Individuals using assistive technology may not be able to fully access the information contained in this file. For assistance, please call 800-835-4709 or 240-402-8010, extension 1. CBER Consumer Affairs Branch or send an e-mail to: ocod@fda.hhs.gov and include 508 Accommodation and the title of the document in the subject line of your e-mail.

SeCore™ CDx HLA Sequencing System

REF

Catalog ID	Product Name
SECCDX-A	SeCore™ CDx HLA A Locus Sequencing Kit
SECCDX-GSSP	SeCore™ CDx HLA GSSP Kit
UTPCDX-PGR	uTYPE™ CDx HLA Sequence Analysis Software

IVD In Vitro Diagnostic Medical Device

INTENDED USE

The SeCore CDx HLA Sequencing System is intended for the detection of human leukocyte antigen A-locus (HLA-A) alleles using genomic DNA isolated from whole blood samples. The device is intended to be used as a companion diagnostic (CDx) to aid in the selection of HLA-A*02:01 positive patients with unresectable or metastatic uveal melanoma who may benefit from treatment with KIMMTRAK® (*tebentafusp*-tebn) when used in accordance with approved therapeutic labeling.

INTENDED PURPOSE

The SeCore CDx HLA Sequencing System is an in vitro diagnostic medical device intended to be used by healthcare professionals for the qualitative detection of HLA-A alleles in DNA samples purified from whole blood specimens using the Applied Biosystems 3500 Dx/3500xL Dx Genetic Analyzer Instrument System.

Detection of HLA-A alleles with the SeCore CDx HLA Sequencing System is intended to be used as a companion diagnostic for the selection of patients to receive treatment with specific medicinal products in accordance with approved therapeutic labelling.

SUMMARY AND EXPLANATION

The SeCore CDx HLA Sequencing System consists of SeCore CDx HLA Sequencing Kits, SeCore™ CDx HLA GSSP (Group Specific Sequencing Primers) Kits, and uTYPE™ CDx HLA Sequence Analysis Software.

A. The SeCore CDx HLA Sequencing kit is used to directly sequence the DNA of target HLA genes.

When using the SeCore CDx Sequencing Kit, you will:

1. Amplify locus-specific DNA by polymerase chain reaction (PCR) using an amplification mix containing locus-specific primers, FastStart™ Taq DNA Polymerase, and sample genomic DNA.
2. Treat the resulting amplicon product with ExoSAP-IT™ reagent to degrade the unincorporated primers and hydrolyze free nucleotides prior to the Sanger sequencing reaction.
3. Determine the nucleotide sequence and resulting HLA-subtype by multicolor, fluorescence-based, BigDye™ Terminator sequencing.
4. Purify the final reactions with an ethanol precipitation prior to loading.
5. Denature the purified product with Hi-Di™ formamide, load samples on an automated sequencing instrument, and detect results.
6. Analyze the results with uTYPE CDx HLA Sequence Analysis Software to determine the HLA allele type.

B. The SeCore CDx HLA GSSP kit is used to resolve ambiguous HLA heterozygous allele combinations in conjunction with the SeCore CDx HLA Sequencing Kits. These reagents are for post-amplification sequencing only.

Note: If the allele of interest is not listed in the sequencing results, ambiguity resolution is not needed.

For instructions on how to set up the locus specific genomic DNA amplification, refer to the ***Procedure*** section.

When using the SeCore CDx GSSP Kit, you will:

1. Use the ExoSAP-IT-treated amplicon, prepared from the SeCore CDx Sequencing Kit, as the template for GSSP reactions.
2. Create a reaction mix containing GSPP mix, Big Dye Terminator, and PPT Buffer for each SeCore CDx GSSP test.
3. Use uTYPE CDx HLA Sequence Analysis Software to compare the sequencing data generated from the GSSP kit with the original data from the corresponding SeCore CDx Sequencing Kit to resolve the cis-trans ambiguity and determine the HLA allele type.

PRINCIPLE(S)

SeCore CDx HLA Sequencing Kits directly identify the DNA sequence of target HLA genes. Polymerase chain reaction (PCR) is used with SeCore primers to perform locus-specific DNA amplification. Sanger sequencing is then used to determine the nucleotide sequence of the amplified product. Resulting data files are analyzed with uTYPE CDx HLA Sequence Analysis Software, which compares sample sequences to reference sequences in the IPD-IMGT/HLA database for HLA allele assignment.

REAGENTS

A. Product Components

See the ***Summary and Explanation*** and ***Materials Provided*** sections for a list of SeCore CDx HLA Sequencing System components.

B. Warnings and Cautions

IVD

- For in vitro diagnostic use
- For professional use only

Rx Only

- For prescription use only

- To ensure the best performance of the SeCore CDx HLA Sequencing Kit and the SeCore CDx HLA GSSP Kit, use the products with the materials, reagents, and equipment recommended in this document.

- To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions. Refer to the Safety Data Sheets for detailed information. Individual Safety Data Sheets can be downloaded at www.onelambda.com.

C. Preparing Reagents for Use

Refer to the ***Procedure*** section.

D. Storage/Handling Instructions

Upon receipt, immediately store SeCore CDx HLA Sequencing and GSSP Kit components at –30°C to –10°C in a non-frost-free freezer.

After opening, SeCore CDx HLA Sequencing and GSSP Kit reagents should be stored separately for use in pre- and post-PCR locations to minimize risk of DNA cross-contamination.

BigDye Terminator and Sequencing Mixtures should be protected from exposure to light.

E. Instability Indications

If package or components are damaged, leaking, or arrive in an unfrozen state or product has leaked, do not use. In such a case, contact One Lambda Technical Support at 1lambda-TechSupport@thermofisher.com.

INSTRUMENT REQUIREMENTS

Refer to the ***Procedure*** section.

SPECIMEN COLLECTION AND SAMPLE PREPARATION

A. Specimen Collection

1. Collect blood samples in ACD or EDTA- anticoagulated tubes. Do not use heparinized samples.

   IMPORTANT: Perform DNA extraction according to DNA extraction kit manufacturer instructions. Phenol, ethanol, and SDS may cause inhibition of the PCR reaction.

WARNING/CAUTION: Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials.

