C.H.U. *de Charleroi*

Intérêt de la détection de l'antigène du virus de l'hépatite C en complément de la sérologie HCV

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Virus de l'hépatite C

- HCV
  - Virus enveloppé, famille des *Flaviviridae* (genre *Hepacivirus*)
  - ARN monocaténaire
  - Polyprotéine
    - Nucleocapsid Core protein
    - Envelope proteins

    (E1, E2/NS1)
    - Nonstructural proteins

    (NS2, NS3, NS4A et B, NS5A et B)

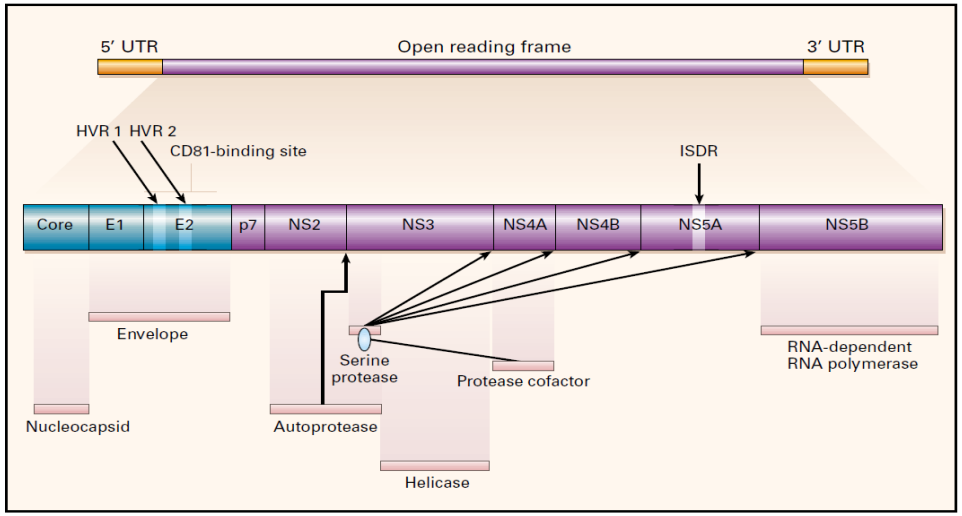


Figure 1. The HCV Genome and Expressed Polyprotein.

  - Six génotypes avec plusieurs sous-types (a, b …)

Virus de l'hépatite C

- Histoire naturelle
  - Transmission: Voie sanguine, sexuellement, périnatale
  - Incubation ≈ 7 semaines (2 – 30)
    - Souvent asymptomatique
    - Tableau d'hépatite aigue

    peu fréquent
  - Chronicité fréquente

    (50 – 80%)
  - Évolution tardive
    - Cirrhose (15 – 20%)
    - Hépatocarcinome

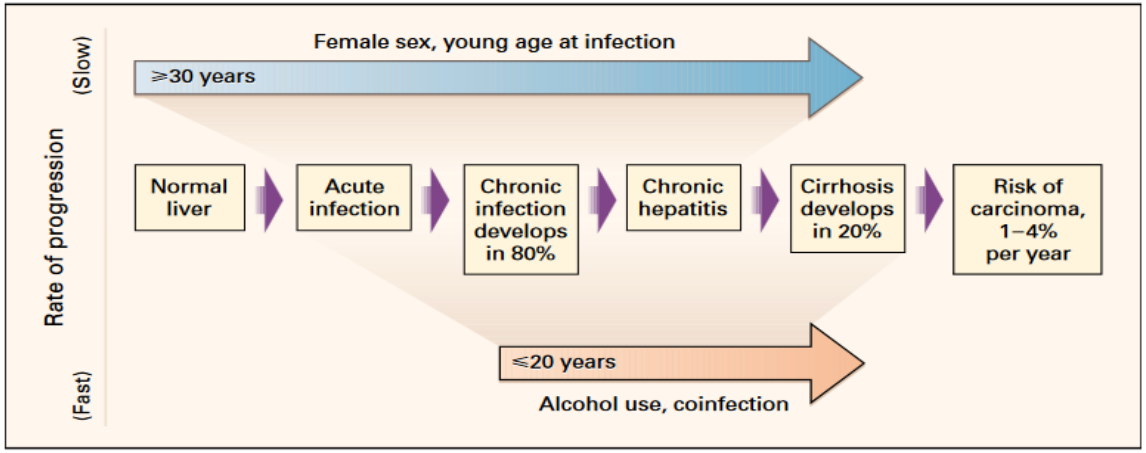


Figure 2. The Natural History of HCV Infection and Its Variability from Person to Person.

The course of infection varies widely among persons. Factors that decrease the risk of progression include female sex and a younger age at infection; factors that increase the risk include alcohol intake, an older age at infection, male sex, and coinfection with other viruses. Persons with a favorable risk profile often do not have progressive liver disease until 30 or more years after infection. In contrast, 20 percent of persons with chronic hepatitis C will eventually have cirrhosis, and this can occur 20 years or less after infection, especially in those with alcohol abuse or coinfection with human immunodeficiency virus type 1 or hepatitis B virus. Once cirrhosis is established, the risk of hepatocellular carcinoma is 1 to 4 percent per year.

