School of Public Health
UNIVERSITY OF CALIFORNIA, BERKELEY

Forum for Collaborative HIV Research

LOD/LLOQ Discussion
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Roche

Definition of Terms

- **Target not detected (TND):**
  - No PCR amplification
  - Equivalent to **0 IU/ml** HCV RNA
  - Validated via Analytical Specificity studies

- **Limit of Detection (LOD)**
  - Lowest amount of analyte that can be detected 95% of the time as a positive PCR signal
  - Varies with matrix and genotype
  - **Not** reported on validated test software

- **Lower limit of Quantification (LLOQ):**
  - Lowest amount of analyte to be both linear and accurate
  - Validated in linearity and total analytical error studies

Commercially Available HCV RNA PCR Tests

LOD and LLOQ assay performance

Assay	Linear Range	LLOQ	Overall LOD[1]
COBAS TaqMan HCV Test, v2.0 for use with the High Pure System	25 – 300,000,000 IU/mL	25 IU/mL	20 IU/mL
COBAS AmpliPrep/COBAS TaqMan HCV Test Version 1	43 – 69,000,000 IU/mL	43 IU/mL	CE-IVD 15 IU/mL US-IVD 18 IU/mL
VERSANT® HCV RNA 3.0 Assay (bDNA)[2]	615 - 7,690,000 IU/mL	615 IU/mL	1,000 IU/mL
Abbott RealTi*me* HCV Test	12 IU – 8.00 Log_{10} IU/mL	12 IU/mL	12 IU/mL
COBAS AmpliPrep/COBAS TaqMan *HCV Test Version 2* ***In Development***	*Targeted Range: 15 - 8.00 Log_{10} IU/mL*	*LOD=LLOQ*	

[1] Performance for LOD is based on overall genotype data

[2] Assay LOD at 1,000 IU/mL (95% detection rate), LLOQ and range determined per package insert, 615 IU/mL is <95% detection rate

High Pure TaqMan HCV v2 Test Results

Validated software read out and lab report guidance

Titer Result	Results Interpretation
Target not Detected	Report results as "**HCV RNA not detected**"
< 25 IU/ml	Calculated IU/ml is below the lower limit of quantification (LLOQ) of the assay. Report results as "**HCV RNA detected, <25IU/ml, HCV RNA is not quantifiable**"
≥25IU/ml and ≤ 3.0E+08 IU/ml	A **quantifiable number** in the linear range of the assay
> 3.0E+08 IU/ml	Results are above the range of the assay. Report results as **"> 3.0E+08 IU/ml HCV RNA**"

* FDA approved COBAS® TaqMan® HCV Test, v2.0 for use with the High Pure System, package insert

