

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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Early Discovery of HCV Core Antigen as an Serologic Marker

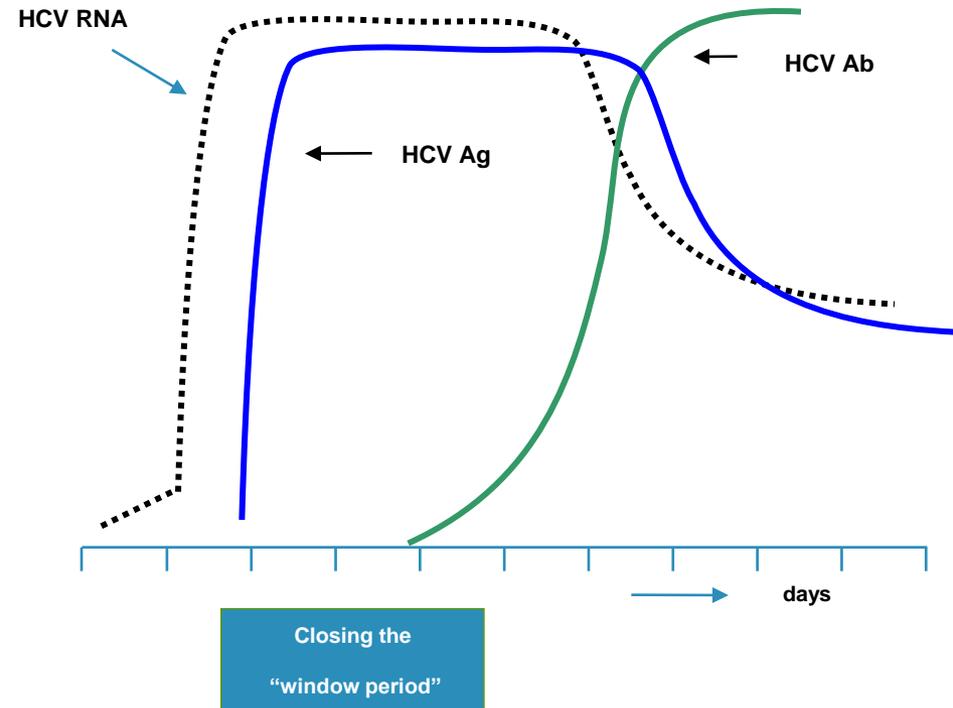
- **1988 – discovery** of HCV as the main cause of non-A, non-B hepatitis: a recombinant protein in the non-structural gene region of the genome (NS4) was utilized c100-3 in antibody tests as a marker of chronic HCV infection
- 1990 – 1992-** Additional recombinant proteins were added to c100-3 (NS3, 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 ^{1,2} or as an antigen/antibody combination test ^{3,4}



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)

