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HCV Genotyping with Concurrent Profiling of Resistance-Associated Variants by NGS Analysis

Kok-Siong Poon, Julian Wei-Tze Tang
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Abstract

Determination of viral characteristics including genotype (GT), subtype (ST) and resistance-associated variants (RAVs) profile is important in assigning direct-acting antivirals regimes in HCV patients. To help achieve the best clinical management of HCV patients, a routine diagnostic laboratory should aim at reporting accurate viral GT/ST and RAVs using a reliable diagnostic platform of choice. A laboratory study was conducted to evaluate performance characteristics of a new commercial next-generation sequencing (NGS)-based HCV genotyping assay in comparison to another widely used commercial line probe assay for HCV genotyping. Information on RAVs from deeply sequenced NS3, NS5A and NS5B regions in samples classified as HCV 1a and 1b was harnessed from the fully automated software. Perfect (100%) concordance at HCV genotype level was achieved in GT2 (N = 13), GT3 (N = 55) and GT5 (N = 7). NGS refined the ST assignment in GTs 1, 4 and 6, and resolved previously indeterminate GTs reported by line probe assay. NGS was found to have consistent intra- and inter-run reproducibility in terms of genotyping, subtyping and RAVs identification. Detection of infections with multiple HCV GTs or STs is feasible by NGS. Deep sequencing allows sensitive identification of RAVs in the GT 1a and 1b NS3, NS5A and NS5B regions, but the list of target RAVs is not exhaustive.

Keywords: resistance-associated variants, next-generation sequencing, hepatitis C, HCV genotyping, NGS

1. Introduction

Due to the genetic diversity of the hepatitis C virus (HCV), its accurate genotyping is still currently challenging despite the use of modern molecular techniques. In addition to the six widely-recognised HCV genotypes, a newly identified genotype (GT) 7 was reported in 2015 [1]. Molecular methods including reverse hybridization, real-time PCR and Sanger sequencing are commonly utilised for HCV genotyping and subtyping in clinical laboratories. HCV genotype and subtype (ST) have been the critical factors in decision-making for administering interferon-based therapies for the past decade [2]. According to the latest AASLD guidelines [3], determination of viral characteristics including GT, ST and resistance-associated variants (RAVs) profile is important in assigning direct-acting antivirals (DAAs) regimes in HCV patients.

To help achieve the best clinical management of HCV patients, a routine diagnostic laboratory should aim at minimising reporting out non-informative HCV genotyping results which are due to inherent limitations of the diagnostic platform of choice. In general, about 2–8.5% of HCV positive samples have been reported to carry "indeterminate" GTs by several commercial assays [4–9]. To tackle uncertainties in determining HCV GT and ST, Sanger sequencing could be utilised to resolve indeterminate or discordant GTs or ST results produced by commercial assays [10, 11]. Despite the ability to provide definitive genotyping information most of the time, unfavourable features of Sanger sequencing including low throughput, time-consuming procedures and relatively high costs, pose a barrier to it becoming routinely adopted as a first-line genotyping method. With the advent of next-generation sequencing (NGS), limitations of probe-based genotyping assays and Sanger sequencing for HCV genotyping can be overcome. NGS provides a high-resolution means for direct sequence-based interrogation of the HCV genome. Moreover, NGS also allows concurrent profiling of RAVs where such value-added feature is highly relevant for the clinical management of HCV infection with appropriate use of DAAs.

In the present study, the Sentosa SQ HCV genotyping assay (hereinafter referred to as Vela NGS) (Vela Diagnostics, Singapore) which primarily interrogates the NS5B region of HCV GTs 1–6 by ion torrent-based NGS technology, was evaluated in comparison to the VERSANT HCV Genotype 2.0 Assay (hereinafter referred to as LiPA) (Siemens Healthineers, Erlangen, Germany). HCV indeterminate GTs previously reported in clinical samples by LiPA were resolved using Vela NGS assay with further confirmation by Sanger sequencing. Information on RAVs was also harnessed from deeply sequenced NS3, NS5A and NS5B regions in samples classified as HCV 1a and 1b using Vela NGS.