Virus de l'hépatite C

- Épidémiologie
  - Positivité de la sérologie HCV estimée à 2% de la population mondiale
  - Positivité pour la virémie HCV estimée à 80 millions de personnes
  - Belgique: prévalence (sérologie) estimée à 0,87% (année 1997)

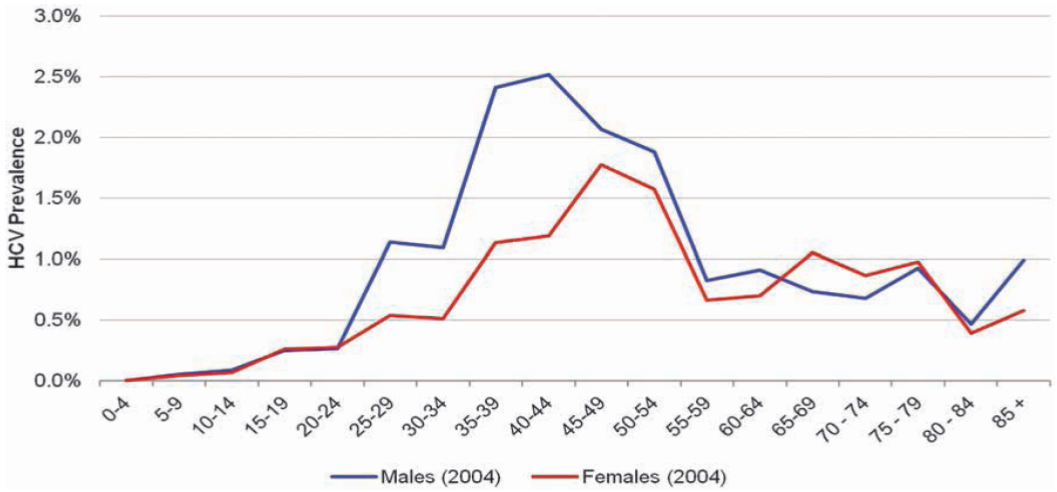


Fig. 1. — Age and gender distribution of anti-HCV prevalence, Belgium, 2004

Table 1. — **Genotype Distribution, Belgium, 2004 (8)**

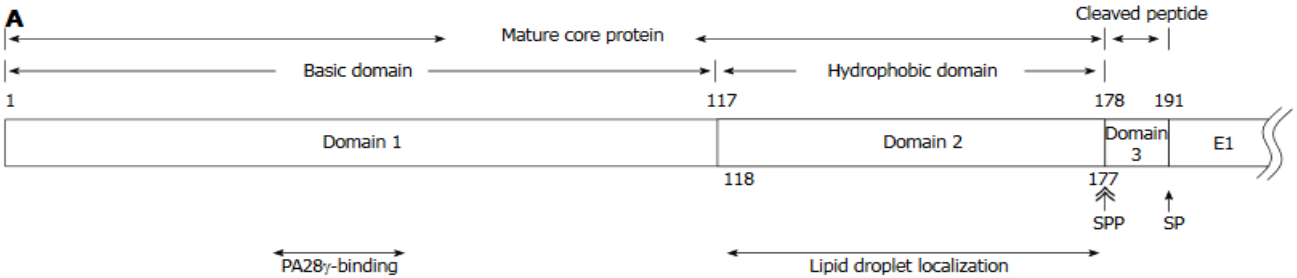
Genotype	1	2	3	4
Percent	61.0%	6.0%	19.0%	14.0%

Virus de l'hépatite C

- Diagnostic
  - Sérologie
  - Biologie moléculaire
    - Qualitative
    - Quantitative
  - Antigénémie: Core protein