B. Sample Preparation

2. Prepare purified DNA with an OD260/280 between 1.7 and 1.9.
3. Prepare a working solution of the purified DNA at 15 to 30 ng/µL by diluting the DNA in nuclease free molecular-biology grade water

   IMPORTANT: Change pipet tips between pipetting each sample and each different mix or reagent to prevent cross contamination. The same pipet tip may be used to dispense the same mix or reagent into multiple tubes or wells as long as the tip does not contact the genomic DNA or PCR product. If this may have occurred, change the pipet tip to prevent contamination.

   Note: Discard DNA aliquoted in water after use.

MATERIALS

A. Materials Provided

Component	SKU	Quantity	Amount
SeCore CDx HLA Sequencing Kits			
A Locus Amplification Mixture	STS017	1 vial	1200 µL
FastStart Taq DNA Polymerase	STS012-2	1 vial	14 µL
ExoSAP-IT Reagent	STS016-1	1 vial	200 µL
Sequencing Mixtures	STS107 (A Exon 1 Fwd) STS108 (A Exon 1 Rev) STS019 (A Exon 2 Fwd) STS020 (A Exon 2 Rev) STS021 (A Exon 3 Fwd) STS022 (A Exon 3 Rev) STS023 (A Exon 4 Fwd) STS024 (A Exon 4 Rev) STS109 (A Exon 5 Fwd) STS110 (A Exon 5 Rev)	10 vials total	200 µL per vial
Sequencing PPT Buffer	STS-015-3	1 vial	500 µL
SeCore CDx HLA GSSP Kits			
Z# Sequencing Mixture	Product-dependent	1 vial/primer	100 µL
BigDye Terminator	100006936	1 vial/primer	100 µL
Sequencing PPT Buffer	B04736	1 vial/primer	50 µL

B. Materials Required, but not Provided

1. Instruments and Software

Instruments, accessories, and/or reagents are available through Applied Biosystems. To order uTYPE CDx HLA Sequence Analysis Software, contact your One Lambda representative.

Item	Part Number
Veriti™ Dx 96-Well Thermal Cycler Model 9912 with 0.2–mL sample wells	4452300
3500 Dx Series Genetic Analyzer CS2 with 3500 Dx Series Software 2011 v1.0	• 3500 Dx (8-capillary) Part No. 4461450 • 3500xL Dx (24-capillary) Part No. 4461447
Consumables for 3500 Dx Series Genetic analyzer: • POP-6™ polymer • Anode buffer • Cathode buffer • CBC septa • 50 cm Capillary array • 96-well plate bases/clips (8-capillary array or 24-capillary array) • 96-well septa • Hi-Di formamide	See the 3500 Dx Series Genetic Analyzer CS2 IVD User Guide (Pub. No. 4457397) for part numbers
uTYPE CDx HLA Sequence Analysis Software	UTPCDX-PGR
uTYPE™ Dx Allele Library Update	53999103

2. General Purpose Supplies

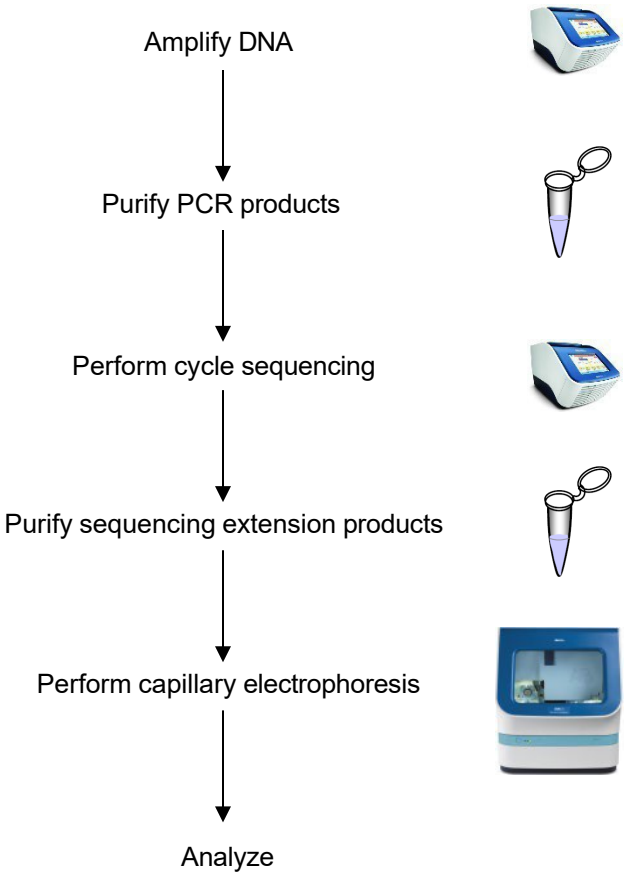
IMPORTANT: The customer is responsible for validation of all general-purpose supplies before use, and for compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

All of the items in this table can be purchased from any major laboratory supplier.

Item	Description
Plates	• 96-well PCR microtiter plate, standard or optical-grade polypropylene, 0.2 mL, half- or semi-skirted design, with or without barcode
Strip tubes, strip caps, silicone mat	• 8-strip full-height PCR tubes, standard- or optical-grade polypropylene, 0.2 mL • 8-tube PCR strip caps, domed, standard- or optical-grade polypropylene, for 0.2 mL 8-strip PCR tubes • 96-well Silicone PCR Plate Mat (can be used instead of caps to cover plates in cycle sequencing and purification
General lab supplies	• Tabletop centrifuge (2500 x *g*) with plate adapters for 96-well plates or reaction tubes • Ice or cooling system for 0.2 ml tubes to keep samples cold before cycle sequencing • Water, molecular-biology grade • Pipettors: o Pipettors and tips: 1 to 10 µL, 10 to 200 µL, 100 to 1000 µL o Dispensing electronic pipettors: 1 to 125 µL aliquots o Multichannel pipettors (8 or 12 channels): 1 to 100 µL, adjustable volume • Absolute ethanol (100%), molecular-biology grade
Gel electrophoresis	• Agarose gel electrophoresis apparatus • Molecular-biology-grade agarose (2%) • PCR marker for 300 to 1300 bp • Loading dye, without Bromophenol Blue or Xylene Cyanol

PROCEDURE - SeCore CDx HLA Sequencing Kit

A. Workflow



B. Prepare PCR Master Mix

Prepare PCR master mix containing Amp Mix and FastStart Taq DNA Polymerase as described in this section.

