510(k) Summary
TABLE OF CONTENTS

1. General Information

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21CFR807.92

Submitter's Name & Address:	Visible Genetics Inc. 700 Bay Street, Suite 1000 Toronto, Ontario M5G 1Z6 Canada
Premarket Notification 510(k) Number:	BK000038
Date of Summary Preparation:	October 22, 2001
Device Trade Name(s):	TRUGENE™ *HIV-1* Genotyping Kit and OpenGene™ DNA Sequencing System
	The major components of the system are: OpenGene™ Software System Long-Read Tower™ Sequencer Gel Toaster™ Polymerization Unit MicroCel™ 500 Cassette
	Additional components that are required for the successful operation of the device are: SureFill™ 6% Sequencing Gel Cartridge GeneObjects™ software GeneLibrarian™ module TRUGENE™ *HIV-1* Resistance Reporter GuideLines™ Resistance Rules OPENSTEP user environment
Common or Usual Name:	*In vitro* HIV Drug Resistance Genotype Assay
Classification Name:	Not known
Class of Device:	II
Identification of the legally marketed device to which the submitter claims equivalence:	There is no predicate device for this device. As a result of a petition filed on July 13, 2001, FDA determined that the TRUGENE *HIV-1* Genotyping Kit and OpenGene DNA Sequencing System can be classified in class II with the establishment of special controls.

2. Intended Use / Indications for Use

The TRUGENE *HIV-1* Genotyping Kit and the OpenGene DNA Sequencing System is intended for use in detecting HIV genomic mutations (in the protease and part of the reverse transcriptase regions of HIV) that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection. These two regions code for the main targets of antiretroviral drug treatment. In order to use the TRUGENE *HIV-1* Genotyping Kit and the OpenGene DNA Sequencing System, an operator (technologist) must first be trained and certified by Visible Genetics. This qualitative assay is used in conjunction with clinical presentation, laboratory markers and antiretroviral history as an adjunct to the therapeutic management of patients with HIV-1, subtype B infection, and a minimum viral load of 1000 RNA copies per mL. Interpretation and application of the results should be done by a qualified physician.

The TRUGENE *HIV-1* Genotyping Kit and OpenGene DNA Sequencing System is not indicated for use as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection.

3. Device Description

General Description

The TRUGENE *HIV-1* Genotyping Kit is a sequence-based assay targeted at the Protease region (codons 1 to 99) and RT region (codon 40 to 247) of the HIV-1 genome. These regions have been selected as they code for resistance against antiretroviral drugs: the protease inhibitors and the reverse transcriptase non-nucleosides and nucleoside inhibitors. The OpenGene DNA Sequencing System, a complete platform to perform DNA sequencing consists of the Long-Read Tower Automated DNA sequencer, a Gel Toaster polymerization unit and the OpenGene HIV-1 Software System to acquire and analyze the data.

The device is comprised therefore of several components, which include chemistry, hardware and software.

Function

The chemistry kit contains all the reagents necessary to perform reverse transcription and amplification of the extracted RNA virion and CLIP sequencing reactions of the protease and reverse transcriptase portions of the HIV-1 genome.

The hardware components are the Long-Read Tower Automated DNA sequencer which is an electrophoresis unit, MicroCel™ cassettes which, when filled with acrylamide monomer, form the stationary phase of the electrophoretic plate and a Gel Toaster™ polymerization unit which polymerizes the acrylamide to form the electrophoresis medium.

The software then assembles the data, aligns the sequences and determines the DNA sequence by looking at the order of the bases. The software is provided to analyze the electrophoretic products by identifying every base in the sequence. Through a process of comparison to an HIV-1 database, the software identifies discordant bases, to produce the final genotyping report, the TRUGENE *HIV-1* Resistance Report for each patient sample. This interpretive report is based upon a defined set of criteria developed by an international panel of HIV-1 clinical and research experts and coded by the Visible Genetics Research & Development group, based on the GuideLines™ Rules interpretation algorithm.

