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A Newly Modified HCV Replicon Assay with an Expanded Capability to Evaluate the Susceptibility of Genotype 1a and 1b Patient Viruses to Polymerase Inhibitors

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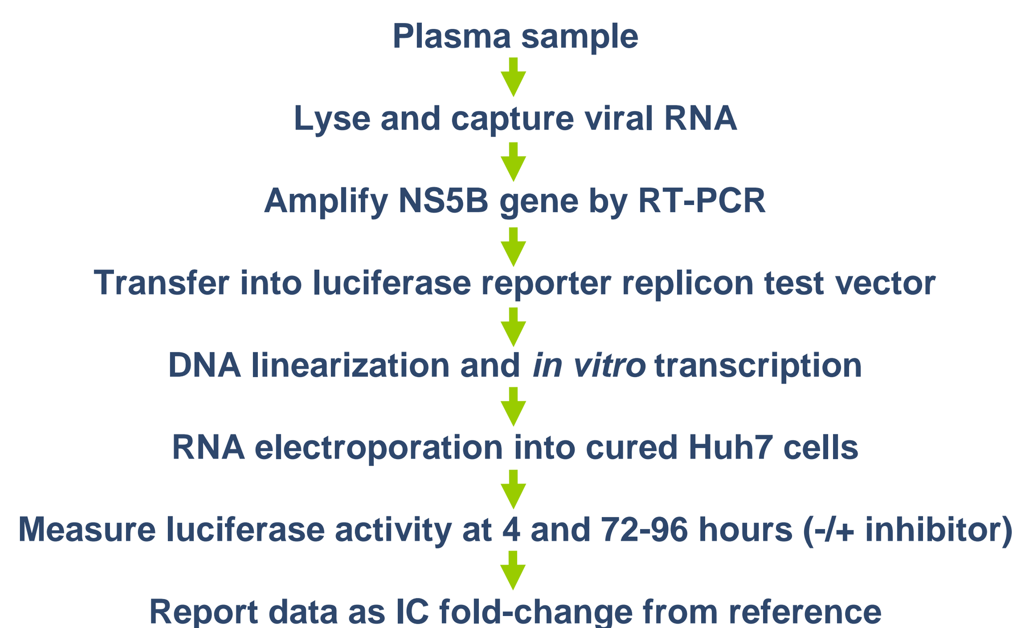
BACKGROUND and AIMS

• Numerous direct-acting antiviral (DAA) agents that target HCV polymerase are in various stages of clinical development. To evaluate DAA resistance, we developed a cell-based phenotypic susceptibility assay for HCV polymerase inhibitors based upon the analysis of resistance test vectors (RTVs) containing NS5B regions derived from genotype 1a (GT1a) and genotype 1b (GT1b) patient viruses (PhenoSense® HCV NS5B Assay). Using a prototypic assay, phenotypic susceptibility was measurable in only 55/94 (59%) patient samples. Consequently, we explored and implemented assay modifications to increase the proportion of patient samples amenable to phenotypic analysis.

METHODS

• The entire NS5B region of GT1a or GT1b HCV is amplified from patient plasma by RT-PCR using subtype-specific primers. Amplification products are cloned into a HCV replicon containing a luciferase-reporter gene. Replicon RNA is introduced into Huh7 cells by electroporation. Replication is determined in the presence and absence of polymerase inhibitors by measuring luminescence (relative light units (RLU)). IC_{50} fold-changes (IC_{50}-FC) from a reference replicon (Con1) are reported.



Sample ID	IC50 Fold-Change from Reference (Con1)				
	Interferon	Ribavirin	Inhibitor A	Inhibitor B	Inhibitor C
10_XXXX	1.2	0.9	1.3	9.5	32.4

• Assay modifications were selected based on individual and combined contributions to assay performance. The technical performance of the enhanced assay was characterized utilizing reference replicons, replicons containing site-directed mutations (SDMs) that confer resistance to polymerase inhibitors and patient samples.

