EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR *SENTOSA*® SQ HIV-1 GENOTYPING ASSAY AND ASSOCIATED *SENTOSA*® NGS (NEXT GENERATION SEQUENCING) WORKFLOW SYSTEM.

DECISION SUMMARY

A. STN:

BR190330

B. Purpose of The Submission:

De Novo petition for evaluation of automatic class III designation for the *Sentosa*® SQ HIV-1 Genotyping Assay.

C. Measurands:

Drug resistance mutations in the protease, reverse transcriptase and integrase genomic regions of the HIV-1 pol gene.

D. Type of Test:

Next generation sequencing test for detection of genomic HIV-1 drug resistance mutations

E. Applicant:

Vela Diagnostics USA, Inc.

F. Proprietary and Established Names:

Sentosa ® SQ HIV Genotyping Assay.

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3955

2. Classification:

   Class II

3. Product code(s):

   QIC

4. Panel:

   Microbiology (31)

H. Intended for use:

1. Intended for use:

   The *Sentosa*® SQ HIV-1 Genotyping Assay is a next generation sequencing (NGS) - based in vitro diagnostic (IVD) test intended for use in detecting HIV-1 genomic mutations (in the protease, reverse transcriptase and integrase regions of the pol gene) as an aid in monitoring and treating HIV-1 infection. This test is used in adjunct to the therapeutic management of patients diagnosed with HIV-1 Group M infection with viral loads of at least 1,000 RNA copies per mL in EDTA plasma specimens.

   The *Sentosa*® SQ HIV-1 Genotyping Assay is used in conjunction with the *Sentosa*® SX Virus Total Nucleic Acid Plus (4x24) and *Sentosa*® SX IA Template Prep kits on the *Sentosa*® SX101 instrument and *Sentosa*® SQ Sequencing instrument and *Sentosa*® SQ 318 Chip kit.

   Results should be used in conjunction with other available laboratory and clinical information and are not intended for use as an aid in the diagnosis of infection with HIV or to confirm the presence of HIV infection, or for screening donors of blood, plasma or human cells, tissues and cellular and tissue-based products (HCT/Ps).

2. Special Conditions for use statements:

   For *in vitro* diagnostic use only
   Prescription use only

3. Special instrument requirements:

   Sentosa® SX101 instrument and *Sentosa*® SQ Sequencing and *Sentosa*® SQ 318 Chip

I. Device Description

The *Sentosa*® SQ HIV Genotyping Assay is a ready-to-use kit for automated next generation sequencing (NGS) that detects HIV-1 drug resistance mutations in HIV-1 pol genomic regions (Protease (PR), Reverse Transcriptase (RT) and Integrase (IN)). The *Sentosa*® SQ HIV-1 Genotyping Assay contains reagents and solutions for the sample preparation, purification, and normalization of up to 22 HIV clinical samples per run including one system control and one positive control. Additionally, an extraction control (EC) containing non-HIV ((b) (4)) is spiked into clinical samples and the positive control to monitor reagent failure and/or the presence of enzymatic reaction inhibitors in clinical samples. The *Sentosa*® SQ HIV Genotyping Assay uses the *Sentosa*® Link from sample processing to final report generation. The function of components of the device are listed below:

- *Sentosa*® SQ Virus Testing solutions and *Sentosa*® SX Virus Total Nucleic Acid Plus (4x24) reagents are non-assay-specific reagents used for nucleic acid extraction and library preparation.
- *Sentosa*® *SX 101* is an automated liquid handling system which uses *Sentosa*®

reagents for extraction of RNA from clinical samples and the preparation of DNA libraries for NGS sequencing.

- Veriti® Dx is a 96-Well thermal cycler that performs 1-step RT-PCR amplification on the extracted RNA
- *Sentosa*® *ST301 Sequencer* sequences the PCR products using *Sentosa*® reagents for DNA sequencing.
- *Sentosa*® *Link* is software that integrates the workflow between *Sentosa*® SX 101 and *Sentosa*® ST301.
- *Sentosa*® *Reporter* is software that automatically performs secondary analysis on primary sequencing data from the *Sentosa*® SQ301 servers for the *Sentosa*® SQ HIV Genotyping Assay and generates a report to the physician.

  The report lists the antiviral drug resistance mutations that were detected in the patient's viral sequences as compared to a reference wild type HIV-1 genome. The report also provides mutation (variant) frequency and the coverage of sequence reads for that mutation. The treating physician uses the report information to adjust therapy as needed in conjunction with other clinical and laboratory information.

- In addition to the equipment listed above, several consumables and general purpose lab equipment listed in package insert are required and are purchased separately.

J. Standards/Guidance Documents Referenced

- MM17-A: Verification and Validation of Multiplex Nucleic Acid Assays; Approved Guideline.

- Food and Drug Administration. Center for Biologics Evaluation and Research (2011). *Class II Special Controls Guidance Document: In Vitro HIV Drug Resistance Genotype Assay*. Available from: https://www.fda.gov/media/72159/download ; Accessed 9/24/2019.

K. Test Principle

The *Sentosa*® SQ HIV-1 genotyping assay detects HIV-1 drug resistance mutations in patients taking or about to start antiviral therapy.

The *Sentosa*® work flow process begins with loading plasma samples and the *Sentosa*® SQ Virus Testing solutions into the *Sentosa*® SX101 for automated nucleic acid extraction. Subsequently, extracted nucleic acids are mixed with the master mix containing 1-step RT-PCR amplification reagents. After the master mix and nucleic acid mixing is complete, the user transfers eluted nucleic acid to the Veriti® Dx 96-Well Thermal Cycler for gene-specific PCR amplification ((b) (4) PR/RT and (b) (4) IN regions of pol gene). Multiple primer sets amplify two HIV-1 fragments and the internal control. PCR amplicons are fragmented enzymatically, followed by ligation of adapters with barcodes of DNA sequences specific for *Sentosa*® ST301 Sequencer technology. DNA libraries are prepared, normalized, and enriched using the Isothermal Amplification (IA) system. All 24 equalized reactions are then pooled at a relatively equal molar ratio into one pooled library to be sequenced using the *Sentosa*® ST301 Sequencer. The DNA barcode in the barcoded adapters are used to

differentiate patient samples by post-sequencing bioinformatics analysis. *Sentosa*® link software integrates the workflow between the instruments and *Sentosa*® Reporter reports mutations associated with drug resistance.

