



# HCV Genotyping in Clinical Trials

## Perspectives from a Central Laboratory

Dwight DuBois, MD

Cenetron Central Laboratories

Amsterdam      April 23, 2013



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## Background

Performed HCV genotyping tests on > 20,000 samples with the INNO-LiPA HCV genotyping assays (Versions 1 and 2), since 2001.

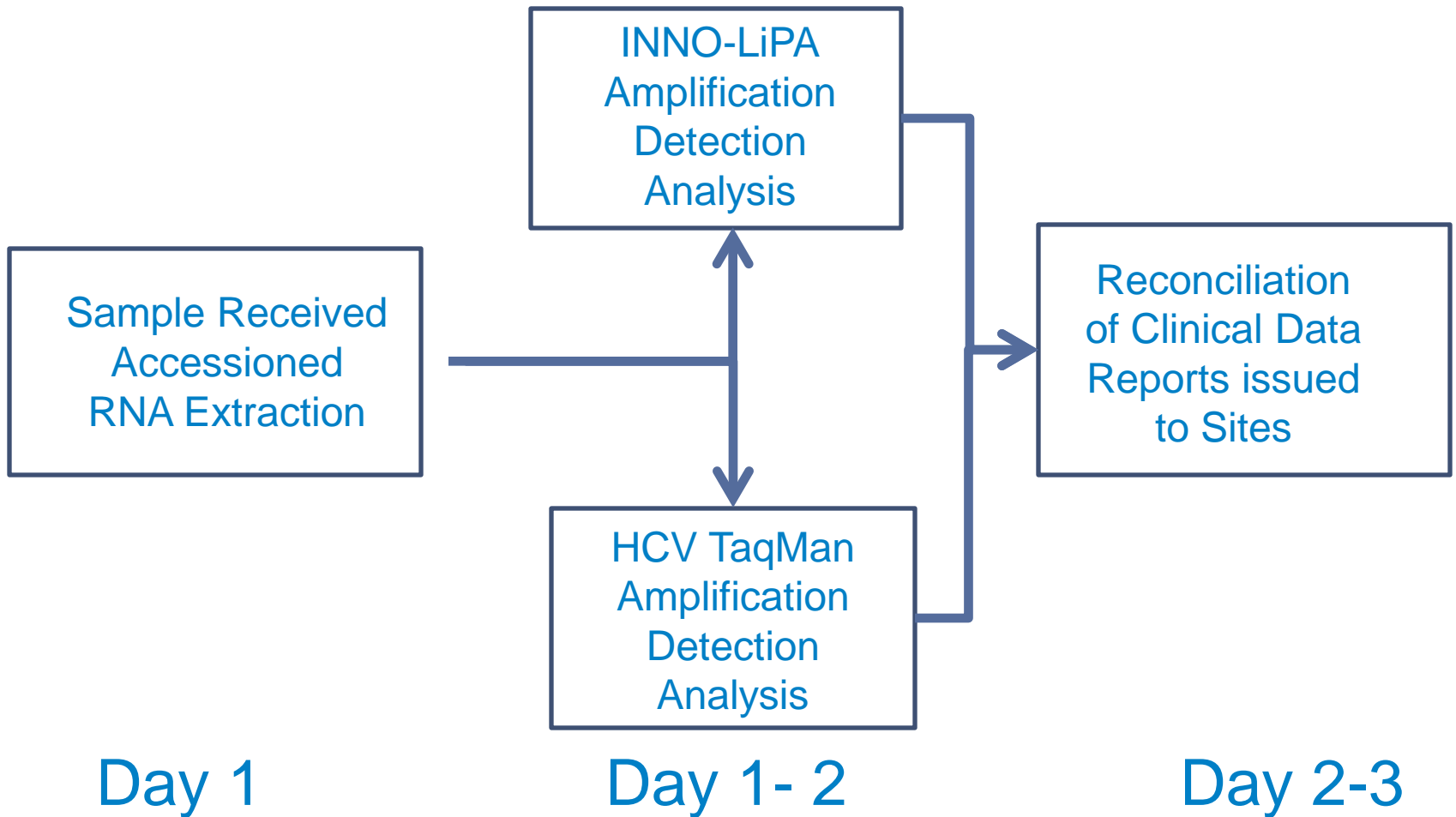
Since 2006, INNO-LiPA HCV Genotyping Assay, Version 2.0, has been in use exclusively for Screening

