

# Detection of HCV Core Antigen

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# Early Discovery of HCV Core Antigen as an Serologic Marker

**1988:** discovery of HCV as the main cause of non-A, non-B hepatitis: a recombinant protein (c100-3) in the non-structural gene region (NS4) of the genome (NS4) was utilized in antibody tests as a marker of chronic HCV infection

**1990 – 1992:** Additional recombinant proteins (NS3, NS5, core) were added to c100-3 to enhance antibody detection. There were additional efforts to detect native viral proteins in serum – to determine if the actual viral antigens may represent better markers than the recombinant proteins.

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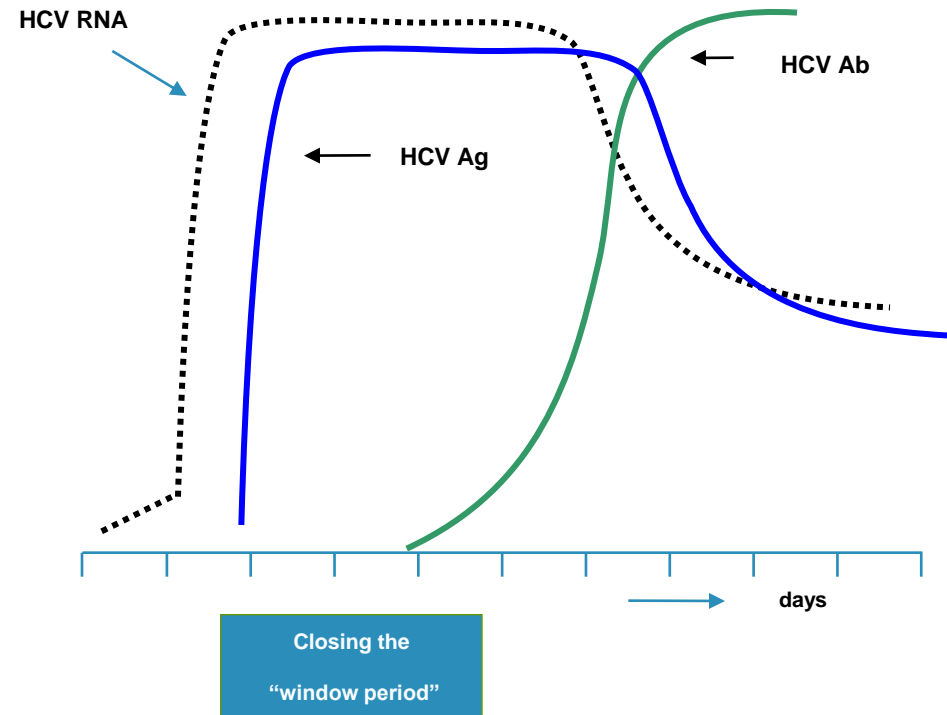
- **1988 – discovery** of HCV as the main cause of non-A, non-B hepatitis: a recombinant protein in the non-structural gene region of the genome (NS4) was utilized c100-3 in antibody tests as a marker of chronic HCV infection
- 1990 – 1992-** Additional recombinant proteins were added to c100-3 (NS3, NS5 and core) to enhance antibody detection. While this was occurring there were additional efforts to detect native viral proteins in serum – to determine if the actual viral antigens may represent better markers than the recombinant proteins.
- 1995 – Tanaka et al**, indicated that HCV core proteins can be detected in the serum of individuals with HCV chronic infection
  - early assay prototype utilized a PEG treatment plus centrifugation
  - two monoclonal antibodies from conserved regions were utilized



# Two Types of HCV Core Antigen Tests Have Shown Utility

## Detection of HCV Core Antigen during the Pre-seroconversion Window Period (blood screening)

HCV antigens can be detected either as a stand alone antigen test <sup>1, 2</sup> or as an antigen/antibody combination test <sup>3, 4</sup>



1. Lee et al., Vox Sang 80: 19-23. 2001
2. Muerhoff et al., Transfusion 42: 349-356. 2002
3. Shah et al., Transfusion 43: 1067-1074. 2003.
4. Laperche et al., Transfusion 45: 1965-1972. 2005.

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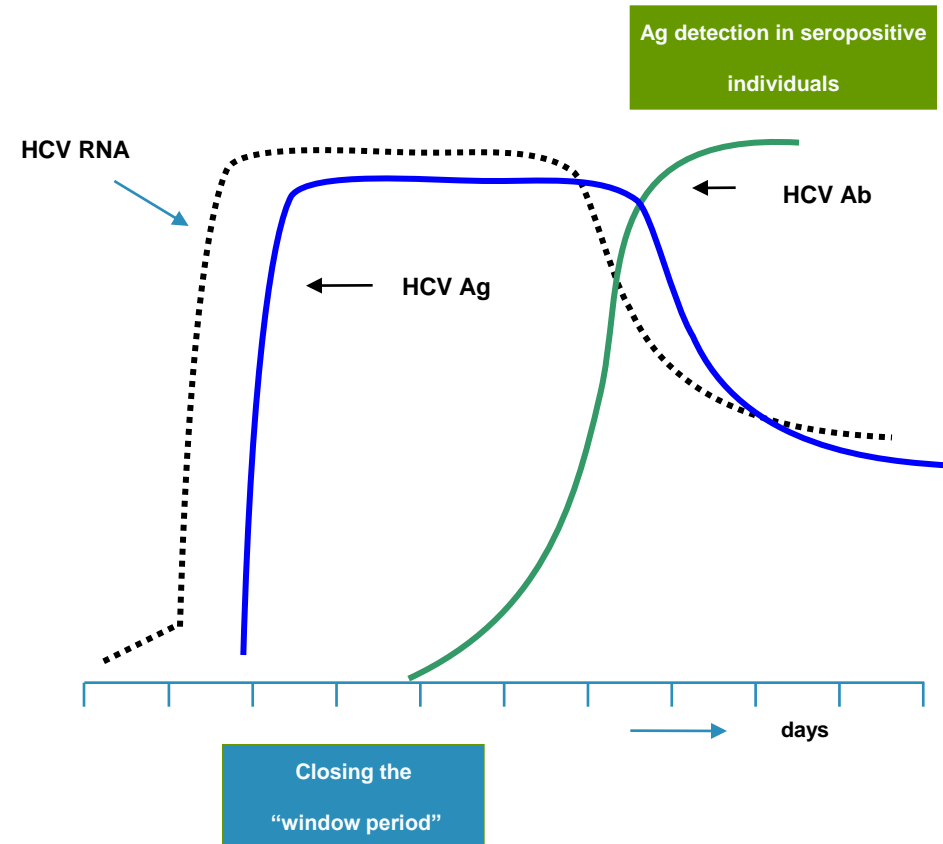
## HCV Antigen Detection in the Presence of Anti-HCV in Host (diagnostics)

