Novel Diagnostics for HCV infection: *Where are we Now And Where are we Headed?*

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Outline

- Virological tools for screening HCV infection
- Virological tools for diagnosing ongoing HCV infection and treatment decision
- Virological tools for monitoring HCV infection

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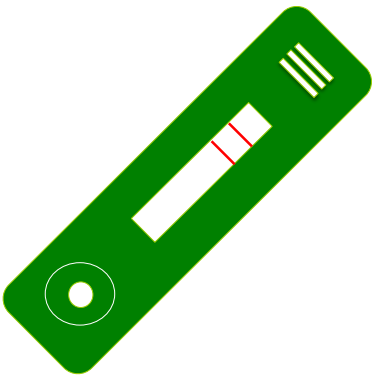
- **Virological tools for screening HCV infection**
- **Virological tools for diagnosing ongoing HCV infection and treatment decision**
- **Virological tools for monitoring HCV infection**

EASL and AASLD/IDSA Recommendations

- **Screening of HCV infection is based on the detection of anti-HCV Ab (reference method)**
- **In addition to EIAs, rapid diagnostic tests (RDT) can be used to screen for anti-HCV Ab**
- **Dried blood spots (DBS) can also be used to collect whole blood specimens in order to perform EIA detection of anti-HCV Ab**

EASL Recommendations on Treatment of Hepatitis C 2016. Available at: http://www.easl.eu/medias/cpg/HCV2016/English-report.pdf; AASLD/IDSA Recommendations for Testing, Managing, and Treating Hepatitis C. Available at: http://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance_April_12_2017_b.pdf.

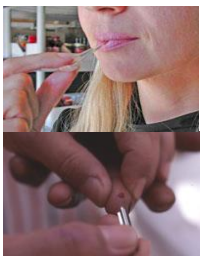


Screening HCV infection

RAPID DIAGNOSTIC TEST

Rapid Diagnostic Tests (RDT)

- **Can be used at the site of patient care**
  - Physician' office
  - Emergency room, ICU
  - Outpatient clinics, rural areas, eventually patients' home
- **Can use original specimen matrices in addition to serum or plasma**
  - Oral fluid
  - Fingerstick whole blood



Available RDTs for anti-HCV Detection

(CE-marked, FDA-approved or Eligible for Procurement by WHO)

	Oraquick® HCV rapid Ab test	Toyo® anti-HCV	Labmen® HCV test*	Multisure HCV	Assure® HCV rapid test
Manufacturer	Orasure Technologies USA	Turklab Turkey	Turklab Turkey	MP Biomedicals Singapore	MP Biomedicals Singapore
Specimen type	**oral fluid**, **whole blood**, serum, plasma	**whole blood**, serum, plasma	**whole blood**, serum, plasma	**whole blood**, serum, plasma	**whole blood**, serum, plasma
Volume needed (µL)	40 (OF) 20	30 10 (S,P)	10	25	50 5 (S,P)
Time required (min)	20-40	5-15	15	15	15

*Compatible with automatic reader ICA-R Turklab

Available RDTs for anti-HCV Detection

(CE-marked, FDA-approved or Eligible for Procurement by WHO)

	First Response HCV card Test	Signal HCV Ver 2.0	SD Bioline HCV
Manufacturer	Premier Medical Corporation Ltd India	Span Diagnostic India	Standard Diagnostics Korea
Specimen type	**whole blood** serum, plasma	serum, plasma	**whole blood**, serum, plasma
Volume needed (µL)	35	100	10
Time required(min)	20-30	10	5-20

Performance of RDT
Capillary Whole Blood

- **318 patients with chronic HCV infection; 25 patients with resolved HCV infection; 170 HCV-seronegative subjects (N=513)**

Tests	Specificity	Sensitivity
OraQuick® HCV Rapid Ab Test	100%	99.4%
TOYO® anti-HCV test	98.8%	96.2%
Labmen® HCV test	100%	63.1%

Chevaliez et al., Clin Microbiol Infect 2016;22(5):459.e1-6.

Performance of RDT
Crevicular fluid

- **318 patients with chronic HCV infection; 25 patients with resolved HCV infection; 170 HCV-seronegative subjects (N=513)**

Test	Specificity	Sensitivity
OraQuick® HCV Rapid Ab Test	100%	97.6%

false-negative results could be explained by the lower amount of anti-HCV antibodies in crevicular fluid

Chevaliez et al., Clin Microbiol Infect 2016;22(5):459.e1-6.

