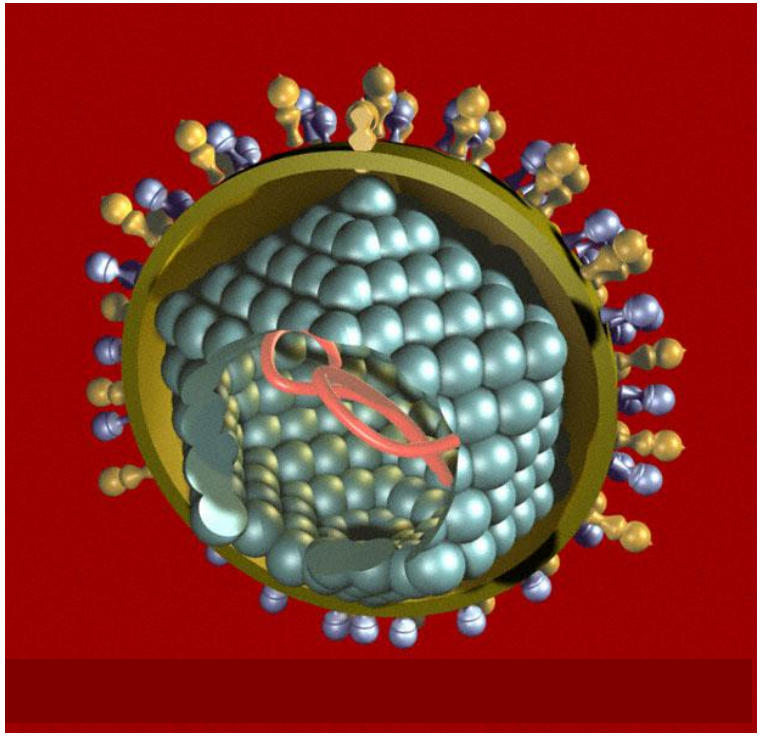
Hepatit C enfeksiyonlarında yeni bir laboratuvar testi: HCV kor (özyapı) antijeni

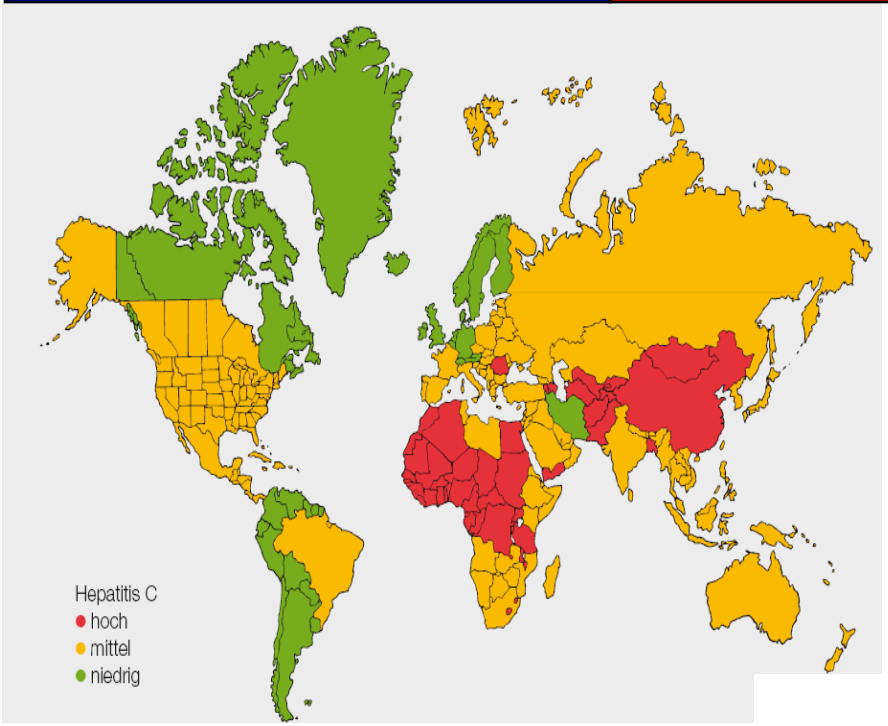


Yrd. Doç. Dr. Koray Ergünay *MD PhD*

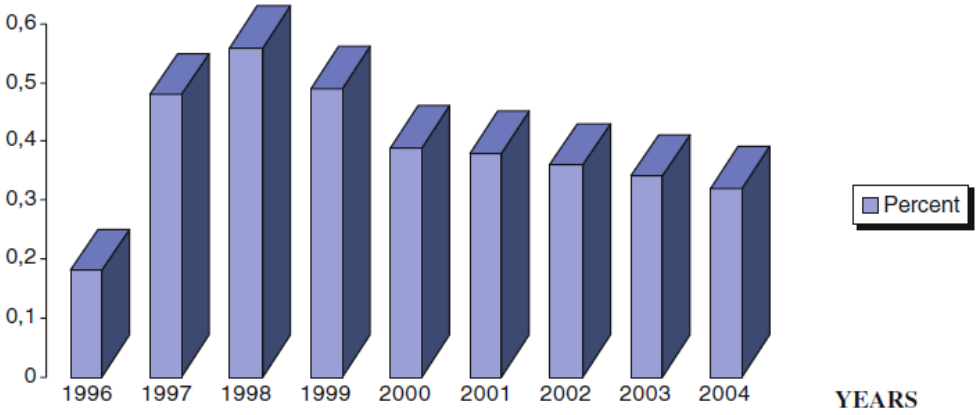
Hacettepe Üniversitesi Tıp Fakültesi

Tıbbi Mikrobiyoloji AD. Viroloji Ünitesi

Hepatit C Virus: Epidemiyoloji



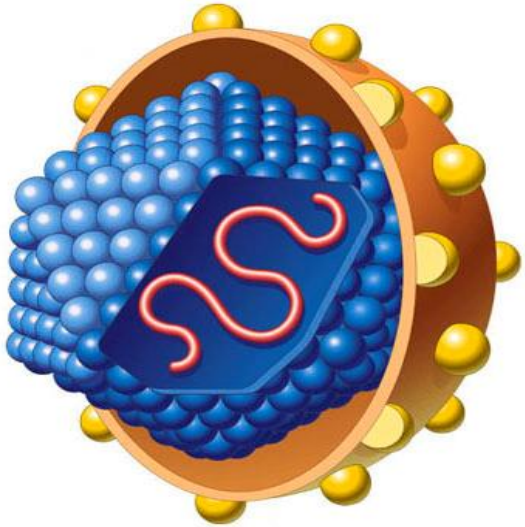
- Global sağlık sorunu (DSÖ)
- ~ 227 milyon enfekte kişi
- ~150 milyon kronik enfeksiyon
- Yıllık insidans: 30.000
- Yıllık prevalans % 0,5 - 10
- Kan donörlerinde yıllık prevalans
  - İskandinav ülkelerinde % 0,23
  - İtalya'da % 1,15
  - Türkiye: ~ % 0.38

