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BACKGROUND

Hepatitis C virus (HCV) genotyping is required for therapeutic strategies. Sequencing of core/E1 or NS5B has been recommended but amplification of these highly variable regions may be difficult and relies on in-house and time consuming techniques not adapted for routine diagnosis.

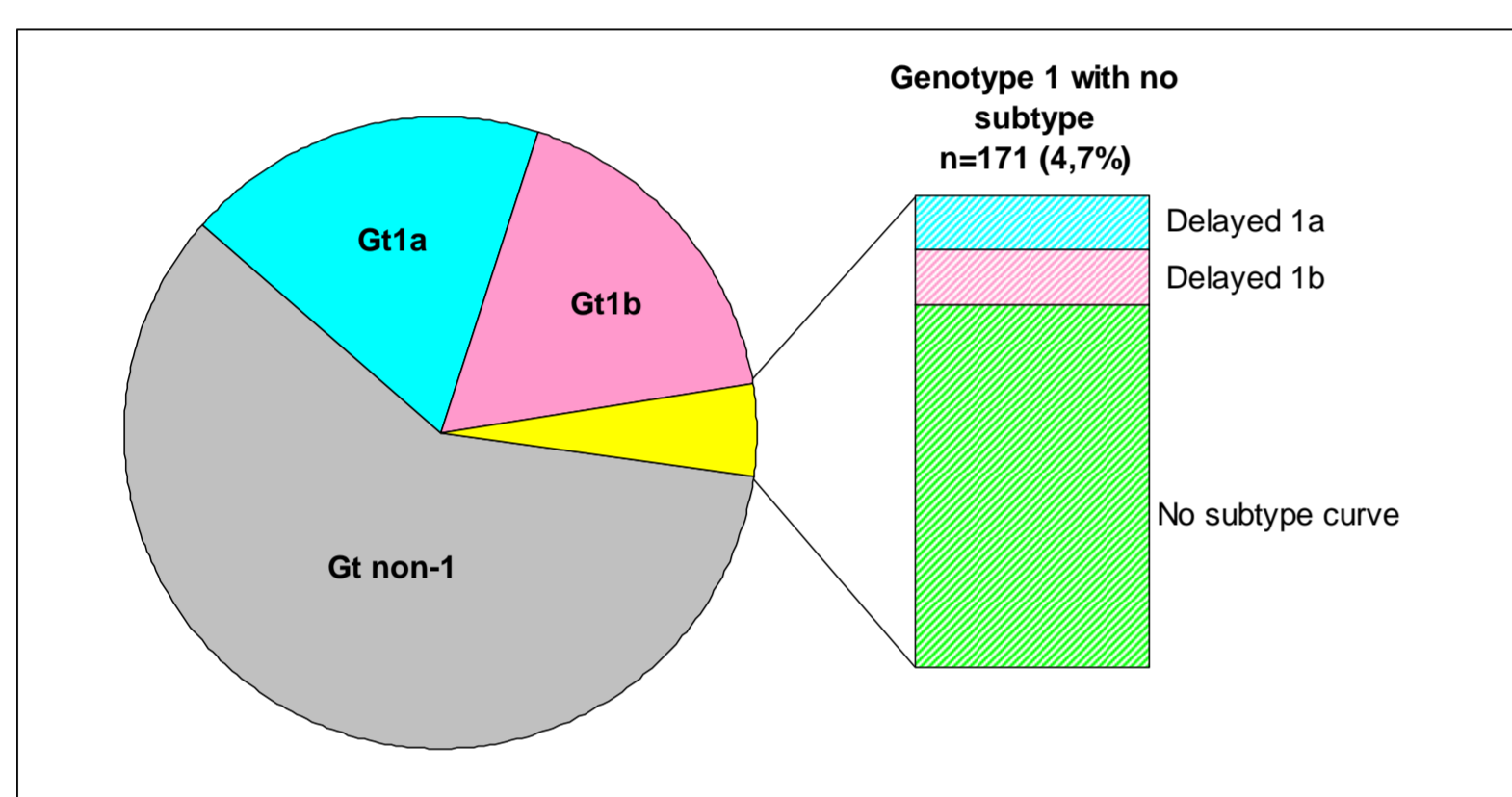
The Abbott RealTime HCV Genotype II test is a commercially available automated real-time PCR technique using probes targeting the 5'untranslated region (5'UTR) for genotype assignment and the NS5B region for genotype 1 (gt1) subtyping (1a or 1b). However, in 3.7-10.5% of genotype 1 results no subtype 1a or 1b can't be identified and might indicate genotype 1 subtypes other than 1a or 1b or genotype 6 subtypes with 5'UTR sequences identical to subtype 1b.

