Molecular Diagnostic Tools for HCV Geno(sub)typing

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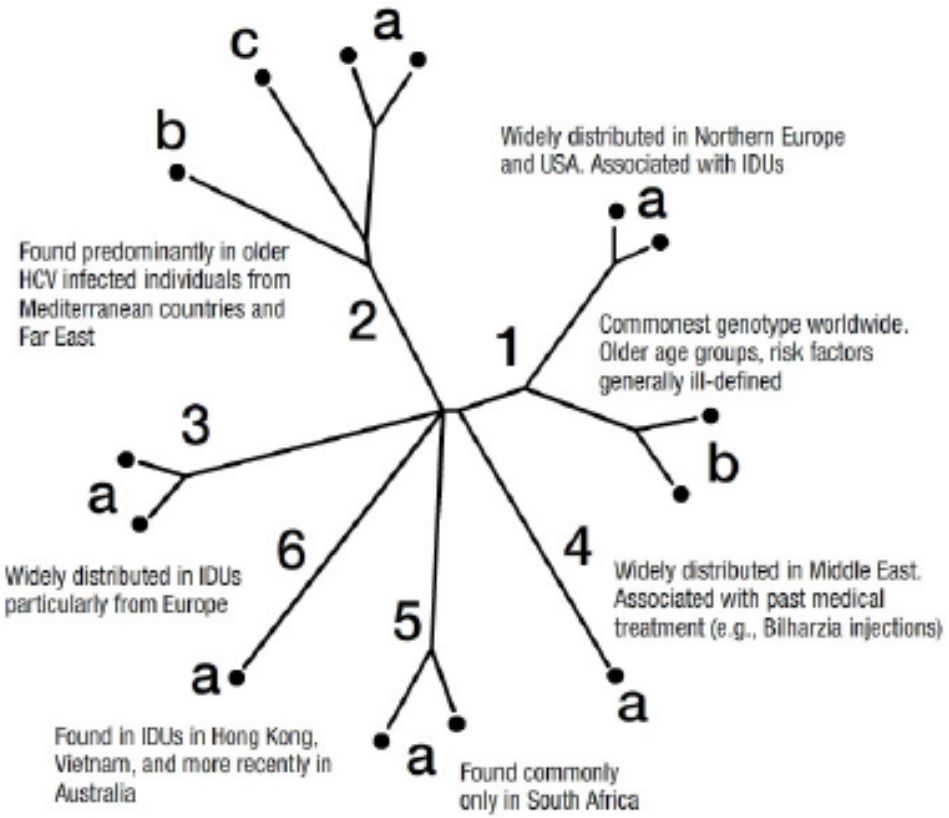
HCV Genomic Diversity

- **Genotype is key factor in HCV patient management**
- **Highly genetically diverse virus**
  - Polymerase lacks proofreading capability
- **6 major genotypes and more than 50 subtypes**
  - 30% sequence difference between genotypes
  - 20% sequence difference between subtypes



Source: Journal of General Virology Web site. http://vir.sgmjournals.org/content/85/11/3173.full. Accessed October 27, 2011.

Commercially available HCV GT Assays

Assay	Technology	Gene Target	Genotype Detection	Automation	Accuracy	Regulatory Status
Abbott RealTIme	RealTime	5'UTR/NS5B	1 – 6 (1a/1b)	High	97%	CE / PMA Submission
Trugene	CLIP Sequencing	5'UTR	1 – 6 w/subtype	Low	90 - 95%	RUO
LiPa v 2.0	Reverse Hybridization	5'UTR/Core	1 – 6 w/subtype	Low-Mid	97 - 99%	CE/ASR
GenMark HCVg Direct	eSensor	5'UTR/Core	1 – 6 (1a/1b,2a/2b /2c)	Low	TBD	RUO
Lab Developed Sequencing	Sanger	Variable	1 – 6 w/subtype	Low	Variable	LDA

Abbott RealTi*me* GTII vs Sequencing

Table 1

HCV Genotype/ Subtype	Number Samples Tested	Number Samples Correctly Identified	Detection Rate (%)
1[a]	169	169	100.00
1a[b]	110	107	97.27
1b[c]	58	56	96.55
2	41	41	100.00
3	27	27	100.00
4[d]	28	25	89.29
5	14	14	100.00
6	12	10	83.33
1 through 6[e]	291	286	98.28

Abbott RealTime HCV GT II package insert.