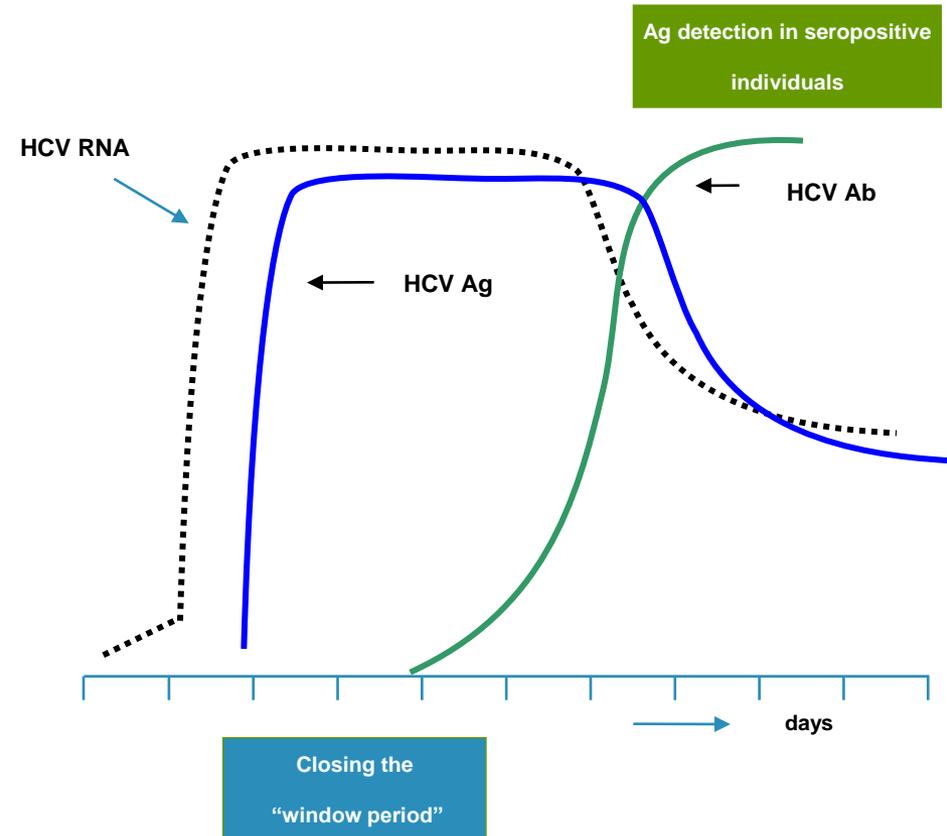
Antibody inactivation step required

Tests may be quantitative or qualitative

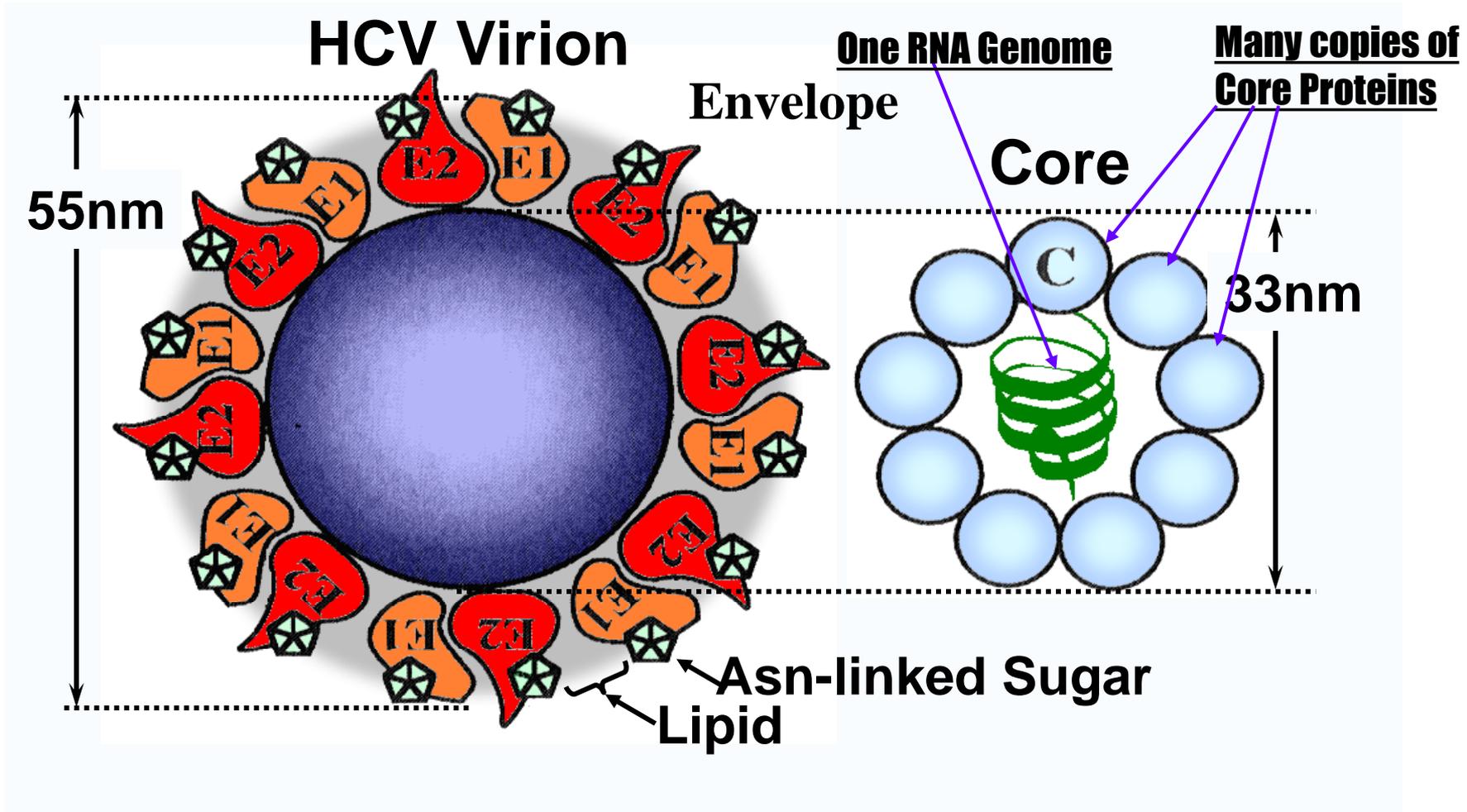
-May be used determine active HCV infection ^{5, 6}

