Poster Number: Monday-30

Detection of HBV Drug Resistance Mutations using *Sentosa*® NGS Platform

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Introduction

350 million people are chronically infected with Hepatitis B virus (HBV) every year with 800,000 deaths every year due to the lack of diagnosis and treatment. Treatment of chronic hepatitis B with nucleoside analogs has been shown to prolonged viral suppression leading to patient survival. However, the occurrence of drug resistance mutations has resulted in the need for genotypic resistance testing. Here, we developed an automated Next Generation Sequencing (NGS)-based system that could be coupled with either classical emulsion PCR templating or rapid isothermal amplification templating for HBV genotyping and detecting drug resistance mutations in HBV-infected patients.

Methods

Components of the HBV NGS system

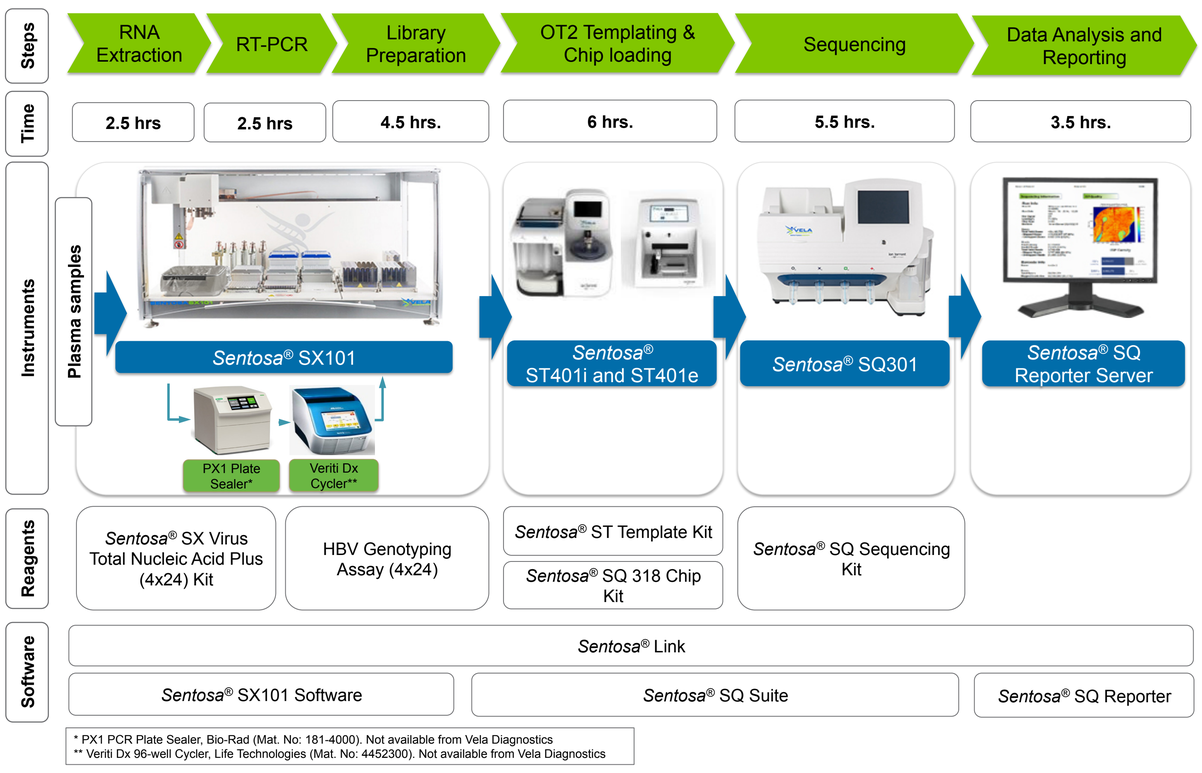
1) **Sentosa® SX101 robotic liquid handling system**
   - Viral RNA extraction & PCR setup
   - NGS library preparation
   - Automated Isothermal Amplification (IA) templating
   - Automated chip loading
2) **Sentosa® ST401 system**
   - Emulsion PCR templating (OT2)
3) **Sentosa® SQ301 (Ion Torrent) NGS platform**
4) **Kits for RNA extraction, library preparation, template preparation and sequencing**
5) **Proprietary software for data analysis, reporting and interpretation**

Characterizing the HBV NGS system

1. **Limit of detection (LOD)**
   - Lowest viral load detectable in a 730uL sample
   - Minimum coverage of 500x for Reverse Transcription and Pre-Core/Core genes
   - Tested at 100, 1000, 2000 IU/mL
2. **Assay Reactivity**
   - HBV genotypes (B, C, D, E, F, G and H) tested at LOD
   - Able to detect (A, B, C, D, E, F, G and H)
3. **Range of detection**
   - Tested at 100, 1000, 2000, 10000, 50000, 100000 IU/mL
   - 3 replicates each
4. **Comparison with MiSeq-based laboratory-developed test (LDT)**
   - 17 samples between 2,200 to 1,000,000 IU/mL
   - Multiple HBV genotypes

Workflow

HBV Genotyping Assay Workflow



* PX1 PCR Plate Sealer, Bio-Rad (Mat. No: 181-4000). Not available from Vela Diagnostics
** Veriti Dx 96-well Cycler, Life Technologies (Mat. No: 4452300). Not available from Vela Diagnostics