Scientific Concepts and Technological Considerations

The TRUGENE *HIV-1* Genotyping Assay is based upon several processes:

- Reverse Transcription and PCR Amplification: Reverse transcription of target RNA to generate cDNA using Reverse Transcriptase (RT) and PCR amplification of target cDNA using HIV-1 subtype B specific primers;
- CLIP Reaction: CLIP sequencing of the PCR amplicons using HIV-1 subtype B specific primers;
- Gel Preparation, Polymerization, and Electrophoresis of CLIP Reaction Products: Separation of the CLIP sequencing reactions by electrophoresis on a polyacrylamide gel, and detection by laser-induced fluorescence; and
- Data Analysis - Genotyping: Analysis of the forward and reverse CLIP sequences using the OpenGene system software. The end result is a TRUGENE *HIV-1* Resistance Report for each sample.

Specimen Collection, Preparation and Storage are standard processes not provided in the kit, however several methods for extraction of RNA are recommended for use in the product labeling.

4. Potential Risks associated with use of the Device

FDA identified risks to health associated with the use of the device included inaccurate detection of resistance mutations present in a patient's viral swarm. This could result in continuance of therapies that are no longer appropriate or it could result in changes to new inadequate therapies. In both cases the patient's viral load may increase, worsening the clinical prognosis and accelerating the development of drug resistant viruses. Patients may be needlessly subjected to serious, deleterious side effects of inappropriate antiviral drugs. Furthermore, failure of the assay to give any results at all (sequence failure) can deny or delay beneficial, appropriate therapies, which may also result in high viral loads.

These risks are controlled by adherence to the procedures identified in the FDA draft guidance document, "Guidance for Industry: Premarket Notifications [510(k)s] for *In Vitro* HIV Drug Resistance Genotype Assays: Special Controls", including the analytical and clinical studies required to determine sensitivity, reproducibility and accuracy of detecting reporting and interpreting sequences of HIV genomes present in the blood of patients already known to be infected with HIV. VGI believes that with the special controls identified by FDA these risks have been addressed with the clinical and non-clinical studies for the TRUGENE *HIV-1* Genotyping Kit and OpenGene DNA sequencing system. There have been no reportable events currently documented for this device.

5. Non-Clinical Studies

Potential user hazards were evaluated and the device hardware (Long-Read Tower Sequencer and Gel Toaster Polymerization unit) met the following safety standards:

Certificate of Compliance, Certificate Number LR 109279-6

- CAN/CSA-C22.2 No. 1010.1-92 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part I: General Requirements (Includes Amendment 1)

- CAN/CSA-C2.2 No. 1010.1B-97 Amendment 2 to CAN/CSA-C22.2 No. 1010.1-92 Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use, Part I: General Requirements
- ANSI/ISA S82.01-1994 Safety Standards for Electrical and Electronic Test, Measuring, Controlling and Related Equipment – General Requirements

CB Test Certificate, Certificate Number CA 1622 / Test Report Ref. No. CB109279-8,

- IEC Publication 61010-1 Edition 1:1990, including Amendment No. 1 (1992) and Amendment No. 2 (1995), conformity testing and certification of electrical equipment

Verification Certificate for Electromagnetic Compatibility Requirements File No. VIS-014-IEC3-2

- IEC 1000-3-2/EN61000-3-2 Harmonic Current emissions (Input current < 16 Amps per Phase)
- IEC 1000-3-3/EN 61000-3-3 Voltage Fluctuations and Flicker in Low Voltage Supply systems (Input Current < 16 Amps)

6. Clinical Studies

Table 1 gives a summary overview of the clinical studies, and the results of the studies for base calling accuracy, codon agreements, sensitivity and specificity measures of the device.