Figure 1: Assay Modifications can Increase Signal and Dynamic Range

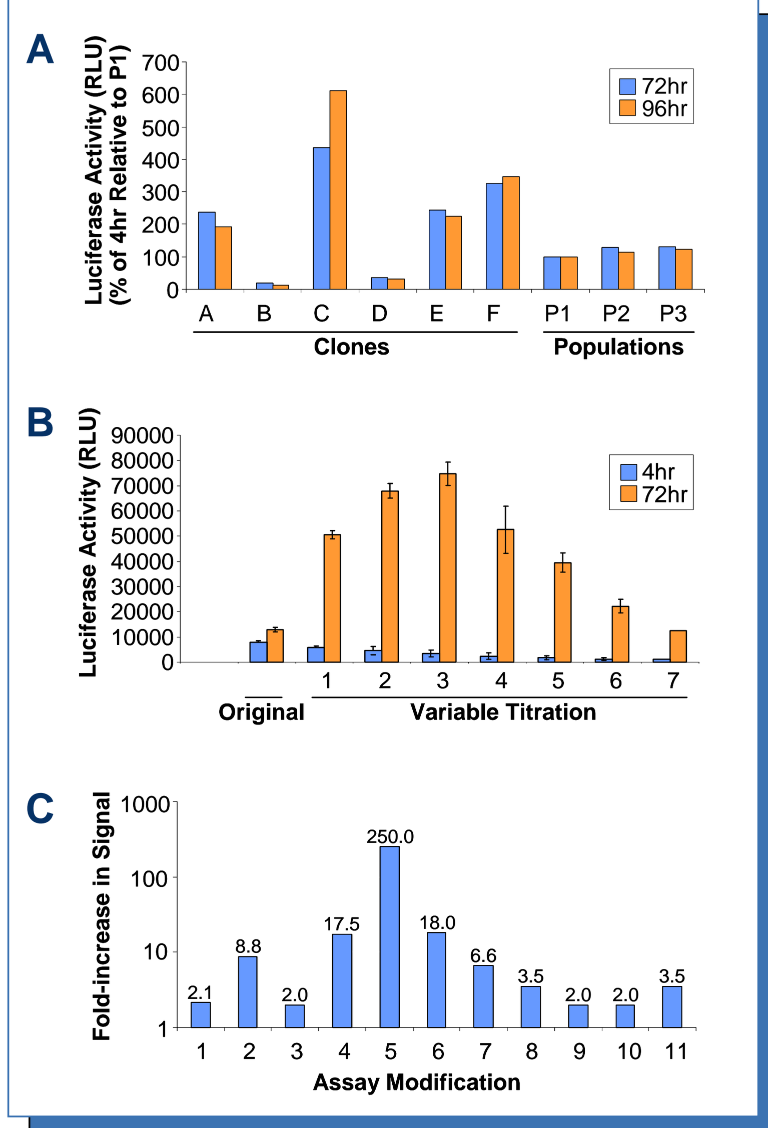


Figure 2: Matrix Optimization of Assay Modifications

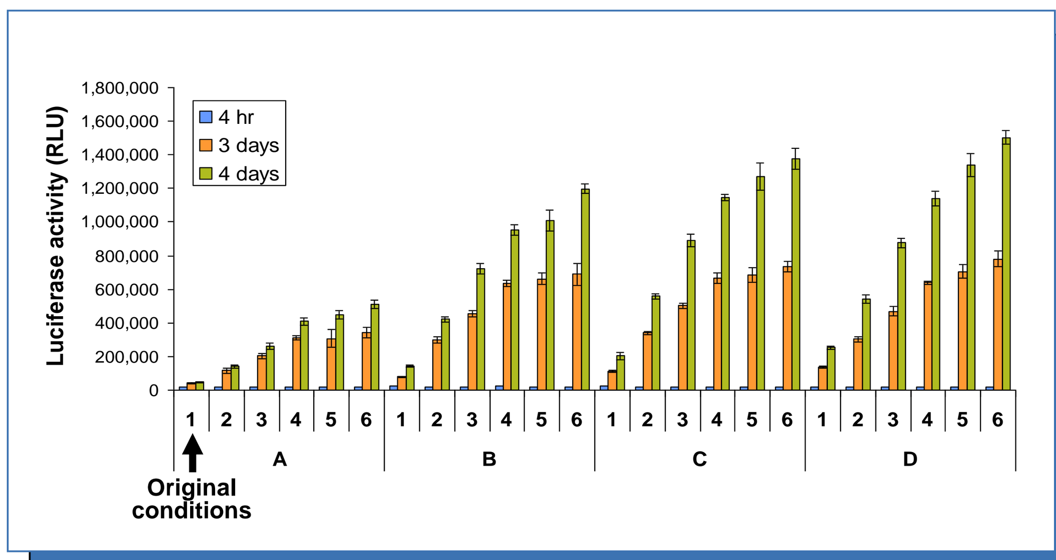


Figure 3: Replicon Activity with Original and Enhanced Assay Conditions