Note: For 25 test kits, prepare a master mix for a minimum of 5 samples to aid in the accuracy of pipetting small volumes of enzyme. Kit reagent volumes are sufficient for 5 sets of such reactions.

IMPORTANT: Due to the viscous nature of FastStart Taq DNA Polymerase, care should be taken to remove excess Taq polymerase that remains on the pipette tip before dispensing the enzyme into the master mix. Taq polymerase should be pipetted from the top of the vial and the user should avoid submerging the tip into the enzyme. Failure to remove excess Taq polymerase will result in a shortage of the enzyme.

1. Use the table below to calculate volumes of Amp Mix and FastStart Taq DNA Polymerase.
2. Add components to a clean tube, pulse-vortex 2 to 3 times to mix thoroughly, then centrifuge briefly to bring the contents to the bottom of the tube.

Note: Set up the appropriate number of amplification reactions to support the sequencing reactions you will perform for each sample. One amplification reaction will provide sufficient volume for 8 sequencing reactions. Prepare sufficient volume for (number of samples + 1 + negative control) to account for pipetting loss.

Component	A Locus per rxn	A Locus 5 rxn	A Locus 20 rxn
Amp Mix	19.8 µL	99 µL	396 µL
FastStart Taq DNA Polymerase	0.2 µL	1.0 µL	4.0 µL

C. Amplify DNA

Note: For information on using the Veriti™ Dx Thermal Cycler, see the *Veriti Dx Thermal Cycler User Guide* (Pub. No. 4453697).

1. Combine the following for each reaction.

Component	A Locus Reactions
Samples: Purified sample DNA (15 to 30 ng/µL) **Negative control**: Molecular-biology-grade water	5 µL
PCR Master Mix	20 µL

2. Cover the plate or tubes, then centrifuge briefly to bring the contents to the bottom of the wells or tubes.
3. Program the thermal cycler.
   a. Set the ramp mode to **Default**.
   b. Set the thermal cycling conditions.

Initial Step	Each of 35 Cycles			Final Extension	Final Step
	Melt	Anneal	Extend		
Soak	CYCLE			HOLD	Soak
95 °C 4 min	95 °C 20 sec	63 °C 20 sec	72 °C 40 sec	72 °C 5 min	4 °C ∞*

*Remove in time to proceed to the next step.

   c. Set the reaction volume to **25 µL**.

4. Start the run. When the thermal cycler block is >80°C, place the tubes or plate in the thermal cycler, then close the lid.

STOPPING POINT: Amplification reactions can be stored at 2°C to 8°C for up to 18 hours.

D. Confirm the Presence of PCR Products

1. Before removing the caps from the tubes or reaction plate containing the PCR products, briefly centrifuge the tubes or plate.
2. Confirm the presence of PCR products by 2.0% agarose gel electrophoresis. Load agarose gel with 5 µL of amplified DNA plus loading dye and run the gel according to your laboratory protocol. Run the gel for a sufficient amount of time to see separation of the bands.

Expected Products for Amplification

Kit	Product Size (bp)	Internal Control Size (bp)
A Locus	~1200 and ~990	None

E. Purify PCR Products

Treat the PCR amplicons with ExoSAP-IT reagent to degrade unincorporated primers and hydrolyze dNTPs.

IMPORTANT: Follow the Taq guidelines listed above for pipetting viscous enzymes when adding ExoSAP-IT.

1. Before removing the caps from the tubes or reaction plate containing the PCR products, briefly centrifuge the tubes or plate.
2. Add 4 µL of ExoSAP-IT reagent to the 20µl of PCR product remaining in the amplification tubes or wells; the final reaction volume should be 24 µl.
   - Add to all tubes or wells containing PCR products.
3. Cover the tubes or plate and centrifuge ~5 seconds to bring contents to the bottom of the tube or well.
4. Vortex the reaction mixtures thoroughly (~10 seconds).

   Note: Make sure that ExoSAP-IT reagent is mixed well with PCR products. This is critical for purification of PCR amplicons.
5. Centrifuge briefly to bring contents to the bottom of the tube.
6. Program the thermal cycler.
   a. Set the ramp mode to **Default**.
   b. Set the thermal cycling conditions.

1 Cycle		
CYCLE		**Soak**
37 °C 20 min	80 °C 20 min	4°C ∞*

*Remove in time to proceed to the next step.

   c. Set the reaction volume to **24 µL**.
7. Place the tubes or plate in the thermal cycler, close the lid, then start the run.

STOPPING POINT: ExoSAP-IT treated amplicons from the SeCore CDx HLA Sequencing Kits can be stored at –20°C for up to two weeks.

Note: ExoSAP-IT-treated amplicons are used for sequencing with SeCore CDx HLA GSSP Kits.

F. Perform Cycle Sequencing

1. Determine the sequencing reactions needed.

Possible Sequencing Reactions

A Locus
Exon1 forward
Exon1 reverse
Exon2 forward
Exon2 reverse
Exon3 forward
Exon3 reverse
Exon4 forward
Exon4 reverse
Exon5 forward
Exon5 reverse

a. SeCore CDx HLA A Locus Sequencing Kit

- Set up forward and reverse sequencing reactions for exons 1-5 for all samples.
- Sequencing reactions can be set up concurrently or within 2 weeks of amplification date if purified reactions are stored at -20°C.

2. Prepare sequencing reactions.

a. Before removing the caps from the reaction tubes or reaction plate, briefly centrifuge the purified PCR products.

b. Place a new set of tubes or plate on ice to hold the sequencing reactions.

c. Add 2 µL of ExoSAP-IT-treated amplicons to the appropriate tube or well (see suggested plate layout below).

Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1F	S1R										
B	S2F	S2R										
C												
D												
E												
F												
G												
H	▼	▼	▼	▼								

Note: S1F and S2F = forward reactions of sample 1 and 2. S1R and S2R = reverse reactions of sample 1 and 2.

Refer to the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397) for illustrations of the injection pattern on 8- and 24-capillary instruments.

d. Add 8 µL of each locus-specific sequencing mix to the appropriate tube or well.

e. Cover the tubes or plate, vortex briefly to mix, then centrifuge the tubes or plate to bring contents to the bottom of the tubes or plate.

f. Program the thermal cycler.