Performance of RDT
Meta-analysis

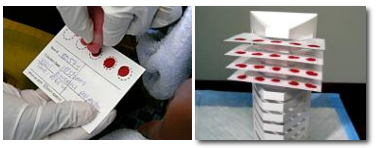
- **More than 13,000 individuals included in 18 studies between 1994 and 2011**
  - Stratification according to matrix specimens
    - Whole blood (venous and capillary): 4,126 specimens
    - Oral fluid: 4,259 specimens

Specimen	Specificity	Sensitivity
Whole blood	99.5%	98.9%
Saliva	98.2%	97.1%

Shivkumar et al., Ann Intern Med 2012;157(8):558-66.

Performance of RDT

- **WHO prequalified tests**
  - OraQuick® HCV Rapid (blood, serum, oral fluid), FDA approved (blood and serum)
  - SD Bioline HCV (blood, serum)



Screening HCV infection

DRIED BLOOD SPOT

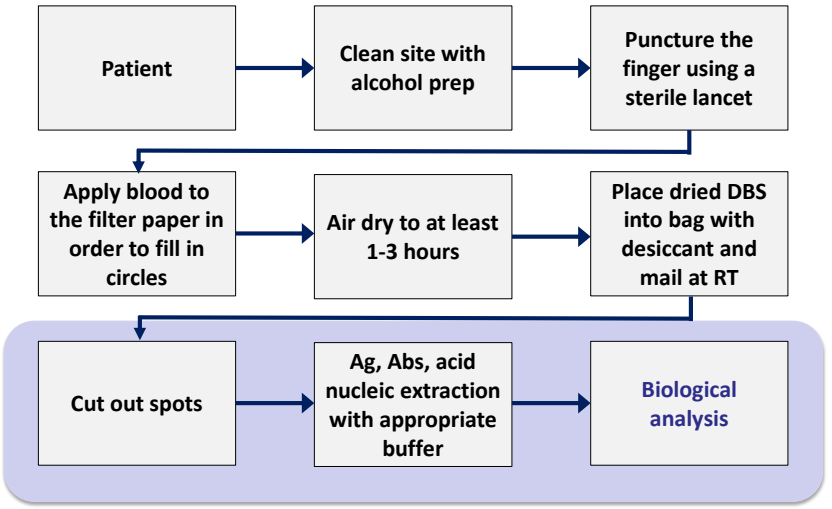
DBS is Recommended by WHO

- **Mapping, monitoring and surveillance of neglected tropical diseases**

- **HIV-1 Drug Resistance genotyping in limited resource settings**

Solomon et al., PLoS Negl Trop Dis 2012;6(7):e1746; WHO manual for HIV drug resistance testing using dried blood spot specimens. Available at: http://apps.who.int/iris/bitstream/10665/75829/1/WHO_HIV_2012.30_eng.pdf?ua=1.

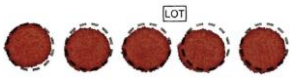
Blood Collection on DBS is a Long Way



Adapted from Hirtz and Lehman., Ann Biol Clin (Paris) 2015;73(1):25-37.

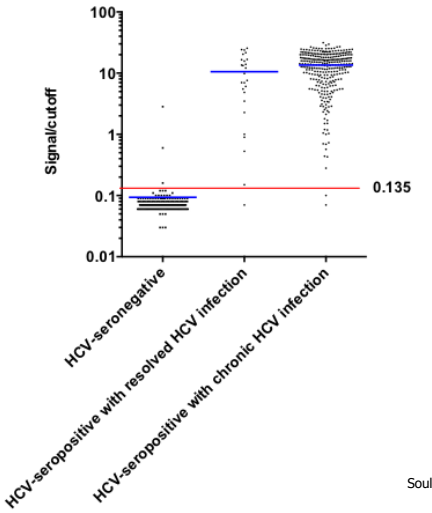
Advantages and Disadvantages of DBS

- **Advantages**
  - "Universal" access to care in particular situations such as geographical remoteness, difficult venous access,…
  - easy to collect, painless and mail at room temperature
  - Good stability of the biological matrix
  - Serological, molecular analysis can be performed : no need for 2nd visit for confirmatory RNA test



- **Disadvantages**
  - Lower analytical sensitivity than classical biological matrices (serum or plasma)
  - No standardized procedures
  - High variability of the results (quality and volume of blood, method used for detection, quality of filter paper, buffer used for extraction, …)
  - No immediate results

Detection of anti-HCV Ab in Whole Blood from DBS by 3rd-generation EIA



Interpretation required the establishment of a new signal-to-cutoff ratio

Sensitivity = 99.1%
Specificity = 98.2%

Soulier et al., J Infect Dis 2016;213(7):1087-95.