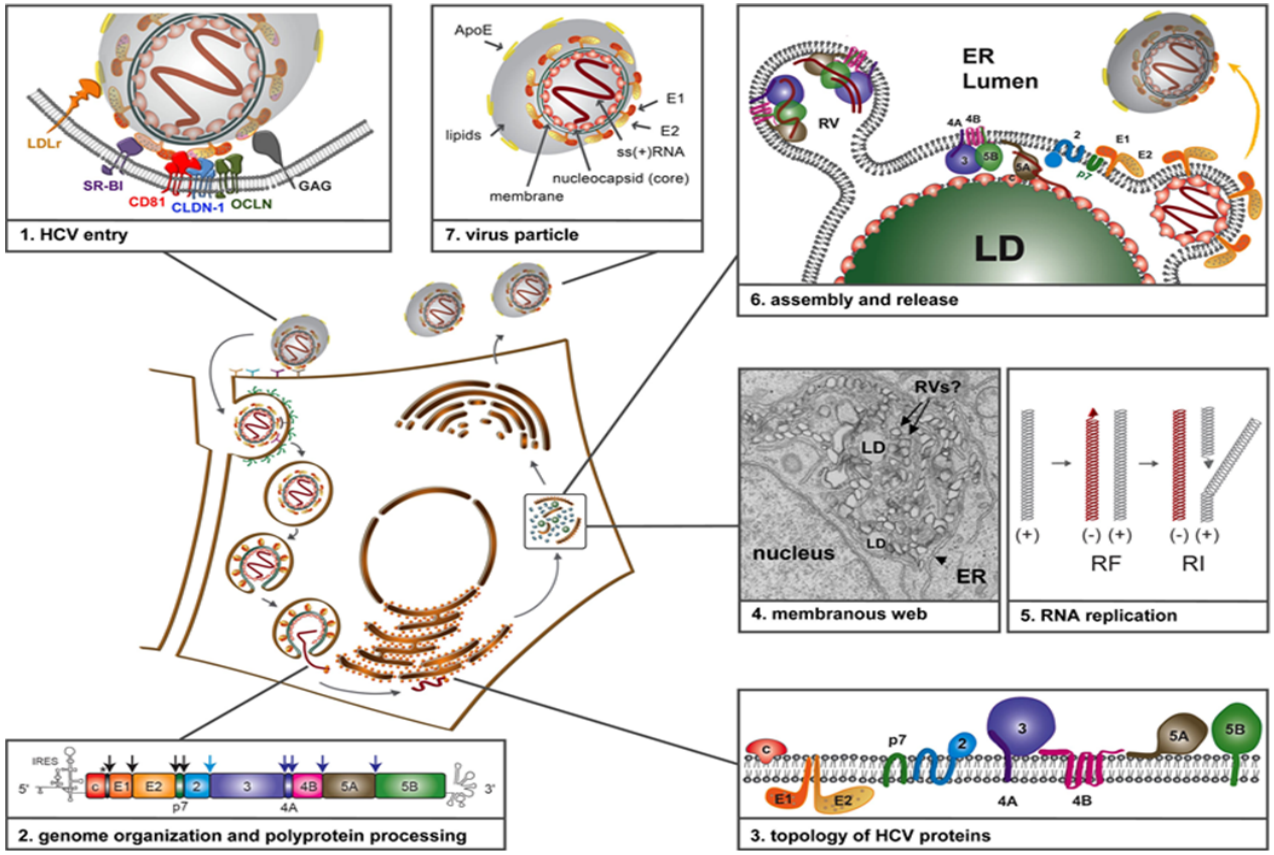
Virus de l'hépatite C

- Core protein
  - Générée par clivage des 191 premiers a.a. de la polyprotéine
  - Peptidase de l'hôte: « mature core protein » (1 → 177) + « Cleaved peptide » (178 → 191)
  - Hautement conservée parmi les différents génotypes
    - Mature core protein
      - Séquence nucléotidique: 81-88%
      - Séquence a.a. : 96%
    - Cleaved peptide: homologie proche de 100% entre les différents génotypes
  - Assemblage des virions
  - (résistance à l'insuline, stéatose hépatique, hépatocarcinome, échappement SI, réponse Tt par Inf)



Virus de l'hépatite C

- Infection par HCV



Virus de l'hépatite C

- Infection par HCV

1. ARN : 1 à 2 semaines
   après infection

2. Core protein : Apparition
   entre 2 à 5 jours après ARN

3. Anticorps HCV : 7 à 8 semaines
   après le début de l'infection
   - Apparition des symptômes:
     50 à 70% de positivité