Data is pooled from 20+ studies (11,077 samples)



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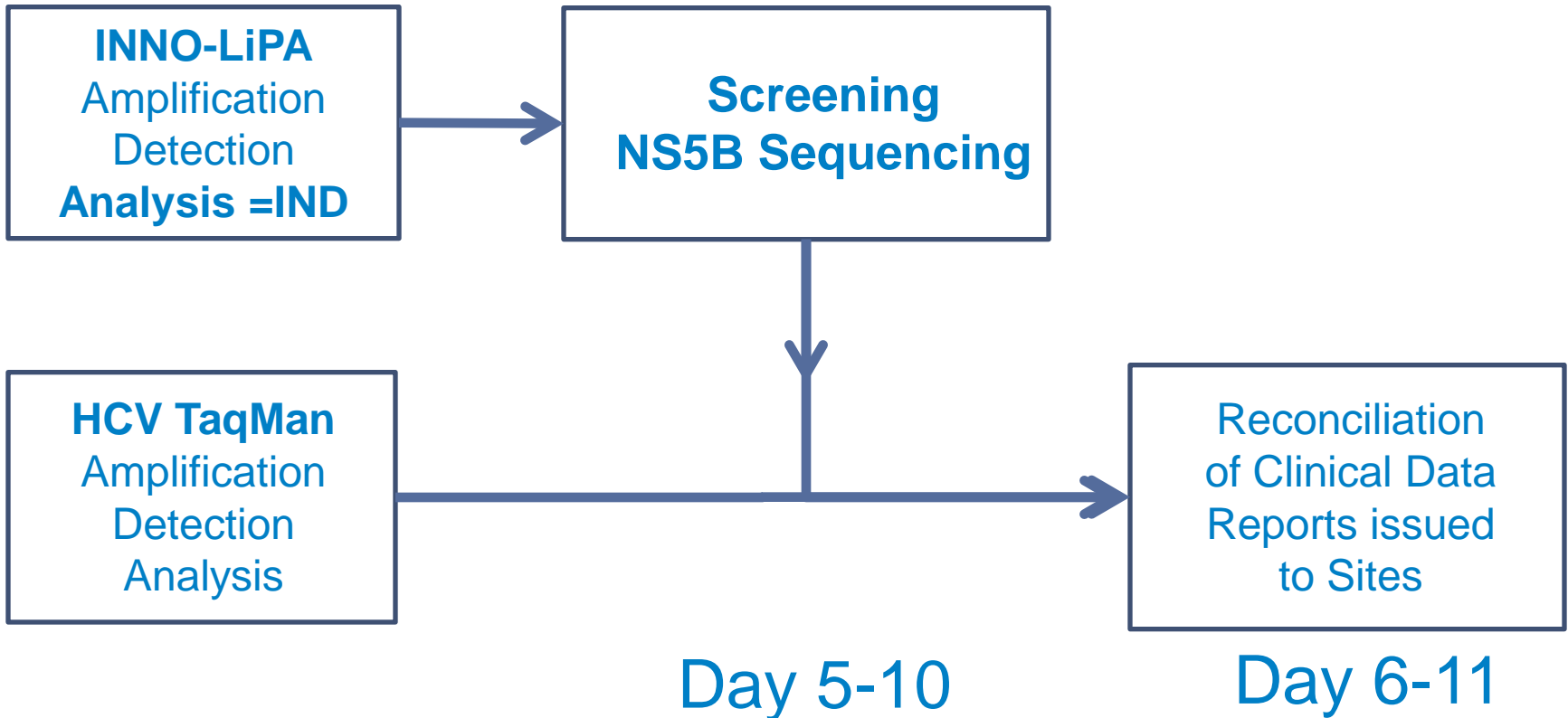
# Typical Workflow for Molecular Testing