To generate the report, the *Sentosa*® SQ Reporter uses a bio-informatic pipeline to compare sequences generated by the *Sentosa*® ST301 Sequencer to the reference HIV sequences and the Stanford University HIV Drug Resistance Database and reports the genotype and mutations detected. This comparison is reported to the physician as a list of drug-resistance mutations detected by the assay.

L. Performance Characteristics

1. Analytical performance:

a) Limit of Detection (LOD)
The purpose of this study was to determine the LOD for the *Sentosa*® SQ HIV-1 Genotyping Assay. This study demonstrates minimal viral load for a reliable detection of HIV-1 target genes and detection of drug resistant mutants at claimed variant frequencies. Three LODs were determined (LOD1, LOD2 and LOD3).

LOD1: the lowest concentration at which (b) (4) of the replicates that were tested detected PR/RT and IN genomic targets. Multiple dilutions of HIV-1 genotype B was tested in replicates of (b) (4). The LOD1 was determined to be 1,000 copies/mL (cps/mL) (Table 1a). LOD1 was also validated using HIV-1 Group M subtypes A, B, C, D, F, G, H, J, and K at 1000 cps/mL (Table 1b).

Table 1a: Analytical sensitivity of *Sentosa*® SQ HIV-1 Genotyping Assay

(b) (4)

Table 1b: Analytical sensitivity of *Sentosa*® SQ HIV-1 Genotyping Assay for all HIV-1 Group M subtypes

	1000 cps/mL															
	A		C		D		F		G		H		J		K	
	PR/RT	IN	PR/RT	IN	PR/RT	IN	PR/RT	IN	PR/RT	IN	PR/RT	IN	PR/RT	IN	PR/RT	IN
Replicates Positive	(b) (4)															
Replicates Negative																

LOD2: the minimum variant frequency of the targeted mutations that were detected with a sensitivity of (b) (4)% when the viral load is equal to LOD1 (1,000 cps/mL). In this study, HIV-1 subtype (b) (4) samples were mixed to generate samples with drug-resistance mutations (DRMs)

at variant frequencies of (b) (4), 20%, (b) (4) at LOD1. Greater than (b) (4)% of the samples with DRMs at a frequency of (b) (4) 20% were detected, and (b) (4) of the samples with DRMs at frequencies of (b) (4) were detected, respectively (Table 2). The LOD2 of the *Sentosa*® SQ HIV-1 Genotyping Assay is 20% variant frequency at 1,000 copies/mL.

(b) (4)

LOD3: The minimum viral load at which the target variants with a variant frequency of 5% and 10% could be detected with a sensitivity ≥ 90%. For this study, two different HIV-1 subtype (b) (4) samples were mixed to obtain samples with DRM frequencies of 5% or 10%, each with viral loads of 5x, 15x, and (b) (4) LOD1 (5,000, 15,000, (b) (4) cps/mL, respectively).

(b) (4) of samples with a DRM frequency of 5% were detected at 5x LOD1 and >90% were detected at 15x (b) (4) LOD1. Greater than 95% of the samples with a DRM frequency of 10% were detected at all three viral loads (Table 3).

Table 3: Percentage of DRMs detected at 5% and 10% variant frequency at (b) (4), 15x and 5x LOD1

LOD1	(b) (4)	15x		5x	
Variant Frequency		5%	10%	5%	10%
% of samples detected		100	100	82	95

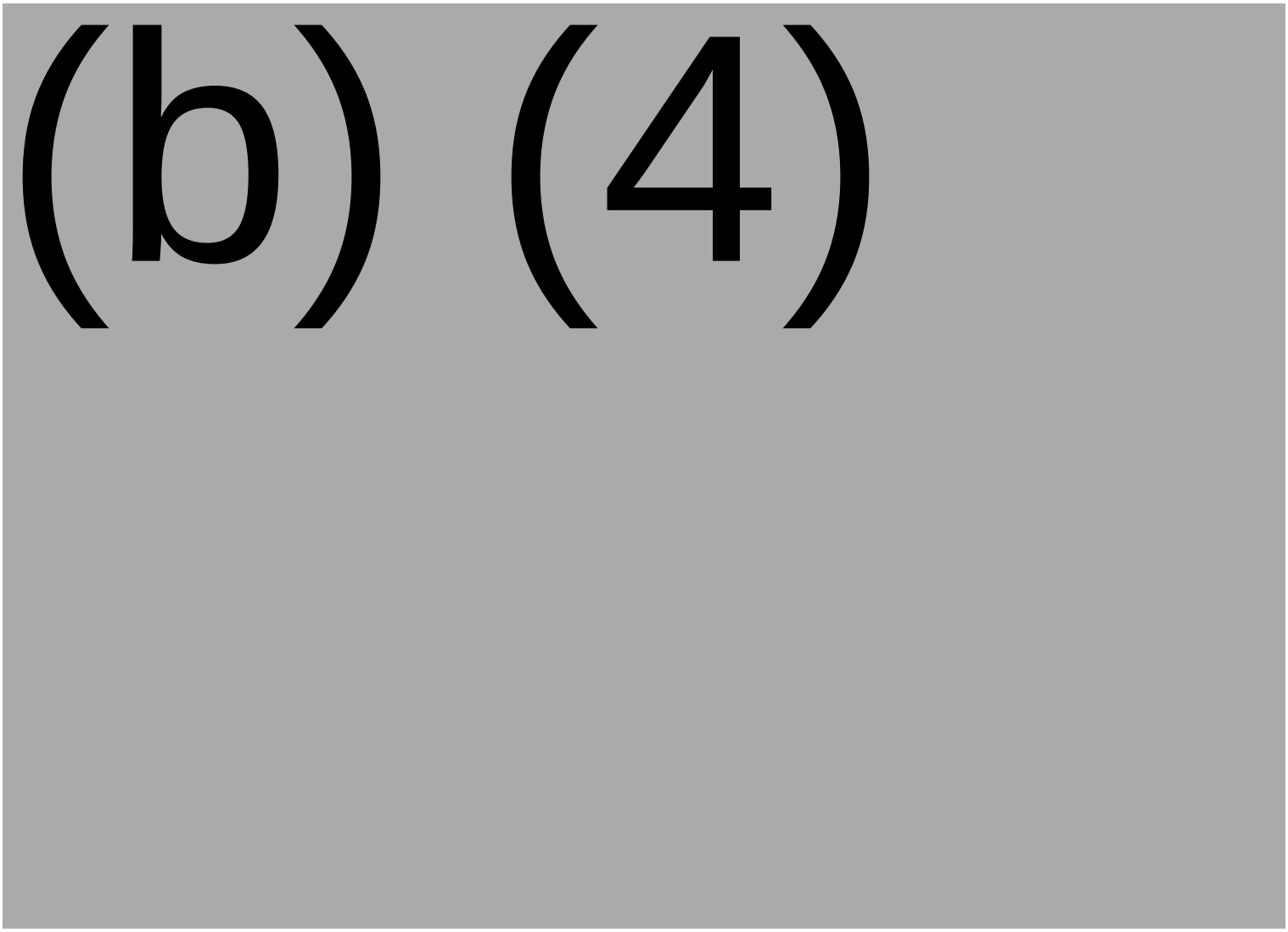
Summary: The LOD1 study demonstrated that the *Sentosa*® SQ HIV-1 Genotyping Assay limit of detection is 1,000 cps/mL. At LOD2, reliable detection of DRMs ≥90% sensitivity is found to be 20% variant frequency. The LOD3 study demonstrated that the minimum viral load at which variant frequencies of 5% and 10% were detected at ≥ 90% was 15x LOD1.