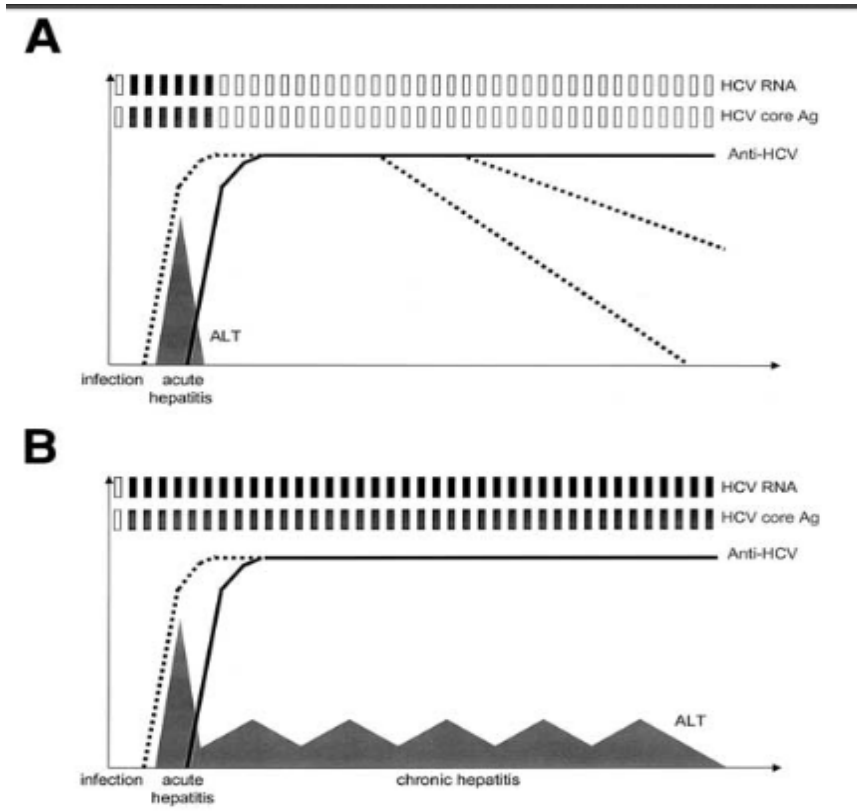


Fig. 1. Time course of HCV RNA, HCV core antigen, and anti-HCV antibodies in patients with (A) acute, spontaneously resolving hepatitis C or (B) evolution to chronic infection. **White boxes:** the marker is absent; **black boxes:** the marker is present. Alanine aminotransferase levels (ALT) are displayed in gray.

Antigénémie HCV

- Historique
  - Kits « maison »
    - Japon, 1990: performances insuffisantes
    - Japon, 1995-96: immunoessais (sandwich)
      - Premières applications cliniques et comparaison avec biologie moléculaire

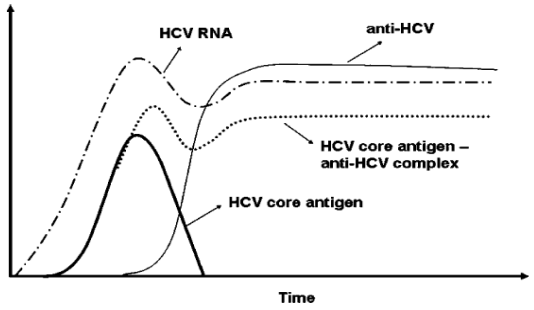


Fig. 1. The kinetics of HCV markers during hepatitis C virus infection.

  - Kits commerciaux : fin 90' – début 2000
    - HCV Core Ag ELISA Test System (Ortho Clinical Diagnostics)
      - Détection uniquement en l'absence dAc
    - Tests de 2ème génération: « Total HCV Core Ag ELISA Test System » aka « Track-C » assay (Ortho Clinical Diagnostics)
      - Détection quantitative en l'absence ou la présence d'Ac anti-HCV
    - 2003: automatisation : Lumispot EIKEN HCV Ag Assay (Eiken Chemical)
    - 2002-2003: microparticle-based chemoluminescent assay (Abbott)
      - Détection unique de l'Ag
      - Combinaison Ag + Ac anti-HCV

Performances analytiques

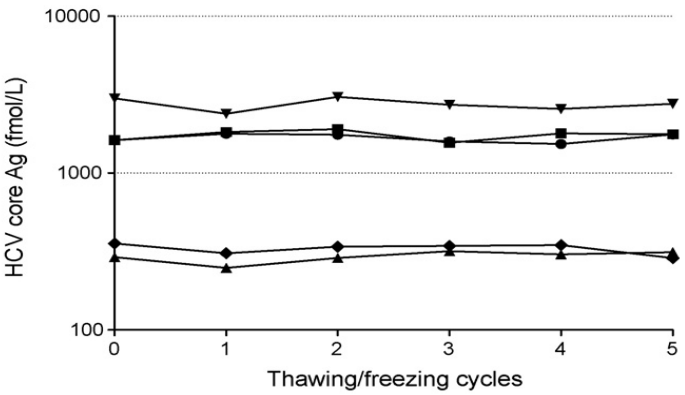
Antigénémie HCV

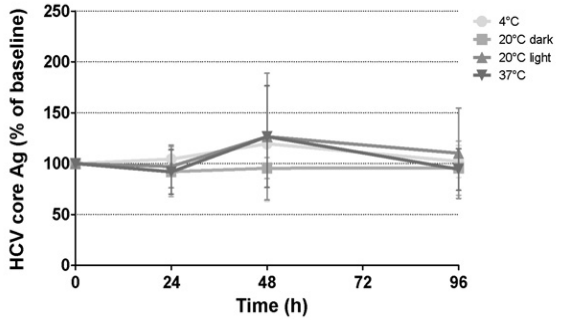
- Performances analytiques

Auteur	Kit	Paramètre	Résultats
Miedouge et al (2004)	Architect® HCV assay	Reproductibilité (QCI)	Niveau bas: 7,7% Niveau haut: 5,8%
Medici et al (2011)	Architect® HCV assay	Répétabilité (1:1; 1:10; 1:100) Reproductibilité (QCI)	3,33%; 3,84%; 2,88% Niv bas: 8,58% Niv haut:9,14%
Ansaldi et al (2004)	Monolisa HCV Ag–Ab Ultra assay	Répétabilité (2 éch) Reproductibilité (2 éch)	7,1%; 2,58% 8,1%; 1,7%
	Ortho Track-C	Répétabilité Reproductibilité	5,4% - 18,9% 11,9% - 21,5%