Antibody inactivation step required

Tests may be quantitative or qualitative

-May be used determine active HCV infection <sup>5, 6</sup>

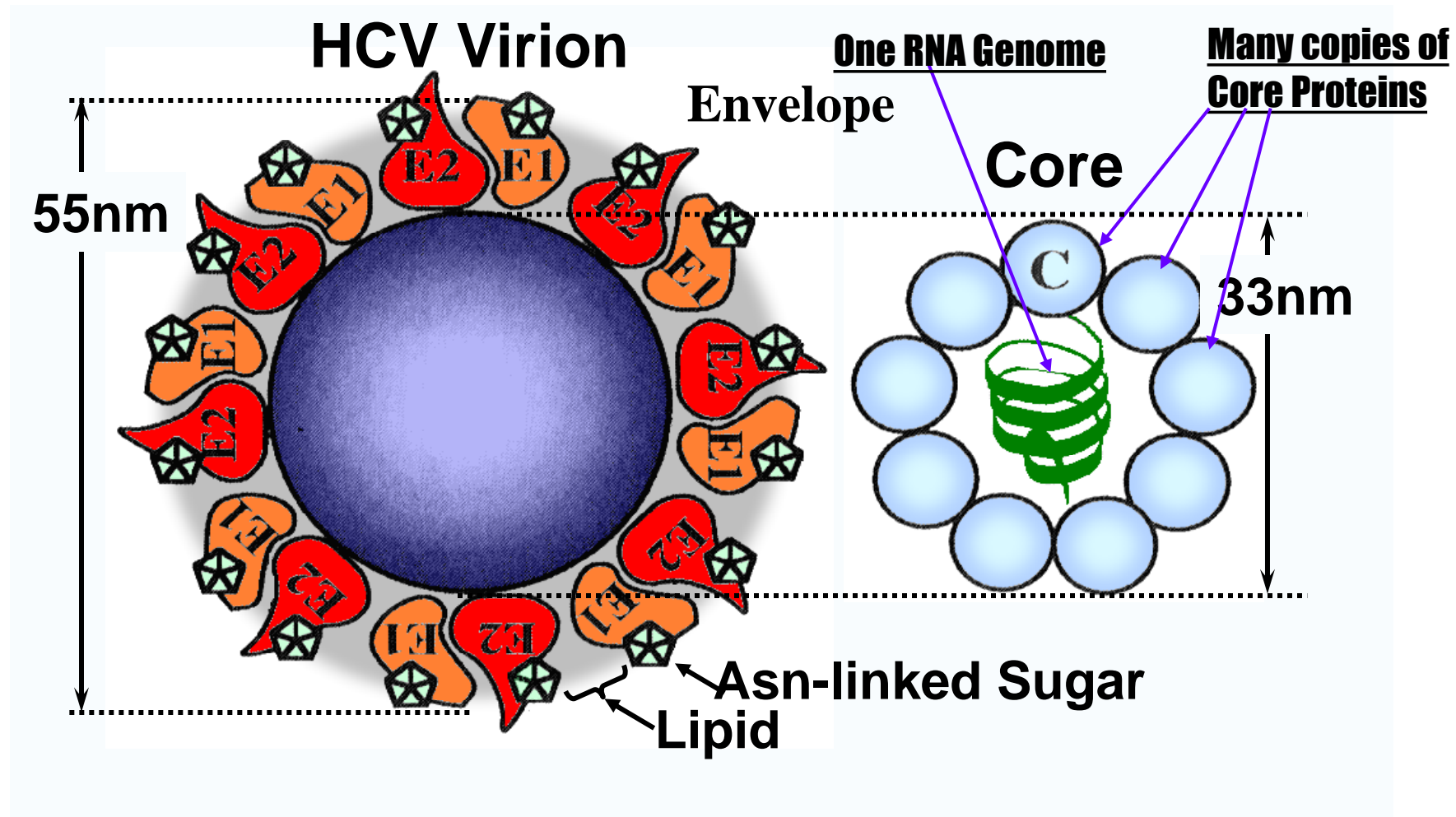
-May be utilized to monitor individuals on antiviral therapy <sup>7, 8</sup>



1. Lee et al., Vox Sang 80: 19-23. 2001
2. Muerhoff et al., Transfusion 42: 349-356. 2002
3. Shah et al., Transfusion 43: 1067-1074. 2003.
4. Laperche et al., Transfusion 45: 1965-1972. 2005.
5. Ayogi et al., J Clin Microbiol 37: 1802-1808. 1999
6. Hayashi et al., J Viral Hepatitis 12: 106-110. 2005.
7. Maynard et al., J Viral Hepatitis 10: 318-323. 2003.
8. Gonzalez et al., J Viral Hepatitis 12: 481-487. 2005.