(a) Set the ramp mode to **Default**.

(b) Set the thermal cycling conditions.

Each of 25 Cycles			Final Step	Total Reaction Time
Melt	Anneal	Extend		
CYCLE			Soak	~1.5 hours
95 °C 20 sec	50 °C 15 sec	60 °C 1 min	4 °C □□	

*Remove in time to proceed to the next step.

(c) Set the reaction volume to **10 µL**.

g. Start the run. When the thermal cycler block is >80 °C, place the tubes or plate in the thermal cycler, then close the lid.

STOPPING POINT: Completed sequencing reactions can be stored at -10°C to -30°C for up to 24 hours.

G. Purify Sequencing Extension Products

IMPORTANT: Perform this procedure away from direct air flow (e.g., exhaust from a centrifuge or other equipment), which may cause ethanol evaporation.

After cycle sequencing, remove excess terminators by ethanol precipitation.

1. Vortex PPT Buffer to dissolve particles until solution is clear.
2. Add 2 µL of PPT Buffer to each sequencing reaction mixture.

   Note: Use of a single channel pipette or single channel repeater pipette is recommended for this step. Dispensing PPT Buffer into a reagent trough or boat to aid in multichannel pipetting can result in significant reagent loss.
3. Centrifuge briefly to bring contents to the bottom of the tubes or plate.
4. Add 40 µL of 100% ethanol to each mixture.
5. Cover the plate and vortex for 60 seconds.

   Note: If you are using a silicone mat, place a flat object over the mat to secure the mat during vortexing. Make sure the contents of all tubes or wells are mixed thoroughly.
6. Fit the centrifuge with a plate-adaptor. Centrifuge the plate for 30 minutes at 2,000 x *g*.
7. Remove the cover, cover the plate with a paper towel, and invert. Centrifuge with the paper towel briefly (10 to 60 seconds at 500 x *g*) in the inverted position to remove as much liquid as possible.
8. Prepare fresh 80% ethanol daily. Add 100 µL of 80% ethanol to the DNA pellets in the plate. Do not vortex the plate.
9. Centrifuge the plate for 5 minutes at 2000 x *g*.
10. Remove the cover, cover the plate with a paper towel, and invert. Centrifuge with the paper towel briefly (1 minute at 500 x *g*) in the inverted position to remove as much liquid as possible.

H. Perform Capillary Electrophoresis

1. Prepare the 3500 Dx Series Genetic Analyzer CS2 for a run as described in the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397).
2. Create a plate.

   The 3500 Dx Series Software contains plate templates for use with samples prepared with the SeCore CDx HLA Sequencing Kits. These templates contain an instrument protocol/run module (POP-6™ polymer) and a base calling protocol optimized for HLA applications.

   For detailed information on creating a plate, refer to the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397).

   a. In the Dashboard, click **Create Plate From Template**.

   b. Select a plate template appropriate for your instrument, then click **Open**.

      For typical, shorter (~68 minutes) sequencing runs:

      - **RapidSeq50_BDTv1.1_HLA-POP6_Dx** for 8-capillary instruments
      - **RapidSeq50_BDTv1.1_HLA_xL-POP6_Dx** for 24-capillary instruments

      For longer (~78 minutes) sequencing runs applicable to GSSPs that sequence through multiple exons:

      - **FastSeq50_BDTv1.1_HLA-POP6_Dx** for 8-capillary instruments
      - **FastSeq50_BDTv1.1_HLA_xL-POP6_Dx** for 24-capillary instruments

   c. Enter plate settings, then click **Assign Plate Contents**.

   d. Specify sample names (SampleID_Locus_xx) in the Plate view.

      For more information on sample-naming conventions, refer to *uTYPE CDx HLA Sequence Analysis Software User Manual* (TDX-OLI-DMR-PS-3202).

   e. Click **Save**.

3. Prepare samples.

   Note: Refer to the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397) for information on preparing the plate assembly.

   a. Add 15 µL of Hi-Di Formamide to each DNA pellet in the plate.

   b. Cover the plate with 96-well septa and briefly centrifuge to bring contents to the bottom of the wells.

   c. Denature the samples by placing the plate in the thermal cycler at 95 °C for 2 minutes.

   d. Centrifuge briefly to remove any air bubbles in the samples.

   e. Perform the following steps as described in the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397):

      (1) Load the plate in the instrument.

      (2) Link the plate, then start the run.

I. Analyze Data

Use the uTYPE CDx HLA Sequence Analysis Software to process the .ab1 sequence files generated on the 3500 Dx Series Genetic Analyzer CS2 and create an HLA typing report.

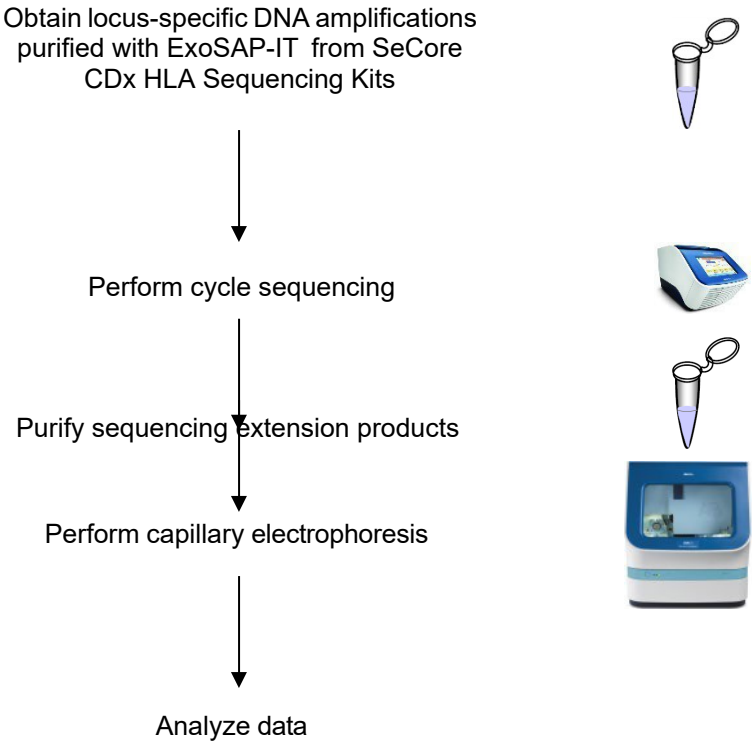
Refer to the *uTYPE CDx HLA Sequence Analysis Software User Manual* (TDX-OLI-DMR-PS-3202) for typing instructions. The uTYPE CDx software will make recommendations for resolution of ambiguous typing results using the SeCore CDx HLA GSSP Kits.