2. Study design

2.1 Clinical samples

This study was performed on residual sera or plasma from 222 clinical specimens previously received for routine genotyping using the VERSANT HCV Genotype 2.0 Line Probe Assay (Siemens Healthineers, Erlangen, Germany). All samples were stored at -80°C post-LiPA analysis and were only thawed prior to re-analysis by NGS and Sanger sequencing. All samples were de-identified for anonymisation purposes, and hence, the treatment histories remain unknown and cannot be traced. These were all residual samples, which would otherwise be discarded, and were used for the purposes of assay validation only. In such situations, ethics approval is not normally required, as all samples could not be linked back to the original patients after anonymisation.

2.2 NGS by Sentosa SQ HCV genotyping assay

In this study, NGS was performed using Sentosa SQ HCV Genotyping Assay (4 × 16) (Vela Diagnostics, Singapore) according to the manufacturer's instructions. The workflow started with automated extraction of total nucleic acids from 530 μL of sera or plasma using Sentosa SX Virus Total Nucleic Acid Plus II kit (Vela Diagnostics) on Sentosa SX101 (Vela Diagnostics). PCR amplification of the HCV NS3, NS5A and NS5B regions was performed on Veriti 96-Well Thermal Cycler (Applied Biosystems, CA, USA). In every individual run, a pooled library containing barcoded amplicons of 15 clinical samples and one system control, was prepared by Sentosa SX101. The pooled library was subject to sequencing template preparation and enrichment on Sentosa ST401 (Vela Diagnostics). Sequencing data generated

by Sentosa SQ301 (Vela Diagnostics) was automatically channelled for primary and subsequent secondary analyses using Sentosa SQ Suite (Vela Diagnostics) and Sentosa SQ Reporter (Vela Diagnostics), respectively. Auto-generated quality control and pathology reports containing technical information, viral typing, and RAVs (available only for GTs 1a and 1b) results were manually reviewed, respectively.

2.3 VERSANT HCV Genotype 2.0 Line Probe Assay

Total nucleic acids were extracted from 200 μL sera or plasma using EZ1 Virus Mini Kit v2.0 (QIAGEN, Hilden, Germany) on Biorobot EZ1 (QIAGEN). Using VERSANT HCV Genotype 2.0 Line Probe Assay (LiPA) (Siemens Healthineers), a one-step reverse transcription-polymerase chain reaction (RT-PCR) amplifying the 5'UTR and core regions was performed on GeneAmp PCR System 9700 (Applied Biosystems). Reverse hybridisation, washing and colour development steps were performed on Autoblot 3000H (Fujirebio Europe, Gent, Belgium). For GT and ST determination, band patterns were manually scored by aligning the strips to an interpretation chart provided by the manufacturer.

2.4 Sanger sequencing

Sanger sequencing was performed on samples previously reported by LiPA as indeterminate genotype. A primary PCR amplification of a 454 bp fragment of the NS5B region was initially attempted using primers 5Bo8254 and 5Bo8707 [12]. In samples with PCR failure using the above-mentioned primers, a secondary PCR amplifying a 446 bp fragment of the 5'UTR/core regions was subsequently performed using primers UTR45 and Cor490 [12]. PCR products from the amplifiable gene segments were subjected to direct sequencing with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) using the respective PCR primers on a 3130XL Genetic Analyzer (Applied Biosystems).

2.5 Sequence analysis

Sequence analysis was performed by querying the nucleotide sequences obtained from Sanger sequencing in the Los Alamos hepatitis C sequence database [13]. For Vela NGS, assembled contigs were downloaded from the Sentosa SQ Reporter software. In samples with discordant results between LiPA and Vela NGS, NGS contigs were uploaded to the Los Alamos hepatitis C sequence database [13] to verify Vela NGS results.

3. Results

3.1 Concordance between results generated by the Vela NGS and Versant platforms at GT and ST levels

The Vela NGS results at both GT and ST levels were tabulated in **Table 1** for 170 clinical samples with GT and/or ST results from LiPA. Perfect (100%) concordance at HCV genotype level was achieved in GT 2 (N = 13), GT 3 (N = 55) and GT 5 (N = 7). For samples reported by LiPA as GT 1 (N = 40), 20% (N = 8) gave discrepant results when compared to Vela NGS. These samples had been previously classified by LiPA as either GT 1a with core inconclusive, GT 1b with 96.1% homology, GT 1b with core inconclusive, or GT 1b with core not available, due to their unconventional band patterns. There was no discrepancy between samples firmly reported as GT 1a and GT 1b by LiPA. In samples reported as GT 4 (N = 16)

by LiPA, 43.8% (N = 7) were found to be GT 3 by Vela NGS. Two samples (5.1%) originally reported by LiPA as GT 3 were classified by Vela NGS as GT 6 samples.