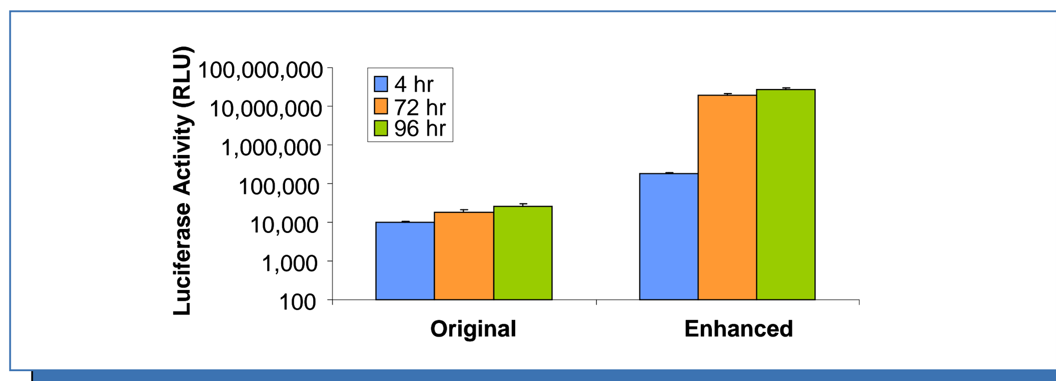


Figure 4: Evaluation of Assay Accuracy Using Replicons with mutations in NS5B

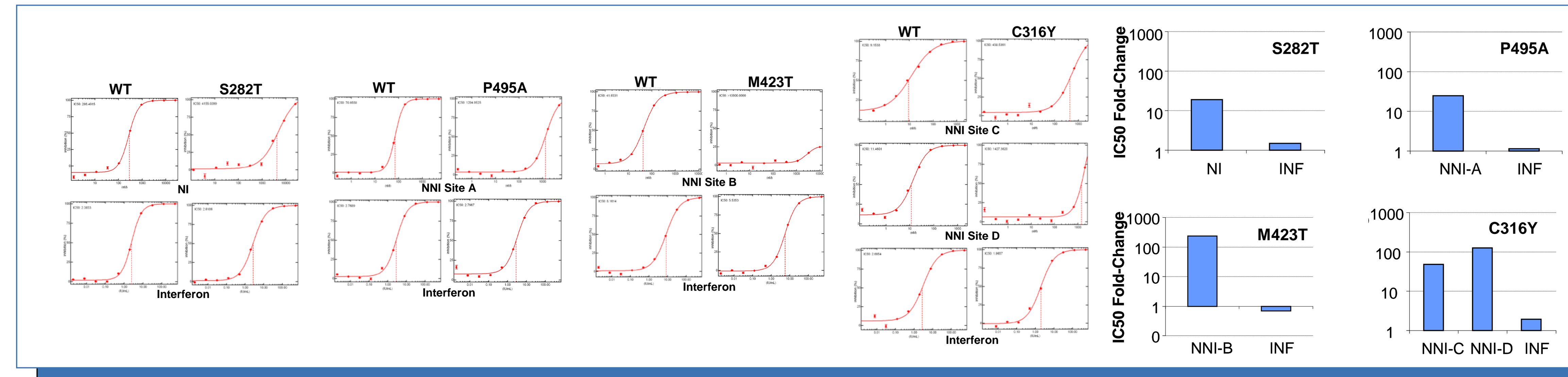
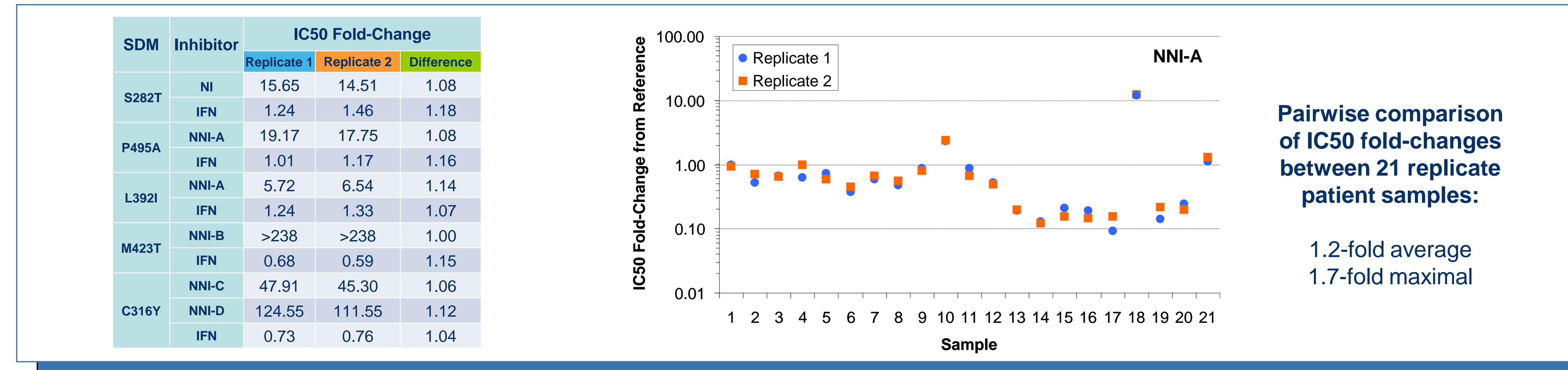


Figure 5: Evaluation of Intra-assay Variation with NS5B Mutants and Patient Samples



SDM	Inhibitor	IC50 Fold-Change		
		Replicate 1	Replicate 2	Difference
S282T	NI	15.65	14.51	1.08
	IFN	1.24	1.46	1.18
P495A	NNI-A	19.17	17.75	1.08
	IFN	1.01	1.17	1.16
L392I	NNI-A	5.72	6.54	1.14
	IFN	1.24	1.33	1.07
M423T	NNI-B	>238	>238	1.00
	IFN	0.68	0.59	1.15
C316Y	NNI-C	47.91	45.30	1.06
	NNI-D	124.55	111.55	1.12
	IFN	0.73	0.76	1.04