AIM

To further characterize such samples, the real-time RT-PCR test (Abbott HCV Genotype Plus RUO) targeting the core region for gt1a, gt1b and gt6 detection was evaluated as reflex test in reference to NS5B or 5'UTR/core sequencing

MATERIALS AND METHODS

Routine Results of Abbott RealTime HCV Genotype II (n=3626)



Among 171 routine samples displaying gt1 results without subtype during a 14 months period, 140 were subjected to the reflex test along with 5 gt1a and 5 gt1b control samples.

NS5B or 5'UTR/core sequencing was performed on 99 selected samples.

RESULTS

RealTime HCV Genotype II assay		HCV Genotype Plus RUO assay					Total
		Subtype 1a	Subtype 1b	Genotype 6	Subtype 1a + 1b	Not detected	
Gt1 without subtype	Delayed 1a	18					18
	Delayed 1b	1	13			2	16
	No subtype	18	38	14		36	106
Control samples	1a	4			1		5
	1b		5				5
		41	56	14	1	38	150

↓ Selection for sequencing

RealTime HCV Genotype II assay		HCV Genotype Plus RUO assay					Total selected
		Subtype 1a	Subtype 1b	Subtype 1a + 1b	Genotype 6	Not detected	
Gt1 without subtype	Delayed 1a	15/18					15/18
	Delayed 1b	0/1	12/13			2/2	14/16
	No subtype	11/18	16/38		12/14	30/36	69/106
Control samples	1a	0/4		1/1			1/5
	1b		0/5				0/5
		26	28	1	12	32	99/150

↓ Sequencing

HCV Genotype Plus RUO assay	NS5B or 5'UTR/core sequencing					Reflex test results confirmed by sequencing (%)
	Gt 1a	Gt 1b	Gt 6	Non-Gt 1a/1b/6*	No sequence	
Gt 1a	19			4	3	19/23 (82.6)
Gt 1b		24	1	3		24/28 (85.7)
Gt 6			11	1		11/12 (91.7)
Not detected		3		25	4	25/28 (89.3)
Gt 1a + Gt 1b	1					0/1
Total						79/92 (85.9)

Reflex Test Results (n=150)

- **Samples with delayed amplification curves :**
Confirmation of 1a or 1b in 91.2% of cases
- **Samples without subtype amplification curves**
Identification of 1a, 1b or 6 in 66% of cases
"Not detected" results in 34% of cases: non-gt1a/1b/6 results?

Sequence availability

NS5B sequencing was successful in 87.9% of cases and 5'UTR/core sequences were available for 5 additional samples : **overall sequencing efficiency was 92.9%**

Comparison of reflex test and Sequencing Results (n=99)

- Concordance : 85.9%, including
- not detected reflex test results = non-gt1a/1b/6 in 75.8%
 - identification of 11/12 gt6 samples, previously misclassified

CONCLUSION

The high genetic variability of HCV remains challenging for genotype and subtype assignment by commercial assays but also for sequencing. For the remaining equivocal samples additional testing is needed. The real-time PCR-based assay presented here is able to successfully resolve gt1 results without subtype of the Abbott RealTime HCV Genotype II assay. Therefore, it is an easy to implement alternative to sequencing-based approaches for supplementary testing.