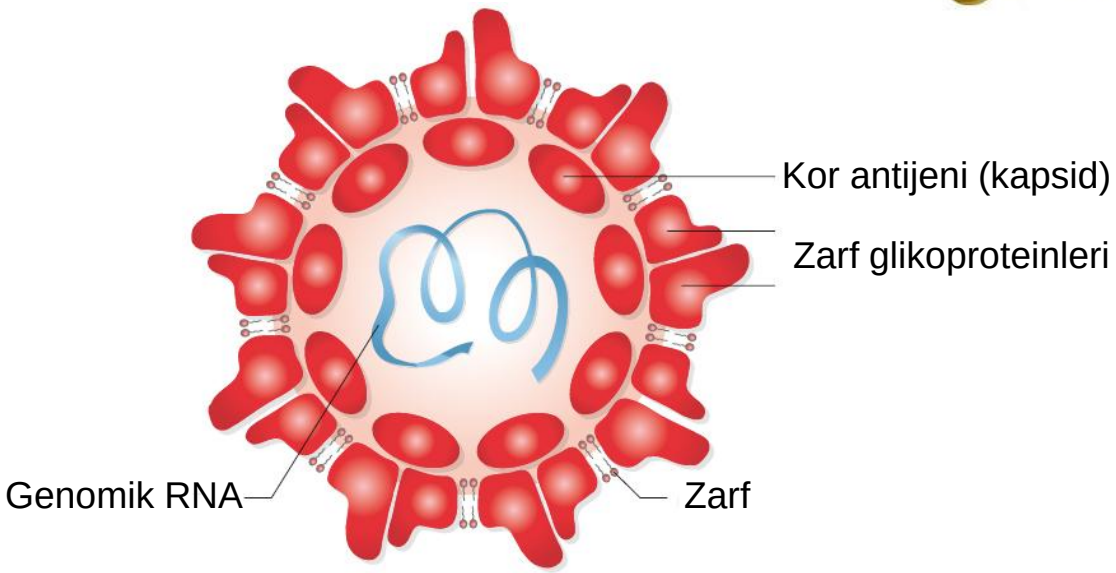


Emekdas et al., Eur J Epidemiol, 21:299-305, 2006

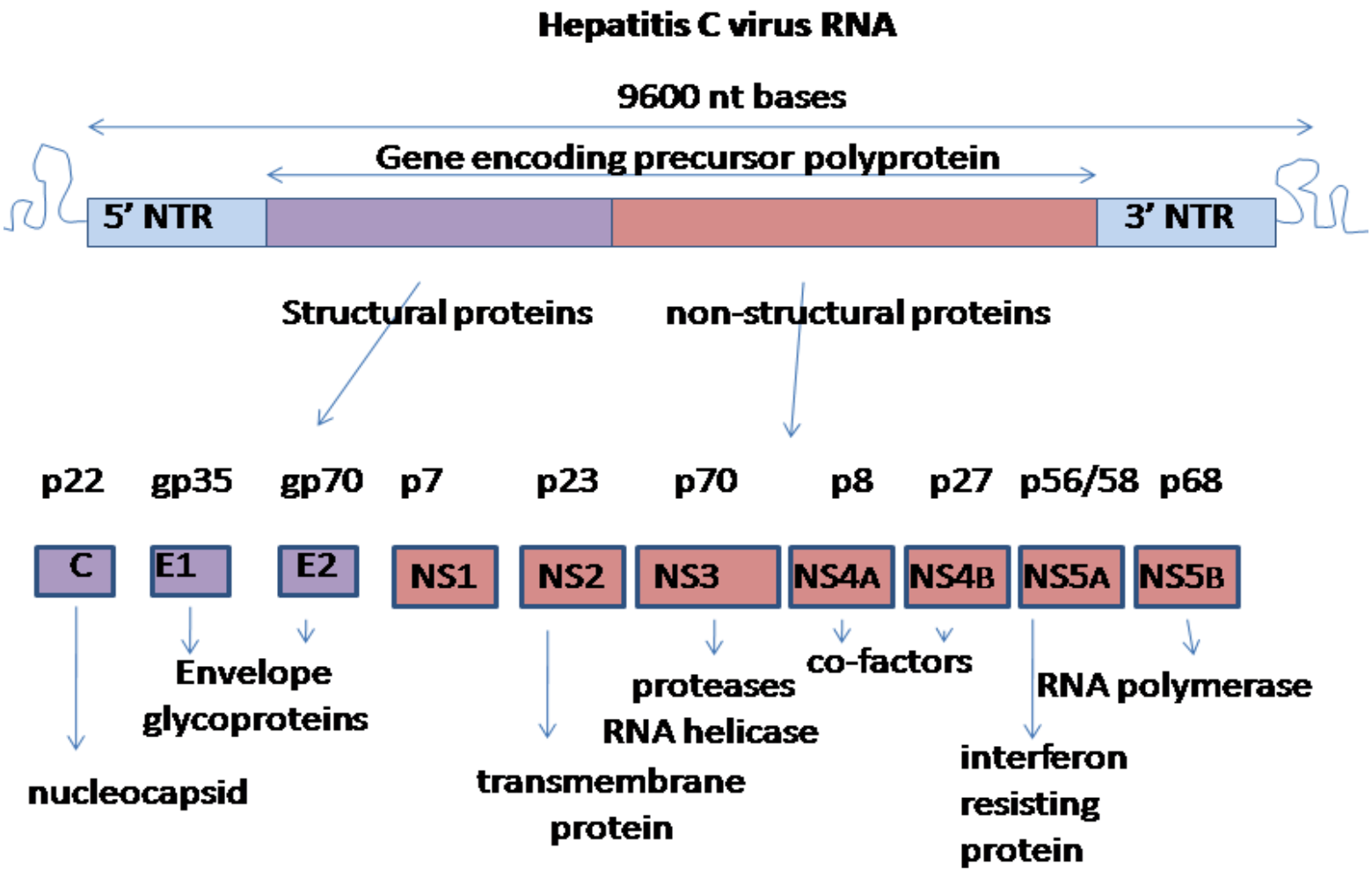
Hepatit C Virus: Yapı ve Sınıflandırma

- 1989: Non-A Non-B Hepatit etkeni
- Zarflı, 55-65 nm çaplı, pozitif tek iplikli RNA virusu,
- *Flaviviridae*, Hepacivirus

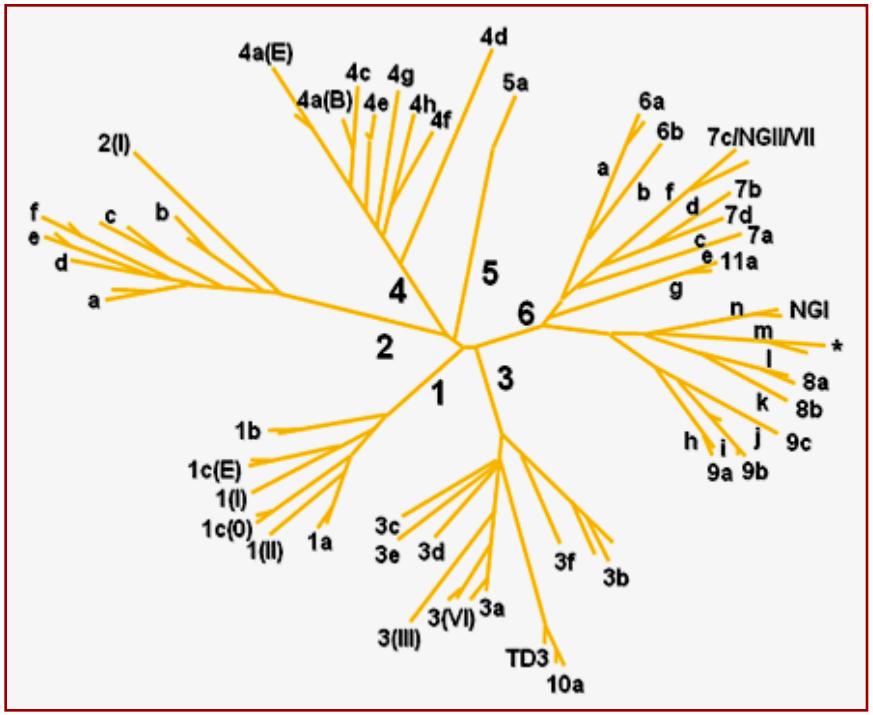




Hepatit C Virus: Genom ve Viral Proteinler



Hepatit C Virus: Genetik Çeşitlilik

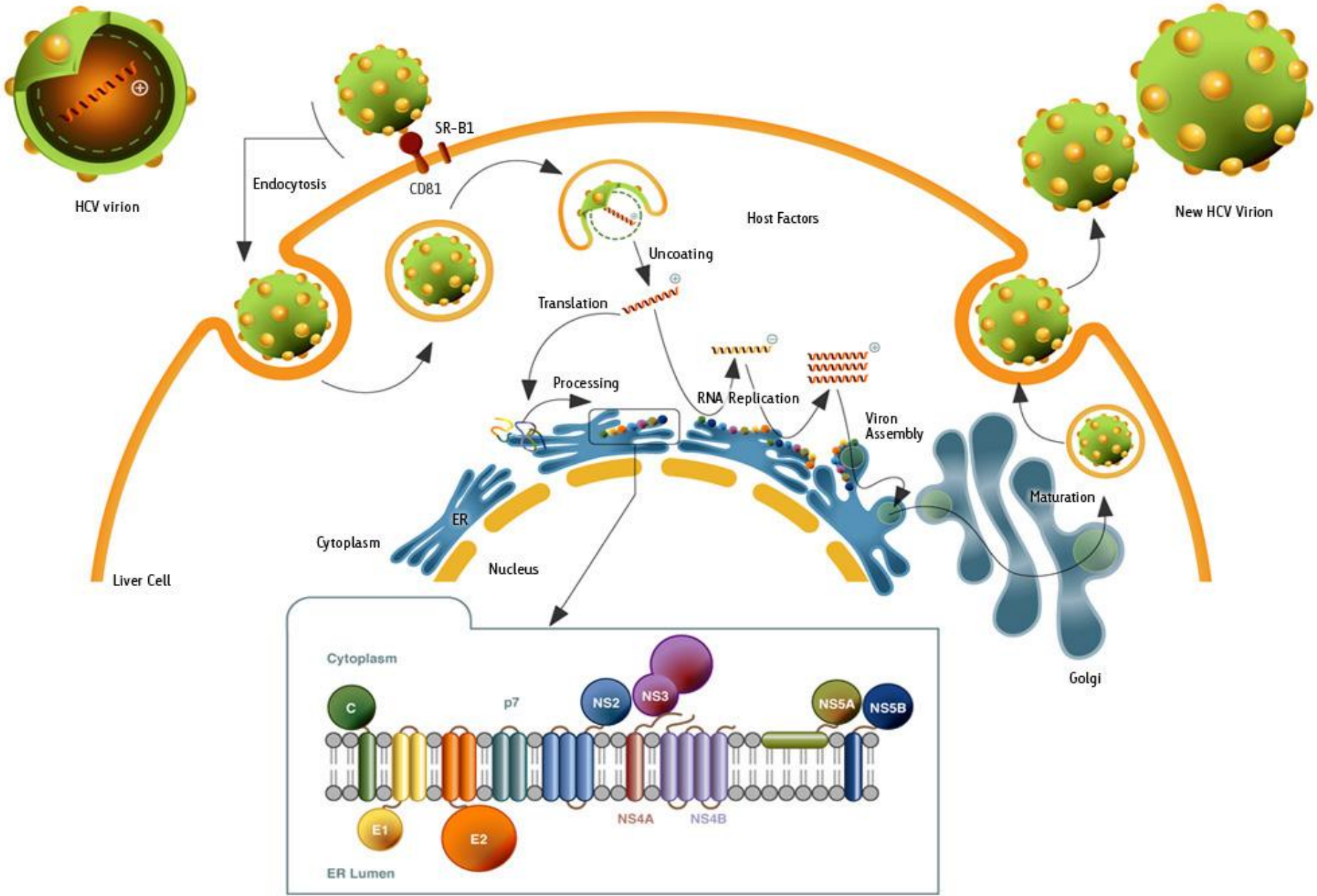


- 6 ana HCV genotipi (1-6) ve birçok alttip
- Genotip dağılımı bölgesel farklılıklar gösterir

Ülke	Baskın Genotip/alttip
ABD/Kanada	1a, 1b, 2a, 2b, 3a
Latin Amerika	1a, 1b, 2, 3a
Avrupa	1a, 1b, 3a
Kuzey ve Orta Afrika	4
Güney Afrika	5a, 1, 2, 3
Japonya, Tayvan, Çin	1b, 2a, 2b
Vietnam, Tayland	6, 1b, 2
Türkiye	1b, 1a, 2,3,4

Genotipler arasında interferon duyarlılığı açısından fark vardır!!!
(örn. Genotip 1 diğerlerine göre tedaviye daha dirençlidir)

Hepatit C Virus: Replikasyon



Hepatit C Virus: Bulaşma

Perkütan yol

- İğne batması, iğne paylaşımı
- Hemodiyaliz
- İnsan ısırığı
- Transplantasyon
- Kan ve kan ürünlerinin transfüzyonu
  (Avrupa ülkelerinde risk: < 1/5 milyon)
- Akupunktur, Dövme, Piercing