# What is HCV Core Antigen ?



# HCV Core Assay Development Activities

A prototype ARCHITECT HCV core antigen test was developed as a collaboration between Abbott Japan and scientists at Advanced Life Sciences, Inc (ALSI) formerly known as Tonen.



# ARCHITECT HCV Ag - Assay Overview\*

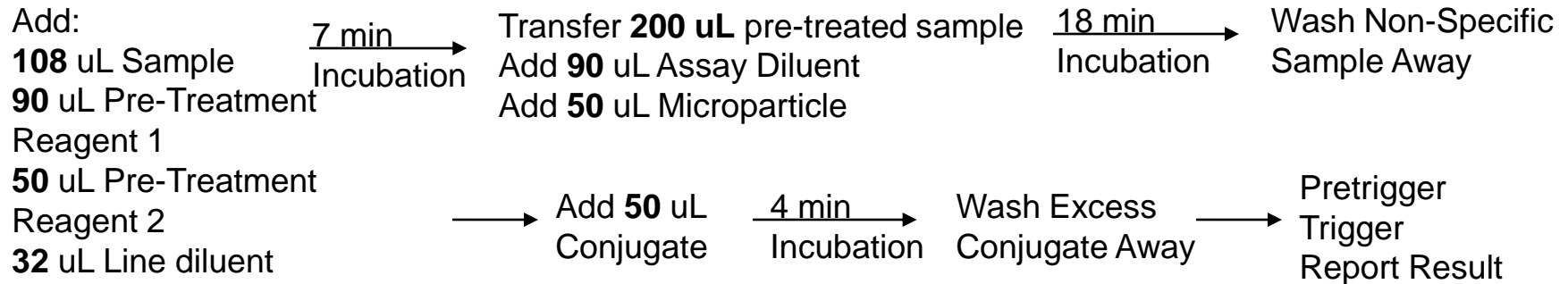
|                      |  |
|----------------------|--|
| Assay Name           | ARCHITECT HCV Ag (LN 6L45-25), 100t                        |
| Test principle       | Chemiluminescent, two-step assay with pretreatment         |
| Time to First Result | 36 minutes – in <i>i</i> 2000/ <i>i</i> 2000SR             |
| Dynamic Range        | 3 – 20.000 fmol/L; alternative unit: pg/mL,                |
| Calibrator (6L45-01) | 6 point calibration (Cal. A 0 – Cal. F 20.000 fmol/L)      |
| Controls (6L45-10)   | 3 Levels: NC (0 fmol/L), PC1 (50 fmol/L), PC2 (300 fmol/L) |

**\*This assay is not commercially available in the United States**

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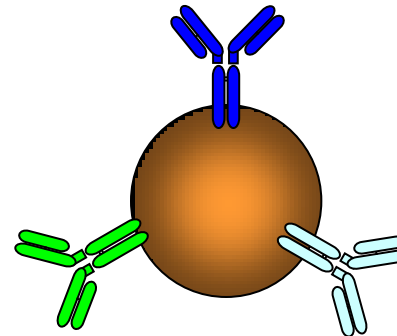
# ARCHITECT HCV Ag Assay\* protocol

## 2 step assay protocol with pre treatment:

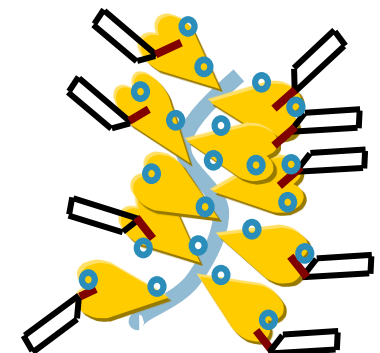


### Objectives of the pretreatment (automated):

- to dissociate antibody-bound core antigen
- to lyse viral particles and expose core antigen
- to inactivate antibody



Microparticle with 3 MAb's



Conjugate (with 2 Mab's)

**\*This assay is not commercially available in the United States**

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# Potential Uses for HCV Core Antigen Test

- **Detection of Pre-Serconversion Window Period Samples**

- Universal Blood Screening
- Selected Screening of High-Risk Patients (Diagnostic Laboratory)

- **Diagnostic Test –**

- Reflex test after antibody testing to distinguish infected from non-infected seropositive individuals

- **Monitoring Antiviral Therapy –** to complement NAT

- HCV antigen test can be successfully utilized to monitor antiviral therapy

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## Window Period - Seroconversion Panel: ZeptoMetrix 6225 – Genotype 1a

| Sample  | Day | HCV RNA<br>copies/mL<br>(Vendor) | ARCHITECT Anti-<br>HCV<br>S/CO | ARCHITECT HCV<br>Antigen Test<br>S/CO |
|---------|-----|----------------------------------|--------------------------------|---------------------------------------|
| 6225-5  | 14  | < 100                            | 0.06                           | 0.09                                  |
| 6225-6  | 19  | < 100                            | 0.07                           | 0.12                                  |
| 6225-7  | 25  | < 100                            | 0.08                           | 0.08                                  |
| 6225-8  | 28  | < 100                            | 0.07                           | 0.07                                  |
| 6225-9  | 32  | < 100                            | 0.06                           | 0.09                                  |
| 6225-10 | 35  | < 100                            | 0.08                           | 0.11                                  |
| 6225-11 | 39  | < 100                            | 0.08                           | 0.11                                  |
| 6225-12 | 45  | 3,000,000                        | 0.08                           | 13.27                                 |
| 6225-13 | 47  | 4,300,000                        | 0.08                           | 65.58                                 |
| 6225-14 | 62  | 2,900,000                        | 0.08                           | 50.32                                 |
| 6225-15 | 56  | 3,100,000                        | 0.07                           | 47.27                                 |
| 6225-16 | 60  | 5,000,000                        | 0.07                           | 127.39                                |
| 6225-17 | 73  | 4,200,000                        | 0.19                           | 16.12                                 |
| 6225-18 | 78  | 1,700,000                        | 1.81                           | 7.74                                  |