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# Workflow when Genotypes are IND by INNO-LiPA





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## Genotype Reports

Genotype and Sub-type: (*" Genotype 1A"*)

Genotype Only: (*" Genotype 1, unable to subtype"*)

Indeterminate Results: (*" Indeterminate"*)



## Screening and Longitudinal Reports

**CENETRON CENTRAL LABORATORIES** 2111 West Braker Lane Suite 300  
Austin, Texas USA 78758  
Phone: USA/Canada (888) 834-6632  
International: (512) 439-2000  
Fax: (512) 439-5000

**LABORATORY REPORT**

<b>Subject</b>	INITIALS: <b>TST</b> GENDER: <b>Male</b> DATE OF BIRTH: <b>01-Jan-1980</b> SCREEN No: <b>01</b>	Site: <b>000</b> INVESTIGATOR: <b>Dr. Paige Turner</b>	VISIT: <b>Screening</b> SPONSOR: PROTOCOL:	ACCESSION No: CENETRON STUDY:
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**SPECIMEN: 12132316-01**

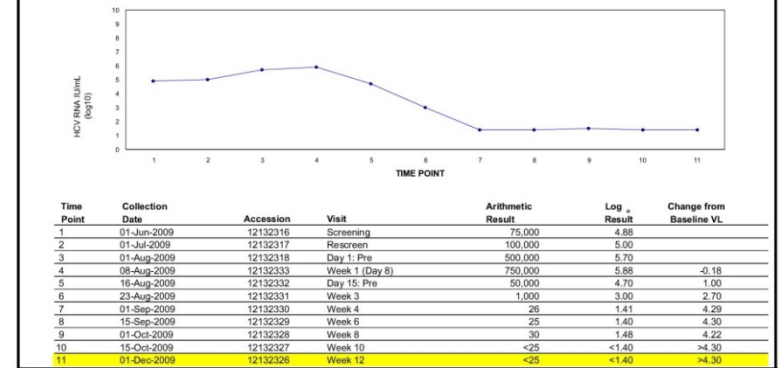
SPECIMEN TYPE: <b>Serum</b>	DATE/TIME COLLECTED: <b>01-Jun-2009 12:00</b>	DATE RECEIVED: <b>06-Jun-2012</b>	DATE REPORTED: <b>06-Jun-2012</b>
TEST NAME	RESULT	FLAG	EXPECTED RESULT UNITS
<b>Genotype</b>	<b>TYPE 1A</b>		
<b>COBAS® TaqMan® HCV Test v2.0 - High Pure System</b>	<b>75,000</b>		<b>IU/mL</b>
	(log <sub>10</sub> = 4.88)		

**SPECIMEN: 12132326-01**

SPECIMEN TYPE: <b>Serum</b>	DATE/TIME COLLECTED: <b>01-Dec-2009 12:00</b>	DATE RECEIVED: <b>06-Jun-2012</b>	DATE REPORTED: <b>06-Jun-2012</b>
TEST NAME	RESULT	FLAG	EXPECTED RESULT UNITS
<b>Comment: Patient does not exhibit signs of virological breakthrough.</b>			
<b>COBAS® TaqMan® HCV Test v2.0 - High Pure System</b>	<b>&lt;25</b>		<b>IU/mL</b>
	(log <sub>10</sub> = <1.40)		