IMPORTANT: If your database is not current, order the latest version of the uTYPE™ Dx Allele Library Update (Part No. 53999103) at: **www.onelambda.com**. Contact your One Lambda representative for more information.

PROCEDURE - SeCore CDx GSSP Kit

This procedure is followed when the uTYPE CDx HLA Sequencing software recommends using GSSPs to resolve ambiguous allele combinations.

A. Workflow



B. Obtain DNA Samples

Use locus-specific genomic DNA amplifications purified with ExoSAP-IT from the SeCore CDx HLA Sequencing Kits (see the ***Purify PCR Products*** section).

IMPORTANT: Change pipet tips between pipetting each sample and each different mix or reagent to prevent cross contamination. The same pipet tip may be used to dispense the same mix or reagent into multiple tubes or wells as long as the tip does not contact the genomic DNA or PCR product. If this may have occurred, change the pipet tip to prevent contamination.

STOPPING POINT: ExoSAP-IT treated amplicons from the SeCore CDx HLA Sequencing Kits can be stored at –20°C for up to two weeks.

C. Perform Cycle Sequencing

This procedure uses ExoSAP-IT-treated amplicons obtained with a SeCore CDx HLA Sequencing Kit.

1. Before removing the caps from the reaction tubes or wells, briefly centrifuge.
2. Place a new set of tubes or plate on ice to hold the sequencing reactions.
3. Add 2 µL of ExoSAP-IT-treated amplicons to a tube or well.
4. Mix equal volumes of the Sequencing Primer Mix and BigDye Terminator.

   IMPORTANT: Volumes in the vials may not be equal. Measure the volumes before combining the components.

   Note: After mixing, store the Sequencing Primer/BigDye Terminator vial at –20°C up to the expiration date on the kit label.
5. Add 8 µL of Sequencing Primer/BigDye Terminator mix to the appropriate tube or well.
6. Cover the tubes or plate, vortex briefly to mix, then centrifuge the tubes or plate to bring contents to the bottom of the tubes or plate.
7. Program the thermal cycler.
   a. Set the ramp mode to **Default**.
   b. Set the thermal cycling conditions.

Each of 25 Cycles			Final Step	Total Reaction Time
Melt	Anneal	Extend		
CYCLE			Soak	~1.5 hours
95°C 20 sec	50°C 15 sec	60°C 1 min	4°C ∞*	

*Remove in time to proceed to the next step.

   c. Set the reaction volume to **10 µL**.
8. Start the run. When the thermal cycler block is >80 °C, place the tubes or plate in the thermal cycler, then close the lid.

D. Purify Sequencing Extension Products

IMPORTANT: Perform this procedure away from direct air flow (e.g., exhaust from a centrifuge or other equipment), which may cause ethanol evaporation.

After cycle sequencing, remove excess terminators with ethanol precipitation.

1. Vortex PPT buffer to dissolve particles until solution is clear.

2. Add 2 µL of PPT Buffer to each sequencing reaction mixture.

   Note: Use of a single channel pipette or single channel repeater pipette is recommended for this step. Dispensing PPT Buffer into a reagent trough or boat to aid in multichannel pipetting can result in significant reagent loss.

3. Centrifuge briefly to ensure all contents are mixed and at the bottom of the well.
4. Add 40 µL of 100% ethanol to each mixture.
5. Cover the plate and vortex for 60 seconds.

   Note: Make sure the contents of all tubes or wells are mixed thoroughly.

6. Centrifuge the plate in a centrifuge fitted with a plate-adaptor for 30 minutes at 2,000 x *g*.
7. Remove cover, cover with a paper towel, and invert. Centrifuge with the paper towel briefly (10 to 60 seconds at 500 x *g*) in the inverted position to remove as much liquid as possible.
8. Prepare fresh 80% ethanol daily. Add 100 µL of 80% ethanol to the DNA pellets. Do not vortex the plate.
9. Centrifuge the plate for 5 minutes at 2,000 x *g*.
10. Remove the supernatant by an inverted spin according to Step 7.

E. Perform Capillary Electrophoresis

1. Prepare the 3500 Dx Series Genetic Analyzer CS2 for a run as described in the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397).
2. Create a plate.

   The 3500 Dx Series Software contains plate templates for use with samples prepared with the SeCore CDx HLA GSSP Kit. These templates contain an instrument protocol/run module (POP-6 polymer) and a base calling protocol optimized for HLA applications.

   For detailed information on creating a plate, refer to the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397).

   a. In the Dashboard, select **Create Plate From Template**.

   b. Select a plate template appropriate for your instrument, then click **Open**:

      For typical, shorter (~68 minutes) sequencing runs:

      - **RapidSeq50_BDTv1.1_HLA-POP6_Dx** for 8-capillary instruments
      - **RapidSeq50_BDTv1.1_HLA_xL-POP6_Dx** for 24-capillary instruments

      For longer (~78 minutes) sequencing runs applicable to GSSPs that sequence through multiple exons:

      - **FastSeq50_BDTv1.1_HLA-POP6_Dx** for 8-capillary instruments
      - **FastSeq50_BDTv1.1_HLA_xL-POP6_Dx** for 24-capillary instruments

   c. Enter plate settings, then click **Assign Plate Contents**.

   d. Specify sample names (SampleID_Locus_Z##) in the Plate view. For sample naming conventions, see the *uTYPE CDx HLA Sequencing Software User Manual*.

   e. Click **Save**.

3. Prepare samples.

   a. Add 15 µL of Hi-Di Formamide to each DNA pellet.

   b. Cover the plate and briefly centrifuge to bring contents to the bottom of the wells.

   c. Denature the samples by placing them in the thermal cycler at 95 °C for 2 minutes.

d. Centrifuge briefly to remove any air bubbles in the samples.

e. Perform the following steps as described in the *3500 Dx Series Genetic Analyzer CS2 IVD User Guide* (Pub. No. 4457397):

(1) Load the plate in the instrument.

(2) Link the plate, then start the run.