-May be utilized to monitor individuals on antiviral therapy ^{7, 8}

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*

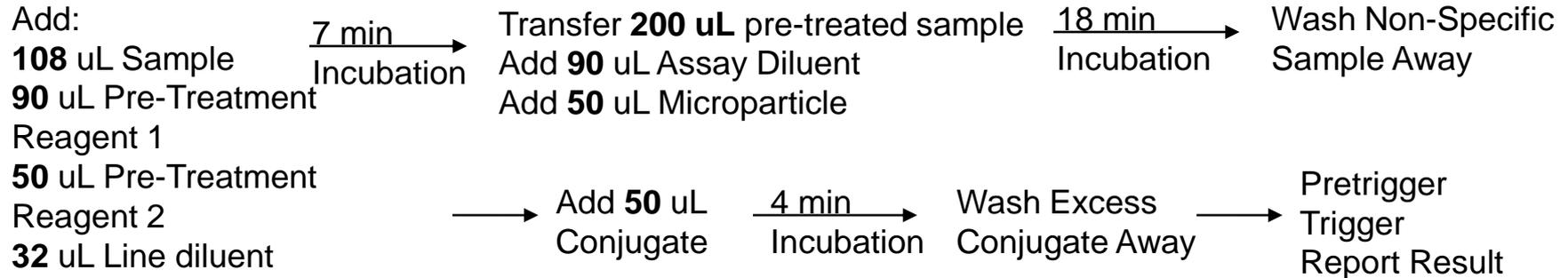
Assay Name	ARCHITECT HCV Ag (LN 6L45-25), 100t
Test principle	Chemiluminescent, two-step assay with pretreatment
Time to First Result	36 minutes – in <i>i</i> 2000/ <i>i</i> 2000SR
Dynamic Range	3 – 20.000 fmol/L; alternative unit: pg/mL,
Calibrator (6L45-01)	6 point calibration (Cal. A 0 – Cal. F 20.000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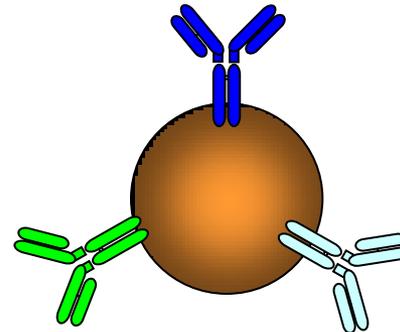
ARCHITECT HCV Ag Assay* protocol

2 step assay protocol with pre treatment:

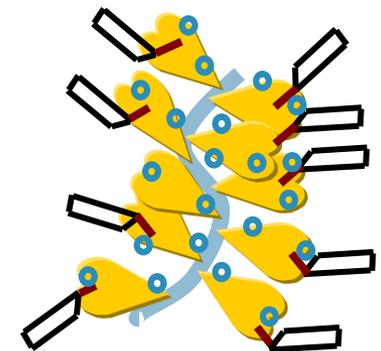


Objectives of the pretreatment (automated):

- 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 copies/mL (Vendor)	ARCHITECT Anti- HCV S/CO	ARCHITECT HCV Antigen Test 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

Window Period Detection of HCV Infection

Table 2
Summary of seroconversion panel detection of the HCV Ag assay.