**SPECIMEN: 12132316-34**

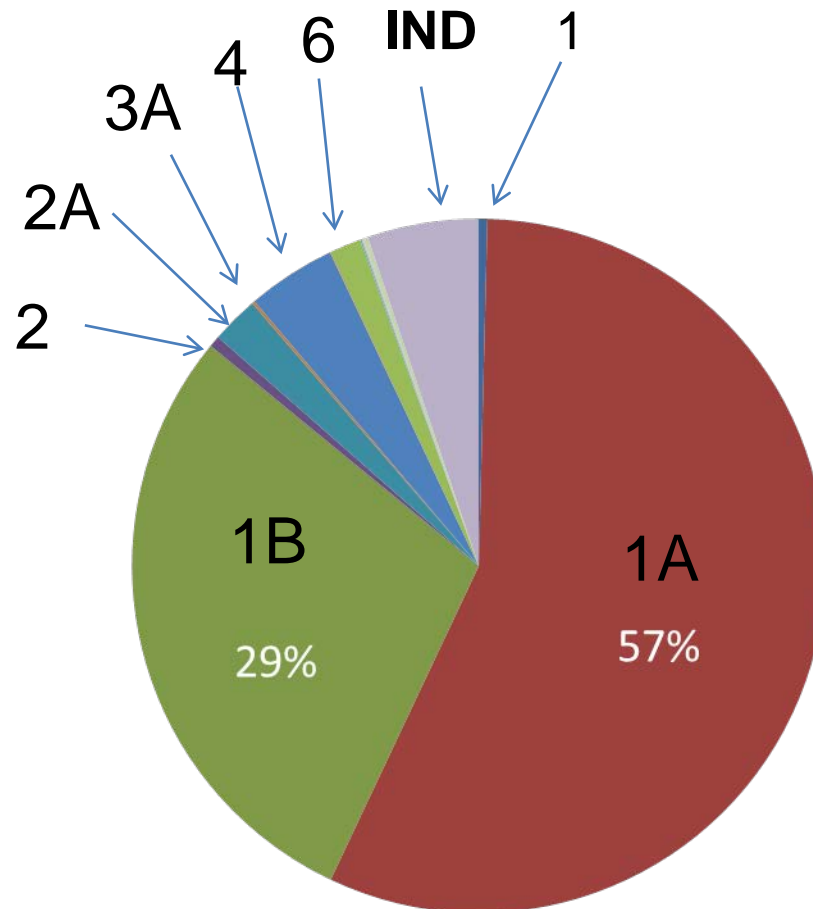
SPECIMEN TYPE: <b>Whole Blood</b>	DATE/TIME COLLECTED: <b>01-Jun-2009 12:00</b>	DATE RECEIVED: <b>06-Jun-2012</b>	DATE REPORTED: <b>06-Jun-2012</b>
TEST NAME	RESULT	FLAG	EXPECTED RESULT UNITS
<b>IL28b (rs12979860) Genotype</b>	<b>CC</b>		