Performance of RDT

Whole Blood Collected on DBS

- **129 patients with chronic HCV infection, 10 patients with resolved HCV infection and 68 HCV-seronegative subjects (N=207)**

Tests	Specificity	Sensitivity*
OraQuick® HCV Rapid Ab Test	100%	100%
First Response® HCV Card test	100%	99.3%
Assure HCV Rapid Test	100%	98.6%
MultiSure HCV	100%	98.6%

*using EIA result in serum as the reference

Poiteau et al.,. J Viral Hepat 2016;23(5):399-401.

Outline

- Virological tools for screening HCV infection
- **Virological tools for diagnosing ongoing HCV infection and treatment decision**
- Virological tools for monitoring HCV infection

EASL and AASLD/IDSA Recommendations

- **If anti-HCV Ab are present, HCV RNA (alternatively, HCV core antigen [Core Ag] if HCV RNA is not available or not affordable*) should be determined to identify patients with ongoing infection**
- **The HCV genotype, including genotype 1 subtype, should be assessed prior to treatment initiation**
- **Systematic resistance testing prior to treatment is not recommended. However, physicians who have easy access to reliable resistance tests can use these results to guide their decisions**

EASL Recommendations on Treatment of Hepatitis C 2016. Available at: http://www.easl.eu/medias/cpg/HCV2016/English-report.pdf; AASLD/IDSA Recommendations for Testing, Managing, and Treating Hepatitis C. Available at: http://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance_April_12_2017_b.pdf.

Diagnosis ongoing HCV infection and decision to treat

HCV RNA DETECTION/QUANTIFICATION

Assessment of HCV RNA Quantification in the Era of DAA

- **In clinical practice, HCV RNA quantification should be based on an accurate real-time molecular assay**
  - With a detection level of 25 IU/mL or lower (10-15 IU/mL)
  - An identical LOD and lower limit of quantification (LLOQ)

- **However, In the era of short-course, highly effective therapy, there might be less need for quantification of HCV RNA for HCV management.**

EASL Recommendations on Treatment of Hepatitis C 2016. Available at: http://www.easl.eu/medias/cpg/HCV2016/English-report.pdf; AASLD/IDSA Recommendations for Testing, Managing, and Treating Hepatitis C. Available at: http://www.hcvguidelines.org/sites/default/files/full-guidance-pdf/HCVGuidance_April_12_2017_b.pdf.

Available Molecular Assays

	RealTi*me* HCV (Abbott)	CAP/CTM HCV 2.0 (Roche)
Principle	Real-time PCR	Real-time PCR
Volume required	500 µL	650 µL
Biological matrix	Plasma/serum	Plasma/serum
Extraction procedure	Automated	Automated
Linear range	12-10^8 IU/mL	15-10^8 IU/mL
LLOQ	12 IU/mL	15 IU/mL
LLOD (plasma/serum)	10.5/7.2 IU/mL	9.3/8.8 IU/mL
Time to have results	276 min*	345 min*
Random access	No	No

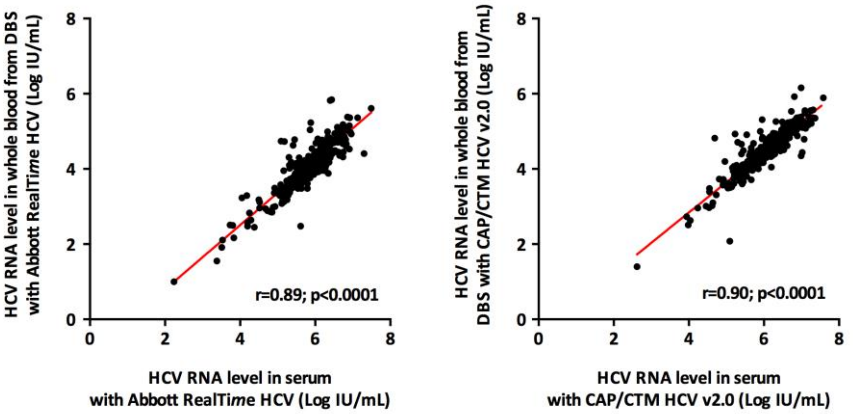
Centralized laboratory structures

-Assays are usually batched

-Labs struggle to provide results within a one full-day window.