	Panel	Genotype ^a	Day first detected			HCV Ag	Antibody/HCV Ag differential (number of days)	HCV Ag/HCV RNA differential (number of days)
			Antibody ^a	HCV RNA				
				Amplicor HCV 2.0	Amplicor HCV 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 subtype	Day on which the following was first detected:			
		Ab ^a	Ag-Ab ^b	Core Ag ^c	RNA ^d
PHV905	1a	11	11	0 ^e	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)

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

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

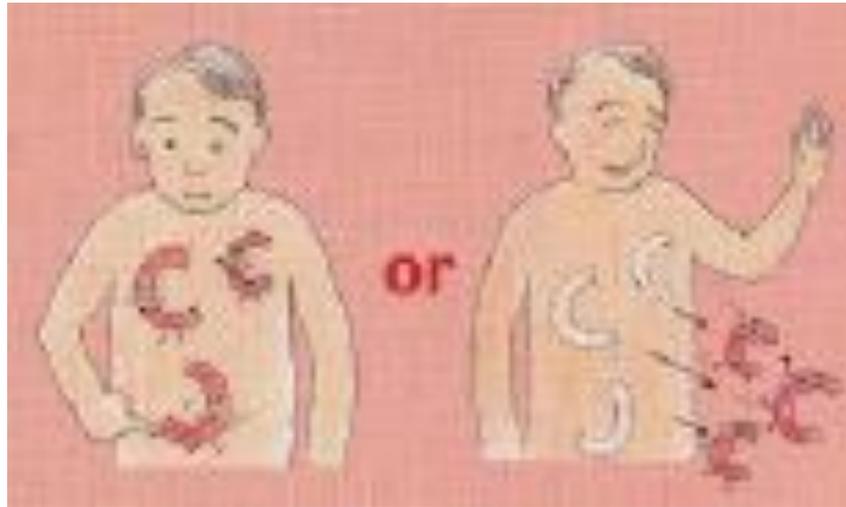
- **Diagnostic Test –**

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- **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