Seksüel yol (nadiren)

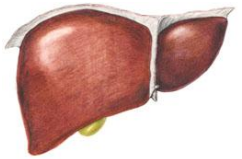
Perinatal yol (nadiren)

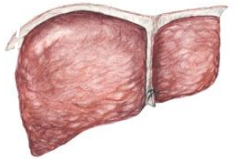
Nosokomiyal

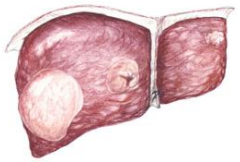
Hepatit C Virus: Klinik

~ % 25 Semptomatik ← **HCV enfeksiyonu** → **~ % 75 Asemptomatik**

- % 20 – 50 Virusun temizlenmesi
- % 50 – 80 Persistan enfeksiyon
  - ↓
  - ~ % 60 Kronik Hepatit
  - ↓ 10 – 30 yıl
  - % 50 – 80 Siroz
  - ↓ 2 - 10 yıl
  - Hepatoselüler Karsinom (yılda %1 – 5)





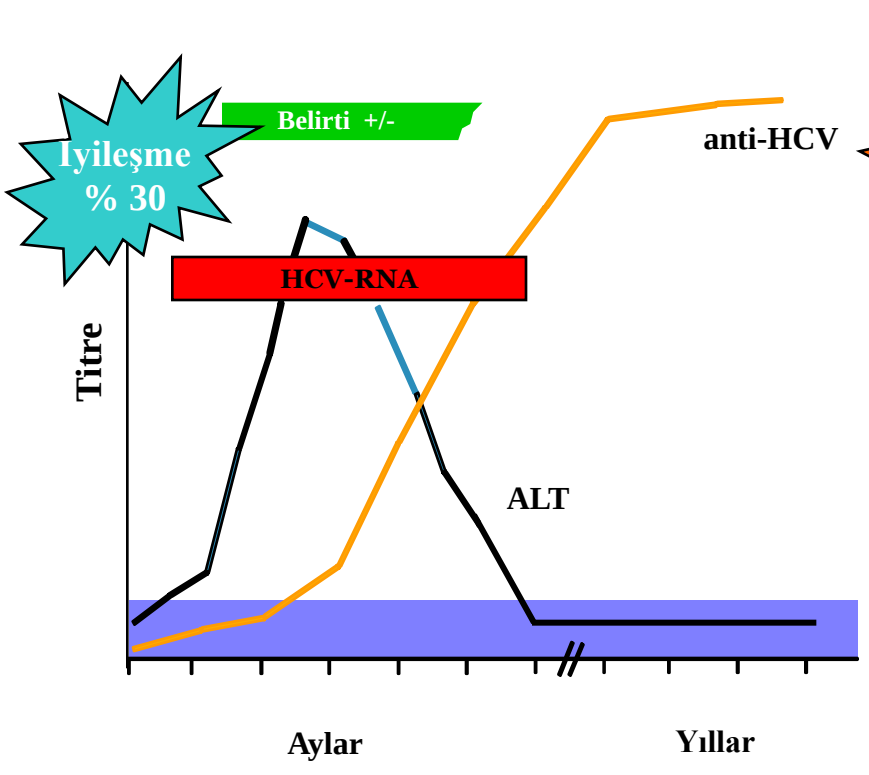


- **İnkübasyon: 2 – 26 hafta (sıklıkla 6-7 hafta)**
- **Çoğunlukla belirtisiz veya özgül olmayan belirtiler**