b) Precision

The precision of the *Sentosa*® SQ HIV-1 Genotyping assay was evaluated using (b) (4) HIV-1 samples. Reliable detection of mutations with a variant frequency of 5% was demonstrated at (b) (4) LOD3. In this study, samples were serially diluted to a variant proportion of 5% and 95% at concentrations equal to (b) (4), 3x (b) (4) LOD3 ((b) (4) LOD3 at 5% variant frequency is (b) (4) copies/mL) using HIV-1 negative EDTA plasma. HIV genotyping was performed with 10 replicates of each sample across the three viral titers using three different lots of the *Sentosa*® SQ HIV Genotyping Assay. A total of (b) (4) ((b) (4) samples x 10 replicates x 3 kits) were generated in this study. Precision was evaluated by determining the coefficient of variation (CV) of the frequency of detection of each mutation. Variants expected to be present as 5% or 95% were considered for the primary CV calculation. Minor DRMs with frequencies

below 5% are under-quantified as a consequence of PCR bias, a well-documented phenomenon where an abundant template population is preferentially amplified. These low frequency variants are filtered by the SQ Reporter software because they cannot be detected with 99% confidence.

PCR bias estimates were determined, and the results adjusted by excluding variants with frequency < 5% from CV calculations (PCR-bias adjusted). However, using raw sequencing data, calculation of detection rates and precision of minor proportion mutations with an observed mean variant frequency (b) (4) % were also included as part of the evaluation (unadjusted) (Table 4).



Summary: Variants at 95% frequency were detected with high precision and < 5% CV at (b) (4) LOD3. However, for variants at 5% frequency, before accounting for PCR bias, mutant detection accuracy is (b) (4) %. After adjustment for PCR bias, detection was > 99% at (b) (4) LOD3 and for overall estimates. The average %CV was (b) (4) % for 5% and 95% variant frequencies before adjusting for PCR bias. After adjusting for PCR bias, the average

%CV for the 5%, 95%, and combined variant proportions were (b) (4) %, respectively.

The detection rate of the precision study when all mutations were considered was (b) (4) % ((b) (4)) before adjustment for PCR bias. After adjustment for PCR bias, mutations with frequency < 5% and the resulting detection rate was (b) (4) % ((b) (4)).

c) Analytical Reproducibility

In these and other studies, the criterion “Sample detection” is defined as the percentage of samples in which at least one of the expected mutations in each of the targeted genomic regions was detected, and the criterion “mutation detection” is defined as the percentage of the total expected mutations that were detected.

The analytical reproducibility of the *Sentosa*® SQ HIV-1 Genotyping Assay was demonstrated using a panel of HIV-1 positive samples in 30 runs across 3 test sites in the U.S. where each site used one lot of reagents, one set of instruments and 2 operators per site. Inter- and intra-assay %CV of variant frequency (VF) measurements were used to evaluate analytical reproducibility. For sample detection, a total of 630 replicates were generated. For the 5%, 95%, and 100% variant frequencies, 900, 360, and 180 replicates were generated, respectively. For the HIV-1 Positive Control (PC) sample detection, 60 replicates were generated. For the PC mutation detection, 600 replicates were generated.

Table 5a: Reproducibility of samples and mutation detection

Sample	Site 1		Site 2		Site 3		Percent Agreement
	Detection	95% CI	Detection	95% CI	Detection	95% CI	
Run (n=10/site)	10	-	10	-	10	-	
Sample Detection (21/run)	100% (210/210)	98.2, 100	100% (210/210)	98.2,100	100% (210/210)	98.2,100	100
5% DRM	97.0% (291/ 300)	94.4, 98.4	99.3% (298/ 300)	97.6,99.8	97.0% (291/300)	94.4,98.4	95.01
95% DRM	100% (120/120)	96.9, 100	100% (120/ 120)	96.9,100	100% (120/120)	96.9,100	100
100% DRM	100% (60/60)	94.0, 100	100% (60/60)	94.0,100	100% (60/60)	94.0,100	100
All DRM	98.1 (471/480)	98.5, 99.0	99.6 (478/480)	98.5,99.9	98.1 (471/480)	96.5,99.0	96.01
HIV-1 PC Detection (2 PC/run)	100% (20/20)	83.9, 100	100% (20/20)	83.9,100	100% (20/20)	83.9,100	100
PC mutation	100% (200/200)	98.1, 100	100% (200/ 200)	98.1,100	98% (196/200)	95.0,99.2	98.69

Table 5b: Reproducibility and precision of detected variant frequencies

		% CV at 5% VF				%CV at 95% VF	%CV at 100% VF
		PR	RT	IN	Overall	PR*	PR*
Inter-assay	Site-to-site	5.06	3.17	5.46	4.44	0.39	0.23
	Lot-to-lot						
	Instrument-to-Instrument						
Intra-assay	Site 1	26.85	31.68	18.96	28.38	3.56	0.42
	Site 2	32.60	28.34	21.79	31.40	4.19	0.25
	Site 3	28.99	35.96	16.16	30.50	3.43	0.32

*These samples did not include mutations in RT or IN

Summary: High analytical reproducibility was demonstrated by the percent agreement > 95% for all tests where sample and variant detection rates were ≥ 95% (Table 5a). The site-to-site %CV at all variant frequencies was < 5% (Table 5b). Intra-assay CV, which represents the precision of measurement was 16.6%–35.96% for the 5% variant frequency population and < 5% for the 95% and 100% variant frequency populations. Inter-assay CV, which represented the reproducibility of data generated at different sites and operators, was ~4%, 0.39% and 0.23% for the 5%, 95% and 100% variant frequency populations (Table 5b). There were (b) (4) failed runs in this study; most were attributed to operator error or system control failure that do not affect safety and effectiveness because these samples are flagged for re-test and are not used to interpret results.

d) Interference

The Interference study evaluated the analytical specificity of the *Sentosa*® SQ HIV-1 Genotyping Assay in accordance with the recommendations in CLSI MM17-A. A total of (b) (4) interference substances/microorganisms were evaluated. HIV-1 samples with DRMs at (b) (4) x LOD1 ((b) (4) copies/mL) were used in the study. Potentially interfering substances/microorganisms were spiked into the HIV-1 positive samples and HIV genotyping was performed using the *Sentosa*® SQ HIV Genotyping assay in triplicate for each interfering substance/microorganism listed below.