F. Analyze data

Use uTYPE CDx HLA Sequence Analysis Software to analyze the data generated from the SeCore CDx HLA GSSP Kit together with the original data from the SeCore CDx HLA Sequencing Kit. For information, see the *uTYPE CDx HLA Sequencing Software User Manual* (TDX-OLI-DMR-PS-3202).

IMPORTANT: If your database is not current, order the latest version of the uTYPE Dx Allele Library Update (Part No. 53999103) at: onelambda.com. Contact your One Lambda representative for more information.

Note: GSSP primers may detect polymorphic sites from untargeted alleles. Any peaks detected from untargeted alleles exhibit peak heights less than 33% of the peak height produced by the targeted allele.

TROUBLESHOOTING

Note: For uTYPE CDx Software troubleshooting, refer to the *uTYPE CDx HLA Sequence Analysis Software User Manual* (TDX-OLI-DMR-PS-3202).

A. Amplification

Observation	Possible Causes	Recommended Action
Absence of PCR product or weak PCR product	FastStart Taq was not added to the amplification mix or not mixed properly when added	Repeat amplification paying attention to the addition and mixing of Taq with the amplification mix. Use a tube that is the appropriate size for the amount of mix.
	Thermal cycler failure	Check the cycler run history. Contact One Lambda, Inc.
	DNA concentration is not optimal	Re-quantify the DNA and adjust the concentration to 15–30 ng/µL.
	Poor quality or degraded DNA	Run DNA on a 1% agarose gel to evaluate the quality. Intact genomic DNA should be ~3000 bp. Re-amplify the sample using 0.3 µL of Taq per test.
	Visibility of the product bands on the agarose gel are masked by the dye in the loading buffer	Repeat gel electrophoresis using a different gel loading buffer.
Presence of extra amplification products	Incorrect thermal cycling settings were used	Repeat the amplification using the correct settings.
	Contaminated amplification mix, DNA, or work area	De-contaminate the work area or re-extract DNA. Repeat the amplification.

B. Sequencing

Observation	Possible Causes	Recommended Action
Excessive background (baseline noise)	Poor or incorrect matrix	Repeat the spectral calibration and reinject the samples.
	Poor injection	Reinject the samples.
	The injection time was set too high	Reduce injection time and reinject the samples. (Samples of poor quality may have lower signal strengths but may still be analyzed and typed.)
	Poor sequencing reaction due to pipetting error	Be sure that both the ExoSAP-IT-treated PCR product and the correct sequencing mix are added and combined.
	The incorrect mobility file was chosen- the peaks will be shifted or will be on top of each other	Choose the correct mobility file.
	Excess 100% ethanol was added during precipitation	Re-sequence. Check to be sure that the pipette is set at the correct volume.

Observation	Possible Causes	Recommended Action
	Ethanol evaporation during precipitation	Prepare new sequencing reactions. Purify the sequencing extension products away from direct air flow (e.g., exhaust from a centrifuge or other equipment), which may cause ethanol evaporation.
Weak signal from short fragments Longer fragments show a stronger signal	Did not use fresh 80% ethanol	Prepare fresh 80% ethanol daily.
Strong signal from short fragments Longer fragments show a weaker signal	Over amplification of short fragments during purification	Repeat sequencing reactions. Reduce the amount of ethanol stepwise during the wash step to a minimum concentration of 70%.
Weak Signal	Sequencing reactions were not vortexed thoroughly after adding PPT Buffer and ethanol	Repeat the sequencing reactions. Vortex for a minimum of 60 seconds after adding ethanol. Make sure all reactions are mixed well.
	Precipitate is present in PPT Buffer	Vortex until particles are dissolved and buffer is clear.
Excessive Dye Blobs	The PPT Buffer was not added to the sequencing reactions before adding ethanol	Repeat the sequencing reaction. Add the PPT Buffer before adding ethanol.
	Did not wash the sequencing reactions with 80% ethanol	Repeat sequencing reactions. Make sure to perform the 80% ethanol wash step.
	Poor sequencing reaction due to error in pipetting or weak amplification product	Be sure that the ExoSAP-IT -treated PCR product and the correct sequencing mix are added and combined. In the case of a weak amplification, run the amplification product on a gel to confirm its intensity.
	Did not remove all of the remaining ethanol during the precipitation	Repeat the sequencing reactions. Make sure to perform the 500 x *g* centrifuge step to remove all remaining ethanol from the reaction tubes/wells.
	Ethanol used in wash step was too dilute	Repeat the sequencing reactions. Use 80% ethanol for the wash step.
Random sequence failures	Poor sequencing reaction due to error in pipetting	Be sure that both the ExoSAP-IT™ treated PCR product and the correct sequencing mix are added and combined.

RESULTS

A. Data Acquisition

Refer to the ***Procedure*** section.

B. Data Analysis

uTYPE CDx HLA Sequence Analysis Software matches imported sequences to reference sequences from the IPD-IMGT/HLA Database, which provides a specific database for sequences of the human major histocompatibility complex. The software identifies reference alleles that best match the sample sequence and assigns alleles based on the provided sequence.

LIMITATIONS OF THE PROCEDURE

- The SeCore CDx HLA Sequencing System is intended to provide HLA genotyping data as part of a screening procedures to determine the eligibility of individuals to receive precision therapeutics. The device not intended or labeled to diagnose, treat, or mitigate disease.
- SeCore CDx HLA Sequencing system is intended for use by HLA laboratory professionals following accepted laboratory accreditation standards (ASHI). Accredited HLA laboratory personnel is required to review test results for reliability and accuracy of test. The reviewer examines the data to ensure that the test is valid.
- Before using SeCore CDx HLA Sequencing Kits in your laboratory, perform quality assurance and quality control checks for sequencing-based typing methods using known molecular typed samples. Reference samples are available from the International Workshop Reference Cell Panel and the UCLA DNA Reference Panel.
- Failure to completely read and explicitly follow all of the instructions contained herein may result in invalid test results.
- Perform DNA extraction according to DNA extraction kit manufacturer instructions. Phenol, ethanol, and SDS may cause inhibition of the PCR reaction.
- Every attempt has been made to validate all amplification primers used in SeCore CDx HLA Sequencing Kits with DNA samples typed by molecular methods. Due to the limited access to such reference materials, some rare alleles may not have been tested with these kits. For specific information, see the Certificate of Analysis documents supplied with each kit.
- The A locus Exon 4-Reverse sequencing primer contains a mismatch towards A*32 and A*74 samples. This mismatch also applies to the A*02:65 allele. Samples containing these alleles may exhibit poor heterozygous peak balance in the Exon 4-Reverse sequence data. The Exon 4-Forward sequence data will be unaffected.

