	Philip Cunningham	p.cunningham@amr.org.au	TG, VS
WPRO	Regional	China	Division of Research on Virology and Immunology (DRVI), NCAIDS, Chinese Center for Disease Control and Prevention	Beijing	Shao Yiming	yshao08@gmail.com	VS, TG, IH

TG: TruGene; VS: Viroseq; IH: In-House

CHUM and Ponce School of Medicine are WHO assigned HIV DR testing laboratories based in the Caribbean

APPENDIX C

Key Resources Available Online

Websites for tools that can be quality assurance processes being developed by WHO/ResNet for genotypic HIV drug resistance testing and sequence analysis.

1. RECall: http://pssm.cfenet.ubc.ca/account/login
2. REGA: http://dbpartners.stanford.edu:8080/RegaSubtyping/stanford-hiv/typingtool/
3. Stanford HIV Database: https://hivdb.stanford.edu
4. MEGA: http://www.megasoftware.net

APPENDIX D

Antiretrovirals: Classes, Drugs and Combinations

Drug Class	Mechanism of Action	ARV included in WHO Essential Medicine List (Organization, 2017)
Nucleoside reverse transcriptase inhibitor (NRTI)	Nucleotide analogues the viral enzyme, reverse transcriptase, from copying the HIV genome	Abacavir (ABC) Emtricitabine (FTC) Lamivudine (3TC) Staduvine (d4T) Tenofovir disoproxil fumarate (TDF) Zidovudine (ZDV / AZT) Didanosine (ddI)
Non-nucleoside reverse transcriptase inhibitor (NNRTI)	Stops the viral enzyme, reverse transcriptase, from making HIV DNA	Efavirenz (EFV) Etravirine (ETR) Nevirapine (NVP) Rilpivirine (RPV)
Protease inhibitor (PI)	Stops the viral enzyme, protease, from maturing the HIV virus	Atazanavir (ATV) Darunavir (DRV) Fosamprenavir (FPV) Indinavir (IDV) Lopinavir (LPV) Nelfinavir (NFV) Ritonavir (RTV); boost for PI efficacy (r) Saquinavir (SQV) Tipranavir (TPV)
Integrase inhibitor (INSTI . INI)	Stops the viral enzyme, integrase, from integrating HIV DNA into the DNA of the cell it has infected	Dolutegravir (DTG) Elvitegravir (EVG) Raltegravir (RTG)
Fusion inhibitor	Stops the viral glycoprotein, gp41, from being used for entry of the virus into cell it is trying to infect.	Enfurvitide (T-20)
Entry inhibitor	Stops the HIV virus from entering the cell it is trying to infect by blocking its co-receptor on the cell, CCR5.	Maraviroc (MVC)

Table 1a. Antiretroviral drug classes that are components of ART

Common Fixed-Dose Combination Medications

Combination Medication	Antiretroviral Components
Kaletra	LPVr
Combivir	3TC, ZDV
Truvada	FTC, TDF
Epzicom	ABC, 3TC
Juluca	DTG, RPV
Trizivir	ABC, 3TC, ZDV
Atripla	EFV, FTC, TDF
Complera	FTC, RPV, TDF
Triumeq	ABC, DTG, 3TC

Table 1b. Fixed-dose combination pills (non-cobicistat containing pills)

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