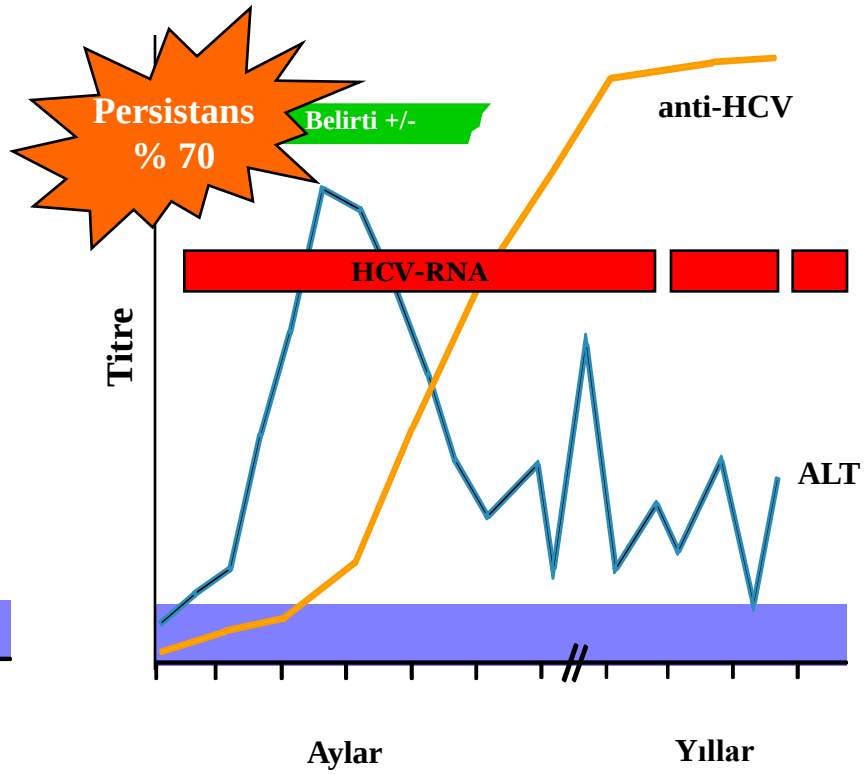
Hepatit C Virus: Laboratuvar Tanısı

1. Anti-HCV antikorlarının saptanması (ELISA, diğer yöntemler)

2. Viral RNA'nın saptanması ve kantitasyonu (NAT)

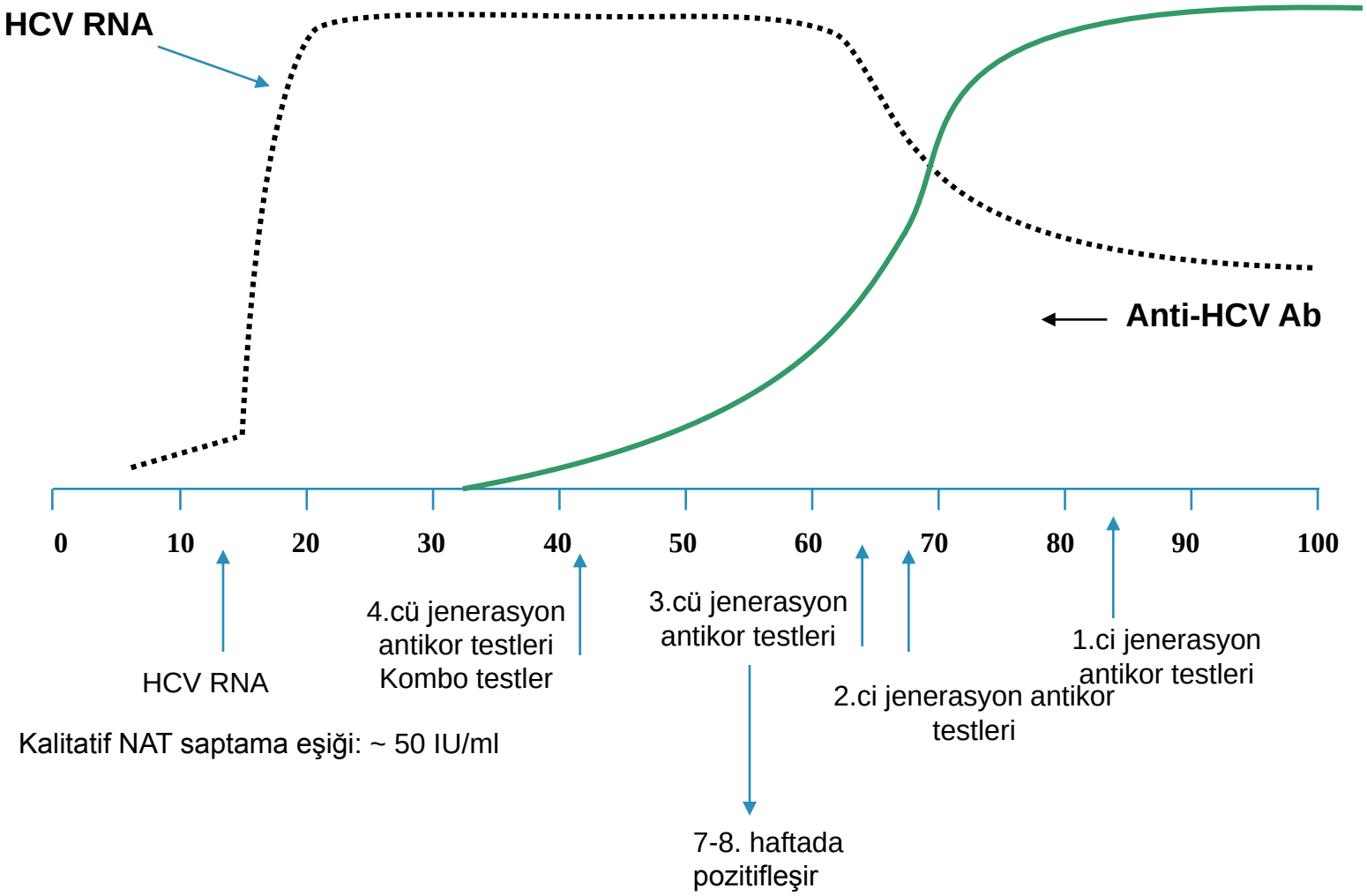


Akut enfeksiyon ve iyileşme



Kronik enfeksiyon

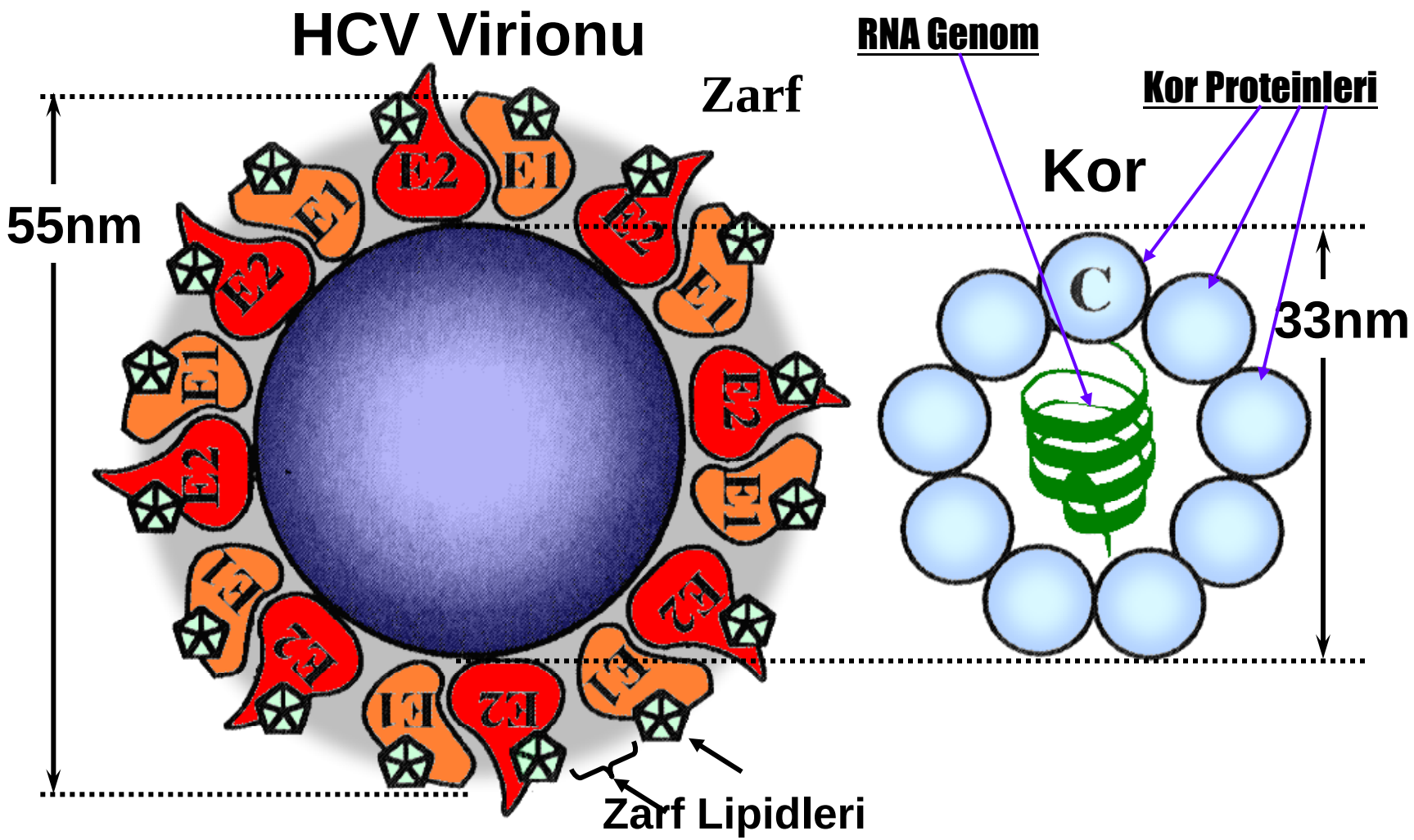
HCV'de Tanı: Laboratuvar Testleri



HCV'de Tanı: Testlerin Değerlendirilmesi

Anti-HCV	HCV RNA	Değerlendirme
Negatif	Negatif	Enfeksiyon yok
Pozitif	Pozitif	Aktif enfeksiyon (akut veya kronik)
Negatif	Pozitif	• Enfeksiyonun erken dönemi • İmmün sistemi baskılanmış kişilerde enfeksiyon
Pozitif	Negatif	• Geçirilmiş enfeksiyon • Düşük ya da geçici viremi izlenen kronik enfeksiyon • Yalancı pozitif anti-HCV sonucu (doğrulama testleri) • Pasif olarak aktarılmış antikor varlığı

HCV kor antijeni: Yapı ve Özellikler



HCV kor antijeni: Tanıda Kullanım

Kor proteinlerinin; *kalitatif* veya *kantitatif* olarak,
"*in house*" veya *ticari* testlerle saptanması

1992- 1994 : **İlk "in house" test (Japonya; Takahashi et al., Moriya et al.)**

1995-1996 : **Geliştirilmiş core antijen testi (Tanaka et al., Kashiwakuma et al.)**

1996: **İlk klinik testler**

1998-2002: **Kapsamlı klinik testler - ticari testler için ön veriler**

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2001, p. 3110–3114 Vol. 39, No. 9
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.9.3110–3114.2001

Novel Approach To Reduce the Hepatitis C Virus (HCV) Window Period: Clinical Evaluation of a New Enzyme-Linked Immunosorbent Assay for HCV Core Antigen

GIANCARLO ICARDI,[1]* FILIPPO ANSALDI,[1] BIANCA MARISA BRUZZONE,[1] PAOLO DURANDO,[1] STEPHEN LEE,[2] CARLA DE LUIGI,[3] AND PIETRO CROVARI[1]

HCV kor antijeni: Ticari Testler

1. Jenerasyon:

HCV Core antigen ELISA Test (Ortho Clinical Diagnostics, NJ, ABD)

Kalitatif, 2 farklı monoklonal antikor,
96 çukurlu ELISA test formatında

Dezavantaj: **sadece Anti-HCV negatif olduğu aşamada saptama – serokonversiyon öncesi dönem !!!**

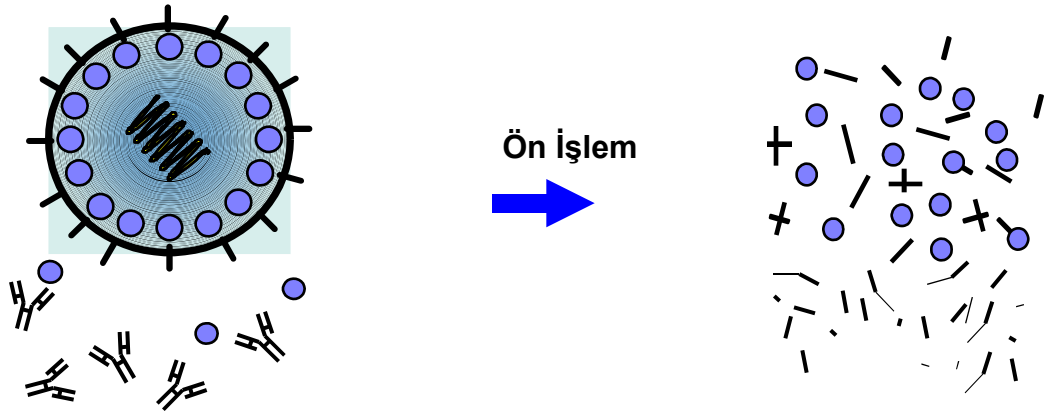
HCV kor antijeni: Ticari Testler

2. Jenerasyon:

Total HCV Core antigen ELISA test system
veya trak-C assay (Ortho Clinical Diagnostics, NJ, ABD)

Kantitatif, 4 farklı monoklonal antikor
96 çukurlu ELISA test formatında

- **Virionları parçalayıp antijenleri ortaya çıkaran,**
- **Anti-(kor) HCV antikorlarının, antijenden ayrıştırılmasını sağlayan ön işlem prosedürü...**



Serokonversiyon öncesine ek olarak antikor varlığında kullanılabilme imkanı!!!

HCV kor antijeni: Ticari Testler

2. Jenerasyon:

- **Lumispot EIKEN HCV antigen assay (Eiken Chemical Diagnostics, Tokyo, Japonya)**

  Trak-C ile benzer özelliklere sahip, otomatize sistem

- **Ortho IRMA HCV Core assay (Ortho Clinical Diagnostics, NJ, ABD)**

  İmmünoradyometrik saptama

- **Lumipulse HCV antigen assay (Ortho Clinical Diagnostics, Tokyo, Japonya)**

  Trak-C'den daha geniş dinamik aralık

2. jenerasyon testler, 1. jenerasyona göre belirgin olarak daha duyarlıdır

HCV kor antijeni: Ticari Testler

Abbott Laboratuvarları (IL, ABD)

Kor antijeni ve Anti-HCV'nin eşzamanlı tespiti için sistem

Shah et al, 2003

Kor antijeninin kalitatif ve otomatize olarak tespiti için sistem

Muerhoff et al, 2002

ARCHITECT HCV Ag assay

HCV kor antijen testleri: Genel Performans

Enfeksiyon varlığı, Anti-HCV antikoru saptanmasına göre daha erken belirlenebilmektedir.

Serokonversiyon öncesi klinik duyarlılık NAT'a yakındır

HCV RNA testlerinden daha ucuzdur, özel laboratuvar ve personel gerektirmez.

Örnekteki antijen, viral RNA'ya göre daha dayanıklı olduğundan, analitik stabilite daha yüksektir.