HCV RNA Quantification in Whole Blood from DBS

315 HCV RNA (+), 308 and 306 patients had HCV RNA quantified in WB specimens from DBS by the Abbott RT and CAP/CTM assay, respectively.



HCV RNA levels lower than in serum specimens (≈1,5 to 2 log IU/mL)→ *only the presence or absence of HCV RNA or changes in the HCV RNA level should be taken into consideration for therapy.*

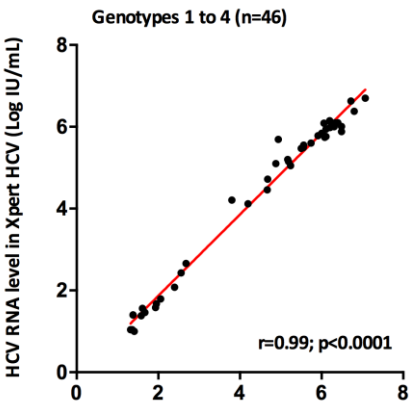
Soulier et al., J Infect Dis 2016;213(7):1087-95.

Available Molecular Assays

	RealTi*me* HCV (Abbott)	**CAP/CTM HCV 2.0** (Roche)	**Xpert® HCV** (Cepheid)	**Aptima® HCV Quant Dx** (Hologic)
Principle	Real-time PCR	Real-time PCR	Real-time PCR	Real-time TMA
Volume required	500 µL	650 µL	1000 µL	500 µL
Biological matrix	Plasma/serum	Plasma/serum	Plasma/serum	Plasma/serum
Extraction procedure	Automated	Automated	Automated	Automated
Linear range	12-10^8 IU/mL	15-10^8 IU/mL	10-10^8 IU/mL	10-10^8 IU/mL
LLOQ	12 IU/mL	15 IU/mL	10 IU/mL	10 IU/mL
LLOD (plasma/serum)	10.5/7.2 IU/mL	9.3/8.8 IU/mL	4.0/6.1 IU/mL	4.3/3.9 IU/mL
Time to obtain results	276 min*	345 min*		
Random access	No	No	Yes	Yes

-No need for batch processing
-One step procedure

HCV RNA Quantification
(*GeneXpert*, *Cepheid*)
WHO prequalified HCV NAAT



Unpublished data.

HCV RNA Quantification
(*GeneXpert, Cepheid*)
Capillary Whole Blood

- **45 patients with chronic HCV infection; 105 HCV-seronegative subjects (N=150)**

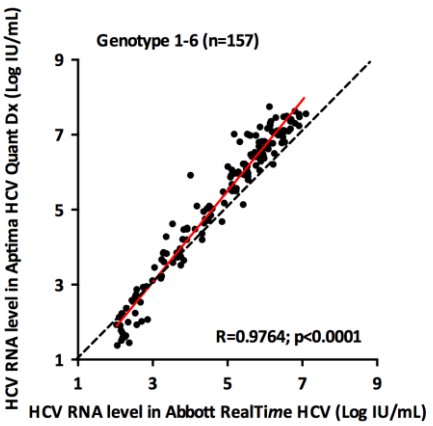
Test	Specificity	Sensitivity*
Xpert HCV viral load finger-stick	98,1%	95,5%

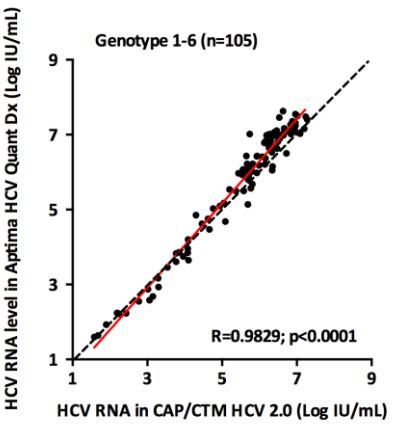
* Based on Abbott RealTime

point of care assay in decentralized settings?

