How different are quantitative HCV RNA assays?

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Disclosures

Consultancies / Advisory boards:
Abbott, BMS, Boehringer-Ingelheim, Gilead, Janssen, Merck/MSD, Novartis, Roche, Rottapharm, Vertex

Research support:
Abbott, Gilead, Janssen, Merck/MSD, Roche, Siemens

Speaker:
Abbott, Astra, BMS, Boehringer-Ingelheim, Gilead, InterMune, Janssen, Merck/MSD, Novartis, Qiagen, Roche, Siemens
Detection of HCV RNA

One target – different methods

Highly conserved area with little variation between different HCV genotypes, subtypes and isolates

5'NTR

Qualitative / quantitative detection of HCV RNA by different methods:

- real-time RT-PCR
- TMA
- bDNA
- conventional RT-PCR
- bDNA
- TaqMan probe
Commercially available HCV RNA assays

- **HCV RT artus™**
  - Qiagen (CE)

- **TMA**
  - Siemens (CE/FDA for TMA/bDNA CE for kPCR)

- **bDNA Versant™**
  - Siemens (CE/FDA)

- **kPCR**
  - Roche Diagnostics (CE/FDA)

- **Qual. Amplicor™ Real-time vs2 TaqMan™ RealTime HCV™**
  - Abbott (CE/FDA)
Characteristics of HCV RNA assays

High sensitivity

The lower detection limit of an HCV RNA assay is a statistical value which depends on the matrix and the HCV genotype.

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<th>HCV RNA IU/ml</th>
<th>Detected (%)</th>
<th>LOD 15 IU/ml</th>
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95% hit probability

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The lower detection limit of an HCV RNA assay is a statistical value which depends on the matrix and the HCV genotype.

### Table 2: LOD confirmation by genotype using the CAP/CTM HCV test, v2.0, in plasma and serum matrices

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Zitzer et al., J Clin Microbiol 2013
Characteristics of HCV RNA assays

Linear amplification

Vermehren et al., J Clin Virol 2011
Characteristics of HCV RNA assays
Standardization to International Units (IU)

Differences for absolute quantification

Cobas TaqMan Roche vs bDNA Siemens
Sarrazin et al., J Clin Microbiol 2006

Cobas TaqMan Roche vs bDNA Siemens
Vermehren et al., J Clin Microbiol 2008

Cobas TaqMan Roche vs bDNA Siemens
Chevaliez et al., Hepatology 2007

Cobas TaqMan Roche vs bDNA Siemens
Sizmann et al., J Clin Virol 2007
Characteristics of HCV RNA assays
Clinically relevant differences between cutoffs and assays?

Baseline viral load
- Predictor of response (dual and triple therapies)
- Shortening treatment 16/24 weeks in GT2/3 & GT1 together with rapid virologic response (RVR) with dual combination therapy

Determination of rapid virologic response for shortening of treatment duration
- Importance for conventional PEG-based triple therapies
- Very early virologic response potentially used to tailor future DAA combination treatments?
Characteristics of HCV RNA assays

Baseline viral load

Classification of low versus high baseline viral load
(< versus ≥ 800 000 IU/ml, n=189 HCV GT1 infected patients)

Discrepancy (%)

Weich et al., EASL 2011
Determination of treatment duration
PI-based Conventional Triple Therapies

**Telaprevir**
- HCV-RNA undetectable
- 12W-TV R
- BL week 4
- week 12
- week 24

**Boceprevir**
- HCV-RNA undetectable
- 4W-LI
- 24W-BOC
- BL week 4
- week 8
- week 24
- week 28

### Study Time Duration SVR

**Boceprevir SPRINT-1**
- <25 IU/ml wk. 8
- 28 wks.
- 5/13 (38%)
- 48 wks.
- 9/12 (75%)
- negative wk. 8
- 28 wks.
- 53/62 (85%)
- 48 wks.
- 62/66 (94%)

Harrington et al., Hepatology 2011

- Undetectable HCV RNA at week 4 of triple therapy is required for shortening treatment duration to 24 weeks

**Simeprevir**
- HCV-RNA undetectable
- 12W-TV R
- BL week 4
- week 12
- week 24

**Faldaprevir**
- HCV-RNA undetectable
- 12W-TV R
- BL week 4
- week 12
- week 24
Determination of treatment duration
Difference between undetectable and <25 IU/ml