Figure 6: Assay Reproducibility with Patient Samples

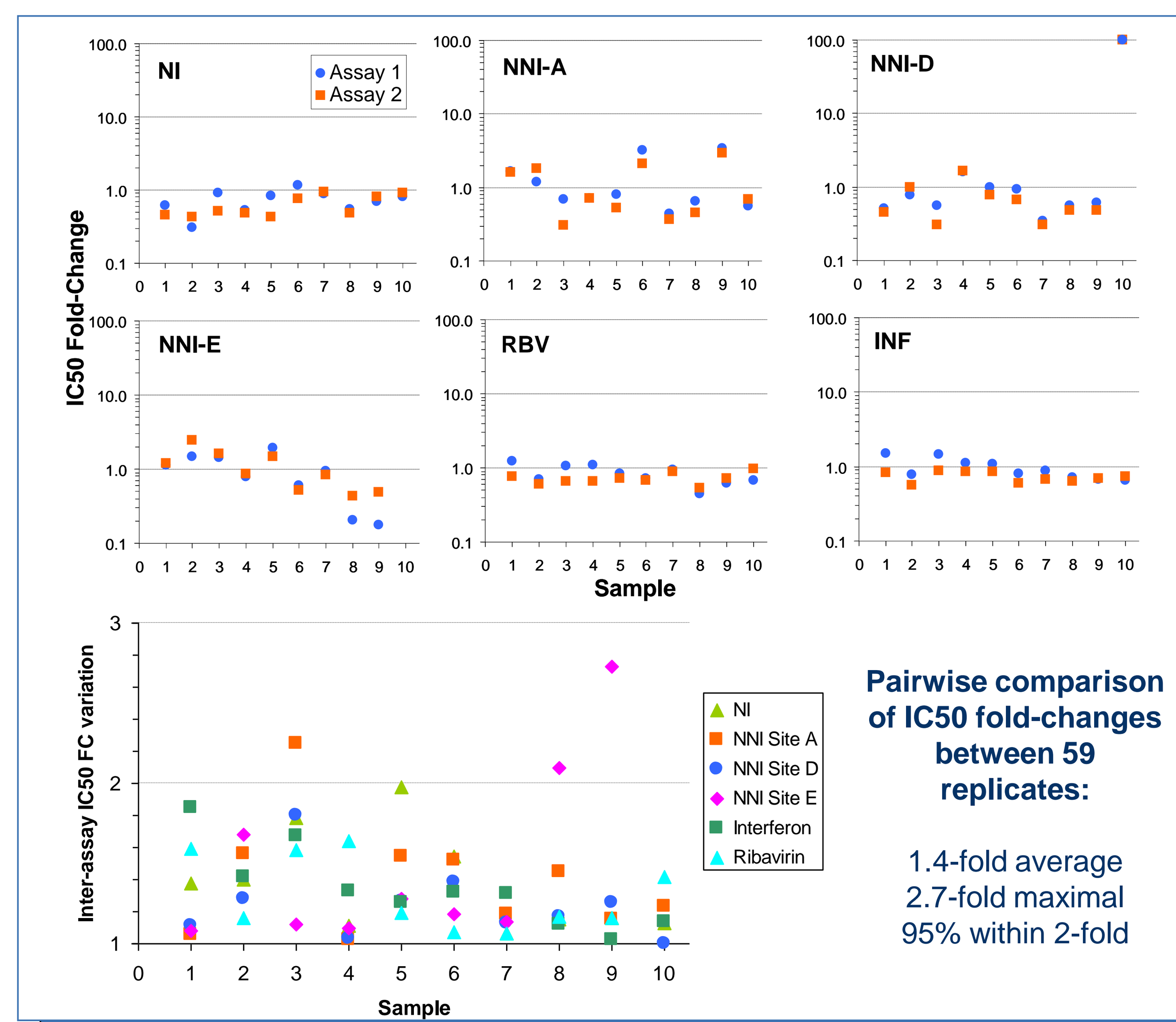
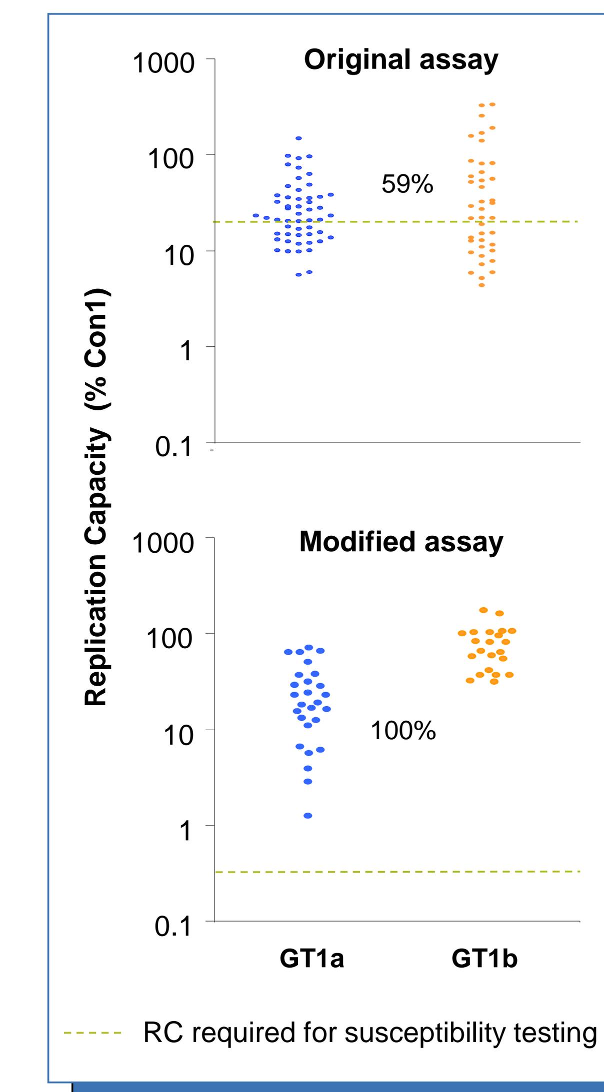