Abbott RealTime GTII vs Sequencing

Results Table 1. Comparison of Genotype Results for 44 Samples Based on Three Methods

Genotype	Number of Samples Interrogated	Abbott RealTime HCV GT II	5'UTR LDT	Core/NS5b LDT
1	29	31*	31*	29
1a	27	25	0**	27
1b	2	2	0**	2
2	4	4	4	4
3	7	7	7	7
4	2	2	2	2
6*	2	0*	0*	2

LDT = Laboratory Developed Assay

*Two samples were identified by the core/NS5b LDT as unusual genotype 6 samples (subtypes 6l and 6u) were identified by the Abbott and 5'UTR LDT assay as genotype 1.

**Subtypes were not determined using the 5'UTR LDT.

Mallory et al AMP 2012

Abbott RealTi*me* GTII vs LiPa 2.0

A multicenter evaluation of the automated Abbott RealTime HCV Genotype II

- 124 HCV positive sera tested previously with the LiPa 2.0 .

Table 1
Comparison of the Abbott RealTime HCV Genotype II and Versant HCV Genotype 2.0 assays at type level (subtype for genotype 1).

	LiPA									
	1	1a	1b	2	3	4	1a+3a	5	IND*	TOT**
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1			2							2
1a	2	16	1				1			20
1b		1	27						1	29
2				33					1	34
3					17					17
4			1			17				18
5								1		1
1b+3			1							1
1a+4		2								2
TOT	2	19	32	33	17	17	1	1	2	124

* Indeterminate.
** Total.

There was good agreement between the two assays.

Genotype concordance was 95.9% (117/122) / Subtype 1a/1b concordance was 95.6% (43/45).

Source: M. Ciotti et al. / Journal of Virological Methods *(2016)*

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Abbott RealTi*me* HCV GT II vs LiPa 2.0

Table 1. Abbott RealTime HCV Genotype II RUO

Versant HCV Genotype 2.0 RUO (LiPA)	1a	1b	1	2	1b, 2	3	4	5	6
1a	18								
1b		21	*1						
1			*1						
2				18	**1				
3						**12			
4							10		
5								1	
6									2

*Non-concordant; not repeated for confirmation.

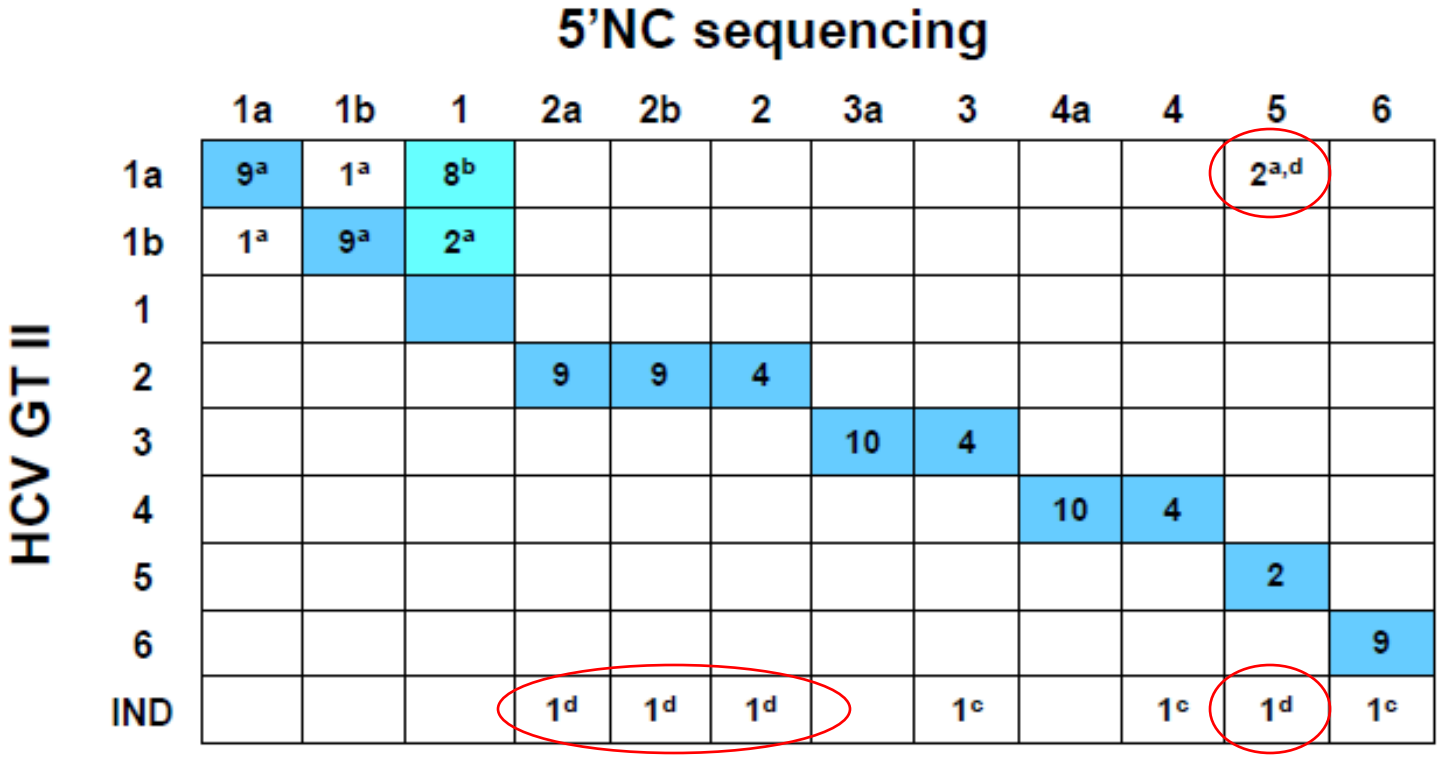
**Non-concordant: Abbott result repeated for confirmation.