Table 6: Microorganisms

Organism tested	Concentration
Human immunodeficiency virus type 2 (HIV-2)	1 x 10^6 cp/mL
Human T-lymphotropic virus type I (HTLV-I)	115.18 S/CO
Human T-lymphotropic virus type II (HTLV-II)	20 Ct
Cytomegalovirus (CMV)	1 x 10^6 cp/mL

Organism tested	Concentration
Epstein Barr virus (EBV)	1 x 10^6 cp/mL
Hepatitis B Virus (HBV)	1 x 10^6 cp/mL
Hepatitis C Virus (HCV)	1 x 10^6 cp/mL
Candida albicans	1 x 10^6 cp/mL
Pneumocystis jirovecii	1 x 10^6 cp/mL
Mycobacterium tuberculosis	1 x 10^6 cp/mL
Mycobacterium avium Strain K-10	1 x 10^6 cp/mL
Mycobacterium intracellular	1 x 10^6 cp/mL
Homo sapiens, genomic DNA	1 µg

Table 7: Interfering substances

Interfering substance		Max concentration
Hemoglobin Human		0.05 mg/mL
Lipid standards - triglycerides		1.9 mg/mL
Albumin, Human Fraction V Powder		55 mg/mL
Bilirubin		0.012 mg/mL
EDTA		6.16 mM
Alpha-interferon		4.27 pg/mL
Interferon-α2a		2580 pg/mL
Interferon-α2b		263 pg/mL
Ribavirin		7.08 µg/mL
Ganciclovir		13.2 µg/mL
Foscarnet		0.08 mg/mL
Antimycobacterial (Rifampin)		32 µg/mL
Antimycobacterial (Isoniazid)		7 µg/mL
Antimycobacterial (Pyrazinamide)		64.6 µg/mL
Ciprofloxacin		11.8 µg/mL
Tenofovir disoproxil fumarate, TNV		0.6 µg/mL
Entecavir		19.1 ng/mL
Valacyclovir		7.55 µg/mL
Azithromycin		9.91 µg/mL
Drug pool 1	Amprenavir	12.47 µg/mL
	Darunavir Ethanolate	13.1 µg/mL
	Enfuvirtide Acetate Salt	14.8 µg/mL
	Efavirenz	10 µg/mL
	(b) (4)	
Drug	Indinavir, European Pharmacopoeia	17.316 µg/mL
	Saquinavir Meslyate	11.2 µg/mL

Interfering substance		Max concentration
pool 2	Lopinavir, European Pharmacopoeia	18.69 µg/mL
	Nelfinavir Mesylate Hydrate	6.7 µg/mL
	Emtricitabine, United States Pharmacopei	4.7 µg/mL
Drug pool 3	Zidovudine, European Pharmacopoeia	26.3 µM
	Didanosine, European Pharmacopoeia	12.1 µg/mL
	Stavudine, European Pharmacopoeia (Zerit)	1.276 µg/mL
	Ritonavir	38.5 µM
	Maraviroc	888 ng/mL
Drug pool 4	Lamivudine	7 ug/mL
	Abacavir Sulfate (Ziagen)	5.55 µg/mL
	Delavirdine Mesylate (Rescriptor)	100 µM
	Nevirapine	20 µg/mL

Summary: 100% of samples and 100% of DRM were detected in the study. The performance of *Sentosa*® SQ HIV-1 Genotyping Assay was not affected by the presence of microorganisms (Table 6) and antiretroviral drugs (Table 7) commonly expected to be present in human plasma specimens tested at the concentrations used.

e) Specimen Stability/Positive Control freeze-thaw Stability

The specimen stability was evaluated using a panel of (b) (4) samples at analyte concentrations equal to (b) (4) at (b) (4) % variant frequency. Stability was evaluated under the conditions below (Table 8):

Table 8: Evaluation of specimen stability after various treatment

(b) (4)	Treatment	Sample Detection	Mutation Detection
	Storage at -20°C for 30 days	(b) (4)	
	Storage at -80°C for 30 days		
	Storage at -20°C for 60 days		
	Storage at -80°C for 60 days		
	Control for Thaw plasma		
	Thawed plasma storage at 4°C (7h)		
	Thawed plasma storage at 4°C (24h)		
	Thawed plasma storage at 4°C (48h)		
	Control for Fresh plasma		
	Fresh plasma storage at 15-30°C (7h)		

(b) (4)	Treatment	Sample Detection	Mutation Detection
	Fresh plasma storage at 15-30°C (24h)	(b) (4)	
	Fresh plasma storage at 4°C (24h)		
	Fresh plasma storage at 4°C (48h)		
	Fresh plasma storage at 4°C (72h)		
	4 Freeze-thaw cycles		
	7 Freeze-thaw cycles		
	10 Freeze-thaw cycles		
	Thawed plasma storage at 4°C (7days)		

Summary: Detection of samples and mutations was > 95% for all conditions tested. These data support HIV-1 specimen stability at 1) storage at -20°C or - 80°C up to 30 and 60 days, respectively, 2) storage of fresh plasma at 2°C to 8°C for up to 72 hours, 3) storage of fresh plasma at 15°C to 30°C for up to 24 hours, and 4) storage of thawed plasma at 2°C to 8°C for up to 7 days and up to 10 freeze-thaw cycles.

Stability of the *Sentosa*® SQ HIV Positive Control (PC) was evaluated for short-term storage at ambient temperature and over multiple freeze-thaw cycles. (b) (4) lots of *Sentosa*® SQ HIV Positive Control were stored at ambient temperature up to three hours (1.5 hours and 3 hours) or underwent multiple freeze-thaw cycles (four, seven, and ten) before HIV genotyping was performed using the *Sentosa*® SQ HIV Genotyping Assay (Table 9).