Figure 7: Patient Sample Replication Capacity



RESULTS

• Figure 1. A; Evaluation of replication in Huh7 cell clones compared to 3 independently maintained cell populations. B; Optimization of cell culture conditions. C; Increased assay signal from individual parameter optimizations. Individual assay modifications (RTV, electroporation, cells and cell culture conditions) were identified that variably increased the assay signal and dynamic range.

• Figure 2 & 3. Matrix evaluations of assay reagents and parameters enabled the identification of optimal conditions that significantly enhanced RTV replication and improved assay dynamic range.

• Figure 4. Assay accuracy was demonstrated using RTVs containing NS5B mutations that confer reduced susceptibility to nucleoside (NI; S282T) and non-nucleoside polymerase inhibitors that target site A (NNI-A; P495A), site B (NNI-B; M423T), site C and D (NNI-C, NNI-D; C316Y).

• Figure 5. Intra-assay variations in inhibitor susceptibility measurements (IC_{50}-FC) were defined at <2-fold using a panel of SDMs and RTVs containing NS5B sequences from GT1a and GT1b patient viruses.

• Figure 6. Inter-assay reproducibility of inhibitor susceptibility measurements (IC_{50}-FC) were defined at <3-fold using a panel of RTVs containing NS5B sequences from GT1a and GT1b patient viruses.

• Figure 7. Using modified assay conditions, the replicons derived from a panel of 49 GT1a and GT1b patient samples all replicated sufficiently to enable phenotypic assessment of polymerase inhibitor susceptibility.

SUMMARY & CONCLUSIONS

• A PhenoSense HCV NS5B assay has been developed to support clinical studies of investigational inhibitors that target HCV polymerase.

• This assay can facilitate phenotypic drug resistance analysis of HCV subtype 1a/b patient virus NS5B populations, clones derived from patient virus populations and SDMs.

• Optimal assay parameters were identified that significantly increased the proportion of patient samples amenable to phenotypic analysis compared to a prototypic assay.