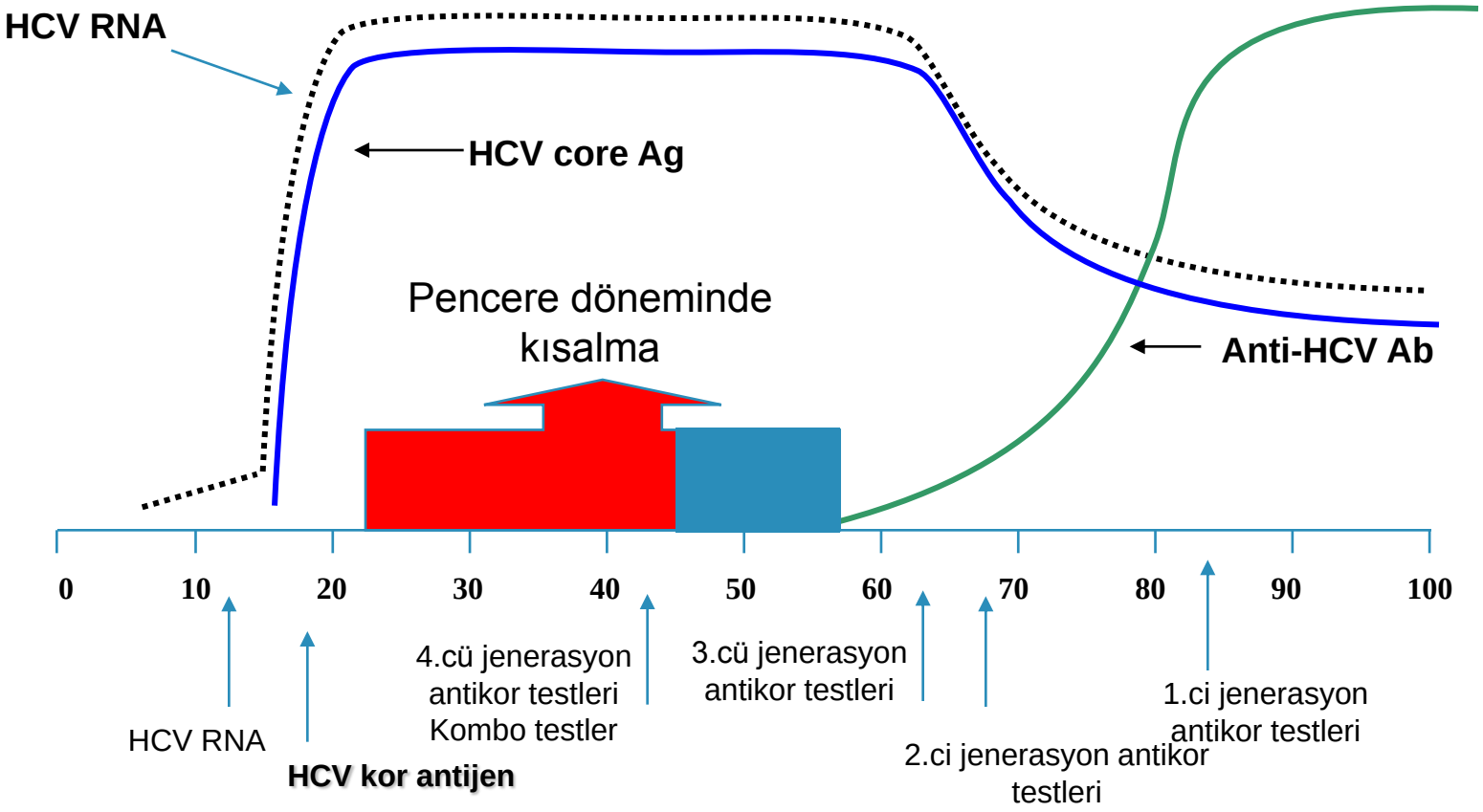
Enfeksiyon modellerinde tanısal açıdan benzer etkinlik göstermektedir.

Sorun: Düşük viral yük izlenen örnekler

HCV RNA ile uyum bozulur

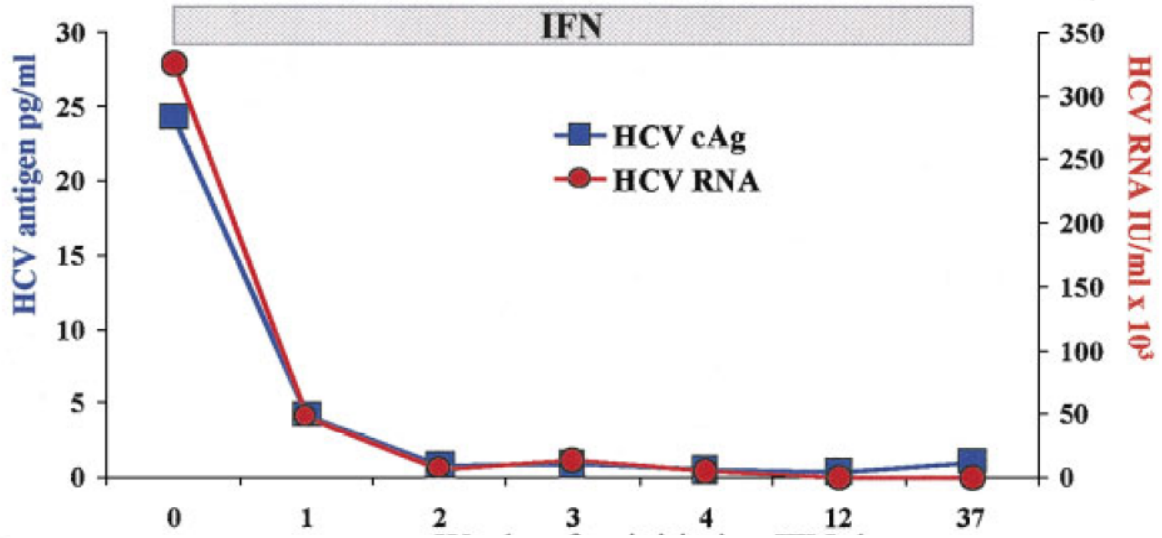
Kor proteini izoformları - mutasyonlar - genotipler???

HCV kor antijen testleri: İzleme Etki

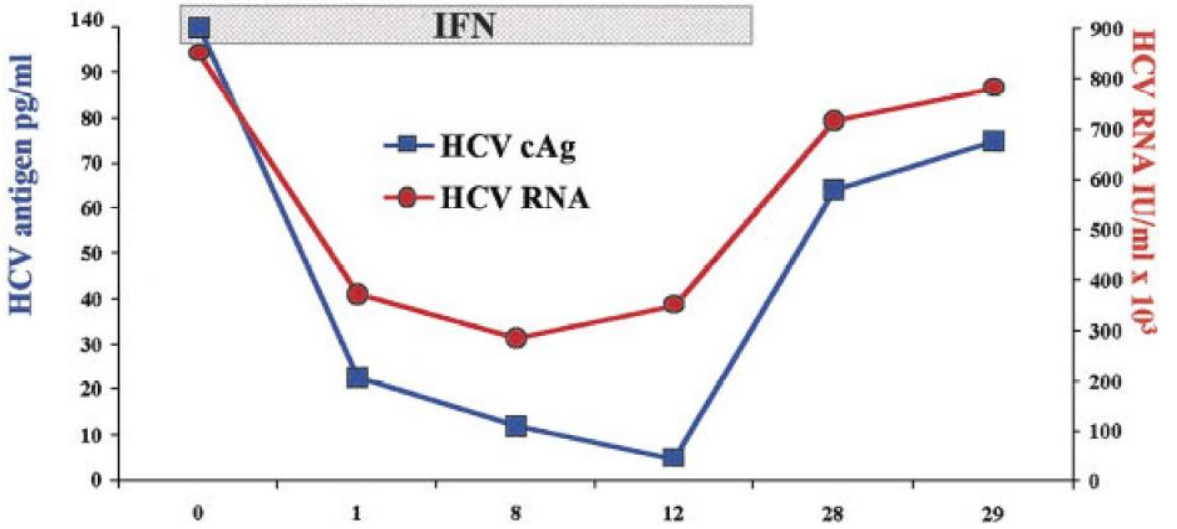


Kantitatif antijen düzeyleri, HCV RNA ile uyumludur...

Tedavi yanıtında profil



Tedavi yanıtsızlığında profil



INF terapisi sonrası hafta

Zanetti et al., J Med Virol, 2003

HCV kor antijen testleri: Uygulama Alanları

Kan bankacılığı - transfüzyon öncesi tarama
(Anti-HCV yerine/birlikte)

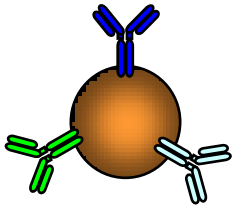
Transfüzyon harici taramalar
(artmış HCV riski içeren durumlar: iv madde, hemodiyaliz hastaları, HIV+)

Anti-HCV pozitif, tedavi almayan kişilerde aktif replikasyon belirteci olarak
(HCV RNA yerine/birlikte)

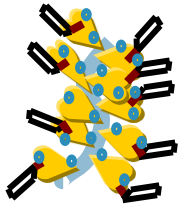
Tedavi alan enfekte kişilerde antiviral tedavinin izleminde kantitatif belirteç olarak
(HCV RNA yerine/birlikte)

ARCHITECT HCV Antijen Testi:

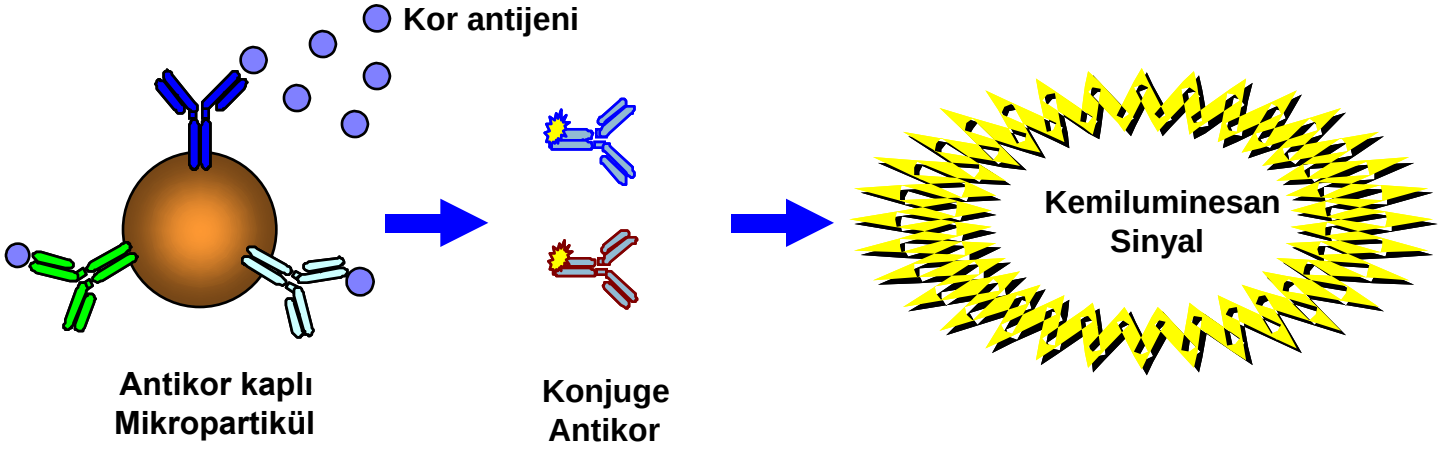
Monoklonal Anti-HCV antikorlarıyla kaplı mikropartiküller ile kemiluminesan immunoassay