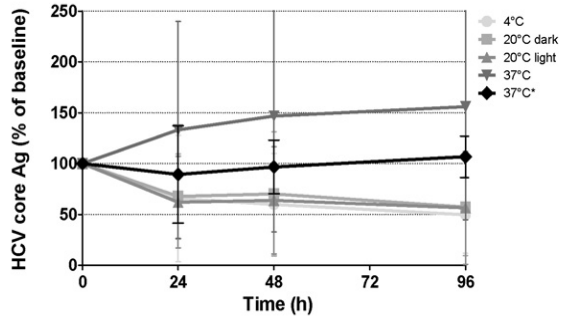
Antigénémie HCV

- Stabilité
  - Plasma > sang total
  - > ARN

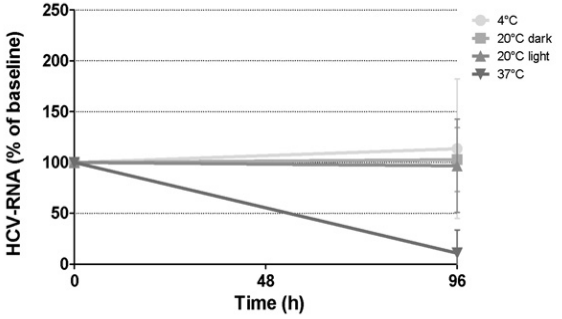




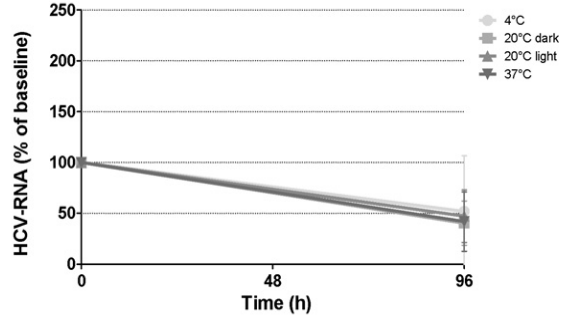
(a) Mean plasma HCV core Ag
(no significant difference for HCV core Ag levels at 4°C, 20°C and 37°C between 0 and 96 h)



(c) Mean whole blood HCV core Ag
(p<0.05 for difference of HCV core Ag level at 4°C between 0 and 96 h, no significant difference for HCV core Ag at 20°C and 37°C between 0 and 96 h)



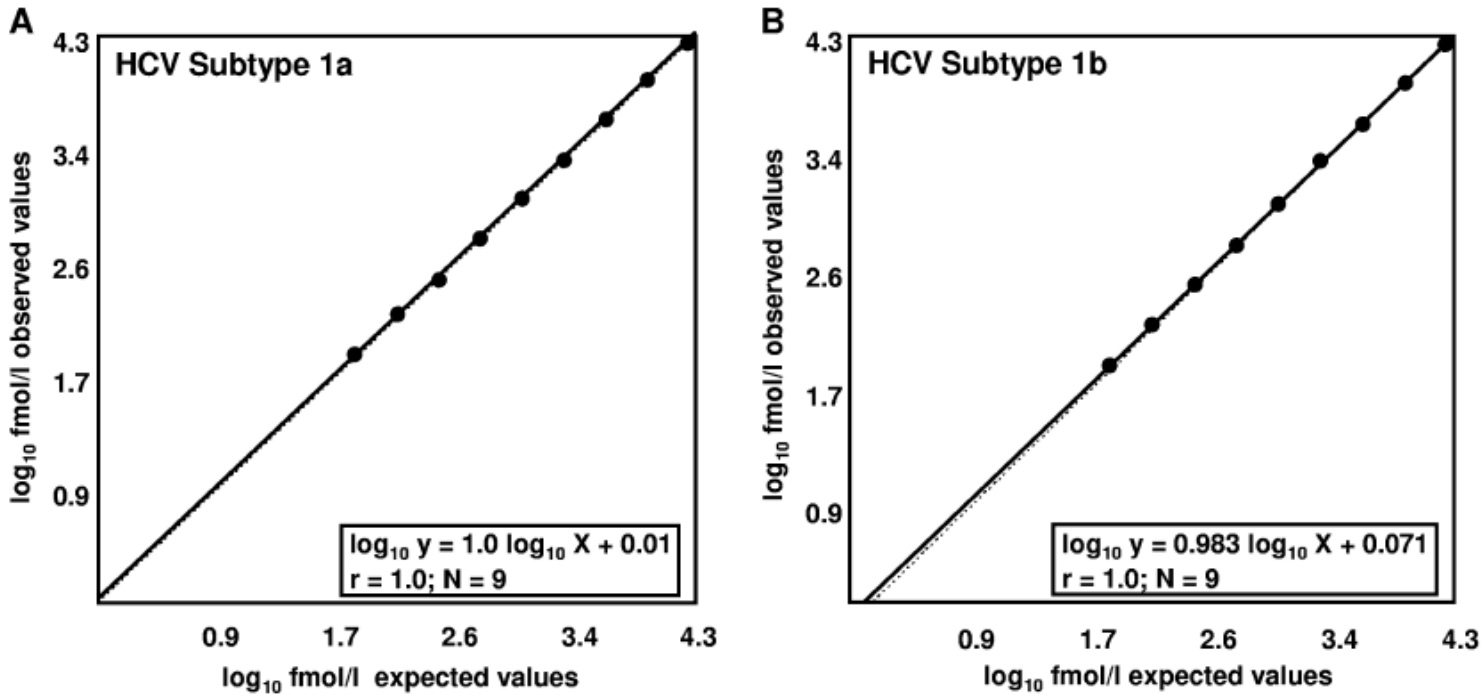
(b) Mean plasma HCV-RNA
(p<0.05 for difference of HCV-RNA at 37°C between 0 and 96 h, no significant difference for HCV-RNA at 4°C and 20°C between 0 and 96h)