HCV Core Antigen – Genotype Detection

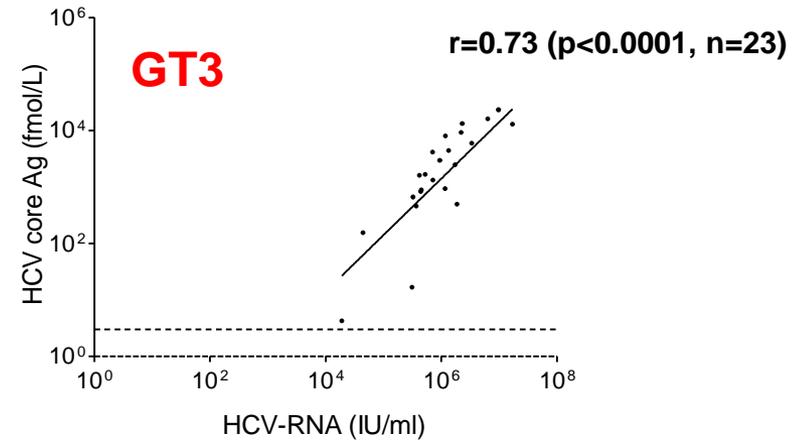
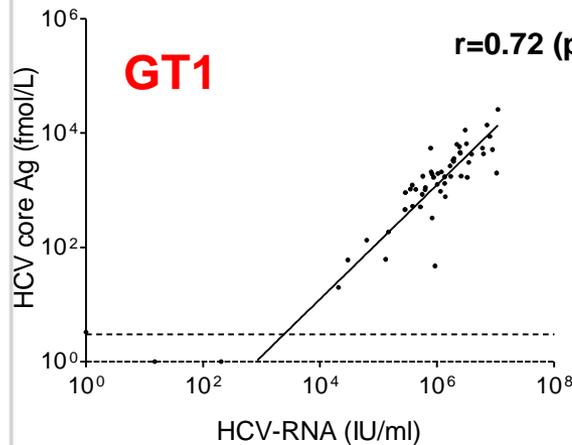
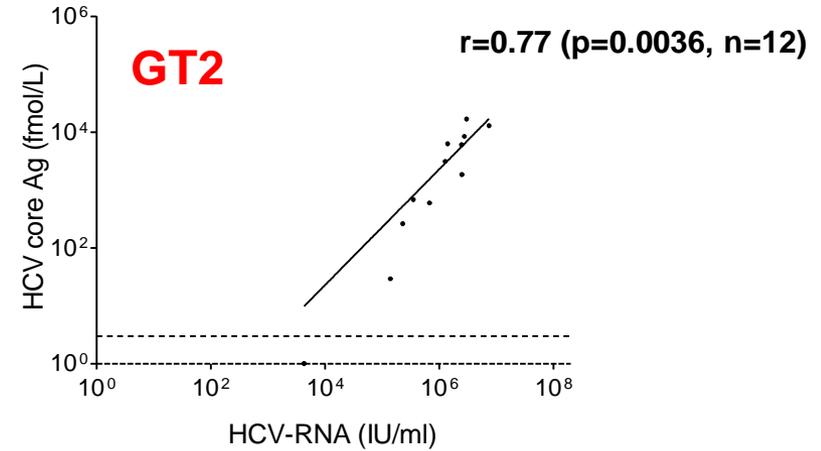
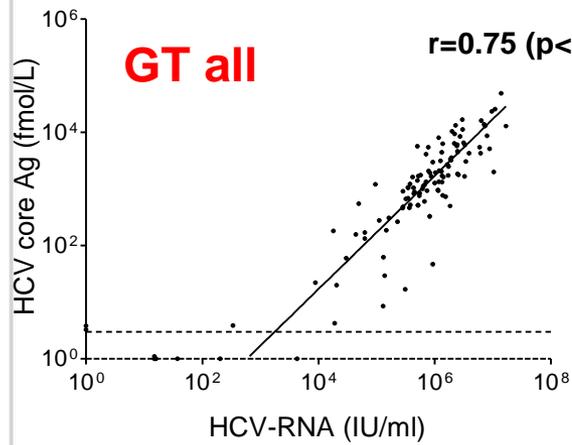
Genotype	# Specimens	% Detection	
		ARCHITECT HCV Core Ag	Amplicor Monitor 2
1	3	100% (3/3)	100% (3/3)
1a	53	100% (53/53)	100% (53/53)
1a/1b	10	100% (10/10)	100% (10/10)
1b	42	100% (42/42)	100% (42/42)
2	1	100% (1/1)	100% (1/1)
2a	2	100% (2/2)	100% (2/2)
2a/2c	4	100% (4/4)	100% (4/4)
2b	20	100% (20/20)	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)
Total	197	99.5% (196/197)	98.0% (193/197)

