HIV-1 Integrase Inhibitor Resistance by Sequencing

Test Name: HIV-1 GeneThink™ INTEGRASE (INI) Select

Laboratory Developed Test (LDT). This test was developed by and its performance characteristics have been determined by Research Think Tank, Inc. Performance characteristics refer to the analytical performance of the test.

Test Code: HIV-GT-0011

CPT Code(s): 87906

Preferred Specimen(s): Frozen plasma collected from an EDTA (lavender-top) tube or Vacutainer PPT® (white-top) tube.

Minimum Volume and Viral Load: 2 mL blood plasma at ≥ 1,500 viral RNA (vRNA) copies/mL or 3 mL blood plasma at ≥ 2,500 viral RNA (vRNA) copies/mL Vacutainer PPT.

Alternative Specimen(s): PPT potassium EDTA (white-top) tube.

Blood Plasma Collection Instructions: Collect blood in sterile tubes containing EDTA (lavender-top) or PPT (white-top) tube. Separate blood plasma from the cells by centrifugation within 6 hours after blood collection. Transfer the plasma to a separate plastic screw-cap cryo-vial or approved “dried transport” tube (ViveST™).

Transport Temperature(s): Frozen for dry ice transport or ambient for dried specimen transport using ViveST™ Transport System.

Specimen Stability: Room temperature, 24 hours, Refrigerated 3 days, Frozen 35 days, and Ambient dried 3-6 months.

Reject Criteria: Minimum volume requirements and viral load Total RNA Equivalents (TRE) not met (see above). Gross hemolysis, Lipemia, Received room temperature (frozen specimen), Received refrigerated, Serum, Non-centrifuged PPT, Frozen PPT (in situ), Heparinized plasma, or non-dried specimen with color indicator (purple/pink) for ViveST Transport System.
**Methodology:** Viral RNA extraction, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) and Sequencing on the FDA approved TRUGENE® System.

**Performing and Receiving Laboratory:**

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**Clinical Significance:** The emergence of integrase drug resistance mutations has been observed in vitro and in patients experiencing virologic failure on raltegravir (brand name Isentress Merck & Co. was approved by U.S. Food and Drug Administration (FDA) in October 2007) and/or elvitegravir (EVG Gilead was approved by the FDA on August 27, 2012) or *dolutegravir* (DTG-ViiVHealthcare www.viiivhealthcare.com (877) 844-8872 and Shionogi www.shionogi-inc.com (800) 844-8872 in expanded access clinical trials March 06, 2012). Further information about inclusion and exclusion criteria for dolutegravir are available online at ClinicalTrials.gov. Twenty three percent of patients receiving integrase inhibitors in a clinical trial experienced virologic failure at 48 weeks, and genotypic analysis detected resistance associated mutations in 68% of virologic failures. This assay amplifies and sequences the HIV-1 integrase gene and reports mutations at positions associated with integrase inhibitor specific drug resistance.

*Note- Dolutegravir is an investigational drug under development as a treatment for HIV-1 infection and is not currently approved by the Food and Drug Administration or the European Medicines Agency. The safety and efficacy of dolutegravir has not been fully established or thoroughly evaluated by regulatory agencies. For more information go to the ViiV expanded access website at http://www.dolutegravir-eap.com/english-general-information.aspx*

**Clinical Use:** Guided selection of HIV-1 antiviral cocktails in order to predict possible drug resistance to the new integrase inhibitor (INI) class of drugs including raltegravir (Isentress) and/or elvitegravir (EVG) and the experimental expanded access drug dolutegravir (DTG) to monitor transmission of INI Resistance Associated Mutations (RAM).
**Clinical Background**

Despite continuing advances in treatment for HIV-1 infection, antiretroviral therapy (ART) may fail due to the accumulation of drug resistance mutations or the transmission of drug resistance mutations pre-therapy. Identifying such mutations can optimize and guide selection of antiretroviral drugs. Thus, detection of mutations associated with antiviral resistance is recommended at entry into care, when ART is initiated or changed, when treatment failure is suspected, or when otherwise clinically indicated.\(^1,2\) When virologic failure is suspected, resistance testing should be performed while the patient is taking the drug or within 3 weeks after discontinuing it.\(^1\)