(d) Mean whole blood HCV-RNA
(p<0.05 for difference of HCV-RNA at 20°C and 37°C between 0 and 96 h, no significant difference at 4°C between 0 and 96h)

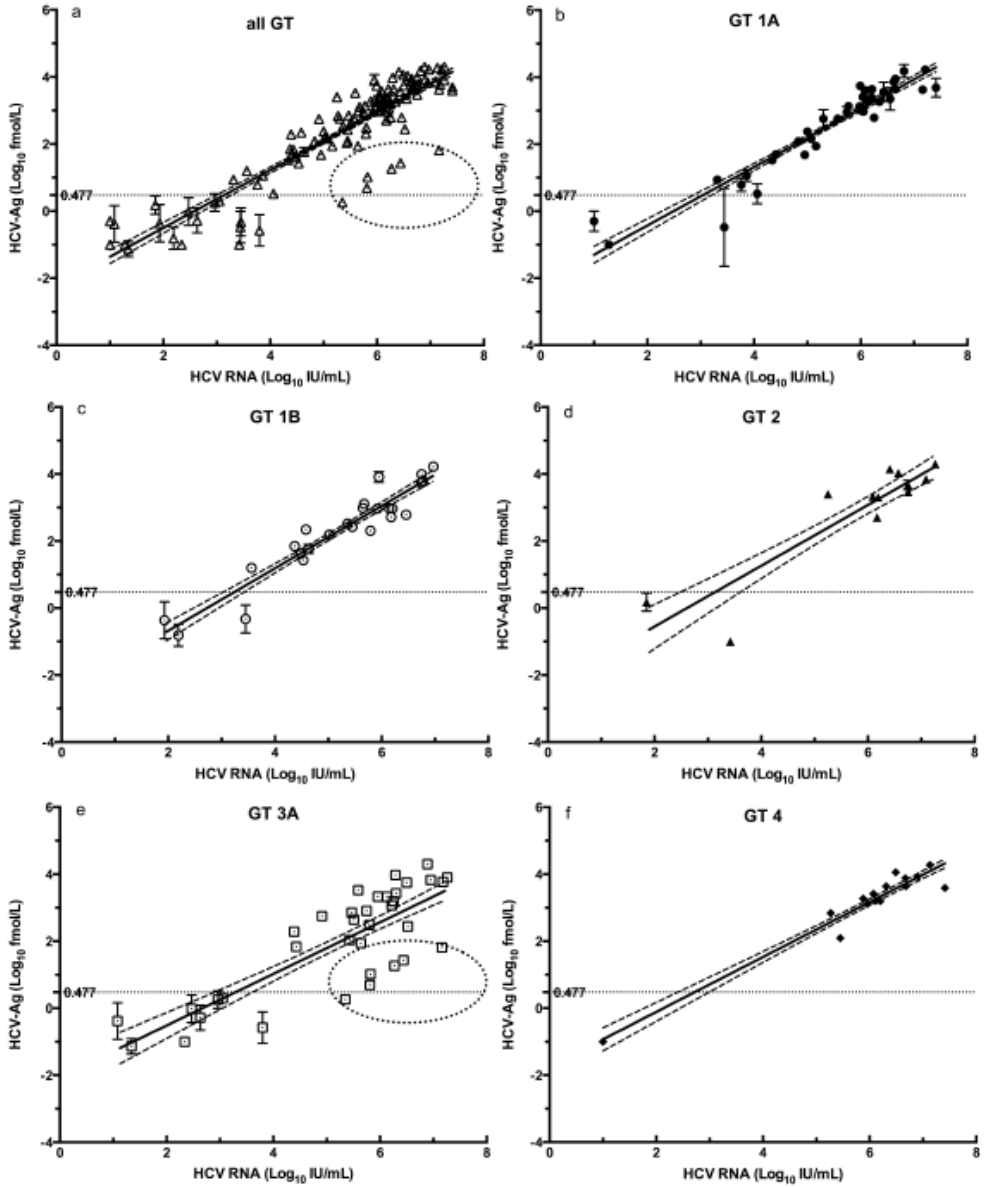
Antigénémie HCV

- Linéarité



Antigénémie HCV

- Relation Ag HCV – ARN HCV
  - Bonne corrélation entre Ag et ARN
  - Pas d'influence majeure du génotype
  - Limitation dans les valeurs basses