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

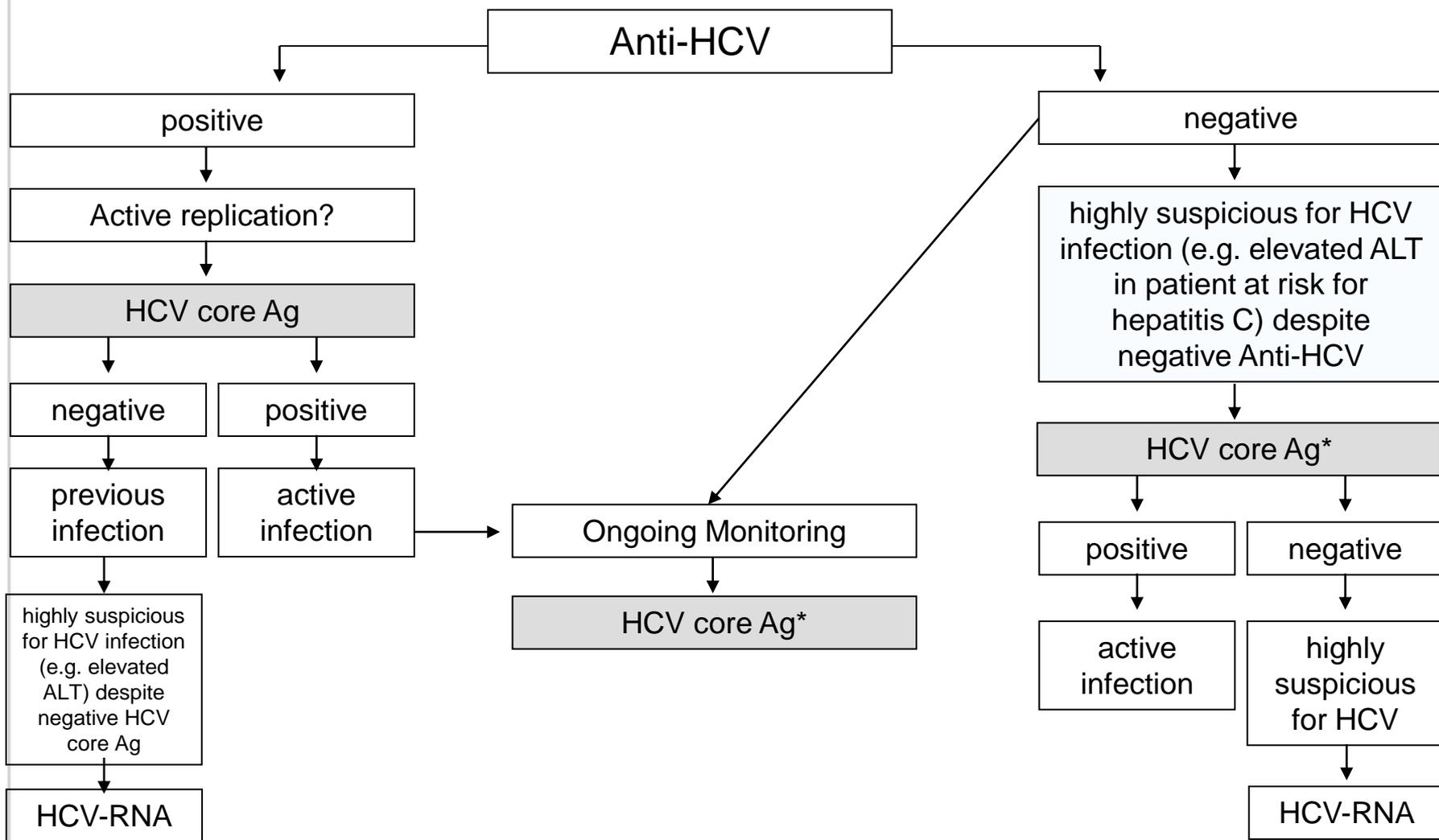
Medici et al., J Clin Virol (2011)