**33 days earlier detection than HCV antibody**

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# Window Period Detection of HCV Infection

**Table 2**

Summary of seroconversion panel detection of the HCV Ag assay.

|     | Panel    | Genotype* | Day first detected |                     |                           | HCV Ag | Antibody/HCV Ag differential<br>(number of days) | HCV Ag/HCV RNA differential<br>(number of days) |
|-----|----------|-----------|--------------------|---------------------|---------------------------|--------|--|---|
|     |          |           | Antibody*          | HCV RNA             |                           |        |  |   |
|     |          |           |                    | Amplicor<br>HCV 2.0 | Amplicor HCV<br>Monitor 2 |        |  |   |
| #1  | HCV 6211 | 1a        | 186                | 140                 | 140                       | 140    | 46   | 0   |
| #2  | HCV 6213 | 1a        | 37                 | 11                  | 11                        | 11     | 26   | 0   |
| #3  | HCV 6222 | 1a        | 40                 | 17                  | 17                        | 17     | 23   | 0   |
| #4  | HCV 6225 | 1a        | 78                 | 45                  | 45                        | 45     | 33   | 0   |
| #5  | HCV 6227 | 1a        | 74                 | 42                  | 42                        | 42     | 32   | 0   |
| #6  | HCV 9041 | 1a        | 62                 | 24                  | 24                        | 24     | 38   | 0   |
| #7  | HCV 9054 | 3a        | 77                 | 52                  | 74                        | 52     | 25   | 0   |
| #8  | HCV 9055 | 3a        | 65                 | 31                  | 31                        | 31     | 34   | 0   |
| #9  | HCV 9057 | 1a        | Not detected       | 17                  | 17                        | 17     | Not applicable                                   | 0   |
| #10 | PHV917   | 2b        | 85                 | 20                  | 20                        | 20     | 65   | 0   |
|     |          |           |                    |                     |                           | Mean   | 35.8   | 0   |

| Panel  | HCV<br>subtype | Day on which the following was first detected: |                    |                      |                  |
|--------|----------------|--|--------------------|----------------------|------------------|
|        |                | Ab <sup>a</sup>                                | Ag-Ab <sup>b</sup> | Core Ag <sup>c</sup> | RNA <sup>d</sup> |
| PHV905 | 1a             | 11   | 11                 | 0 <sup>e</sup>       | 0                |
| PHV914 | 2b             | 16   | 12                 | 0                    | 0                |
| PHV921 | 3a             | 7  | 4                  | 0                    | 0                |

Morota K et al, J Virol Methods 2009;157:8–14.

Ross RS et al, Jour Clin Microb, Apr. 2010, p. 1161–1168



# Window Period - HCV Core Ag Screening of Dialysis Patients

**Objective:** To determine the incidence of acute HCV infection in absence of anti-HCV in dialysis patients

**Methods:** A total of 2,752 anti-HCV negative patients from 37 dialysis centers were tested for HCV Ag and HCV RNA in parallel (HCV RNA testing in minipools of 20, detection limit 600 IU/ml).

## Results :

|          | HCV RNA - | HCV RNA + |
|----------|-----------|-----------|
| HCV Ag - | 2729      | 0         |
| HCV Ag + | 21*       | 2         |



Specificity : 99.2% with GZ or 100% w/o GZ

Sensitivity: 2/2 HCV RNA positive samples

\* HCV Ag Indeterminates had values between 0.06 and 0.2 pg/mL

Midouge et al., J of Clinical Virology 48: 18-21 (2010)

# Potential Uses for HCV Core Antigen Test

- **Detection of Pre-Serconversion Window Period Samples**

- Universal Blood Screening
- Selected Screening of High-Risk Patients (Diagnostic Laboratory)

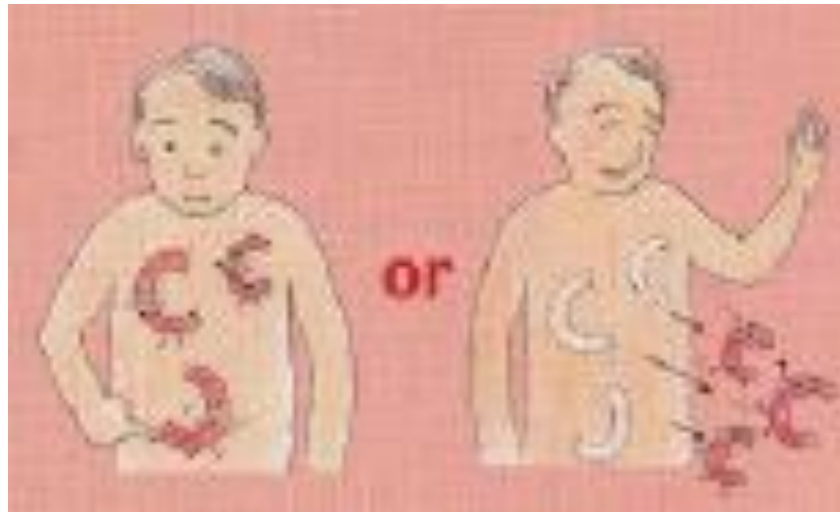
- **Diagnostic Test –**

- Reflex test after antibody testing to distinguish infected from non-infected seropositive individuals

- **Monitoring Antiviral Therapy – to complement NAT**

- HCV antigen test can be successfully utilized to monitor antiviral therapy

# HCV-RNA / HCV Core Antigen in HCV Infected Individuals



Dr. S.Iino, Kiyokawa Hospital, & Dr. H.Yoshizawa, Hiroshima University, Japan

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# HCV Core Antigen – Genotype Detection