Antigénémie HCV

- Relation Ag HCV – ARN HCV
  - Limitation dans les valeurs basses
  - Diminution de la sensibilité vers 10 000 – 20 000 IU/mL
    - Seuil CLIA < ELISA

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

[a] Calculation of the analytical sensitivity, i.e., the concentration of HCV core antigen which could be detected with a probability of 95%, was achieved by probit analysis.

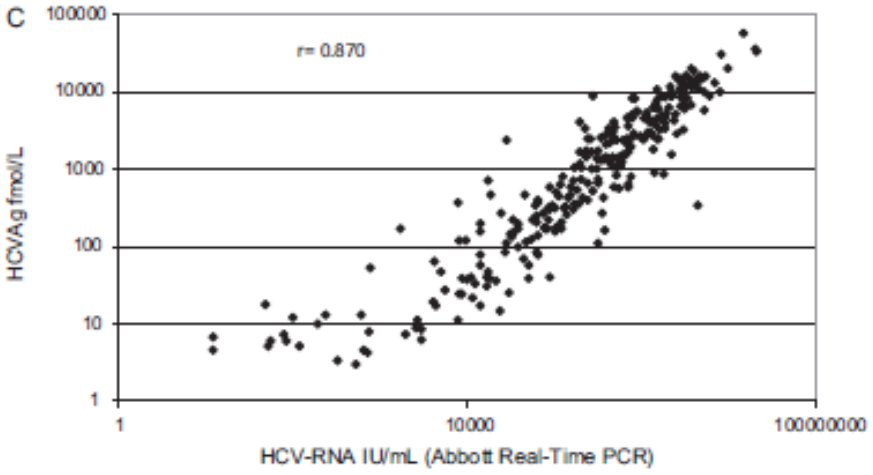
Table 2a
HCV RNA at the cut-off value of HCV-Ag of 0.477 Log_{10} fmol/L, i.e. 3 fmol/L, extrapolated from 120 samples, within 95% confidence interval (95%CI).

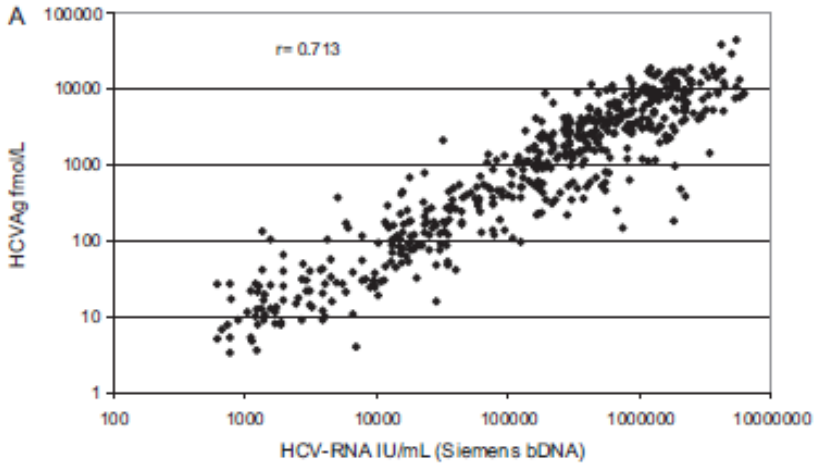
	HCV RNA	
	Log_{10} (IU/mL)	IU/mL
Genotype 1A	2.984 – 3.545	963 – 3507
Genotype 1B	3.184 – 3.805	1527 – 6382
Genotype 2 (A/C, B)	2.755 – 4.548	568 – 35′318
Genotype 3A**	3.037 – 3.692	1088 – 4920
Genotype 4 (A/C/D)	2.400 – 3.458	251 – 2870
All genotypes pooled*	3.359	2288