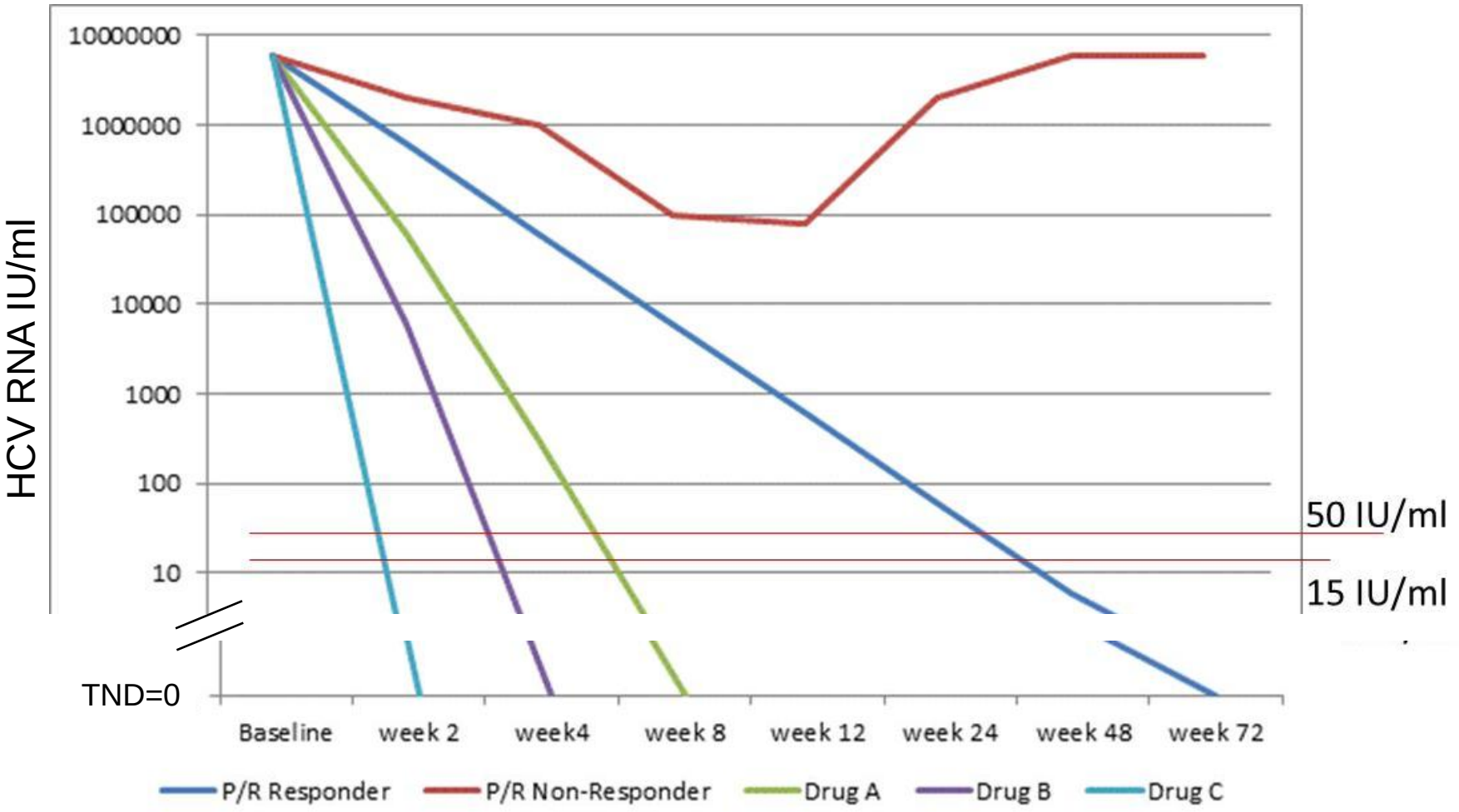
Assay used during clinical studies for boceprevir and telaprevir. Gained FDA approval and global commercialization

Conclusion and Recommendation

- Lab should report out only validated software prompts
  - No combination of TND and <LLOQ with fusion of terms
- LOD not suitable as a clinical parameter as it varies with country/regional approval, matrix and genotypes
- Clinical Trial analysis:
  - **TND** and **fixed cut-off** within linear range (i.e. 25 or 50 IU/ml FDA call June 30th 2011) of several assays most suitable for SVR predictions

Viral Load Decline on Therapy

What does this all mean for drug development and clinical practice?



Backup

High Pure Taqman HCV v2 Test Results

The FDA approved LOD in the US-IVD Package Insert

plasma

Genotype	Geometric Mean of Observed Results at the Lowest Concentration with > 95% Positivity Rate	Number of Replicates Tested	Number of Positive Results	Positivity Rate
1	15.1 IU/mL	66	66	100%
2	5.6 IU/mL	137	131	96%
3	15.3 IU/mL	63	63	100%
4	12.0 IU/mL	63	63	100%
5	3.7 IU/mL	63	60	95%
6	20.4 IU/mL	66	65	98%

serum

Genotype	Geometric Mean of Observed Results at the Lowest Concentration with > 95% Positivity Rate	Number of Replicates Tested	Number of Positive Results	Positivity Rate
1	2.1 IU/mL	66	63	95%
2	10.9 IU/mL	126	124	98%
3	2.5 IU/mL	68	65	96%
4	7.5 IU/mL	71	71	100%
5	12.1 IU/mL	66	65	98%
6	7.9 IU/mL	64	62	97%

LOD differs

- depending on *sample* used (*plasma, serum*)
- between *genotypes 1-6*

Limit of Detection was assigned using highest titer with >95% positivity rate and the most conservative genotype: 20 IU/ml

COBAS® TaqMan® HCV, v2.0 Test for Use with the High Pure System, FDA approved Package Insert

Why LOD is not a General Usable Term
CE vs. US Registration Data Differences

Genotype 1	CE Registration Data	US Registration Data
Plasma	9.3 IU/ml	15.1 IU/ml
Serum	8.8 IU/ml	2.1 IU/ml
	N>148 per matrix and concentration	N>63 per matrix and concentration