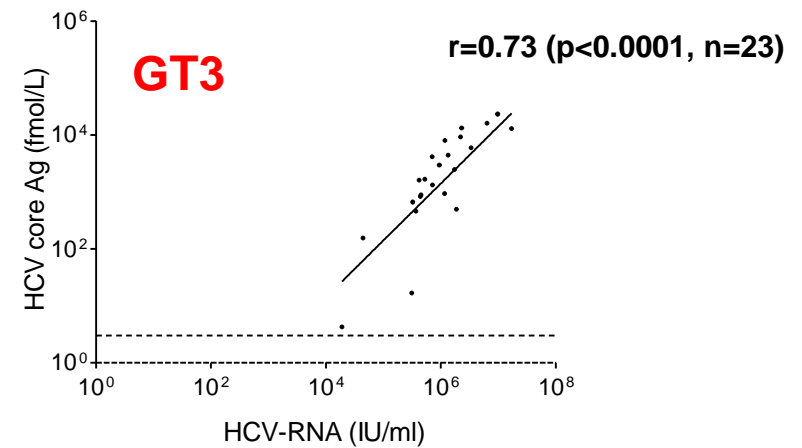
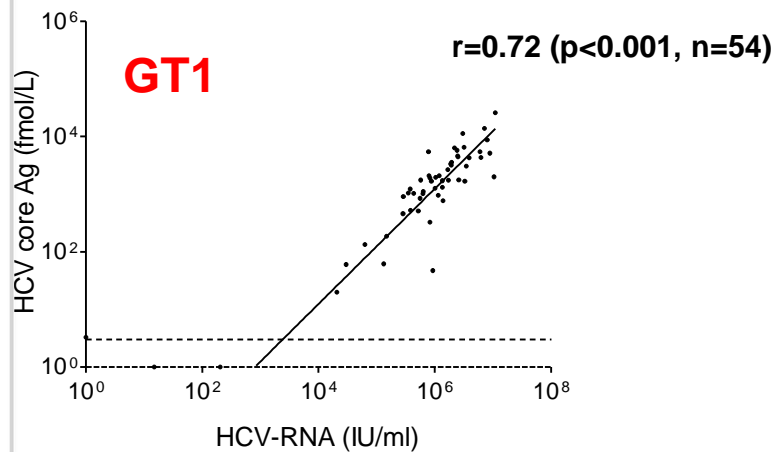
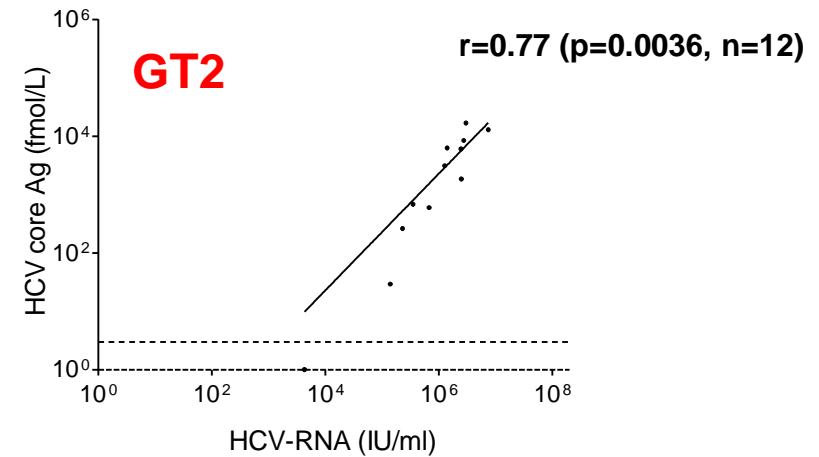
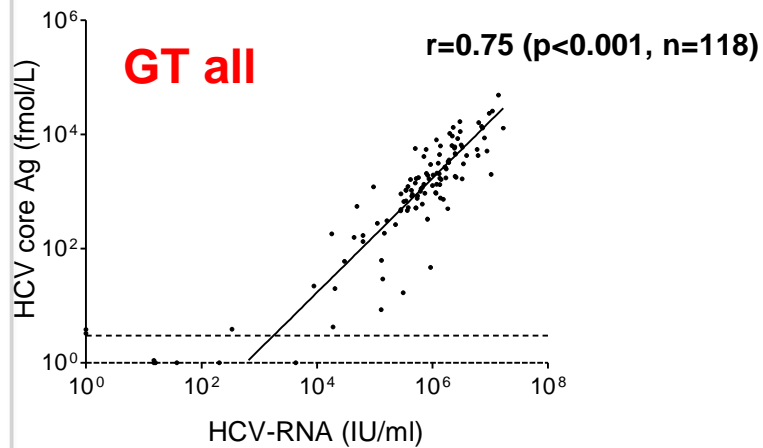
| Genotype     | # Specimens | % Detection            |                        |
|--------------|-------------|------------------------|------------------------|
|              |             | ARCHITECT HCV Core Ag  | Amplicor Monitor 2     |
| 1            | 3           | 100% (3/3)             | 100% (3/3)             |
| 1a           | 53          | 100% (53/53)           | 100% (53/53)           |
| 1a/1b        | 10          | 100% (10/10)           | 100% (10/10)           |
| 1b           | 42          | 100% (42/42)           | 100% (42/42)           |
| 2            | 1           | 100% (1/1)             | 100% (1/1)             |
| 2a           | 2           | 100% (2/2)             | 100% (2/2)             |
| 2a/2c        | 4           | 100% (4/4)             | 100% (4/4)             |
| 2b           | 20          | 100% (20/20)           | 100% (20/20)           |
| 3a           | 24          | 100% (24/24)           | 88% (21/24)            |
| 3k           | 2           | 100% (2/2)             | 100% (2/2)             |
| 4            | 1           | 100% (1/1)             | 100% (1/1)             |
| 4a           | 16          | 100% (16/16)           | 100% (16/16)           |
| 4a/4c        | 3           | 67% (2/3)              | 67% (2/3)              |
| 4c/4d        | 3           | 100% (3/3)             | 100% (3/3)             |
| 4c/4d/4e     | 1           | 100% (1/1)             | 100% (1/1)             |
| 5/5a         | 9           | 100% (9/9)             | 100% (9/9)             |
| 6a           | 2           | 100% (2/2)             | 100% (2/2)             |
| 6i           | 1           | 100% (1/1)             | 100% (1/1)             |
| <b>Total</b> | <b>197</b>  | <b>99.5% (196/197)</b> | <b>98.0% (193/197)</b> |