At ST level, Vela NGS reclassified 1 sample previously assigned as HCV 1a with core inconclusive by LiPA as 1c. Two samples each reported as 4a/4c/4d and 4e by LiPA, respectively, were reclassified as 4n and 4o by Vela NGS. Another 29 GT 6 (ST c-l) samples reported by LiPA were reassigned by Vela NGS as 6e/6u (N = 1), 6j (N = 1), 6m (N = 9), and 6n (N = 18), respectively. One sample with LiPA 6m (77.9% homology) was reassigned as 6u by Vela NGS.

3.2 Verification of contig sequences generated by the Vela NGS in samples with discordant results

Of the 170 samples tested, there were 104 agreements at both GT and ST levels, 49 partial agreements at genotype but not the subtype levels, and 117 discordant results generated by LiPA and Vela NGS (**Table 1**). At GT level, the calculated Cohen's Kappa is 0.869 (95% confidence interval: 0.810–0.928), suggesting good strength of agreement between the two assays. The 66 NGS contig sequences of samples with partial agreement or discordant results were submitted to the online analysis in the Los Alamos hepatitis C sequence database. HCV GT and ST called by Vela NGS were verified in all 66 contigs.

3.3 Intra-run and inter-run reproducibility on GT and ST calling by Vela NGS

HCV genotyping and subtyping results were found to be reproducible for a panel of 5 samples with different HCV GT/ST including 1a, 1b, 2a, 3a and 3b tested in triplicates within a single run on the Vela NGS platform (**Figure 1a**). For inter-run reproducibility testing (**Figure 1b**), GT and ST results were consistently reported in another panel of 7 samples including 1a, 1b, 2b, 3a, 4d, 5a and 6n, which were repeatedly tested in three separate runs on different days. Details of viral load and median coverage of the targeted NS5B region are depicted in **Figure 1a** and **b**, respectively.

3.4 RAV analysis in GT 1 samples reported by the Vela NGS platform

In the current Vela NGS assay, a list of variants differing from the wild-type codons are detectable for HCV 1a and 1b. The 16 target codons in the NS3 gene are 36, 41, 43, 54, 55, 80, 109, 122, 132 (1a only), 138, 155, 156, 158, 168, 170 (1b only) and 175 (1b only). For NS5A, variants at nine codons including 28 (1a only), 30 (1a only), 31, 32, 54 (1b only), 58, 62 (1b only), 92 and 93, are detectable. Eight codons in the NS5B gene including 414, 419, 422, 423, 495, 499 (1b only), 554 and 559, are also covered in this assay.

Of 13 GT 1a samples (**Table 2**), five were found to carry at least one target variant in the NS3 gene. Notably, two samples carried the Q80K RAV. For NS5A, the M28A variant was detected in one sample in which NS3 Q80K was also present. None of the GT 1a samples was found to carry any of target variants in the NS5B gene.