Table 1: Summary Table of Clinical Data for Basecalling Accuracy

Brief description of the study	Result
Mutation Recognition and Accuracy Study Reliability of detection of 72 codons including specific resistance mutations. Virus grown from molecular infectious clones created to contain the 72 mutations were tested at three sites. Each sample was assayed 10 times, thus each mutation was sequenced at least 10 times. Some mutations appeared on more than one MIC and were therefore sequenced 20 times.	Mutations detected with ≥ 99% reliability overall. Protease: L10F/I/R/V, K20M/R, L24I, D30N, V32I, L33F, M36I, M46I/L, I47V, G48V, I50V, I54L/V, L63P, A71T/V, G73A/S, V77I, V82A/F/S/T, I84V, N88D, L90M RT: M41L, I50V, A62V, K65R, D67N, T69D/N, K70E/R, L74V, V75I/T, F77L, A98G/S, L100I, K101E, K103N/T, V106A, V108I, Y115F, F116Y, Q151M, V179D, Y181C/I, M184I/V, Y188C/H/L, G190A/E/S, L210W, T215F/Y, K219E/Q, P236L.
Multi-Center Reproducibility Study Multi-center accuracy, reproducibility and reliability study. Panels of samples constructed from human plasma were tested. Six centers contributed to the site to site comparisons. Three sites contributed to the day to day, technician to technician and lot to lot comparisons. Three kit lots were evaluated.	All wild type codons basecalling[1] accuracy: 99% to 100% agreement with reference sequence; Wild type codons associated with resistance basecalling accuracy 98% to 100% agreement with reference sequence. **All bases:** Site to site accuracy > 98% Technician to technician accuracy > 98% Day to day accuracy > 98% Lot to lot to lot accuracy > 98% No effect seen between sites, users, days or kit lots.[2] **All codons**[3]: 97.95% (1.39) to 98.81% (0.77) agreement **Sensitivity**[4]: 0.94 (0.08) to 0.96 (0.05) **Specificity**[5]: 0.99 (0.01) to 1.00 (0.00)
Plasma Extraction Methodology Study Five different manual extraction methods were evaluated.. Samples with viral loads both >1,000 copies/mL and < 1,000 copies/mL were tested.	**All bases**: basecalling accuracy ≥ 97.8%. **All codons**: 97.45% (2.91) to 99.02% (0.92) agreement. **Sensitivity**: 0.87 (0.2) to 0.96 (0.09) **Specificity**: 0.99 (0.02) to 1.00 (0.01) Plasma extraction methods tested are compatible with the device, when used with patient plasma samples. Viral load minimum 1,000 copies/mL.
Interfering Substances Study Two- center interfering substances study for sensitivity and specificity of detection for HIV-1. Biochemicals: lipids, triglycerides, hemoglobin, bilirubin. ARVs: all FDA-approved drugs (as of 06/1999). Pathogens: HIV-2, HTLV I and II, CMV, HCV, HBV.	Basecalling accuracy >99% for all test samples in the three panels. Effect of interfering biochemicals - NONE Effect of interfering antiretrovial drugs - NONE Effect of interfering co-existing pathogens - NONE