Mikropartikül
- c11-3 Monoklonal antikor
- c11-7 Monoklonal antikor
- AOT-3 Monoklonal antikor



Konjugat (Dextran BSA - SPSP Acridinium Ester - Fab')
- c11-9 Fab' Monoklonal antikor
- c11-14 Fab' Monoklonal antikor



Ön işlemler dahil tam otomatik uygulama

Artmış duyarlılık : 0.06 pg/ml (önceki testlerden 16-25 kat yüksek)

ARCHITECT HCV Ag: Serokonversiyon

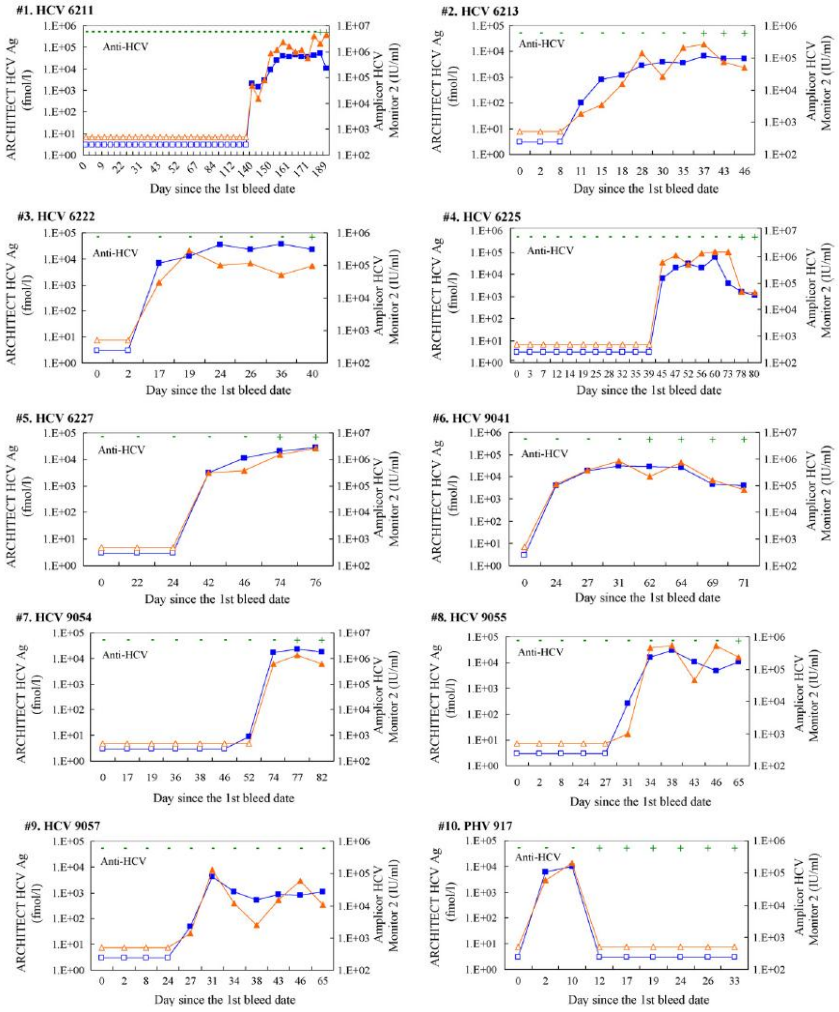


Table 2
Summary of seroconversion panel detection of the HCV Ag assay.

	Panel	Genotype*	Day first detected				Antibody/HCV Ag differential (number of days)	HCV Ag/HCV RNA differential (number of days)
			Antibody*	HCV RNA		HCV Ag		
				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

*Abbott AxSYM Anti-HCV.

Pencere döneminde kısalma: 23-65 gün (ortalama:35.8)

HCV NAT'a eşdeğer performans (RT-PCR)

Morota et al., J Virol Methods, 157(1):8-14, 2009

ARCHITECT HCV Ag: Analitik Duyarlılık

TABLE 1. Impact of HCV genotypic variability on analytical sensitivity of Architect HCV Ag assay

HCV subtype	Analytical sensitivity (fmol/liter)[a]	95% CI (fmol/liter)
1a	5.3	3.8–8.3
1b	3.5	2.5–5.3
2a	13.5	9.9–20.2
3a	3.9	2.8–6.0
4a	4.2	3.1–6.5
5a	7.0	4.9–11.0
6f	7.0	4.9–11.0

HCV genotip 1,3,4,5,6: < 10 fmol/lt
HCV genotip 2: ~ 15 fmol/lt

~500-3000 IU/ml RNA

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

ARCHITECT HCV Ag: Serokonversiyon

TABLE 2. Performance of Architect HCV Ag assay with serum samples from seroconversion panels

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

[a] Antibody (Ab) determined by the Architect anti-HCV assay (Abbott Diagnostics).
[b] Antigen and antibody (Ag-Ab) determined by the Monolisa HCV Ag-Ab Ultra assay (Bio-Rad).
[c] Core antigen (Ag) determined by the Architect HCV Ag assay (Abbott Diagnostics).
[d] HCV RNA determined by the Versant HCV RNA qualitative (TMA) test (Siemens Diagnostics).
[e] Zero represents the first sample from the panel.

Pencere döneminde kısalma: 4-16 gün

HCV NAT'a eşdeğer performans (TMA)

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

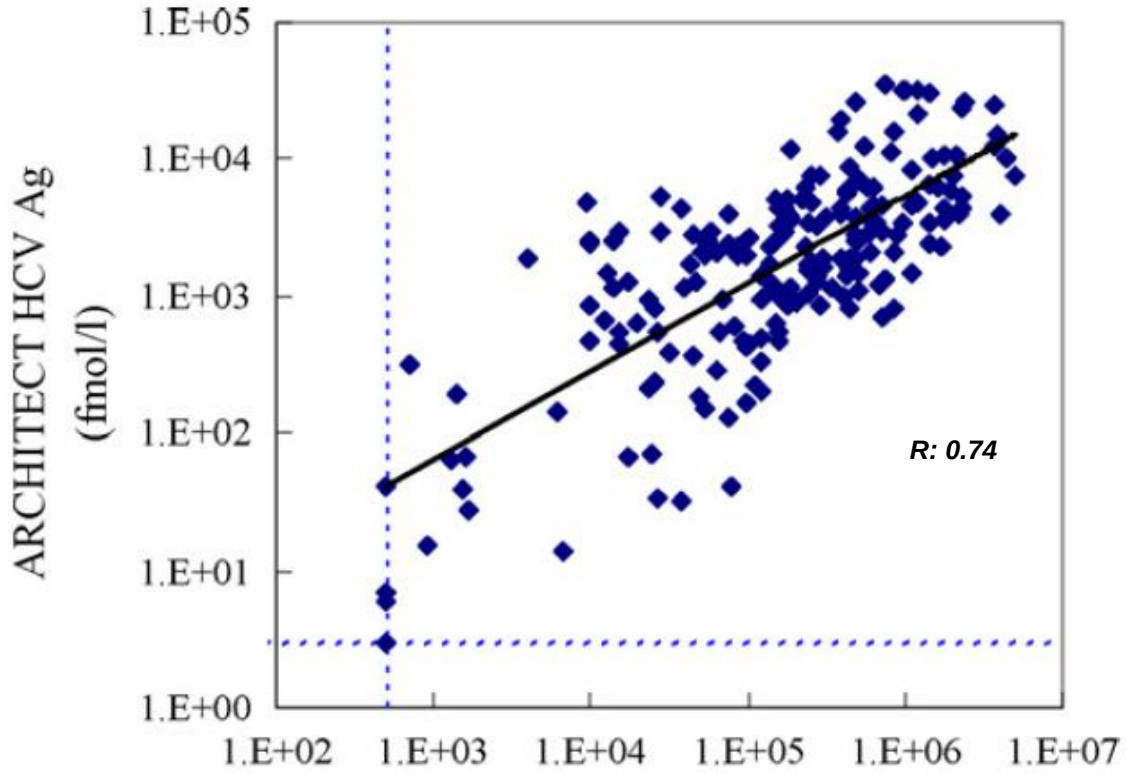
ARCHITECT HCV Ag: Genotip Duyarlılığı

Table 1
Genotype detection of the HCV Ag, IRMA HCV Ag and Amplicor HCV Monitor 2 assays.