Of 18 HCV 1b samples (**Table 2**), five were detected with at least one target variant in the NS3 gene. Twelve samples were identified with at least one target variant in the NS5A gene. For NS5B, the P495A and V499A variants were detected in one and eight samples, respectively. Notably, there were four samples detected with at least one target variant in each of the NS3, NS5A and NS5B genes.
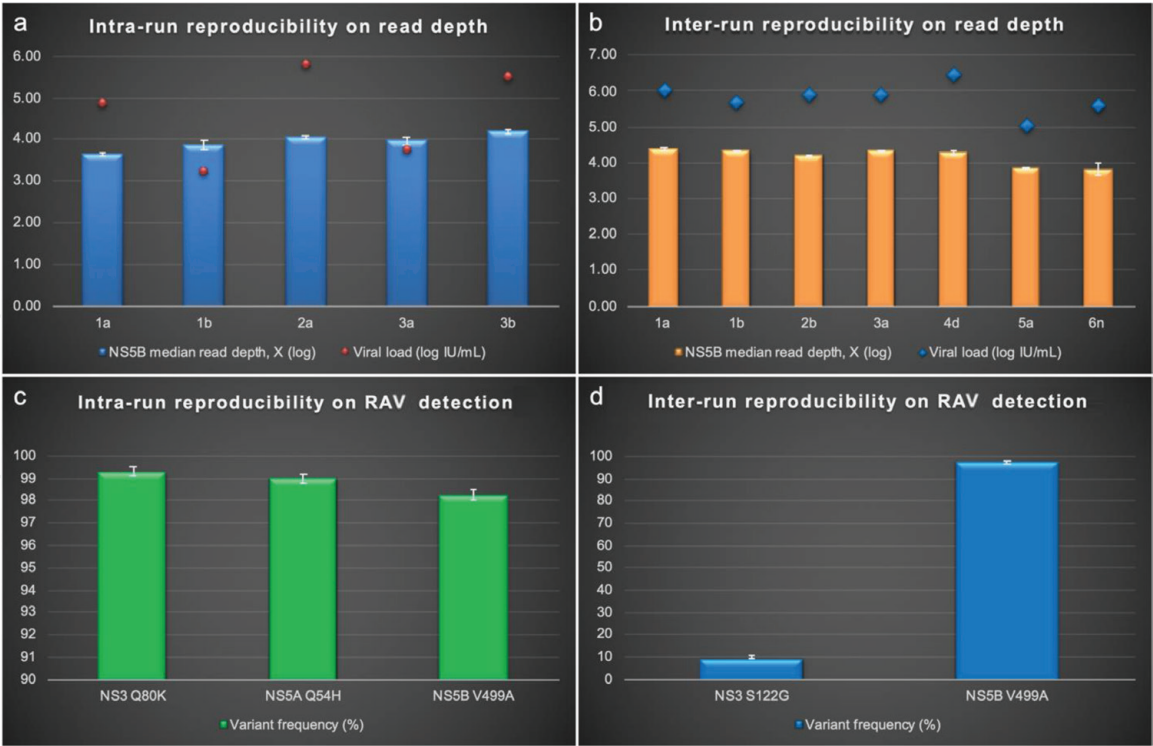
3.5 Intra-run and inter-run reproducibility on variant calling and frequency

In intra-run reproducibility analysis, the Q80K variant was reproducibly detected in the NS3 gene of the GT 1a samples. Another two variants, namely Q54H

Platform			LiPA																								Total
Genotype			1						2			3						4				5	6				
	Genotype	Subtype	1a	1a (core inconclusive)[a]	1b	1b (96.1% homology)[b]	1b (core inconclusive)[a]	1b (core not available)[a]	2	2a /2c	2b	3	3a	3b	3b (96.6% homology)[c]	3b (93.6% homology)[d]	3k	4	4a /4c /4d	4e	4h	5a	6a /6b	6e (83.3% homology)[e]	6 (c-l)	6m (77.9% homology)[f]	
Vela NGS	1	1a	12	1																							13
		1b			13	1	4																				18
		1c		1																							1
	2	2a							3	7																	10
		2b									3																3
	3	3a											11														11
		3b										9		24	8	1											46
		3k															2										7
	4	4a																1	1								3
		4d																	1								1
		4n																1	2								3
		4o																		2							2
	5	5a																				7					7
	6	6a																					4				4
		6e																						1			1
		6e/6u																							1		1
		6j																							1		1
		6m																							9	1	10
		6m/6u																								1	1
		6m/6n/6u																								2	2
		6n																							18	1	19
		6u					1	3																			4
		6v						2																			2
Total			12	2	13	1	6	6	3	7	3	9	11	24	8	1	2	3	5	2	6	7	4	1	29	5	170

a In LiPA, the possibility of GT 6 (STs c-l) can not be excluded in HCV 1a or 1b with inconclusive or unavailable core regions.
b LiPA bands 3,4,6, 16& 26.
c Bands 13, 17& 24.
d Bands 6, 7, 17, 18 & 24.
e Bands 3, 4, 13& 24. f Bands 6& 24.

Table 1.
Comparison of GT and ST distribution in 170 samples tested by both LiPA and Vela NGS.