Investigator's Initials \_\_\_\_\_ Date \_\_\_\_\_

Investigator's Initials \_\_\_\_\_ Date \_\_\_\_\_

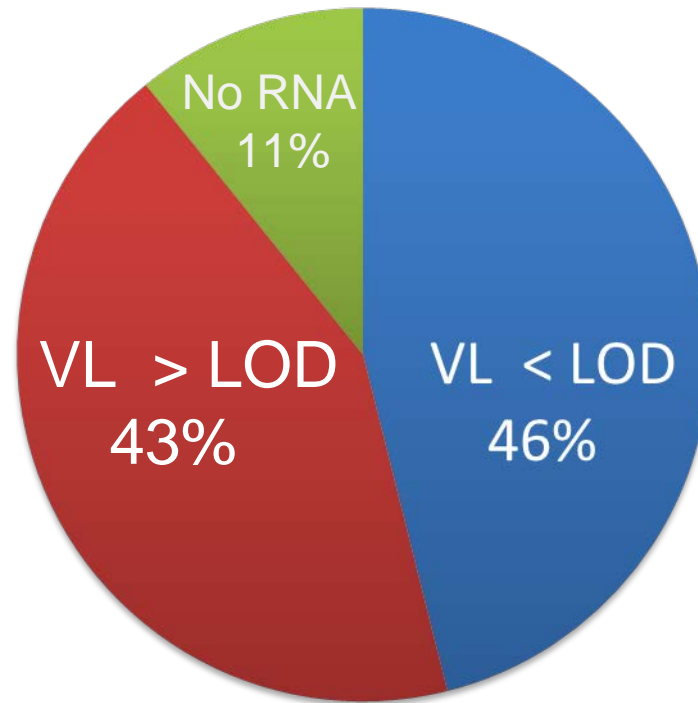
Total Patients Screened = 11,077





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## Viral Load Values in Indeterminate Group



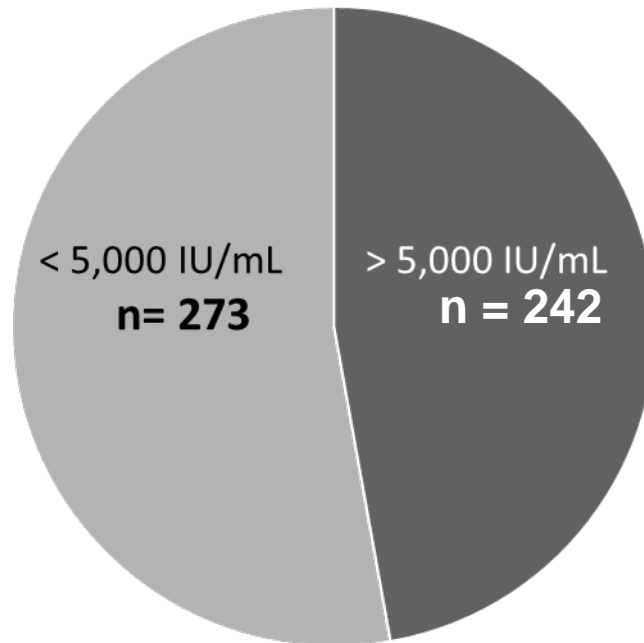
Total Indeterminate n=575 (5.2%)





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## Total Indeterminates with VL > LOD (n=515)



166 Patients  
Sequenced  
NS5B

Total Indeterminates n=575 (5.2%)



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## Causes for Indeterminate Results

- Low viral loads (no or very low signal)
- Genotype 1 samples with low signal in the Core region hybridization (appear to be GT1, but cannot rule out GT6) (90%)
- INNO-LiPA banding patterns does not fit into band pattern interpretation algorithm (rare genotypes) (10%)



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# Concordance between INNO-LiPA Testing and NS5B Sequencing

Pooled data from 9 clinical trials (1882 patients screened)

1806 Paired Results were analyzed

Analysis included only specimens with a reported genotype or sub-genotype with the INNO-LiPA assay *AND* with a sub-genotype determined by sequencing.

Sequencing performed *after* enrollment at outside specialty laboratory (not part of screening protocol)



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# Concordance between INNO-LiPA Testing and NS5B Sequencing

## Definitions

*Major Discordance:* Discordance at the genotype level, that could result in enrollment or randomization errors

*Minor Discordance:* Discordance at the subtype level



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# Concordance between INNO-LiPA Testing and NS5B Sequencing

## Genotype 1 Results

INNO-LiPA	No. of Samples	Major Discordance	Minor Discordance	Comments
1	14	0	N/A	
1A	1165	0	2	1B by sequencing
1B	617	0	2	1A by sequencing
Total	1796	0	4	



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# Concordance between INNO-LiPA Testing and NS5B Sequencing