*Acrometrix 1 control : Abbott assay first reported a 1a,1b mix but only 1b when repeated. The banding pattern on LiPA indicated a 1a,1b mix. We were unable to obtain confirmation of these findings from Acrometrix.

**One clinical sample was initially Indeterminate but was GT3 when rerun.

Shinol et al CVS 2012

Abbott RealTi*me* HCV GT II vs Trugene



N=100

IND; Indeterminate.

[a] HCV GT II result(s) supported by NS5B sequencing result(s).

[b] HCV GT II result is not supported by NS5B sequencing result in a single specimen.

[c] HCV GT II result "Indeterminate".

[d] 5'NC and NS5B sequencing results discordant.

Yao et al CVS 2012

HCV Genotype 1 Subtype Identification

Table 1. Ability of the different molecular methods tested in this study to correctly identify HCV subtypes 1a and 1b in a series of 500 patients infected by one or the other of these subtypes.

Assay		Trugene HCV 5′NC Genotyping Assay	INNO-LiPA HCV 1.0	INNO-LiPA HCV 2.0	Abbott RealTi*m*e HCV Genotype II assay
Manufacturer		Siemens Medical Solutions Diagnostic	Innogenetics	Innogenetics	Abbott Molecular
Method		Sequence analysis of the 5′NCR followed by sequence comparison	Reverse hybridization targeting the 5′NCR	Reverse hybridization targeting the 5′NCR and the core-coding region	Real-time PCR assay targeting the 5′NCR and NS5B-coding region
All samples	Subtype 1a* (N = 237), n/N (%)	183/237 (77.2%)	167/237 (70.5%)	231/237 (97.5%)	220/236** (93.2%)
	Subtype 1b* (N = 263), n/N (%)	238/263 (90.5%)	240/263 (91.3%)	253/263 (96.2%)	232/261** (88.9%)
Samples that could be PCR-amplified only	Subtype 1a*, n/N (%)	183/235(77.9%)	167/236 (70.8%)	231/232 (99.6%)	220/236 (93.2%)
	Subtype 1b*, n/N (%)	238/258 (92.2%)	240/260 (92.3%)	253/255 (99.2%)	232/259 (89.6%)

Correct identification with the different techniques tested is shown for all samples, and for samples that could be amplified by PCR in the assay.
*The correct HCV genotype 1 subtype was identified by means of direct sequence analysis of a portion of the NS5B gene followed by phylogenetic analysis, the reference method.
**In one 1a case and two 1b cases, not enough serum volume was available for testing in the Abbott RealTime HCV Genotype II assay.

Conclusions/Significance: In the context of new HCV drug development, HCV genotyping methods based on the exclusive analysis of the 5′NCR should be avoided. The second-generation line probe assay is currently the best commercial assay for determination of HCV genotype 1 subtypes 1a and 1b in clinical trials and practice.

Chevaliez S, et al., (2009)PLoS ONE 4(12): e8209. doi:10.1371/journal.pone.0008209

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Summary

- Sanger sequencing “Gold Standard” for HCV GT determination
  - Primer selection critical
  - Two targets recommended for sub-type determination
  - Quality Control important
- Commercially available tests vary and no test is perfect
  - Overall accuracy 95% - 97%
  - Alternate reflex method may be necessary to resolve indeterminate results.