EXPECTED VALUES

A. Acceptance Criteria

1. Signal Intensity
   - SeCore CDx HLA Sequencing Kit: ≥ 300 RFU
   - SeCore CDx HLA GSSP Kit: ≥ 100 RFU
2. Noise-to-Signal Intensity
   - Both Kits: ≤ 8%

SPECIFIC PERFORMANCE CHARACTERISTICS

A. Analytical Performance Characteristics

1. Accuracy Studies

a. Accuracy Study 1: Detection of HLA-A*02:01

An accuracy study was conducted to evaluate the SeCore CDx HLA Sequencing Systems ability to detect and discriminate the HLA-A*02:01 allele. The study utilized specimens from individuals with metastatic or unresectable uveal melanoma (mUM) and well-characterized DNA reference samples. Results generated from the SeCore CDx assay were compared to either an established reference sample typing or a validated reference method. A total of 33 mUM samples and 72 well-characterized reference samples were evaluated during the study.

The SeCore CDx HLA Sequencing System detected the HLA-A*02:01 allele in a total of 40 samples and did not detect the HLA-A*02:01 allele in the remaining 65 samples. A 100% overall percent agreement (OPA) was demonstrated for the device.

See Table 1 for a summary of the accuracy study results.

Table 1. Results of Accuracy Study 1

	Samples		Total
	Metastatic or Unresectable Uveal Melanoma	Well-Characterized Reference Samples	
HLA-A*02:01 Detected	22	18	40
HLA-A*02:01 Not Detected	11	54	65
Total	33	72	105
PPA[1]	40/40 = 100%		
NPA (LB 95% CI)[2]	65/65 = 100% (95.5%)		
OPA (LB 95% CI)[2]	105/105 = 100% (97.2%)		

PPA = Percent Positive Agreement; NPA = Percent Negative Agreement; OPA= Overall Percent Agreement; CI = Confidence Interval; LB = Lower Bound; [1]PPA 100% concordance by point estimate sample size not large enough to calculate 95% CI; [2]LB 95% CI calculated by Clopper-Pearson method

Based on these results, the SeCore CDx HLA Sequencing system can accurately define and identify the HLA-A*02:01 allele in human genomic DNA samples purified from whole blood specimens.

b. Accuracy Study 2: Other Analytical Accuracy Studies

Accuracy studies for the SeCore Sequencing System were previously performed on the HLA-A locus. Well-characterized reference samples collected from the UCLA Histocompatibility Panel (N=71) were evaluated. Allele detection data from the SeCore System matched 100% with the established HLA A-locus reference typing on the DNA panel (see Table 2).

Table 2. Results of Accuracy Study 2

HLA-Locus	Number of Samples Tested (N)	Number of Concordant Samples	OPA (LB 95% CI)
A	71	71	71/71 = 100% (98.3%)

OPA = Overall Percent agreement; CI = Confidence Interval; LB = Lower Bound

c. Accuracy Study 3: Expanded Exon Coverage

Evaluations were conducted on the SeCore HLA-A Locus Sequencing Kit to verify the accuracy of kits with the expanded exon coverage (exon 1 - 5). A total of 73 well characterized HLA-A samples were tested at 20ng/µL All 73 samples were concordant with the established HLA reference typing (see Table 3).

Table 3. Results of Accuracy Study 3

SeCore Kit	Concordance[1]	90% Confidence Interval[2]	Success[3]
HLA-A Locus	100.0% (73/73)	95.98%, 100%	YES

[1] Concordance is reported as Percent (Number Concordant/Number of Samples).
[2] Confidence interval calculated by the Clopper-Pearson exact method.
[3] Success is determined by a lower bound on the 90% confidence interval greater than 0.95.

2. Measuring Range Studies

Measuring range studies were performed to evaluate the optimal DNA input concentration for proper function of the device. Six well-characterized samples were tested against a representative Class I sequencing kit at various DNA input concentrations. This study demonstrated a large functional range of DNA input concentrations for the device and established the recommend DNA concentration range of 15-30ng/µL.

3. Precision Studies

a. Site-to-Site Reproducibility

Site-to-site reproducibility testing was conducted using one lot of each SeCore Sequencing Kit and representative GSSP Kit.

Four previously characterized DNA samples were tested in triplicate at three external sites using the 3500xL Dx Genetic Analyzer CS2 instrument. Two operators at each site tested the samples six times over six non-consecutive days using one 24-capillary instrument.

The total number of genotyping events for the three external sites combined was 432 for A Locus. The genotyping results are all concordant with the known genotype (100%) and the ambiguous results for each sample are reproducible, meaning that the same ambiguous results are obtained per site, operator, run, and replicate for each sample.

b. Lot-to-Lot Reproducibility

Two studies were conducted for lot-to-lot reproducibility of the SeCore HLA Sequencing Kit and the 3500xL Dx Genetic Analyzer CS2 instrument consumables.

- Study 1 tested three SeCore Sequencing Kit lots with one instrument consumable lot.
- Study 2 tested three instrument consumables lots and one SeCore Sequencing Kit lot.

For each study, five replicates each of three previously characterized DNA samples were tested over five non-consecutive days using one 24-capillary instrument for representative Class I HLA Loci sequencing kits.

The genotyping results were all concordant with the known genotype (100%) and the ambiguous results for each sample were reproducible (i.e., the same ambiguous results were obtained per lot, run, and replicate for each sample).

Note: Genotyping was considered concordant if at least one pair of alleles matched between the SeCore Sequencing Kit result and the known DNA genotype.

c. Reproducibility for Expanded Exon Coverage

Reproducibility of the SeCore HLA-A Locus Sequencing Kit with expanded exon coverage was conducted. Four well characterized HLA-A samples were tested at 20ng/µL in quadruplicate over four non-consecutive days by two operators for a total of 64 genetic typing events per operator yielding a total of 128 genetic typing events. All test cases were 100% concordant with the reference typing results (See Table 4).