Genotype	Number of specimens	%Detection		
		ARCHITECT HCV Ag	IRMA HCV Ag	Amplicor HCV Monitor 2
1	3	100% (3/3)	100% (3/3)	100% (3/3)
1a	53	100% (53/53)	100% (53/53)	100% (53/53)
1a/1b	10	100% (10/10)	90% (9/10)	100% (10/10)
1b	42	100% (42/42)	100% (42/42)	100% (42/42)
2	1	100% (1/1)	100% (1/1)	100% (1/1)
2a	2	100% (2/2)	100% (2/2)	100% (2/2)
2a/2c	4	100% (4/4)	100% (4/4)	100% (4/4)
2b	20	100% (20/20)	100% (20/20)	100% (20/20)
3a	24	100% (24/24)	88% (21/24)	88% (21/24)
3k	2	100% (2/2)	100% (2/2)	100% (2/2)
4	1	100% (1/1)	100% (1/1)	100% (1/1)
4a	16	100% (16/16)	94% (15/16)	100% (16/16)
4a/4c	3	67% (2/3)	67% (2/3)	67% (2/3)
4c/4d	3	100% (3/3)	100% (3/3)	100% (3/3)
4c/4d/4e	1	100% (1/1)	100% (1/1)	100% (1/1)
5/5a	9	100% (9/9)	100% (9/9)	100% (9/9)
6a	2	100% (2/2)	100% (2/2)	100% (2/2)
6i	1	100% (1/1)	100% (1/1)	100% (1/1)
Total	197	99.5% (196/197)	97.0% (191/197)	98.0% (193/197)

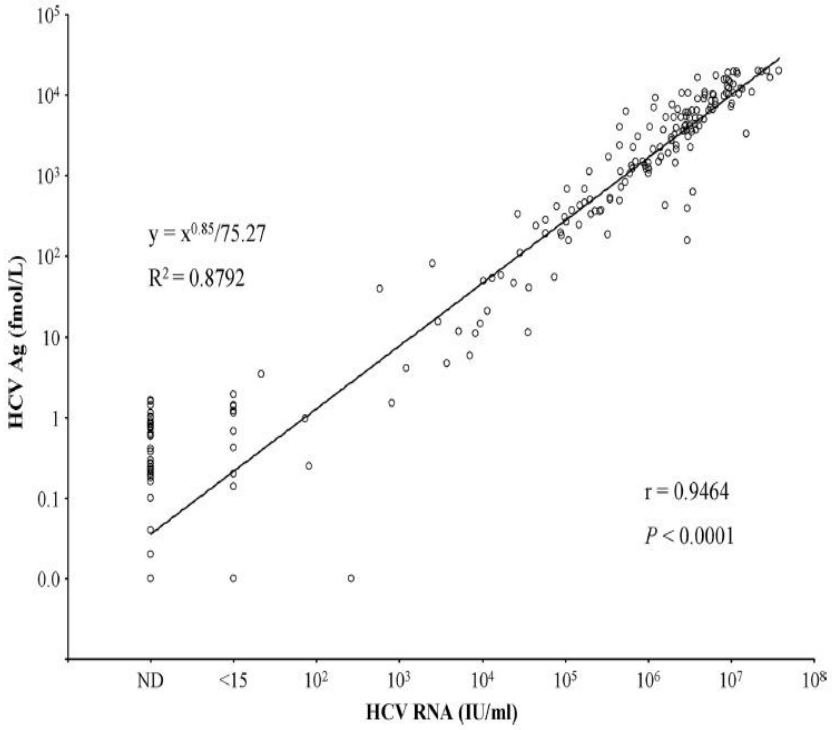
Morota et al., J Virol Methods, 157(1):8-14, 2009

ARCHITECT HCV Ag: Viral Yük ile Uyum



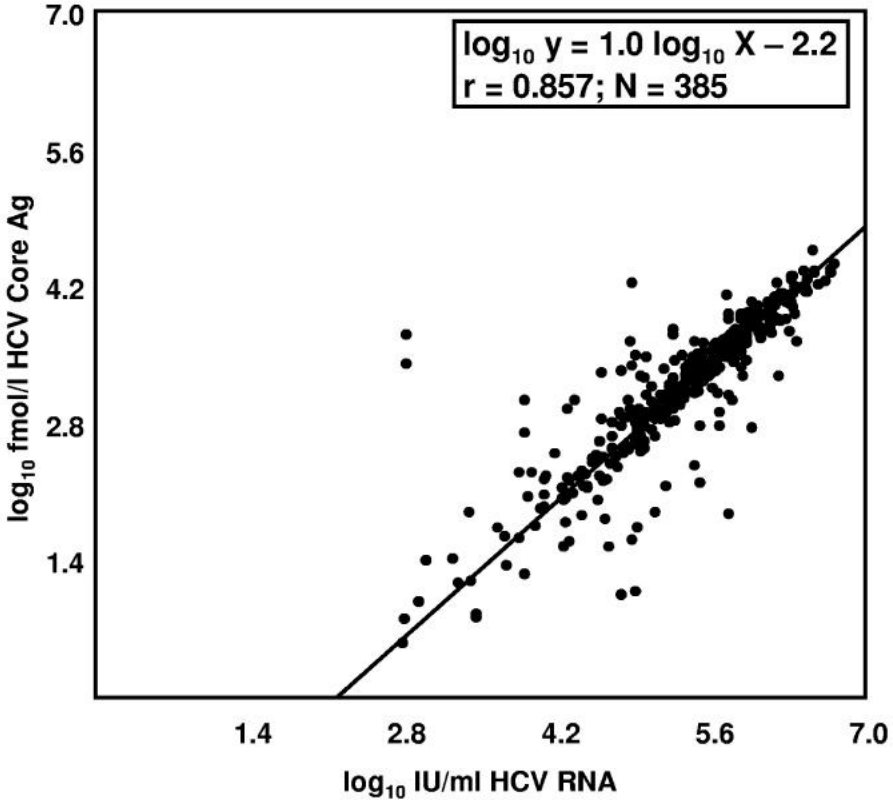
Morota et al., J Virol Methods, 157(1):8-14, 2009

ARCHITECT HCV Ag: Viral Yük ile Uyum



Roche Cobas TaqMan (Real-time PCR)

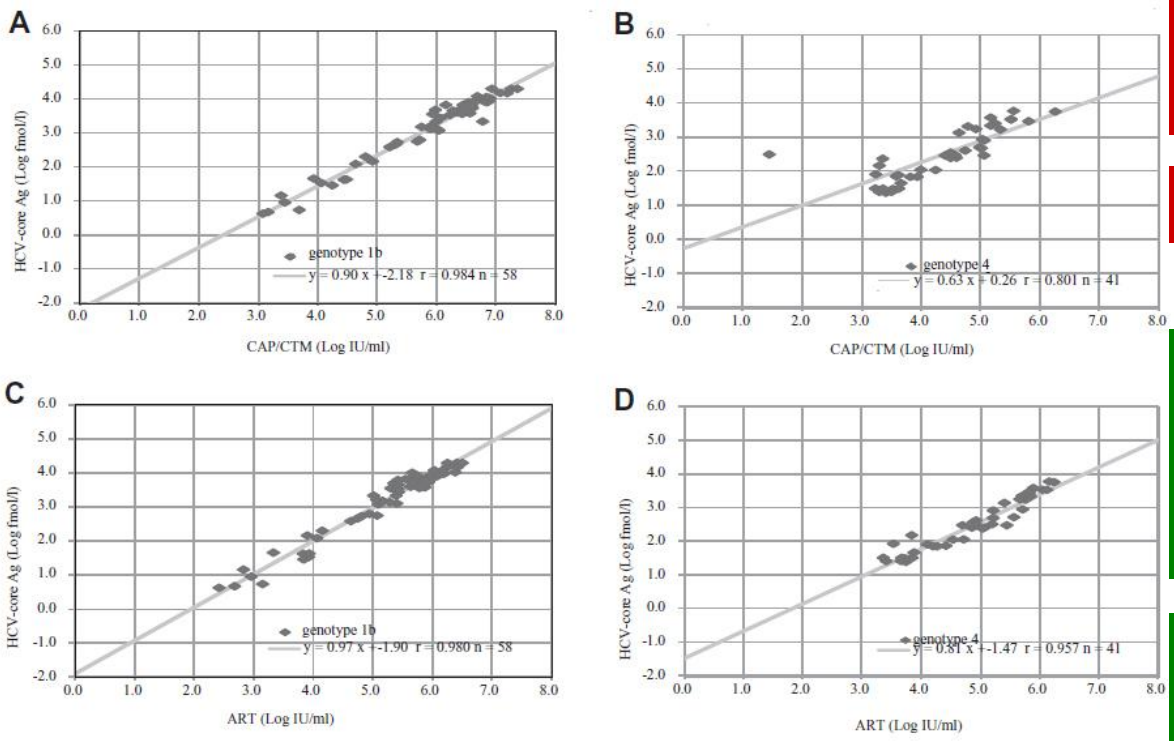
Park et al., J Clin Microbiol, 48(6):2253-6. 2010



Versant HCV RNA 3.0 (bDNA)

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

ARCHITECT HCV Ag: Viral Yük ile Uyum



Cobas Ampliprep/Cobas TaqMan (CAP/CTM); Abbott RealTime HCV (ART)

Her iki viral yük testinde genotip 1b, 2a, 2b ve 3a için korrelasyon: 0.98-0.99

genotip 4 : 0.78

genotip 1b, 2a, 2b ve 3a için viral yük-kor antijeni arasında yüksek düzey korrelasyon

genotip 4:
0.95 (ART)
0.80 (CAP/CTM)