Brief description of the study	Result
Anticoagulants Study Effect of blood collection methods. Single site study. Acid Citrate Dextrose (ACD), Heparin and EthyleneDiamine TetraAcetic Acid, (EDTA) were tested using freshly collected plasma samples. Samples were assayed at 30 days and 3 months post collection. Plasma samples were stored at -70°C.	Basecalling accuracy > 99% when samples are collected in EDTA or ACD and show no deterioration over a 3 month storage period at –70°C. EDTA and ACD are compatible anticoagulant media. Heparin is not a compatible anticoagulant media. **Heparin is contraindicated for use as an anticoagulant.**
GART Reanalysis Study A re-analysis by genotyping using the TRUGENE *HIV-1* Genotyping Kit and OpenGene DNA Sequencing System of clinical plasma samples obtained during the GART clinical study. Single-center study, 153 banked plasma samples collected at baseline in the GART study were reanalyzed using the device, and the results compared to the original sequence data.	General agreement between the methodologies for the major protease resistance mutations (30N-99%, 46I-91%, 46L-92.2%, 48V-100%, 82A-93.5%, 82F-100%, 82T-98.7%, 84V-99.3%, and 90M-91.5%) with all kappa statistics exceeding 0.72. Similarly, there was agreement for the major nucleoside reverse transcriptase resistance mutations (65R-99.3%, 69D-100%, 74V-99.3%, 151M-99.3%, 184V-97.4%, 215F-96.1%, and 215Y-90.8%) with kappa statistic ranging from 0.66 to 1.0.
Freeze-thaw Study Freeze-thaw cycling of plasma specimens – up to 10 freeze-thaw cycles were investigated for two plasma samples: one with a viral load 1,200 copies/mL and one with a viral load 43,000 copies/mL.	**All bases**: basecalling accuracy > 98% for each of the 10 freeze-thaw cycles, for both samples. **All codons**: 98.04% (0.46) to 98.86% (0.69) **Sensitivity**: 1.00 (0.00) **Specificity**: 0.98 (0.03) to 1.00 (0.00) No deterioration in device performance measures after 10 freeze-thaw cycles of two plasma specimens.

1. Base calling accuracy: # of bases in test sequence that match the gold standard sequence. Total # of bases in the sequence = 925.
2. Data reported for manually edited data only.
3. Total number of codons in the sequence = 306, data reported is % agreement between test sequence and gold standard. Data presented as the range of mean and (standard deviation) values reported.
4. Sensitivity defined as mutation recognition agreement rate for all codons. Calculated as the # of codons with mutations on which the test sequence and gold standard agree/ # of codons with mutations on the gold standard. Data presented as the range of mean and (standard deviation) values reported.
5. Specificity defined as wild type recognition agreement rate and calculated as the number of codons without mutations on which the the test sequence and gold standard agree/ # of codons without mutations on the gold standard. Data presented as the range of mean and (standard deviation) values reported.

Summary Data for Reliability, Reproducibility and Sensitivity of Detection

Plasma HIV-1 test panels were assayed at eight clinical sites in three different studies (Multi-Center Reproducibility, Plasma Extraction Methodology and Freeze-thaw studies as described in Table 1). The panels contained varying number of samples depending upon the study but in total, 340 assays were performed. These samples varied in HIV-1 RNA viral load from 1,200 copies/mL to 350,000 copies/mL. The data from the three studies were pooled and analyzed.

The mean base agreement between the test assay sequence and the gold standard sequence for all 340 assay sequences was 98.65%, and ranged from 97.7% to 99.61%. The mean codon agreement between the test assay sequence and the gold standard sequence was 98.6% for all codons in the sequence, and ranged from 92.81% to 100%. Two samples, containing approximately 1,000 copies/mL viral RNA were assayed a total of 92 times. For these two samples the mean base agreement rate and codon agreement rate was > 98%.

7. Conclusions Drawn from the Clinical and Non-Clinical Studies

The clinical and non-clinical studies described in the preceding sections established the key safety and performance characteristics of the TRUGENE *HIV-1* Genotyping Kit and the OpenGene DNA Sequencing System. Together, these studies show that the TRUGENE *HIV-1* Genotyping Kit and the OpenGene DNA Sequencing System provides a practical HIV-1 genotyping method which is safe and effective for clinical use. The GART re-analysis study confirms that the clinical utility of HIV-1 genotyping indicated in the original GART study can be achieved with the TRUGENE *HIV-1* Genotyping Kit and the OpenGene DNA Sequencing System for use in detecting HIV genomic mutations (in the protease and part of the reverse transcriptase regions of HIV) that confer resistance to specific types of antiretroviral drugs, as an aid in monitoring and treating HIV infection.