Figure 1.
Precision studies on the Vela NGS. (a) Intra-run and (b) inter-run reproducibility on median read depth were tested on 5 and 7 clinical specimens, respectively. For RAV analysis, variants were called with reproducible frequency (c) within a run (intra-run) and (d) between runs (inter-run).

No	ID	GT1 STs	RAVs (variant frequency)		
			NS3	**NS5A**	**NS5B**
1	R02-BC02	1a	S122G (99.21%), D168E (97.07%)	–	–
2	R02-BC03	1a	V55A (91.44%)	–	–
3	R02-BC04	1a	Q80K (25.63%)	M28V (99.47%)	–
4	R02-BC05	1a	Q80K (4.84%)	–	–
5	R13-BC13	1a	D168E (51.43%)	–	–
6	R01-BC02	1b	Q80K (55.29%) M175L (87.81%)	–	V499A (98.15%)
7	R01-BC03	1b	–	–	V499A (97.03%)
8	R01-BC04	1b	–	L31M (22.03%), Q54H (98.82%)	V499A (33.65%)
9	R01-BC05	1b	–	Q54H (99.11%), Y93H (99.73%)	–
10	R01-BC06	1b	Q80L (99.52%), S122G (9.99%)	Q54H (99.05%)	V499A (97.91%)
11	R01-BC07	1b	–	Q54H (98.76%), Y93H (99.61%)	–
12	R01-BC08	1b	–	Q54H (99.22%), Q62E (99.04%)	–
13	R01-BC09	1b	S122G (97.69%)	Q54H (99.21%), Q62E (51.64%)	P495A 8.83%
14	R01-BC11	1b	–	Y93H (99.24%)	–

No	ID	GT1 STs	RAVs (variant frequency)		
			NS3	NS5A	NS5B
15	R02-BC07	1b	–	Q54H (99.37%)	V499A 98.9%
16	R02-BC-11	1b	Q80R (92.29%)	Q62E (5.61%)	V499A 95.15%
17	R11-BC14	1b	M175L (99.97%)	Y93H (99.8%)	V499A 98.7%
18	R12-BC14	1b	–	Q54H (80.35%), Y93H (8.07%)	–
19	R12-BC15	1b	–	Q54H (98.82%), Q62R (99.79%)	–

In this study, RAVs with variant frequency less than 1% are not shown.

Table 2.
List of resistance-associated variants (RAVs) identified in GT 1a and 1b samples by Vela NGS.

No	LiPA results (bands)	Vela NGS	Sanger sequencing		Concordance at GT or ST level
			NS5B	5'UTR/core	
1	Indeterminate (3,6,16,24)	6n	Not amplified	6n	GT & ST
2	Indeterminate (3,6,16,24)	6n	Not amplified	6n	GT & ST
3	Indeterminate (3,6,16,24)	6n	Not amplified	6n	GT & ST
4	Indeterminate (6,7,24)	3b	3b	Not done	GT & ST
5	Indeterminate (6,7,24)	6m/6u	Not amplified	6e/6d	**GT only**
6	Indeterminate (6,7)	6u	6u/6n	Not done	GT & ST
7	Indeterminate (6,7)	6u	6m/6n	Not done	**GT only**
8	Indeterminate (6,7)	6u	6n/6a	Not done	**GT only**
9	Indeterminate (6)	6m/6l	6d/6e	Not done	**GT only**
10	Indeterminate (17,24)	3b	3b	Not done	GT & ST
11	Indeterminate (17,18,24)	3b	3b	Not done	GT & ST
12	Indeterminate (6,17,24)	3b	3b	Not done	GT & ST
13	Indeterminate (7,8,14,15,24)	3a	3a	Not done	GT & ST
14	Indeterminate (7,13,17,18)	3b	3b	Not done	GT & ST
15	Indeterminate (7,13,17,18,24)	3b	3b	Not done	GT & ST
16	Indeterminate (13,16,17,18,24)	3b	3b	Not done	GT & ST
17	Indeterminate (13,14,15,18,24)	3b	3b	Not done	GT & ST
18	Indeterminate (3,4,13,25)	1a	1a	Not done	GT & ST
19	Indeterminate (3,4,7,13,25)	1a	1a	Not done	GT & ST
20	Indeterminate (3,4,7,13,24)	6e	6e	Not done	GT & ST
21	Indeterminate (3,4,6,7,13,24)	6e	Not amplified	6e/6d	GT & ST
22	Indeterminate (5,9,21,24)	6a	Not amplified	6a	GT & ST
23	Indeterminate (5,6,9,17,18)	4a	Not amplified	4a	GT & ST
24	Indeterminate (5,9,10,13,14,15,24)	2a & 3a	Two mixed sequences	Not done	Likely mixed infections
25	Indeterminate (5,8,9,11)	2a	2a	Not done	GT & ST
26	Indeterminate (24)	3b	3b	Not done	GT & ST