Acknowledgments

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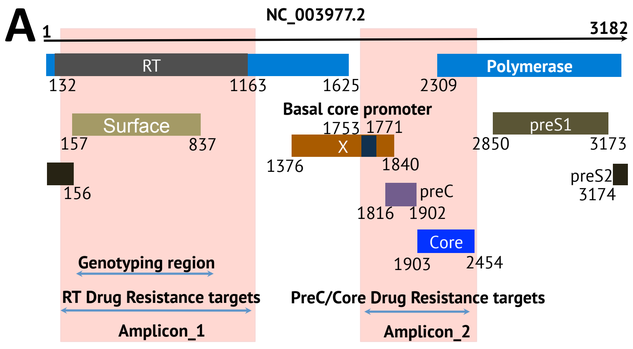
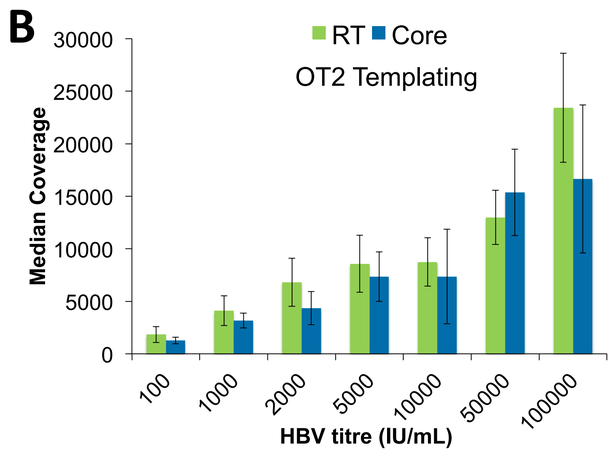
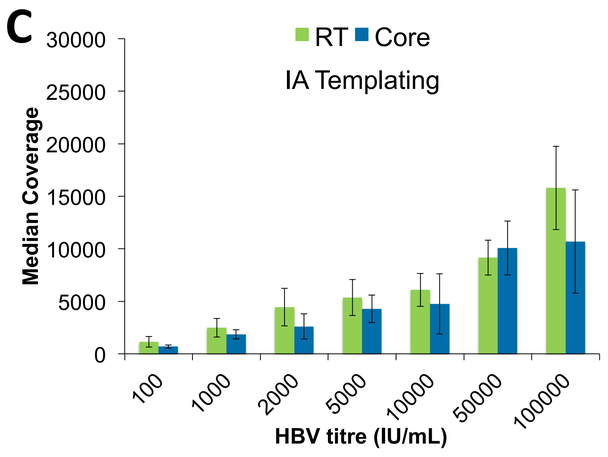
Results

Figure 1 Assay characteristics.

(A) Target regions of the HBV Genotyping Assay. HBV clinical samples were tested in triplicates from 100 to 100000 IU/mL and sequenced using **(B)** emulsion PCR templating on OT2 or **(C)** automated isothermal amplification templating. Both templating methods had identical LOD and ROD albeit slight differences in sequencing depth due to technical limitations. Error bars represent ±SD of n=3.







Workflow Characteristics

- **Throughput:** 23 Samples and System Control
- **Turn-around time:** ~2.5 days (OT2) or ~26 hours (IA)
- **Hands-on time:** ~3.5 hours (OT2) or ~1 Hour (IA)

Assay Performance

- Interrogates RT and Pre-Core/Core genes (Fig 1A)
- Limit of detection: 100 IU/mL
- Range of detection: 100 – 100000 IU/mL (Fig 1B,C)
- Readily detects HBV Genotypes A, B, C, D, E, F, G and H

Comparison Study

- High concordance between HBV Genotyping Assay (4x24) and MiSeq-based LDT (Table 1).
- 100% concordance in genotyping and 90% in variant detection.

Table 1: Comparison between HBV Genotyping Assay and MiSeq LDT.

		HBV Genotyping Assay			MiSeq LDT		
S/N	Titer (IU/mL)	Mutation(s)		Genotype	Mutation(s)		Genotype
		RT	Pre-Core/ Core		RT	Pre-Core/ Core	
1	2,200	No	T118V P127T A128V	D	No	128V	D
2	10,000	No	T118A P120T C137CF	E	No	P120T	E
3	13,000	No	No	A	No	No	A2
4	16,000	No	No	E	No	No	E
5	37,000	No	No	C	No	No	C
6	110,000	L180M S202G M204V	No	B	L180M S202 M204V	Q101S	B
7	170,000	No	No	D	No	No	D
8	530,000	No	M133T	B	No	S114T(5%) M133T T140S	B
9	4,500,000	No	P127T F134L	B	No	G119EG P127T F134L	B
10	7,800,000	L180M A181AG S202G M204V	No	C	L180M S202 M204V	No	C
11	39,000,000	No	No	B	No	No	B
12	81,000,000	No	L127P	E	No	L127P	E
13	270,000,000	No	No	D	No	No	D
14	>100,000,000	I233IV	No	B	I233V	No	B
15	RV	No	No	A	No	No	A
16	RV	No	No	A	No	No	A
17	RV	No	No	D	No	No	D1

Conclusions

The HBV Genotyping Assay is an accurate and sensitive automated assay for the detection of HBV genotypes and drug resistance mutations (within the Reverse Transcription and Pre-Core/Core regions) for epidemiology or prognostic purposes.