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



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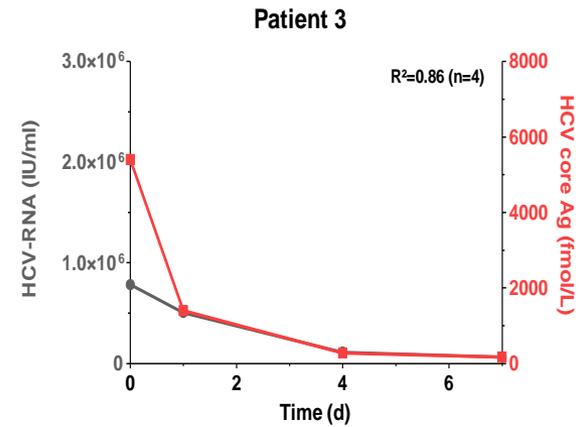
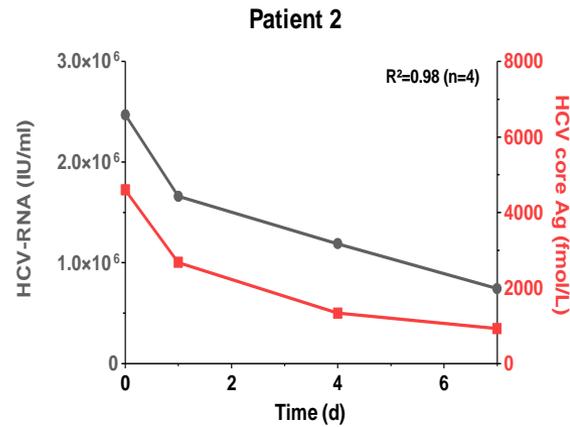
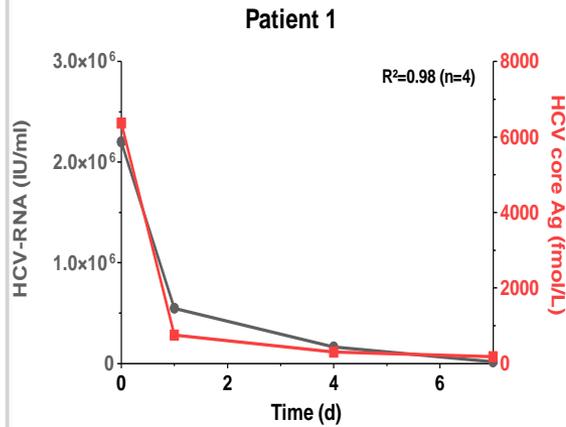
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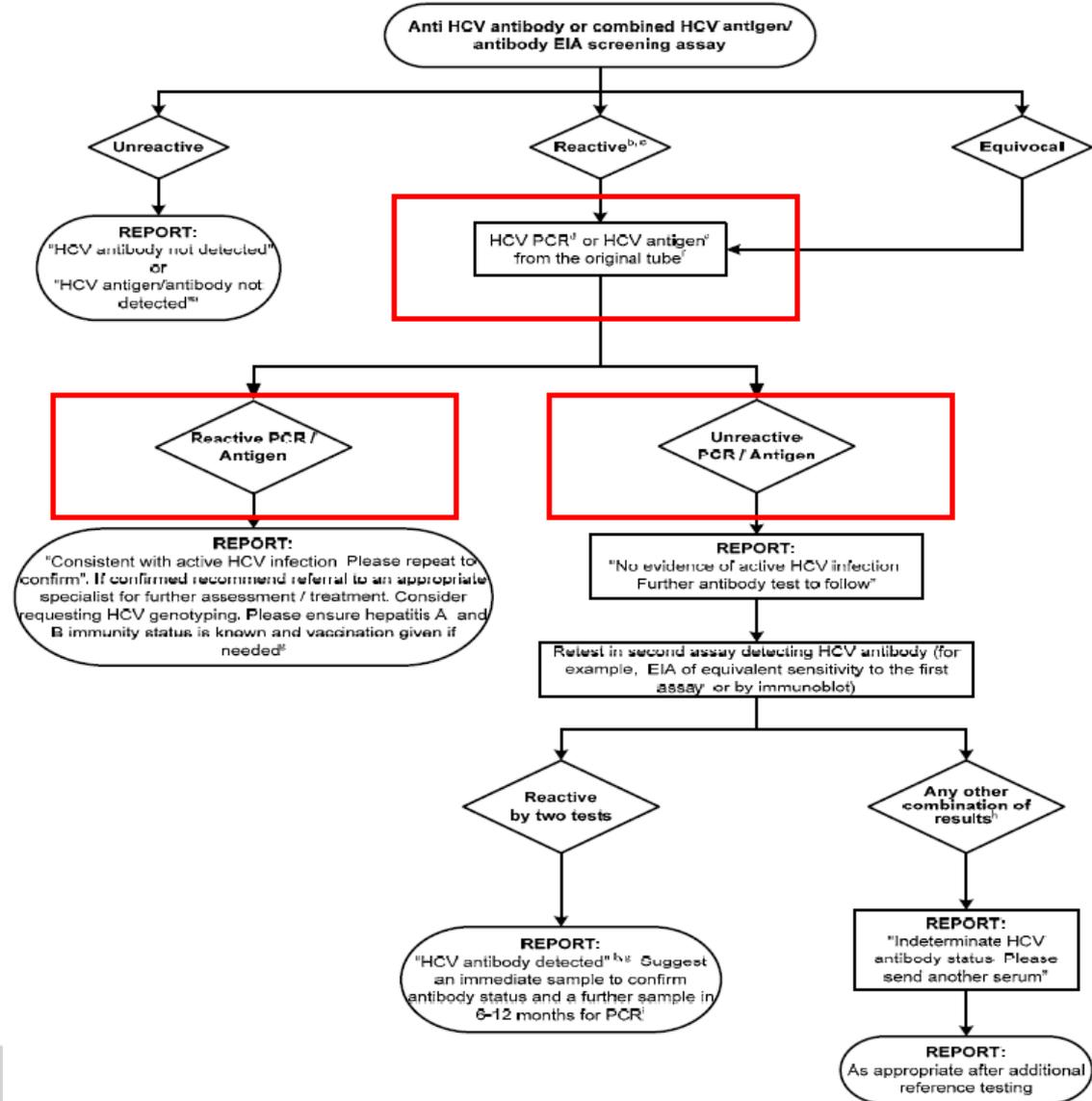
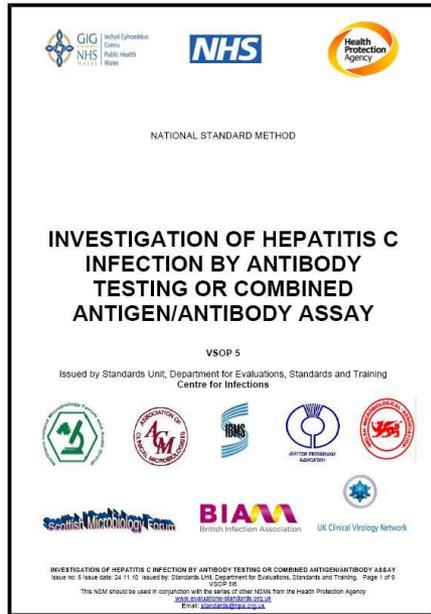
- **Monitoring Antiviral Therapy – to complement NAT**