Elkady et al., J Med Virol, 82:1878-88, 2010

ARCHITECT HCV Ag: Kantitasyonda Doğrusallık

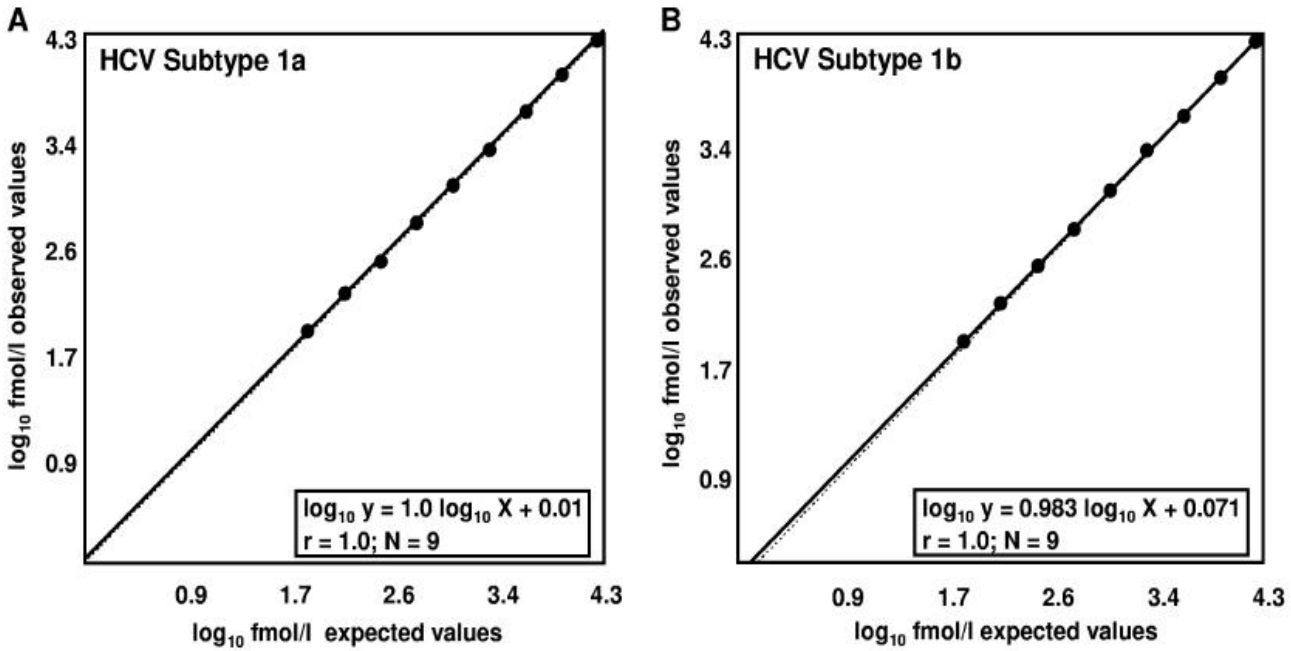


FIG. 1. Linear range of quantification by the Architect HCV Ag assay. Nine serial dilutions of two high-titer samples containing either HCV subtype 1a (A) or 1b (B) isolates were tested in triplicate in three separate runs. The observed mean values (filled circles) were plotted against the expected values (dotted lines) by using logarithmic scales.

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

ARCHITECT HCV Ag: Klinik İzlem

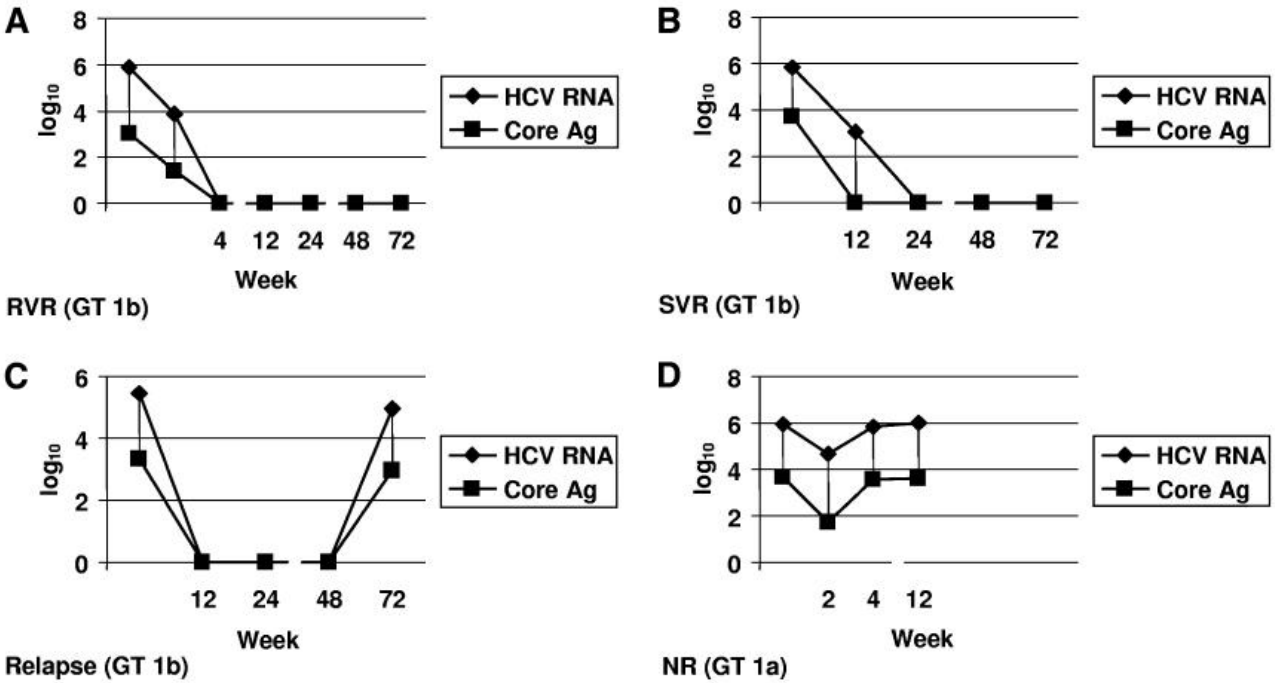


FIG. 3. Viral kinetics estimated by testing for HCV core antigen (■) and RNA (◆) in four individuals receiving antiviral combination therapy with pegylated alpha interferon and ribavirin. (A) Rapid virological response (RVR); (B) sustained virological response (SVR); (C) results for a patient who developed a relapse after the cessation of antiviral treatment; (D) results for a subject not responding to therapy. GT, HCV genotype.

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

ARCHITECT HCV Ag: Klinik İzlem

TABLE 3. Predictive values of sustained virological response to pegylated alpha interferon-ribavirin on the basis of a 2-log decrease in total HCV core antigen and HCV RNA concentrations at week 12 after initiation of antiviral treatment

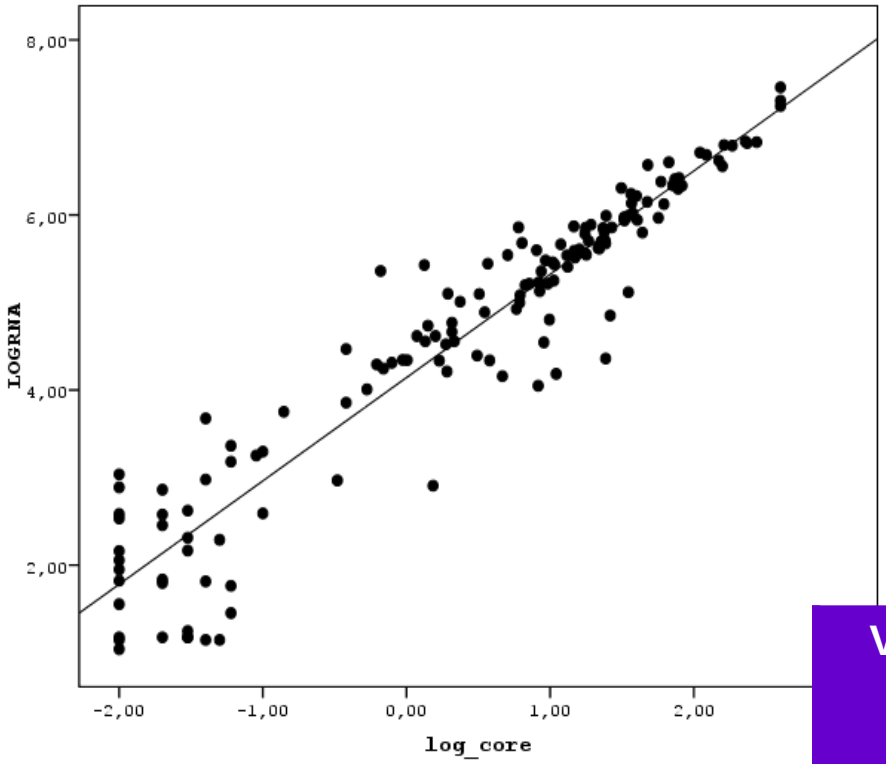
Assay target and HCV genotype	No. of specimens with the following concn changes at wk 12:		PPV[a]		NPV[b]	
	<2 log	≥2 log	Ratio	%	Ratio	%
HCV core antigen						
GT[c] 1/4 (n = 29)	9	20	9/20	45.0	9/9	100
GT 2/3 (n = 9)	1	8	7/8	87.5	1/1	100
HCV RNA						
GT 1/4 (n = 29)	9	20	9/20	45.0	9/9	100
GT 2/3 (n = 9)	1	8	7/8	87.5	1/1	100

[a] PPV, positive predictive value.
[b] NPV, negative predictive value.
[c] GT, HCV genotype.

Takipte viral yüke benzer etkinlik!!!

Ross et al., J Clin Microbiol, 48(4):1161-8. 2010

ARCHITECT HCV Ag: Hacettepe Deneyimi



R: 0.949

Viral Yük (IU/ml)	HCV kor antijeni		
	N	*Spearman' s rho*	*P*
$10^6 \geq$	30	0.891	<0.001
10^5-10^6	53	0.730	<0.001
10^4-10^5	28	0.274	0.143
10^3-10^4	8	0.659	0.076
10^2-10^3	24	0.459	0.024
15-10^2	17	0.049	0.667

Viral Yük (IU/ml)	HCV kor antijeni		Toplam
	Pozitif # (%)	*Negatif* # (%)	
$10^4 \leq$	110 (99.1)	1 (0.9)	112
10^3 - 10^4	5 (62.5)	3 (37.5)	8
10^2 - 10^3	4 (16.7)	20 (83.3)	24
1.5 x 10^{1-} 10^2	2 (11.8)	15 (88.2)	17
Toplam	121	39	160

Ergünay K et al. ASM Congress, 2010;
TMC 2010, Poster - PB307

ARCHITECT HCV Ag: Diğer Merkezler

İstanbul Üniversitesi Cerrahpaşa Tıp Fakültesi, Mikrobiyoloji ve Klinik Mikrobiyoloji Anabilim Dalı ve Düzen Laboratuvarları

HCV RNA (IU/ml)	HCV RNA	HCV Kor Ag.	
	Pozitif	Pozitif	Negatif
0-1000	4	0	4
1001-2000	2	0	2
2001-10000	2	2	0
10001-100000	13	12	1
100001 ve üstü	102	102	0
Toplam	123	116	7

Yüksel P et al., ESCV Congress, 2008

ARCHITECT HCV Ag: Sonuç

- HCV replikasyonunun saptanmasında güvenilir ve etkin bir sistem....
- Uygulama ve maliyet avantajları...
- Virusa maruziyetin saptanması ve klinik izlemde uygulama potansiyeli