This HIV-1 Integrase Genotype test is designed to detect mutations associated with resistance to integrase class of inhibitors (INI) also known as HIV integrase strand transfer inhibitor (INSTI). These INI’s represent a new antiretroviral drug class in which the catalytic activity of integrase enzyme is inhibited, thereby preventing formation of the HIV provirus required for viral propagation and integration into host chromosome. The major mutations associated with raltegravir (RTG) resistance include N155H, Q148H/K/R, and Y143C/H/R.\(^3,4\) and for elvitegravir (EVG) resistance mutations include T66A/K/I, E92Q/G, T97A, S147G, Q148H/K/R, N155H\(^5,6\) (**Note cross-resistant profiles of INI\(^6,7,8\) and the mutations E92Q, L101I, T124A, Q148H/R, and S153Y/F\(^7,8,9\) for dolutegravir, the experimental expanded access second generation INI. In addition, minor mutations that may contribute to drug resistance as compensatory, Resistance Associated Mutations (RAM), are reported, as well as non-RAM polymorphisms (mutations that do not have reported resistance effects) which make up the virus “Polymorphic Fingerprint”.

**Note- See cross-resistance profiles for raltegravir (RTG) and elvitegravir (EVG).**
Individuals Suitable for Testing

- HIV-1–infected individuals receiving raltegravir (RTG) or elvitegravir (EVG) or dolutegravir (DTG) and showing evidence of virologic failure.

- HIV-1–infected individuals for whom raltegravir (RTG) or elvitegravir (EVG) or dolutegravir (DTG) treatment is being considered, i.e., drug naïve to Integrase inhibitors (INHI).

Method

- Reverse transcription and PCR amplification of the HIV-1 integrase gene.
- DNA sequencing of the clinically relevant portions of the integrase gene (codons 19-182).
- Report includes detected mutations and predicted drug resistance to raltegravir and etrivorine.
- Analytical sensitivity: 1,000 copies/mL with 1 ml of blood plasma.
- Analytical specificity: detects mutations in the integrase gene of HIV-1 group M subtype B as well as various non-B subtypes.
- Aliases: Isentress (raltegravir, MK-0518), elvitegravir (EVG), HIV-1 integrase mutations.

Interpretive Information

Lack of mutations associated with raltegravir or elvitegravir (EVG) or dolutegravir (DTG) resistance suggests the patient may be eligible for treatment with raltegravir or elvitegravir (EVG) or dolutegravir (DTG). On the other hand, mutations predictive of raltegravir or elvitegravir (EVG) or dolutegravir (DTG) resistance are associated with drug failure, and alternative therapy should be considered.

Therapeutic failure may be due to factors other than drug resistance, including poor adherence to the drug regimen, suboptimal therapy or drug bioavailability, and immunologic decline. Thus, in clinical practice, physicians select therapeutic regimens on the basis of the patient’s antiretroviral treatment history, viral load, clinical status, and potential metabolic toxicity, as well as resistance information.
References


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6. Olivia Goethals1, Reginald Clayton1;*, Marcia Van Ginderen1, Inge Vereycken1, Elisabeth Wagemans1, Peggy Geluykens1, Koen Dockx1, Rudy Strijbos1, Veerle Smits1, Ann Vos1, Geert Meersseman1, Dirk Jochmans1, Kurt Vermeire2, Dominique Schols2, Sabine Hallenberger1, and Kurt Hertogs1, Resistance Mutations in Human Immunodeficiency Virus Type 1 Integrase Selected with Elvitegravir Confer Reduced Susceptibility to a Wide Range of Integrase Inhibitors, JOURNAL OF VIROLOGY, Nov. 2008, p. 10366–10374.

7. Garrido C, Soriano V, Geretti AM, Zahonero N, Garcia S, Booth C,

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