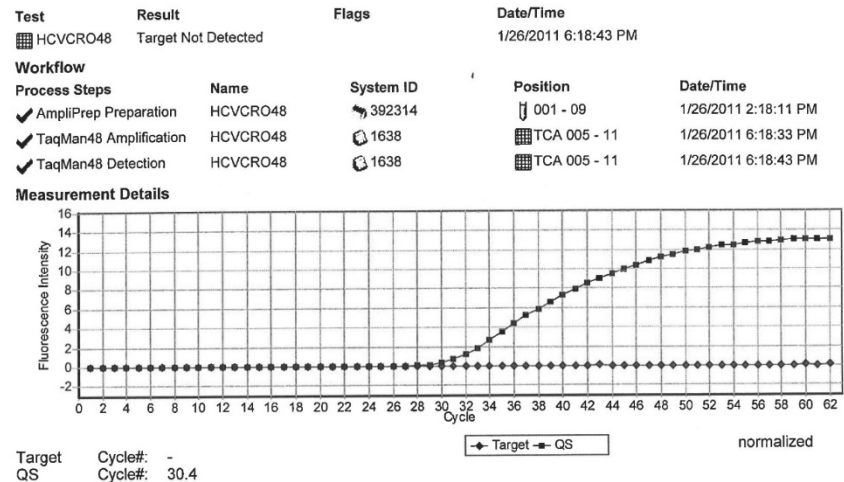
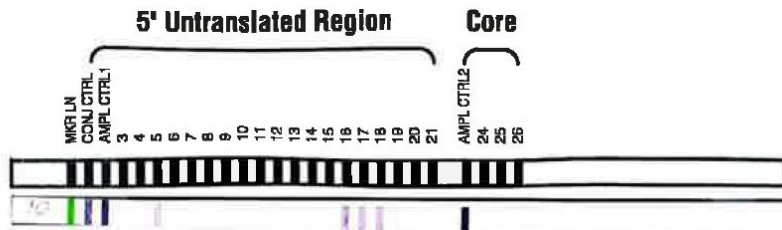
## Genotype 4 Results

INNO-LiPA	No. of Samples	Major Discordance	Minor Discordance	Comments
4	58	0	N/A	
4H	2	0	2	4A by sequencing
4F	1	0	0	4A by sequencing
Total	61	0	2	

## Interesting Cases

HCV genotype at Screening was a clear Genotype 4, but the VL was “No HCV RNA” detected by Roche HCV TaqMan Assay, version 1.0.

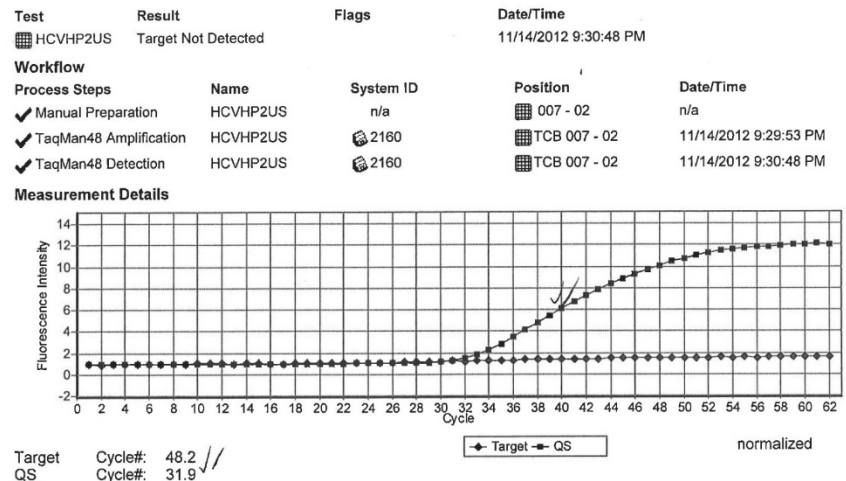
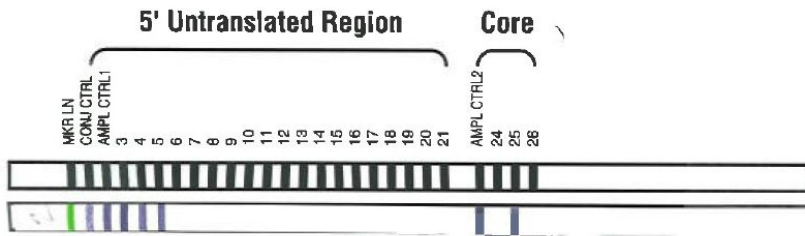
Sequencing of TaqMan primer/probe binding regions revealed unusual polymorphisms. Testing with Version 2 of the same assay: Viral load 450,000 HCV IU/mL.