Table 9: PC Stability at ambient temperature or up to ten (10) freeze-thaw cycles

(b) (4)

Summary: Detection of 10 DRMs in the *Sentosa*® SQ Positive Control was 100% for all (b) (4) lots tested after multiple freeze-thaw cycles. The *Sentosa*® SQ HIV Positive Control as determined to be stable at ambient temperature (19–21°C) for three hours and for up to ten freeze-thaw cycles.

f) Kit Stability

A real-time stability study was performed and is planned to continue for a total of (b) (4) months. Kit stability was evaluated using (b) (4) separate lots each of the *Sentosa*® SQ HIV Genotyping Reagents, *Sentosa*® SQ HIV Positive Control Kit and *Sentosa*® SX IA Reactions Kit. The test kits were stored at the temperatures shown in Table 10 and tested at every testing point for a total of (b) (4) intervals. The stability of the kit reagents were tested with a panel of (b) (4) samples at (b) (4) and a panel of (b) (4) samples with (b) (4) % variant frequency at (b) (4) . Performance data was submitted for four testing intervals covering up to (b) (4) months from the date of manufacture.

Table 10: Recommended storage for *Sentosa*® SQ HIV Genotyping Assay

Product	Recommended Storage Temperature
Sentosa® SQ HIV Genotyping Reagents	-25 to -15°C
Sentosa® SQ Virus Testing Solutions	2 to 8°C
Sentosa® SX IA Reagents	2 to 8°C
Sentosa® SX IA Reactions	-25 to -15°C
Sentosa® SX IA Solutions	15 to 30°C
Sentosa® SQ HIV Positive Control	-25 to -15°C

Summary: 100% of the samples and 100% of the mutations present at (b) (4) were detected at the time points evaluated. All results met the acceptance criteria. Real-time stability data for *Sentosa*® Genotyping Reagents support a stability claim of (b) (4) months from the date of manufacture. The data for the positive control support a stability claim of (b) (4) months from date of manufacture. Therefore, the data support a shelf-life claim of a 6 months at the time of granting this de novo.

g) Simulated extreme conditions shipping validation

Following the manufacture of the *Sentosa*® SQ HIV Genotyping Assay, the kits are shipped from Vela Singapore to the U.S. A shipping study was conducted in an environmentally controlled chamber with temperature and humidity profiles simulating extreme (b) (4) shipment conditions using (b) (4) kit lot. Simulated (b) (4) profiles were evaluated for a range of temperatures (-20 to (b) (4) °C) and humidity ((b) (4)), followed by functional testing of the kits. (b) (4) NGS run from each kit lot was performed with (b) (4) HIV-1 samples at (b) (4) and (b) (4) HIV-1 Positive Controls. Data was then compared to the reference run using the same kit lots at the point of origin at Vela in Singapore.

Table 11: Performance of NGS runs in reference kits and kits exposed to (b) (4) conditions

(b) (4)

Summary: 100% of the samples and mutations were detected before and after shipment. These data indicate the kits are stable under anticipated shipping conditions.

2. Performance Testing – Animal

This premarket De Novo Classification Request submission does not rely on the assessment of animal performance data to demonstrate substantial equivalence. This section is not applicable.

3. Performance Testing – Clinical

Clinical Validation

Following clinical studies were performed to evaluate the clinical performance of the *Sentosa*® HIV-1 Genotyping assay.

(1) Clinical Reproducibility Study,
(2) Clinical Sensitivity Study, and
(3) Population Sensitivity and Specificity Study.

1. Clinical Reproducibility

This study was conducted at two external sites (Cleveland Clinic (CCY, Cleveland, OH), Mayo Clinic (MYO, Rochester, MN)), and one internal site (Vela Fairfield (VFF, Fairfield, NJ)). A panel of twenty retrospective HIV-1 positive samples (4x LOD1) and the positive control (PC) were tested in triplicate in this study. All samples were de-identified and assigned unique study identification numbers. Reproducibility at 10% variant frequency was the primary metric used for evaluation due to the assay's LOD3; however, reproducibility for variant frequencies < 10% also were evaluated. Sample and mutation detection agreement between replicates at each site (S1-S3) were determined. Metrics evaluated were complete agreement (3/3): all three sites detected all mutations or samples; partial agreement (1/3 or 2/3): all three sites detected all mutations or samples in only one or two replicates; no calls (0/3): none of the sites detected any mutations; All Others: additional mutations other than those expected were detected by at least one site. Overall percent agreement between sites was used to evaluate similarity of data generated by the 3 test sites.

Table 12a: Clinical reproducibility

Reproducibility Test	Total	Agreement between sites				All Others	Overall Percent Agreement
		S1(3/3) S2(3/3) S3(3/3)	S1(2/3) S2(2/3) S3(2/3)	S1(1/3) S2(1/3) S3(1/3)	S1(0/3) S2(0/3) S3(0/3)		
Sample detection	20	19	0	0	0	1	95.0% (19/20)
Variant ≥ 20% VF	161	149	0	0	3	9	94.41% (152/161)
Variant 10-20% VF	7	7	0	0	0	0	100% (7/7)
Variant 5-10% VF	22	6	1	2	0	13	40.91% (9/22)
Variant <5% VF	46	0	0	1	0	45	2.17% (1/46)

Table 12b: Positive control reproducibility

Reproducibility Test	Total	Agreement		All Others	Percent Agreement
		S1(9/9) S2(9/9) S3(9/9)	*S1(x/9) S2(x/9) S3(x/9)		
PC detection	1	1	0	0	100% (1/1)
PC mutation detection	10	9	0	1	90.0% (9/10)

**Total counts of complete agreement for x=8, 7, 6, 5, 4, 3, 2, 1 and 0*

Summary: For reproducibility of sample detection, two sites detected all 20 test samples while one site detected 19 of the 20 test samples. Detection of the positive control was 100% for all sites. The overall percent agreement was ≥ 95% for sample detection, indicating highly reproducible sample detection. Evaluation of the reproducibility of mutation detection included all detected variant threshold levels. Greater than 90% agreement was observed at variant frequencies of 10–20% and 90% of positive control mutations were detected. Taken together, these data indicate reproducible sample and mutation detection by the *Sentosa*® SQ HIV Genotyping assay.