197 samples that were positive via Amplicor HCV 2.0 qualitative assay were tested via Amplicor Monitor 2 and ARCH HCV Core Ag. A total of 196 of 197 (99.5%) were detected via ARCHITECT HCV Core Ag: Amplicor Monitor 2 detected 193 of 197 (98.0%) samples.

Morota et al., J Virol Methods 157: 8-14 (2009)

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# Good correlation between HCV-RNA and HCV core Ag in different genotypes



Mederacke et al, J Clin Virol 2009

# Clinical Sensitivity – Reflex Testing

| Assay  | S/CO Range | No Samples | HCV Core Ag + | %HCV Core Ag + |
|--------|------------|------------|---------------|----------------|
| Vitros | 1.00-1.99  | 140        | 1             | 0.7%           |
|        | 2.00-7.99  | 271        | 9             | 3.3%           |
|        | >8         | 185        | 110           | 59.53%         |
| Abbott | 1.00-1.99  | 170        | 1             | 0.6%           |
|        | 2.00-9.99  | 217        | 64            | 29.5%          |
|        | >10        | 213        | 178           | 83.6%          |

NOTE: HCV core ag was detected in:

1 of 122 RIBA negatives

17 of 353 RIBA indeterminates

99 of 165 RIBA positive samples

**Medici et al., J Clin Virol (2011)**

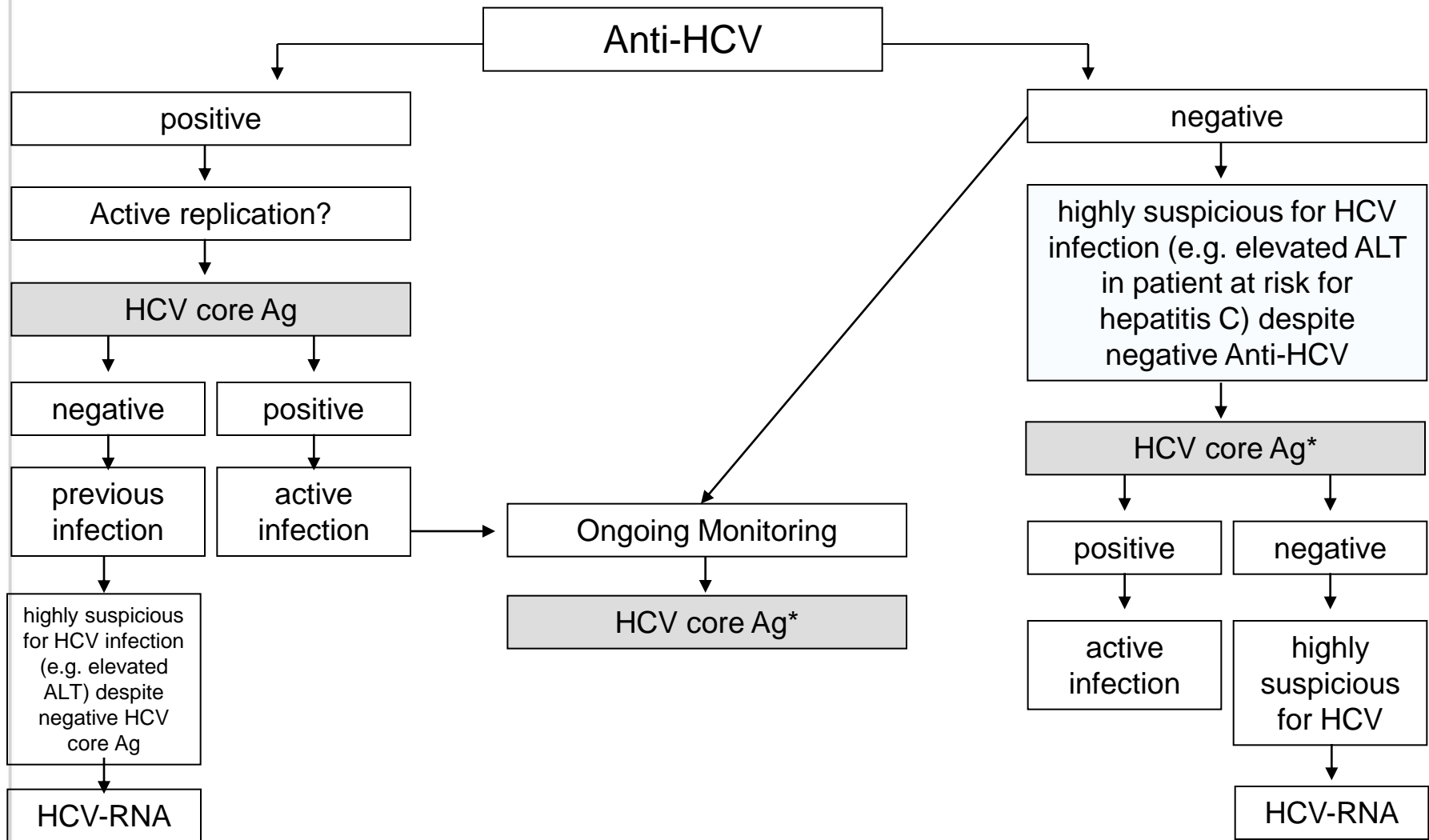


# Clinical Sensitivity – Reflex Testing

| HCV RNA Log IU/ml | No. of Samples | %HCV Core Ag + |
|-------------------|----------------|----------------|
| <3                | 319            | 19.7%          |
| 3-3.99            | 193            | 81.9%          |
| 4-4.99            | 276            | 97.1%          |
| 5-5.99            | 371            | 99.7%          |
| >6                | 321            | 99.7%          |
| Total             | 1480           | 79.7%          |

**Medici et al., J Clin Virol (2011)**

# Diagnostic algorithm



# Potential Uses for HCV Core Antigen Test

- **Detection of Pre-Serconversion Window Period Samples**

- Universal Blood Screening
- Selected Screening of High-Risk Patients (Diagnostic Laboratory)

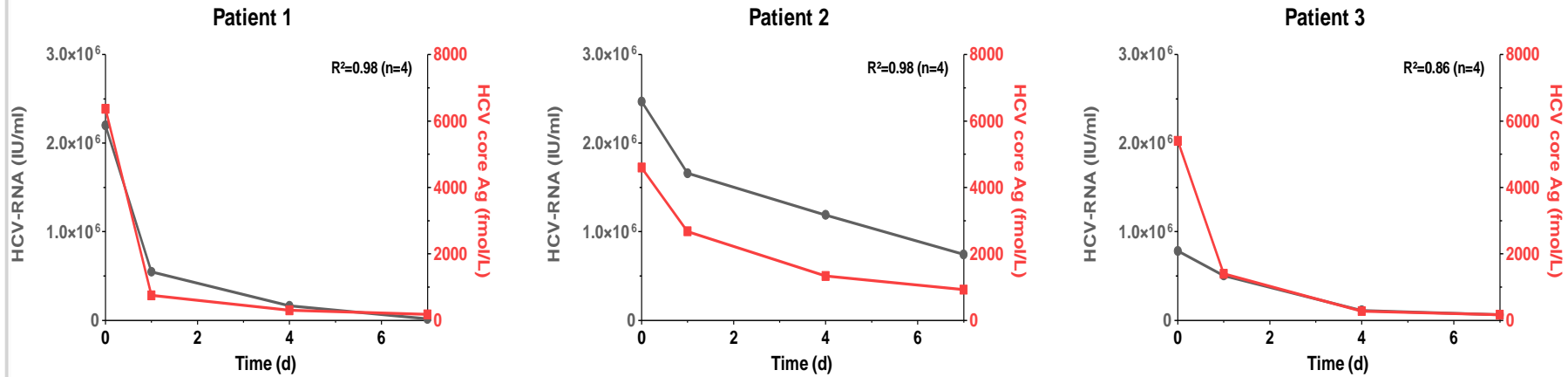
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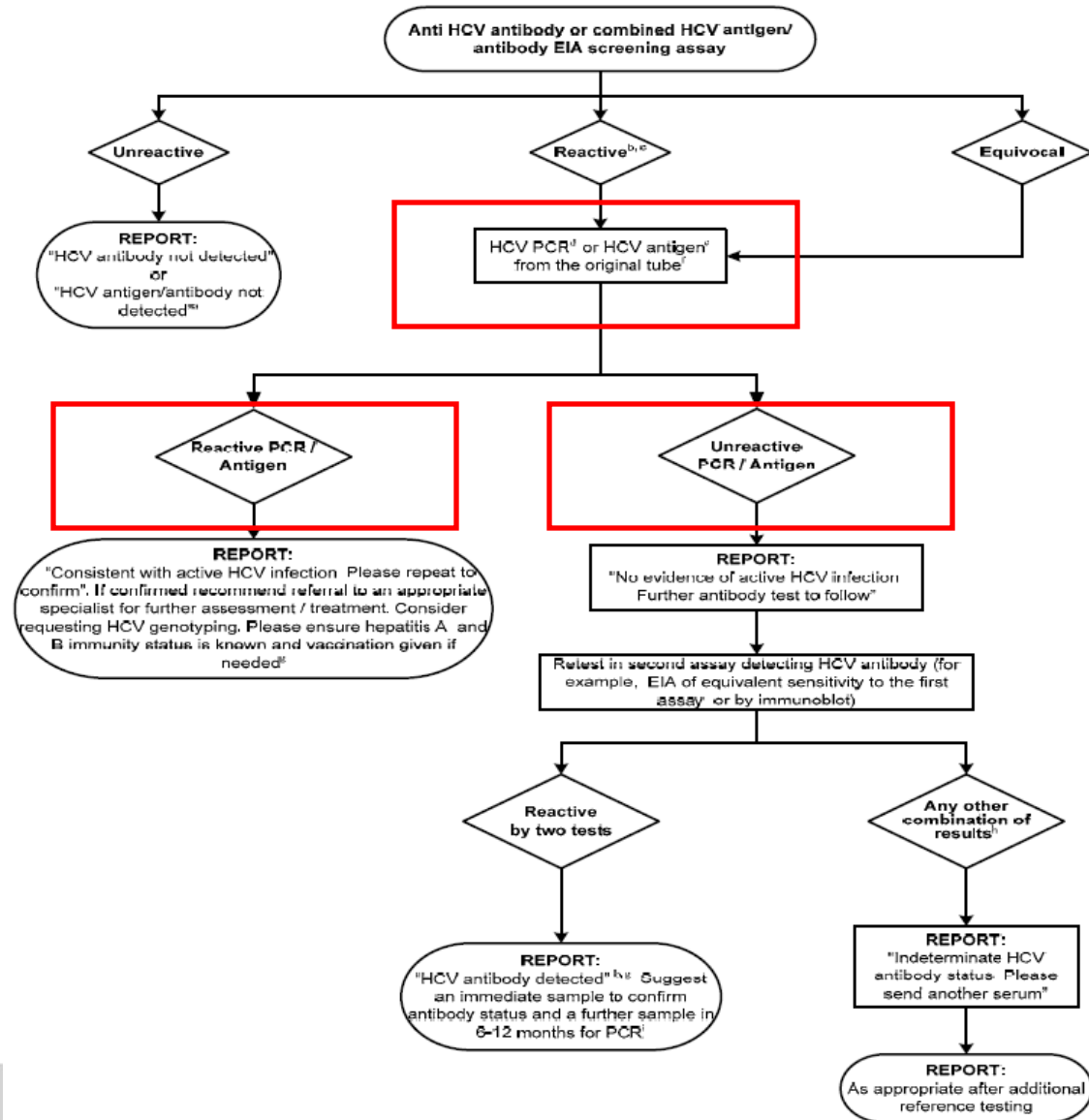
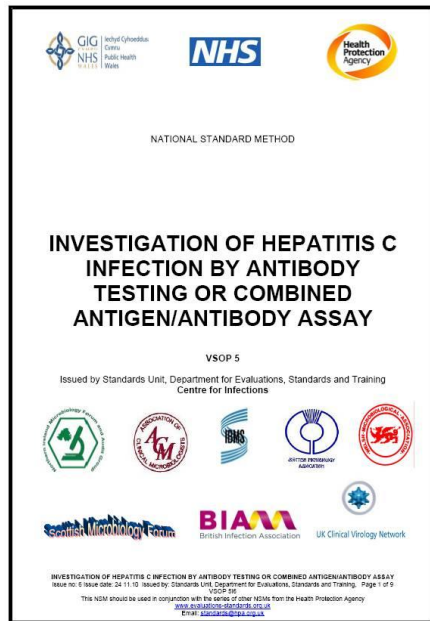
- **Monitoring Antiviral Therapy – to complement NAT**