Grebely et al., Lancet Gatsro Hepatol 2017; 2(7):514-520

HCV RNA Quantification
(*Panther, Hologic*)





Chevaliez et al., J Clin Virol 2017 Jun;91:5-11

Diagnosis ongoing HCV infection and decision to treat

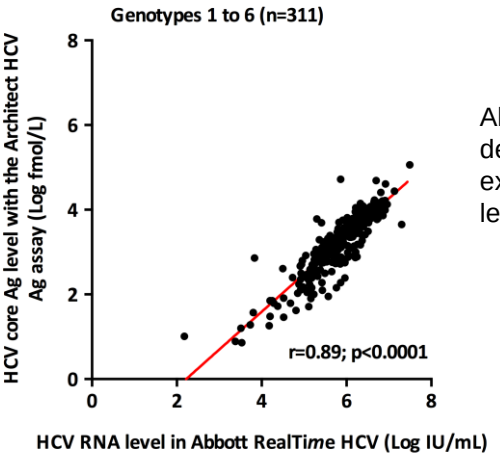
HCV CORE AG DETECTION/QUANTIFICATION

Interest of HCV Core Ag Quantification

- **A surrogate marker of HCV replication**
- **Many advantages over molecular methods**
  - ≈ 30% less expensive than molecular method
  - Stability of marker at RT for 96 hours
  - Short time to result (~60 min) but not a decentralized test
- **New tool to monitor virologic responses during IFN-free DAA-based regimens**
  - Discrimination between patients with or without SVR

Chevaliez et al., Antivir Ther 2016 Apr 26 doi: 10.3851/IMP3042.

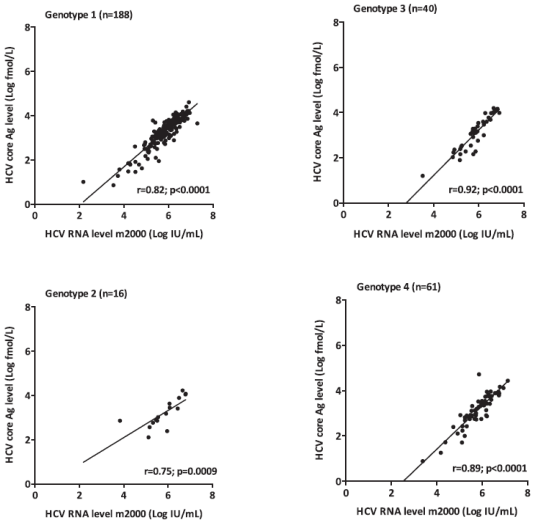
HCV Core Antigen: A Marker of HCV Replication



All HCV RNA (+) were detected for HCVcAg except six with HCV RNA level <2.70 Log IU/mL.

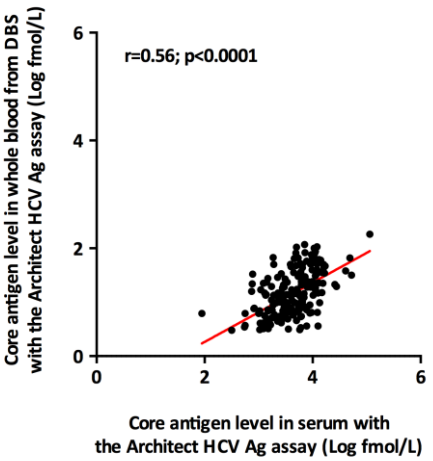
Chevaliez et al., J Clin Virol 2014;61(1):145-8.

HCV Core Antigen: A Marker of HCV Replication



Chevaliez et al., J Clin Virol 2014;61(1):145-8.

HCV Core Ag Quantification by the Architect HCV Assay in Whole Blood from DBS



-mean difference DBS/serum≈ 2,5 log fmol/L

-Reduced sensitivity of detection in DBS/serum

Soulier et al., J Infect Dis 2016;213(7):1087-95.

Diagnosis ongoing HCV infection and decision to treat

HCV RESISTANCE TESTING

Tests Available in the US?