- 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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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, anti-HBc, anti-HBs, anti-HCV, HCV Ag (Abbott Architect), HCV PCR x 2 (2 weeks apart), HIV Ag/Ab ALT			
Anti-HCV negative, HCV Ag/HCV PCR negative		ALT	Anti-HCV, HCV Ag (Abbott Architect) **	HCV PCR**
Anti-HCV positive, HCV Ag/HCV PCR negative		HCV Ag 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

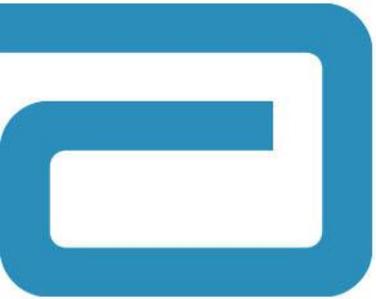
Annual HBsAg, anti-HCV, HCV Ag (Abbott Architect) and HIV Ag/Ab

Summary

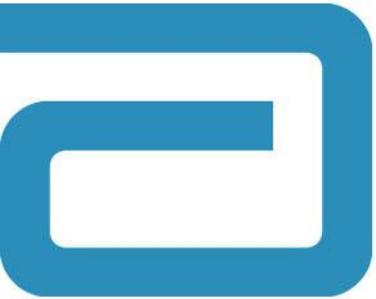
HCV Core Antigen testing can be utilized to:

- 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

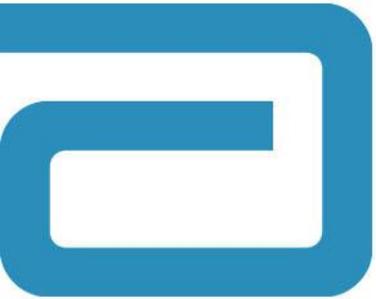
Will HCV Core antigen be adapted as standard of care for HCV infection?



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Thank You!