No	LiPA results (bands)	Vela NGS	Sanger sequencing		Concordance at GT or ST level
			NS5B	5'UTR/core	
27	Indeterminate (24)	3b	3b	Not done	GT & ST
28	Indeterminate (4,5,9,16,21,24)	6a	6a	Not done	GT & ST
29	Indeterminate (4,9,21)	6a	Not amplified	6a	GT & ST
30	Indeterminate (3,4,6,13,17,18,24,26)	3b	3b	Not done	GT & ST
31	Indeterminate (6)	6n/6a	6d/6u	Not done	**GT only**
32	Indeterminate (3,4,13,25)	1a	1a	Not done	GT & ST
33	Indeterminate (6)	6u	6u	Not done	GT & ST
34	Indeterminate (13,16,24)	3b	3b	Not done	GT & ST
35	Indeterminate (7)	6u	Not amplified	6v/6l/6d/6k	**GT only**
36	Indeterminate (17,18,24)	3b	3b	Not done	GT & ST
37	Indeterminate (3,4,5,16,25)	1a	Not amplified	1a	GT & ST
38	Indeterminate (5,6,18,24)	3k	3k	Not done	GT & ST
39	Indeterminate (8,9,21,24)	6a	6a	Not done	GT & ST
40	Indeterminate (3,4,6,16,24)	6q	6q	Not done	GT & ST

PCR amplification for NS5B was first attempted in all 40 specimens. A secondary PCR amplifying 5'UTR/core regions were performed in samples with unsuccessful amplification of NS5B. Sanger sequencing was performed on PCR amplicon obtained.

Table 3.
Comparison of genotyping results produced by the Vela NGS and Sanger sequencing methods in 40 specimens with indeterminate genotypes by LiPA.

and V499A were also repeatedly identified in the NS5A and NS5B genes of the GT 1b sample, respectively. Variant frequencies of the three variants were highly reproducible within run (**Figure 1c**).

In the inter-run reproducibility study, NS3 S122G and NS5B V499A variants were tested. Variant frequencies of the two variants were found to be highly reproducible among the three separate runs (**Figure 1d**).

3.6 Vela NGS assigned HCV GT and ST to samples with indeterminate LiPA results

Forty specimens, which were previously reported as HCV indeterminate GT by LiPA, were subject to Vela NGS analysis. Sanger sequencing were successfully performed on NS5b (N = 30) or 5'UTR/core (N = 10) regions in 40 samples (**Table 3**). Of the 40 samples with Sanger sequencing results, Vela NGS results were confirmed at GT level in 39 samples (97.5%). In a sample with LiPA complex band patterns (5, 9, 10, 13, 14, 15 & 24), a mixed genotypes of GT 2a and GT 3a were assigned by Vela NGS. Sanger sequencing on NS5B showed overlapping nucleotide base calls in the overall sequences, in which putative mixed infection with two different HCV GTs was likely inferred.

4. Discussion

The application of NGS assays to analyse quasispecies HCV genomes has been increasing in recent years. Several laboratory-developed NGS assays had been

previously described in the literature for phylogenetic studies [14], outbreak investigation [15, 16], characterisation of HCV full genome [17, 18] and identification of HCV GT and ST in clinical samples [19, 20]. However, there are fewer reports of adoption of NGS assays in routine HCV genotyping. In 2016, Vela NGS became available as a CE-IVD certified commercial kit for diagnostic use in the clinical laboratories. In this study, we report the performance characteristics of Vela NGS in comparison to the widely used LiPA assay for HCV genotyping.