- **Commercial molecular methods for all three DAA-targeted regions are available**
  - Genotypes 1a/1b and 3 (for NS5A only)
  - HCV RNA VL ≥2,000 IU/mL is required
  - Based on NGS with a cutoff of 10%

HCV NS5A Drug Resistance Assay

HCV NS5B Drug Resistance Assay

HCV GenoSure® Drug Resistance Assay NS3/4A

Monogram BIOSCIENCES LabCorp Specialty Testing Group

Available at: https://www.monogrambio.com/hepatitis-tests.

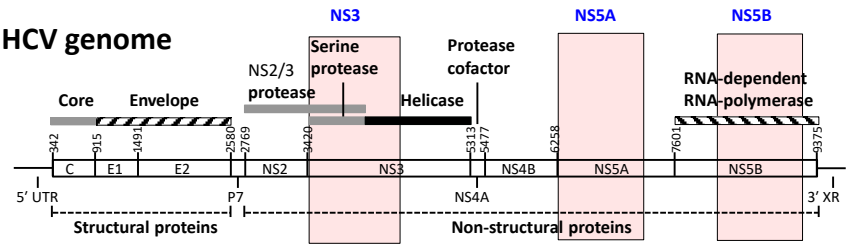
Tests Available in Europe?

- **No commercially standardized tests are available**
  - Except for the detection of NS3 Q80K RAS (Polymorphism kit, Clonit srl, Milan)[1]
- **Only homebrew population sequencing-based methods for NS3^{pro}, NS5A domain I and NS5B**
- **NGS-based tests currently in development**
  - *Sentosa* SQ HCV Genotyping Assay (VELA Diagnostics)[2]

[1]Vicenti et al., J Clin Virol. 2016;76:20-3; [2]Poon et al., AASLD 2015.

Features
(*Sentosa* SQ HCV Genotyping Assay)

- **HCV RNA level >1000 IU/mL**
- **Serum or plasma specimens accepted**
- **Genotypes 1a and 1b**
- **Time to obtain results**
  - 48 hours



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- Virological tools for screening HCV infection
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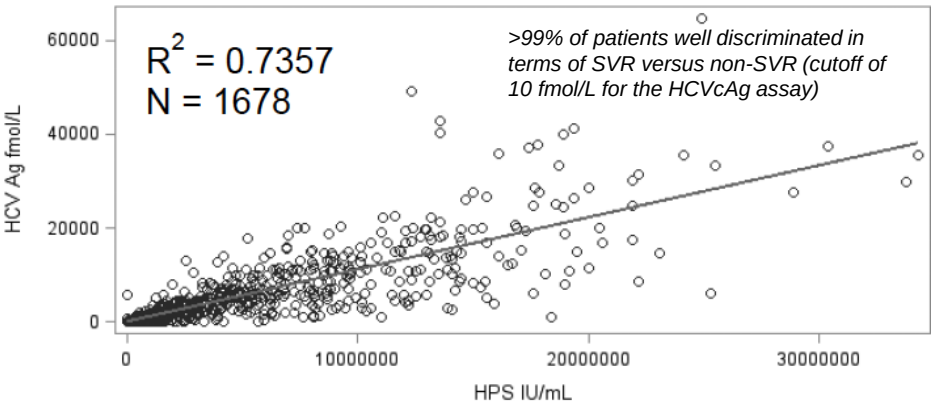
- **Virological tools for monitoring HCV infection**

Monitoring HCV DAA Therapy

- **Using new DAAs, the levels of viral load decline no longer correlate with response**
- **Particularly in LMICs, the number of virological tests could be reduced to a single post treatment virological test to assess cure**
- **Measurements of HCV core Ag levels can be used as an alternative to HCV RNA measurements in settings where HCV RNA assays are not available or not affordable**

Baseline and On-Treatment HCV Core Ag *versus* HCV RNA in SAPPHIRE-I Trial



Chevaliez et al., Antivir Ther. 2016 Apr 26. doi: 10.3851/IMP3042.

Summary

- **RDTs and DBS are reliable tools for the screening and diagnosis of HCV infection**
- **Treatment monitoring can be simplified by the use of DBS, ideally as qualitative tests due to their altered ability to accurately quantify HCV RNA or core antigen**
- **HCV core Antigen quantification may be an attractive alternative tool to monitor patients receiving IFN-free regimens**