## Interesting Cases

HCV genotype at Screening was a clear Genotype 1A, but the VL was “Target Not Detected” by Roche HCV TaqMan Assay, for use with the High Pure System.

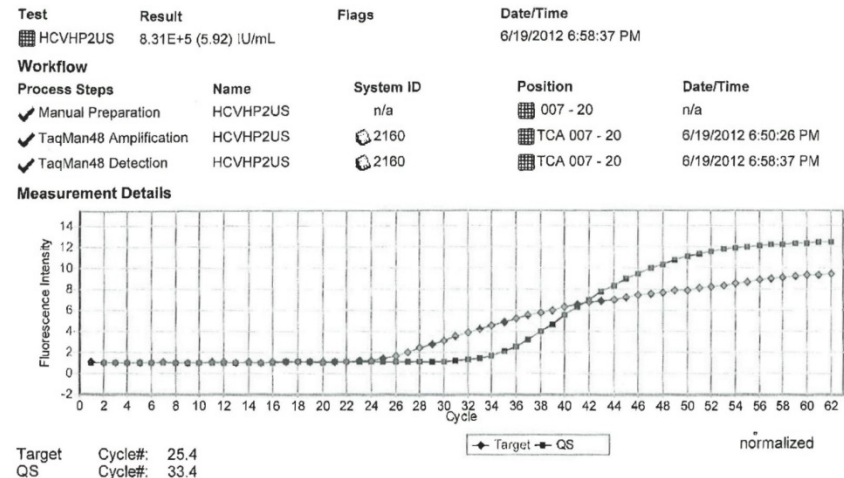
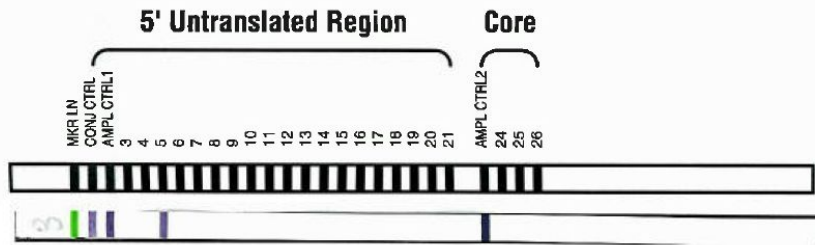
Sequencing of TaqMan primer/probe binding regions revealed 2 mismatches in the probe binding region.





## Interesting Cases

HCV genotype at Screening “Indeterminate”, and the VL was 831,000 IU/mL by Roche HCV TaqMan Assay, for use with the High Pure System. #####





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## Interesting Cases

HCV genotype at Screening was “Indeterminate.” Banding pattern was suggestive of a 1A/1B mixture. NS5B sequencing at 2 different laboratories showed 1A and 1B, respectively.



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## Discordance with Historical Genotype Results and Results for Screening

HCV Genotype results recorded in patients' medical charts are frequently discordant with results in clinical trials.

Invariably, the INNO-LiPA and NS5B sequencing results in the clinical trial agree with each other. Historical GT4 and GT6 are frequently determined to be GT1.

Sequencing is generally performed on **Baseline** samples, and the INNO-LiPA assay is performed on **Screening** samples. Apparent discordance can be a result of chain-of-custody errors.



## Acknowledgements

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Roche

Leen-Jan van Doorn

DDL

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Gilead

Brandon Harper and Yanqun Cai

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## **Slide Set**

On line at [www.cenetron.com](http://www.cenetron.com)

**Questions:**

[ddubois@cenetron.com](mailto:ddubois@cenetron.com)