The performance of Vela NGS in determining the HCV GT and ST in the clinical specimens had been discussed in several previous studies [21–23]. Perfect agreement at GT level was observed between Vela NGS and LiPA in a study by Manee et al. [21]. Samples with unclear ST results in GTs 2, 3, 4 and 6 reported by LiPA were each assigned with a specific subtype after subject to Vela NGS analysis. Dirani et al. [22] also performed a direct comparison of GT and ST calling between Vela NGS and LiPA for samples from patients infected with HCV GTs including GT 1, 2, 3 and 4, and found a high concordance (>99%) at GT level between the two tests. Vela NGS was also found to have better performance in assigning HCV STs among the four GTs when compared to LiPA [22]. In another study by Rodriguez et al. [23], Vela NGS achieved high concordance rates with Sanger sequencing in assigning GTs 1 to 6, 1a and 1b STs, and other STs for GTs 4, 5 and 6. Discrepant calls at ST level was mainly found among HCV GTs 1 and 2 between Vela NGS and Sanger sequencing; the latter was used as the reference method to sequence the 286 bp segment of NS5B for which phylogenetic analysis was performed.

In the present study, discrepancy in results was mainly observed in samples with LiPA GT 1b with incomplete or missing bands at the core region. In this particular result group, GT 6 with different STs were assigned by Vela NGS. This observation was not unexpected as it has been specified in the LiPA interpretation chart that GT 6 (STs c-1) cannot be differentiated from ST 1a and 1b without additional information from the core region sequence. Among LiPA GT 4 samples, all ST 4h were reassigned as GT 3 by Vela NGS. Some geographical regions, for example, Southeast Asia, where GT 6 is highly prevalent [24], could thus be impacted more by this misclassification with the use of LiPA method.

In contrast to LiPA which utilises primarily the 5'UTR in GTs 1-6 and core regions for the discrimination of GT 6 STs c-l from 1a and 1b, Vela NGS targets the non-structural genes implicated in both accurate genotyping/subtyping and resistance to DAAs. The LiPA is known to be poor at detecting and identifying recombinant forms of HCV [25]. Due to the assay design of Vela NGS, this may also pose a problem for this platform, despite the application of NGS technology. The HCV recombinant forms can be accurately detected via sequencing of recombination breakpoint junctions or the whole HCV genome [26]. For example, in our study, one previously LiPA-indeterminate sample was reported by the Vela NGS to have mixed HCV infections with HCV 2a and 3a. This NGS finding was confirmed by Sanger sequencing in which overlapping Sanger electropherograms were observed for NS5B.

The Vela NGS offers information on RAVs in HCV 1a or 1b positive samples, where such profiling will be useful when prescribing DAA regimes, and detecting of baseline or emerging RAVs. Targeted assays had been previously developed to identify a specific RAV [27, 28]. RAVs which are found at levels with at least 15% variant frequency, at baseline, are known to confer resistance to certain DAAs [29], and therefore may impact on the effectiveness of DAA treatment [30]. Vela NGS targets relevant RAVs in three non-structural gene segments (NS3, NS5A and NS5B) of HCV 1a and 1b, and although the RAV profiling is comprehensive but not exhaustive due to the assay design, any baseline RAVs present in any of these DAA target genes, can affect the therapeutic effectiveness [31]. In our study, four HCV 1b samples were found to harbour variants in all three NS3, NS5A and NS5B genes concurrently.

5. Conclusions

In conclusion, the genotyping results of the Vela NGS were found to be highly concordant with those of the LiPA method. Vela NGS refined the ST assignment in GT 6 and resolved previously indeterminate GTs reported by LiPA. Technically, the HCV Vela NGS was found to have consistent intra- and inter-run reproducibility in terms of GT and ST calling and RAVs identification. Detection of infections with multiple HCV GTs or STs is feasible by Vela NGS. Due to the assay design which relies on investigating the HCV sub-genomic regions, HCV recombinant strains may still be potentially missed. Deep sequencing allows sensitive identification of RAVs in the GT1a and 1b NS3, NS5A and NS5B regions, but the list of target RAVs is not exhaustive. We would also suggest the RAVs detection spectrum should be extended to cover GTs other than HCV 1a and 1b, namely GTs 2-6.

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Conflict of interest

The authors have no conflict of interest to declare.

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