



Hepatitis C Genotyping

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On August 1, 2004 the Molecular Virology laboratory will change the method used for genotyping of HCV. This new method is a significant improvement in two major areas –

- 1) The new method uses two different regions of the viral genome to improve accuracy of the result
- 2) The new method is faster and simpler, reducing turn-around time

The order test code, CPT4 code, and charges will remain the same as for the previous method.

Background Information – Traditionally, the 5' UTR region of the viral genome has been used for genotype assignment. However, variation between genotypes is often limited in this region so genotype differences are assigned based on a single base pair difference. Thus, a point mutation could cause the genotype to be assigned in error. Many recent published studies have shown that more accurate HCV genotype results are provided by analyzing the Core, NS5, or E1 region, or by combining analysis of the 5'UTR with a second region. Published studies have shown good agreement between Core, NS5 and E1 region data while each of these has shown greater disagreement when compared with 5'UTR results. For these reasons, we have selected a new method that utilizes both NS5 and 5'UTR sequences. Both of the most commonly used commercially available tests, the Bayer Tru-Gene and LIPA Line Probe assays utilize the 5'UTR region alone.

In-House Validation – Over 400 samples were run with the older method, an RFLP assay utilizing 5'UTR sequence, and a new real-time PCR assay from Abbott Diagnostics. We found that about 95% of samples tested had an identical genotype with both methods. Similar results have been seen in other published studies where discrepant rates of from 2 – 10% have been reported. The highest discrepant rates have been shown when non-sequence based methods using the 5'UTR were compared to direct sequencing of the NS5 or E1 regions.

Comparison of Results 5' UTR RFPL vs Real Time PCR

		In-House		RF		LP		
		1a	1b	2a	2b	3a	4	6
Abbott Real Time	1a	119	19	32				
	1b	3	32	2				
	2a			9	2			1
	2b	1		3	50			
	3					34		
	4	1		3			5	
	6			3				
Total		124	51	52	52	34	5	1

[Note: Genotype distribution in this table does not reflect actual percentages in our population, as the samples tested were significantly augmented with additional Genotype 2a samples.]

Genotype 2a Discrepant – A significant number of discrepant were typed by the older RFLP assay as 2a. Sequencing of many of these samples was done either in-house in the Core region or at Abbott Diagnostics in the NS5 region to determine the most correct genotype result. The sequencing data supported the new method result, which was usually 1a, 4 or 6 genotype. Based on these studies, we determined that a significant number of samples previously typed to be 2a were actually incorrectly typed by the 5' UTR RFLP method. This compromised about 3-4% of all samples submitted to the laboratory.

Genotype 2a Retyping – To correct the identified error in the 5' UTR RFLP method, we have retested all Genotype 2a samples received in the laboratory between 1 June 2003 and 1 June 2004 for which sufficient volume was available. Samples resulted as 2a since 1 June 2004 have been tested with multiple methods so their results have been confirmed with the new method prior to being resulted. A summary of the retesting results are –

**Comparison of Results – 2a Samples
5' UTR RFPL vs Real Time PCR**

		RFLP
		2a
Abbott Real Time	1a	66
	1b	13
	2a	24
	2b	11
	3	
	4	11
	5	1
	6	3
Total		129

Each physician or originating laboratory location should receive a letter containing retesting information specific for each patient whose virus was resulted as Genotype 2a. If you do not receive information for your patient(s), please contact us at 206-667-6999. If desired, we will retest at no charge a new sample from any patient for whom insufficient stored sample was available, or for a patient tested previous to June 1, 2003. Please arrange to have the sample redrawn and then contact us to make the arrangements for the testing to be done.

For any questions concerning this information, please contact either Keith Jerome, M.D., Ph.D. (206-667-6793, kjerome@fhcrc.org) or Linda Cook, Ph.D. (206-667-2378, lcook@fhcrc.org).