- HCV antigen test can be successfully utilized to monitor antiviral therapy

# HCV Core Antigen can be utilized to monitor HCV antiviral therapy



# Guideline for HCV Infection Investigation (UK)



Investigation of Hepatitis C infection by antibody testing or combined antigen/antibody assay – UK Guidelines

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HCV Ag October 2011

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# Guidelines for dialysis (Ireland)

## Irish Renal Guidelines for BBV 2010

Table 1: Schedule for routine testing for HBV, HCV and HIV infections for haemodialysis patients

| Patient status                                      | On admission   | Monthly       | 3 monthly  | Annual    |
|---|--|---------------|--|-----------|
| All patients  | HBsAg,<br>anti-HBc, anti-HBs,<br>anti-HCV,<br>HCV Ag (Abbott Architect),<br>HCV PCR x 2 (2 weeks apart),<br>HIV Ag/Ab<br>ALT |               |  |           |
| Anti-HCV<br>negative, HCV<br>Ag/HCV PCR<br>negative |  | ALT           | Anti-HCV,<br>HCV Ag<br>(Abbott<br>Architect)<br>** | HCV PCR** |
| Anti-HCV<br>positive, HCV<br>Ag/HCV PCR<br>negative |  | HCV Ag<br>ALT |  |           |

\*\* Annual PCR is not necessary provided baseline HCV PCRs negative and HCV Ag (Abbott Architect) are performed every 3 months.

### 4.1 Pre CAPD/CCPD

Before starting CAPD/CCPD patients should be screened for BBVs as follows: HBsAg, anti-HBc, anti-HBs, Anti-HCV, HCV Ag (Abbott Architect), HCV PCR on 2 occasions 2 weeks apart and HIV Ag/Ab.

### 4.2 Regular testing on CAPD/CCPD

Annual HBsAg, anti-HCV, HCV Ag (Abbott Architect) and HIV Ag/Ab

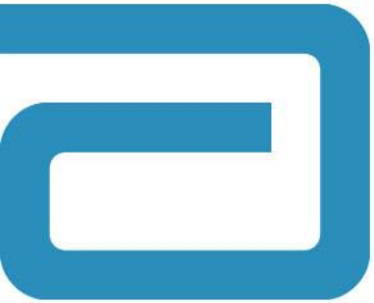


# Summary

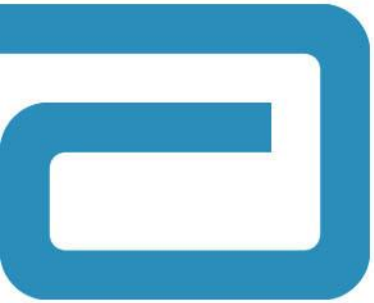
## **HCV Core Antigen testing can be utilized to:**

- identify HCV infection in seronegative individuals (pre-seroconversion window period detection)
- Identify seropositive individuals who are actively infected with HCV
- As a complementary test to HCV NAT to monitor antiviral therapy

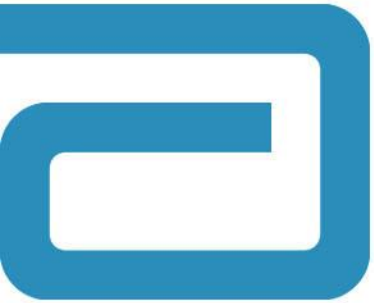
Will HCV Core antigen be adapted as standard of care for HCV infection?



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Thank You!

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