2. Clinical Sensitivity and Specificity

Clinical Sensitivity and specificity were evaluated by comparing the mutation calls generated by the *Sentosa*® SQ HIV Genotyping Assay to Sanger sequencing reference methods ((b) (4) for Protease and Reverse Transcriptase regions, and lab-developed (b) (4) Assay for Integrase region). The panel of 20 samples were tested at two external sites and the internal Vela site. Positive and negative results were defined as mutation and non-mutation, in

comparison with the reference sequence ((b) (4)). The 95% CI was calculated using the Wilson score method. To account for the higher LOD for variant frequency detection of the reference Sanger methods (< 20% VF), sensitivity and specificity for *Sentosa*® SQ HIV-1 genotyping assay was only calculated for mutations with > 20% VF (Table 13).

Table 13: Clinical sensitivity and specificity

Overall		**Sanger Sequencing-based methods (Reference)**		
		> 20% VF		
		Mutation	Non-mutation	Total
Sentosa® SQ HIV-1 Genotyping Assay (Test)	Mutation	1376	36	1412
	Non-mutation	37	60111	60148
	Total	1413	60147	61560
	Sensitivity:	97.38% (1376/1413) 95%CI: (96.41%, 98.09%)		
	Specificity:	99.94% (60111/60147) 95% CI: (99.92%, 99.97%)		

Summary: Overall, 97.38% (1376/1413, 95%CI: 96.41–98.09) of the mutations present at > 20% that were called by the *Sentosa*® SQ HIV Genotyping assay were also called by the reference methods.

3. Population sensitivity and specificity study

The study was conducted at the VFF site. A panel of 107 retrospective HIV-1 positive samples were used for the study. All samples were de-identified and assigned unique study identification numbers. Ten of the 107 samples were diluted with HIV-1 negative plasma; the samples covered a range of viral loads from (b) (4) . Positive and negative results were defined as mutation and non-mutation as compared to the reference sequence (b) (4) and with respect to the DRM target list. Population sensitivity and specificity were evaluated by comparing mutation detection by the *Sentosa*® SQ HIV-1 genotyping assay to the reference method (b) (4) for the Protease and Reverse Transcriptase regions, and to the (b) (4) assay for the Integrase region. The 95% CI was calculated using the Wilson score method . To account for the higher LOD for variant frequency detection of the Sanger methods (< 20% VF), sensitivity and specificity for *Sentosa*® SQ HIV-1 genotyping assay were only calculated for mutations with ≥ 20% VF (Table 14).

Table 14: Summary of population sensitivity and specificity

Overall		Sanger Sequencing-based methods (Reference)		
		> 20% VF		
		Mutation	Non-mutation	Total
Sentosa® SQ HIV-1 Genotyping Assay (Test)	Mutation	819	32	851
	Non-mutation	33	35514	35547
	Total	852	35546	36398
	Sensitivity:	96.13% (819/852) 95%CI: (94.61%, 97.23%)		
	Specificity:	99.91% (35514/35546) 95% CI: (99.87%, 99.94%)		

Summary: All samples (107/107) were detected by all three methods: *Sentosa*® SQ HIV Genotyping, (b) (4) . The overall concordance with the comparator device was > 96.13%.

4. Drug Resistance Mutation (DRM) Target List:

The *Sentosa*® SQ HIV-1 Genotyping Assay has a target list of 342 DRMs that include DRMs listed in the "FDA Guidance Class II Special Controls Guidance Document: In Vitro HIV Drug Resistance Genotype Assay" and mutations listed in the Stanford HIV drug resistance database (v8.5) that are reported by the *Sentosa*® Reporter.

The *Sentosa*® SQ HIV-1 Genotyping Assay DRM target list is classified into four categories:

(1) 121 Verified and Validated DRMs
(2) 53 Verified only DRMs
(3) 36 Validated only DRMs
(4) 132 Not verified and not validated DRMs

Verified DRMs are defined as mutations detected by the *Sentosa*® SQ HIV-1 Genotyping Assay with at least 10 replicates with > 90% accuracy during verification studies presented above that were performed to validate the assay. Validated DRMs were defined as mutations detected by the *Sentosa*® SQ HIV-1 Genotyping Assay in 127 clinical samples and confirmed with Sanger sequencing during clinical validation of the assay. An additional 132 DRMs are reported as not validated and not verified because they were not validated or verified with this assay but are included in the Stanford HIV drug resistance database (v8.5, versioning date 04/16/2018).

The list of DRMs reported by the *Sentosa*® SQ HIV-1 Genotyping Assay is included in Appendix I to IV. The reference sequence used to generate the list was (b) (4)

HIV-1 gag mutations:

The *Sentosa*® SQ HIV-1 Genotyping Assay does not interrogate the HIV-1 Gag gene. However, mutations in gag cleavage sites may confer resistance to all protease inhibitors (PI) and may emerge before mutations in protease. The Sponsor indicated that a large proportion of virus samples from patients with confirmed virologic failure on a PI-containing regimen was not found to have PI resistance–associated mutations. Preliminary data from recent studies suggest that several mutations in the Gag protein may be responsible for reduced PI susceptibility in a subset of these patients.

M. Instrument Name:

Sentosa® SX101, *Sentosa*® SQ 301, *Sentosa*® SQ suite and *Sentosa*® SQ Reporter.

N. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver or mobile device?
   Yes _____ or No ____X ___

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?
   Yes _____ or No ___X_____

2. Software

Version: • *Sentosa*® SX101: v6.1 (OEM v41.0.1.5) • SQ301/SQ Suite: v5.6.15 • *Sentosa*® Workflow Assistant: v1.0 • *Sentosa*® SQ Reporter: v2.0.0012 • Stanford HIV db Algorithm: v8.5 (versioning date 04/16/2018) • Stanford Sierra : 2.2.6 (versioning date 06/11/2018)		
Level of Concern: **Moderate**		
	Yes	**No**
Software description:	x	
Device Hazard Analysis:		x
Software Requirements Specifications:	x	

Architecture Design Chart:	x	
Design Specifications:		x
Traceability Analysis/Matrix:	x	
Development:	x	
Verification & Validation Testing:		x
Revision level history:	x	
Unresolved anomalies:		x
Cybersecurity/Interoperability		x

3. Electromagnetic Compatibility and Electrical, Mechanical and Thermal Safety

Not applicable for the *in vitro* diagnostic assay *Sentosa*® SQ HIV Genotyping Assay. However, the sponsor reported that *Sentosa*® SX101 and *Sentosa*® SQ301 instruments are both devices that meet the following electrical safety standards IEC-61010-1 Safety requirements for electrical equipment for measurement, control, and laboratory use.