**Telaprevir**
*Undetectable at week 4 of triple therapy*

**Boceprevir**

**Simeprevir**
*<25 IU/ml at week 4 of triple therapy*

**Faldaprevir**

-Jacobson et al.; Sherman et al.; Poordad et al.; all NEJM 2011
-Manns et al.; Jacobson et al.; Ferenci et al.; all EASL 2013
Determination of treatment duration
Difference between undetectable and <25 IU/ml

- 73-75% on SMV / FDV triple therapy had undetectable HCV RNA at week 4
- 13-15% on SMV / FDV triple therapy had detectable HCV RNA <25 IU/ml at week 4
- SVR rates after 24 weeks of treatment are significantly higher in patients with undetectable versus <25 IU/ml HCV RNA at week 4 (93% vs. 69-75%)
Determination of treatment duration
Differences between HCV RNA assays

HCV RNA testing at week 4 of treatment by Roche TaqMan HPS and Abbott realtime HCV assays during HCV NS3 Protease-Inhibitor Triple Therapy with Simeprevir (Pillar Study), n=261

**Roche TaqMan (HPS)**
- Not detected: 75.5%
- <25 IU/mL detected: 16.5%
- ≥25 IU/mL: 8.0%

**Abbott RealTime (ART)**
- Not detected: 49.4%
- <12 IU/mL detected: 79.7%
- ≥12 and <25 IU/mL: 30.3%
- ≥25 IU/mL: 7.7%
- ≥25 IU/mL: 12.6%

Fevery et al. J Hepatology 56, Suppl 2, 2012, S26
Determination of treatment duration
Difference between assays: Real world triple therapy

Comparative testing with Roche TaqMan (CAP) and Abbott RealTime (ART) of week 4 and 12 samples of HCV genotype 1 patients undergoing Telaprevir triple therapy (Frankfurt and Stockholm)

Comparison of CAP and ART at week 4 and 12

Cobas TaqMan (CAP) used as primary assay for response guided therapy in FFM and Stockholm
Patients who achieved eRVR according to CAP and ART (FFM/Stockholm)

- CAP: 67% eRVR, 33% no eRVR
- ART: 63% eRVR, 37% no eRVR

Treatment outcome according to week 4 HCV RNA results (RGT according to CAP)*

- CAP not detected: 31/31 (100%) SVR, 1 BT
- ART not detected: 13/31 (42%) SVR, all SVR
- ART <12 positive: 15/31 (48%) SVR, all SVR
- ART quantifiable: 3/31 (10%) SVR, all SVR

*At week 12 HCV RNA was undetectable by CAP and ART in all patients.
**HCV RNA concentrations in 3 pts. with quantifiable viral load: 13, 13 and 15 IU/ml
Determination of treatment duration
Difference between assays: Real world triple therapy

Comparative testing with Abbott RealTime (ART) and Roche TaqMan (CAP) of week 4 and 12 samples of HCV genotype 1 patients undergoing Telaprevir triple therapy (Milan)

RealTime (ART) used as primary assay for response guided therapy in Milan
Determination of treatment duration
SOF IFN-containing and IFN-free treatment schedules

Potent DAA combination therapies
- Viral break-through is uncommon
- Very early viral kinetics for prediction of relapse / optimal treatment duration?

Osinusi et al., JAMA 2013; Jacobson et al., EASL / NEJM 2013
## Determination of treatment duration

**HCV RNA measurement during IFN-free DAA combo**

### PILOT Study

*(ABT450r+ABT072+RBV GT1 CC naive, n=11)*

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**Cloherty et al., APASL 2013**

- HPS < LOD
- ART < 25 IU/ml
- HPS undetectable
- ART < 12 IU/ml
Summary

- Real-time PCR based methods for highly sensitive detection of HCV RNA and linear quantification
- Differences between assays for absolute quantification as well as lower limit of detection
- Determination of treatment duration in PI-based Triple Therapy (TVR, BOC, SMV, FDV):
  - Cobas TaqMan Assay: undetectable HCV RNA at week 4 of triple therapy (<25 IU/ml is associated with relapse)
  - RealTime HCV Assays: <12 IU/ml detectable or undetectable at week 4 of triple therapy
  - Other assays: no data so far
- IFN-free DAA combination therapies
  - Predictability of response by very early viral kinetics?