Table 4. Results Summary of Reproducibility Study

SeCore Kit	**Concordance[1]**	**90% Confidence Interval[2]**	**Success[3]**
HLA-A Locus	100.0% (128/128)	97.69%, 100%	YES

[1] Concordance is reported as Percent (Number Concordant/Number of Samples).
[2] Confidence interval calculated by the Clopper-Pearson exact method.
[3] Success is determined by a lower bound on the 90% confidence interval greater than 0.95.

4. Interfering Substances

An evaluation was performed using the substances in Table 3 in accordance with CLSI document EP7-A2 using one lot of representative SeCore HLA Sequencing and GSSP kits. As part of the study, the interfering substance was added directly to the purified DNA. The concentration where assay inhibition was evident is described in the following table.

Table 5. Summary of Interfering Substance Testing Results

Substance	**Highest Concentration without Inhibition**
SDS (w/v)	0.0050%
100% EtOH	200 mmol/L
Phenol (v/v)	0.0125%
Sucrose	100,000 µmol/L
EDTA	100 µmol/L
ACD (w/v)	0.1%
500x Cholesterol	50x
Bilirubin, conj.	10.7 µmol/L
Hemoglobin	0.0156 g/L
Hemolyzed Blood (w/v)	0.001%

5. Sample Preparation

Eighteen donor samples prepared using three different DNA preparations were tested with the SeCore HLA Sequencing System. HLA typing concordance analysis was performed using the HLA typing obtained from the reference typing. The results showed that in all DNA preparations tested using SeCore, the HLA typing obtained was 100% concordant with the reference typing.

B. Clinical Performance Studies

1. Companion Diagnostic Clinical Trials (IMCgp100-202)

Results from the SeCore CDx HLA Sequencing System were used as inclusion criteria during clinical trials for the use of KIMMTRAK in the treatment of mUM patients. The safety and effectiveness of KIMMTRAK was established in IMCgp100-202, a randomized, open-label, multicenter trial (ClinicalTrials.gov NCT03070392) that enrolled patients with mUM (N=378).

Patients were required to be HLA-A*02:01 genotype positive as identified by the SeCore CDx HLA Sequencing System. Patients were randomized (2:1) to receive KIMMTRAK weekly by intravenous infusion (N=252) (20 mcg on Day 1, 30 mcg on Day 8, 68 mcg on Day 15, and 68 mcg once every week thereafter) or Investigator's Choice (N=126) (pembrolizumab, ipilimumab, or dacarbazine).

Whole blood specimens from mUM patients being considered for treatment were tested with the SeCore CDx HLA Sequencing System. Patients positive for HLA-A*02:01 were eligible for enrollment in the drug trial if they met other eligibility criteria. Patients who tested negative for HLA-A*02:01 were ineligible for drug trial enrollment. Conclusions supporting KIMMTRAK efficacy were based on HLA-A*02:01 positive patients as detected by the SeCore CDx HLA Sequencing System.

The primary efficacy outcome for KIMMTRAK was based on overall survival (OS). Additional efficacy outcomes were investigator-assessed progression free survival (PFS) and objective response rate (ORR; per RECIST 1.1). Efficacy results for KIMMTRAK are shown in Table 2.

Note: Refer to Drugs@FDA for the most recent KIMMTRAK product labeling.

Table 2. Efficacy Results for IMCgp100-202

	KIMMTRAK (N=252)	Investigator's Choice (N=126)
Overall Survival (OS)[1]		
Number of deaths	87 (34.5%)	63 (50%)
Median in months (95% CI)	21.7 (18.6, 28.6)	16 (9.7, 18.4)
HR (95% CI)[2]	0.51 (0.37, 0.71)	
p-value[3, 4]	<0.0001	
Progression-free Survival[5]		
Number (%) of patients with event	198 (78.6%)	97 (77%)
Median in months (95% CI)	3.3 (3, 5)	2.9 (2.8, 3)
HR (95% CI)[2]	0.73 (0.58, 0.94)	
p-value[3, 6]	0.0139	
Objective Response Rate (95% CI)[7]	9.1% (5.9, 13.4)	4.8% (1.8, 10.1)
Complete Response	1 (0.4%)	0
Partial Response	22 (8.7%)	6 (4.8%)

CI = Confidence Interval; HR = Hazard Ratio; [1]Based on prespecified interim analysis; [2]Hazard ratio is from a Cox proportional hazards model stratified by LDH status; [3]Two-sided p-value based on log rank test stratified by LDH; [4]Compared to the interim efficacy boundary of 0.006; [5]Final PFS analysis; [6]Compared to the efficacy boundary of 0.05; [7]Not formally tested

2. Clinical Comparison Studies

Samples from three testing sites (N=299) were tested to evaluate the equivalence between the SeCore Sequencing System and a predicate device (SSP UniTray with UniMatch Plus interpretation software). The overall concordance rate for detection of HLA-A Locus was calculated to be 100% (one-sided 95% lower-bound confidence interval exceeding 95%).

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Should the user observe any serious incident that has occurred in relation to this in vitro medical device, the user shall report the serious incident to the manufacturer, any local regulatory agency and the competent authority of the Member State in which the user is established.

EXPLANATION OF SYMBOLS

Symbol	Description
Medical Device Symbols *(Reference EN ISO 15223-1: medical devices – symbols to be used with medical device labels, labeling and information to be supplied.)*	
 *ISO 7000 Reg No. 1641*	Consult instructions for use.
 *ISO 7000 Reg No. 2493*	Catalog number
	In vitro diagnostic medical device
 *ISO 7000 Reg No. 0434A*	Caution
 *ISO 7000 Reg No. 0659*	Biological risks
 *ISO 7000 Reg No. 0632*	Temperature limitation
 *ISO 7000 Reg No. 2492*	Batch Code
 *ISO 7000 Reg No. 3082*	Manufacturer
 *ISO 7000 Reg No. 0624*	Protect from light sources
 *21 CFR 809.10(a)(4)*	For prescription use only Caution: Federal law restricts this device to sale by or on the order of a physician

Batch field on the label is for traceability of manufacturing event.

REVISION HISTORY

Revision	Date	Revision Description
01	30-OCT-2019	Initial Release
02	Current	Initial IVD Product Release (Refer to BR# *Pending*)