O. Identified Risks to Health and Mitigation Measures:

Identified Risks	Mitigation Measures
Inaccurate detection of resistance mutation(s)	Device description information, including performance characteristics, and performance studies in labeling. Device description validation procedures and performance studies meeting acceptance criteria. Device limitations in labeling for genetic mutation detection.
Incorrect interpretation of test results	Device description information, performance characteristics, and performance studies in labeling.

P. Benefit/Risk Assessment

Summary of the Assessment of Benefit

The current standard of care for HIV-infected patients uses antiretroviral therapy (ART) to suppress the virus by blocking specific stages of viral replication. High viral loads and/or viral load rebound during ART is considered an indication of treatment failure and signals the need to consider changing medications. One of the most common causes of treatment failure is the existence or emergence of virus species that, due to genetic mutation(s), are resistant to the drugs that are included in the patient's treatment regimen. HIV-1 drug resistance monitoring assays have been developed to identify the genetic mutations, or

variants, that are present in the virus. These assays identify nucleic acid sequences in specific targets of the HIV-1 genome, e.g., protease (PR), reverse transcriptase (RT), Integrase (IN) genes, that make up the viral population in a HIV-1 infected patient. The sequence data are used to guide treatment choices for patients.

Next Generation Sequencing (NGS) assays generate sequence data on all amplified sequences they target, including rare mutations that may lead to drug resistance and treatment failure. The *Sentosa*® SQ HIV-1 Genotyping Assay uses an NGS method to detect HIV drug resistance mutations (DRMs) using an automated system to minimize operator-derived errors. This assay identifies DRMs in three genomic regions of HIV-1 to which all currently marketed drugs are used to block viral replication. Performance studies demonstrate that minor variants can be detected at a frequency as low as 5%. Detection of low-frequency variants may enable earlier intervention and treatment modulation to avoid treatment failure.

Summary of the Assessment of Risk

The risks associated with the device, when used as intended, are those related to inaccurate detection of DRMs and incorrect interpretation of test results. Inaccurate detection in this case means sequencing errors that result in not detecting DRMs when present, detecting a mutation where none exists, or result interpretation errors.

The risks to the patient associated with inaccurate detection of DRMs due to procedural or device errors are the continuance of therapies that are no longer appropriate, or changes from appropriate to an inappropriate therapy. In both cases, the patient's viral load may increase, worsening the clinical prognosis and accelerating the development of drug resistant viruses. In addition, treatment with incorrect drugs has the substantial risk of failed viral suppression and unjustified adverse side effects.

The risk to the patient from inaccurate test results are mitigated by the requirement for acceptable performance of the device in analytical and clinical studies. The risks also are mitigated by information in the labeling regarding the specimen type for which testing is indicated and the target population for which the test is intended.

The risk to the patient of inaccurate test result interpretation is mitigated by requirements in the labeling, including a limitation statement that a negative test result in patients may suggest that there are low-frequency virus mutations, and lack of detection of a mutation does not preclude the possibility of drug resistance. The labeling also includes a detailed description of the device targets, as well as a detailed explanation of the interpretation of results. Risks are further mitigated by inclusion of detailed directions for use in the package insert so that the operator can successfully use the instrument. Finally, the labeling stipulates that all results should be interpreted along with other clinical information and laboratory tests such that treatment decisions are not made solely on the basis of the results of this test.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks associated with the device. However, special controls have been written so that the probable clinical benefits outweigh the potential risks for this device. The proposed assay labeling will facilitate accurate assay implementation and interpretation of results. The performance observed in the clinical studies suggests that the assay may provide substantial benefit to patients by detecting drug resistance mutations in integrase, protease, reverse transcriptase genes and minor drug-resistance variants.

Q. Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

R. Conclusion:

The information provided in this de novo submission is sufficient to classify this device into class II under regulation 21 CFR 866.3955. FDA believes that the special controls, in combination with the general controls, provide a reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: QIC
Device Type: HIV Drug Resistance Genotyping Assay Using NGS Technology.
Class: II (special controls)
Regulation: 21 CFR 866.3955

(a) Identification.

The human immunodeficiency virus (HIV) drug resistance genotyping assay using next generation sequencing (NGS) technology is a prescription in vitro diagnostic device intended for use in detecting HIV genomic mutations that confer resistance to specific anti-retroviral drugs. The device is intended to be used as an aid in monitoring and treating HIV infection.

(b) Classification. Class II (special controls). The special controls for this device are:

(1) The intended use of the device must:

(i) Specify the analyte (RNA or DNA), the genes in which mutations are detected, the clinical indications appropriate for test use, the sample type, and the specific population(s) for which the device in intended.

(ii) State that the device in not intended for use as an aid in the diagnosis of infection with HIV or to confirm the presence of HIV infection, or for screening donors of blood, plasma or human cells, tissues and cellular and tissue-based products (HCT/Ps).

(2) The labeling must include:

(i) A detailed device description, including but not limited to, all procedures from collection of the patient sample to reporting the final result, all device components, the control elements incorporated into the test procedure, instrument requirements, and reagents required for use but not provided as part of the device.

(ii) Performance characteristics from analytical studies and all intended specimen types.

(iii) A list of specific mutations detected.

(iv) The name and version of the standardized database used for sequence comparison and results derivation.

(v) A detailed explanation of the interpretation of test results, including acceptance criteria for evaluating the validity of a test run.

(vi) A limitation statement that the device is intended to be used in conjunction with clinical history and other laboratory findings. Results of this test are intended to be interpreted by a physician or equivalent.

(vii) A limitation statement that lack of detection of drug resistance mutations does not preclude the possibility of genetic mutation.

(viii) A limitation statement indicating the relevant genetic mutations that are included in the standardized database of HIV genomic sequences used for comparison and results derivation but that are not detected by the test.

(ix) A limitation statement that detection of a genomic drug resistance mutation may not correlate with phenotypic gene expression.

(x) A limitation statement that the test does not detect all genetic mutations associated with antiviral drugs.

(xi) A limitation statement listing the HIV types for which the test is not intended, if any.

(3) Device verification and validation must include:

(i) Design of primer sequences and rationale for sequence selection.

(ii) Computational path from collection of raw data to reported result.

(iii) Detailed documentation of analytical studies including, but not limited to, characterization of the cut-off, analytical sensitivity, inclusivity, reproducibility, interference, cross reactivity, instrument and method carryover/cross contamination, sample stability and handling for all genomic mutations claimed in the intended use.

(iv) Precision studies that include all genomic mutations claimed in the intended use.

(v) Detailed documentation of a multisite clinical study evaluating the sensitivity and specificity of the device. Clinical study subjects must represent the intended use population and device results for all targets claimed in the intended use must be compared to Sanger sequencing or other methods found acceptable by FDA. Drug resistance-associated mutations at or above the 20 percent frequency level must detect the mutations in greater than 90 percent of at least 10 replicates, for each of drug class evaluated.

(vi) Documentation that variant calling is performed at a level of coverage that supports positive detection of all genomic mutations claimed in the intended use.

(vii) Detailed documentation of limit of detection studies (LoD) in which device performance is evaluated by testing a minimum of 100 HIV-positive clinical samples, including samples with analyte concentrations near the clinical decision points and near the LoD.

(A) The LoD for the device must be determined using a minimum of 10 HIV-1 group M genotypes if applicable. A detection rate at 1x LoD greater than or equal to 95 percent must be demonstrated for mutations with a frequency greater than 20 percent.

(B) The LoD of genetic mutations at frequency levels less than 20 percent must be established.

(viii) A predefined HIV genotyping bioinformatics analysis pipeline (BAP). The BAP must adequately describe the bioinformatic analysis of the sequencing data, including but not limited to read alignment, variant calling, assembly, genotyping, quality control and final result reporting.

(xii) A clear description of the selection and use of the standardized database that is used for sequence comparison and results derivation.

(4) Premarket notification submissions must include the information in 21 CFR 866.3955 (b)(3)(i)-(xii).

APPENDICES

APPENDIX - 1 Verified and Validated DRMs

Protease		RT		Integrase
L10I[C]	D60E	M41L[A,D]	E138Q	T66I
L10V[C]	I62V	E44D[D]	V179D	L74I
V11I	L63P	A62V[A,D]	V179I	L74M
I15V	I64L	K65R[A,D]	Y181C[A,E]	E92Q
G16E	I64M	D67N[A,D]	Y181I[A,E]	T97A
K20I	I64V	S68G[A]	M184I[A,D]	E138K
K20M[C]	H69K	T69D[A,D]	M184V[A,D]	G140S
K20R[C]	H69Q	T69N	Y188L[A,E]	S147G
K20T	H69Y	K70E	V189I	Q148H
V32I[C]	A71T[C]	K70R[A,D]	G190A[E]	Q148R
L33F[C]	A71V[C]	L74I	L210W[A,D]	V151I
L33V	G73C	V75M	T215D	N155H
E34Q	G73S[C]	V90I	T215E	K156N
E35N	G73T	A98G	T215F[A,D]	E157Q
M36I[C]	T74S	A98S	T215H	G163E
M36L	V77I[C]	L100I[A,E]	T215Y[A,D]	G163K
K43R	V82A[B,C]	K101E	K219E[A]	G163Q
K43T	V82I	K101Q	K219N	G193E
M46I[B,C]	I85V	K101R	K219Q[A,D]	T206S
M46L[C]	N88D[B,C]	K103N[A,E]	K219R	S230N
I47A	N88S	K103R	H221Y	R263K
I50L	L89I	K103S	P225H[E]	
I50V[B,C]	L89M	V106M	K238T	
F53L	L90M[B,C]	V108I[A,E]		
I54L[C]	I93L	Y115F[A,D]		
I54V[B,C]		V118I[D]		

[A-E] *DRMs are from referenced FDA guidance document content Tables A-E*

APPENDIX II Verified-only DRMs

Protease		RT		Integrase
L10F[C]	Q58E	D67E	Q151M[A,D]	T66K
V11L	T74P	D67G	I167V	G140A
L23I	L76V	T69S	V179F	Y143R
L24IC	V82C	T69del	V179T	Q148K
M46V	V82L	L74V[A,D]	Y181F	
I47V[C]	V82M	V75I[A,D]	Y188C[A,E]	
G48M	V82S[B,C]	V75L	G190E	
G48Q	V82T[B,C]	K101H	G190S[E]	
G48V[B,C]	N83D	K101T	G190T	
F53Y	I84V[B,C]	K103H	L210S	
I54A		V106A[A,E]	M230L	
I54M		F116Y[A,D]	K238R	
I54S		I132L	N348I	

[A-E] *DRMs are from referenced FDA guidance document content Tables A-E*

APPENDIX III Validated-only DRMs

Protease	RT		Integrase
K20V	T69A	V179L	T66A
D30N[B,C]	K70N	Y181V	L68V
L33I	K70Q	Y188H[E]	A128T
E35G	K70T	L210F	E138D
I66F	K101P	T215A	Y143C
A71I	V106I	T215C	Q146K
T74A	E138A	T215L	G193R
L89V	E138G	T215N	S230R
I93M	E138K	T215S	
	V179E		

[A-E] *DRMs are from referenced FDA guidance document content Tables A-E*

APPENDIX IV Not Verified and Not Validated DRMs

Protease		RT		Integrase	
L10C	A71L	E40F	I132M	A49G	S153F
L10M	G73A[C]	M41I	E138R	H51Y	S153Y
L10R[C]	G73D	E44A	E138S	V54I	N155S
L10Y	G73F	K65E	Q151L	E92G	N155T
I15A	G73V	K65N	V179M	E92V	G163R
K20N	T74E	D67H	Y181G	Q95K	G163S
L24F	V77A	D67Q	Y181S	H114Y	S230G
L24M	V77T	D67S	Y188F	G118R	
V32L	V82F[B,C]	D67T	G190C	F121Y	
L33M	V82G	D67del	G190Q	E138A	
E34V	N83S	T69E	G190V	E138T	
M36V	I84A	T69G	T215G	G140C	
L38W	I84C	T69I	T215I	Y143A	
R41I	N88G	T69K	T215V	Y143G	
R41T	N88T	T69ins[A,D]	K219H	Y143H	
K45I	L89R	K70G	K219W	Y143K	
M46T	L89T	K70S	F227C	Y143S	
G48A	C95F	V75A	F227L	P145S	
G48L		V75S	M230I	Q146I	
G48S		V75T[D]	M230V	Q146L	
G48T		F77L[A,D]	Y232H	Q146P	
F53W		L100V	Y232N	Q146R	
I54T		K101I	L234I	Q148E	
H69I		K101N	P236L[E]	Q148G	
H69N		K103E	K238N	Q148N	
H69R		K103Q	Y318F	V151A	
A71G		K103T		V151L	

[A-E] *DRMs are from referenced FDA guidance document content Tables A-E*