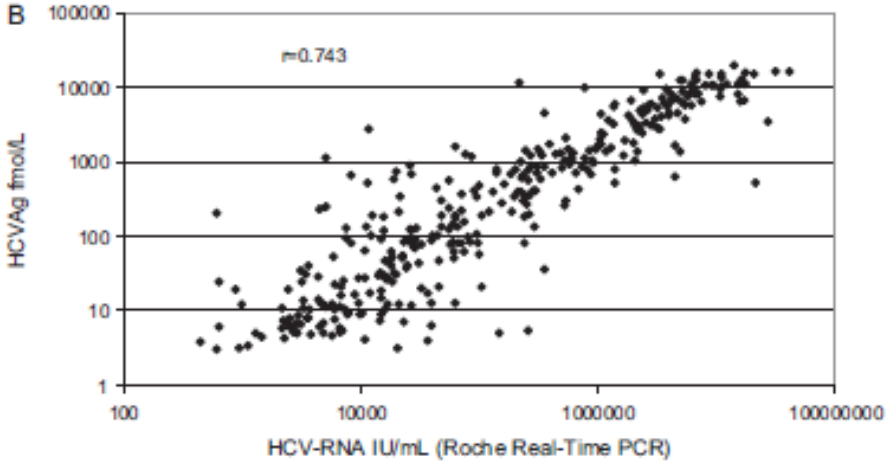
* Correlation data not significant ($P > 0.05$).
** Six samples from 4 patients of genotype 3A were excluded from the calculation.

Antigénémie HCV

- Relation Ag HCV – ARN HCV







En pratique

Antigénémie HCV

- Screening
  - Donneurs, hémodialysés…
  - Patients négatifs pour anti-HCV
    - Très bonne spécificité
    - Quelques cas détectés, confirmés par PCR
    - Rares faux positifs

Table 1
Ag HCV results in 98 serum samples positive for HCV RNA.

HCV RNA (IU/mL)	Ag HCV results	
	Negative	Positive
0–2000	6	7
2001–6000	2	15
6001–10,000	0	7
10,001–20,000	0	14
>20,000	0	47
Total	8	90

Table 2
Comparison of HCV Ag and HCV RNA results in 2752 haemodialysis patients negative for HCV antibodies.

HCV Ag	RNA HCV	
	Negative	Positive
Negative	2729	0
Positive	21	2

Miedouge et al (2010)

Table 1 MONOLISA HCV Ag–Ab ULTRA assay results of 500 and 239 sera evaluated for specificity and sensitivity respectively

					Monolisa HCV Ag–Ab ULTRA (OD/CO ratio)				
					Negative		Grey zone	Positive	
Evaluation	Sample from	Sample (*n*)	Anti-HCV	HCV-RNA	<0.5 (%)	0.5–0.79 (%)	0.8–0.99 (%)	1–1.2 (%)	>1.2 (%)
Specificity	Open population subjects	400	Negative		400 (100)	0	0	0	0
	Difficult and 'sticky' patients	100	Negative	Negative	99 (99)	0	1 (1)	0	0
Sensitivity	Seroconversion patients	76	Negative	Positive	3 (3.9)	1 (1.3)	1 (1.3)	4 (5.3)	67 (88.2)
	Seroconversion panels ($n = 7$)	5	Negative	Negative	5 (100)	0	0	0	0
		17	Negative	Positive	2 (11.8)	1 (5.9)	0	0	14 (82.4)
		31	Positive	Positive	0	0	0	0	31 (100)
	Infected patients	20	Weak positive		0	0	0	0	20 (100)
	Infected patients (different subtypes)	80	Positive	Positive	0	0	0		80 (100)
	Co-infected patients	10	Positive	–	0	0	0	0	10 (100)

Ansaldi et al (2004)

TABLE 1. Summary of HCV Ag, HCV RNA, and anti-HCV test results

HCV Ag	HCV RNA (no. of anti-HCV positive/total)			
	Not detected	<15 IU/ml	>15 IU/ml	Total
Nonreactive (<3 fmol/liter)	108 (35/108)	13 (9/13[a])	4 (4/4)	125 (48/125)
Reactive (>3 fmol/liter)	0 (0/0)	0 (0/0)	157 (154/154[b])	157 (154/154)
Total	108 (35/108)	13 (9/13)	161 (158/158)	282 (202/279[c])

[a] The four anti-HCV-negative and HCV Ag-nonreactive cases were from patients undergoing hemodialysis multiple times.
[b] Anti-HCV was positive in all 154 cases of which HCV Ag was reactive and the HCV RNA level was over 15 IU/ml.
[c] Of the total 282 specimens tested for HCV RNA and Ag, 279 samples were assayed for anti-HCV.

Park et al (2010)

Antigénémie HCV

- Panels de séroconversion
  - Sensibilité: PCR > Ag > Ac
  - Délai: *(Shah et al 2003)*
    - PCR >< Ag: 2 jours
    - Ag >< Ac : 15 jours
    - PCR >< Ac : 50 jours

Panel	Specimens (*n*)	Sampling days since first bleed until first anti-HCV positive sample	Days to detection from the first available bleed		
			Anti-HCV	HCV-RNA	Monolisa HCV Ag–Ab ULTRA
Natural-A	9	0/31/38/52/66	66	31	38
Natural-B	7	0/34/47/69	69	34	47
Natural-C	5	0/42/57/79	79	42	42
Natural-D	6	0/34/47/67	67	34	34
Natural-E	6	0/21	21	0	0
PHV901	11	0/72/104	104	72	104
PHV905	9	0/4/7/11/14/18/21	21	0	0

Table 3 Seroconversion sensitivity calculated with natural and commercial seroconversion panels

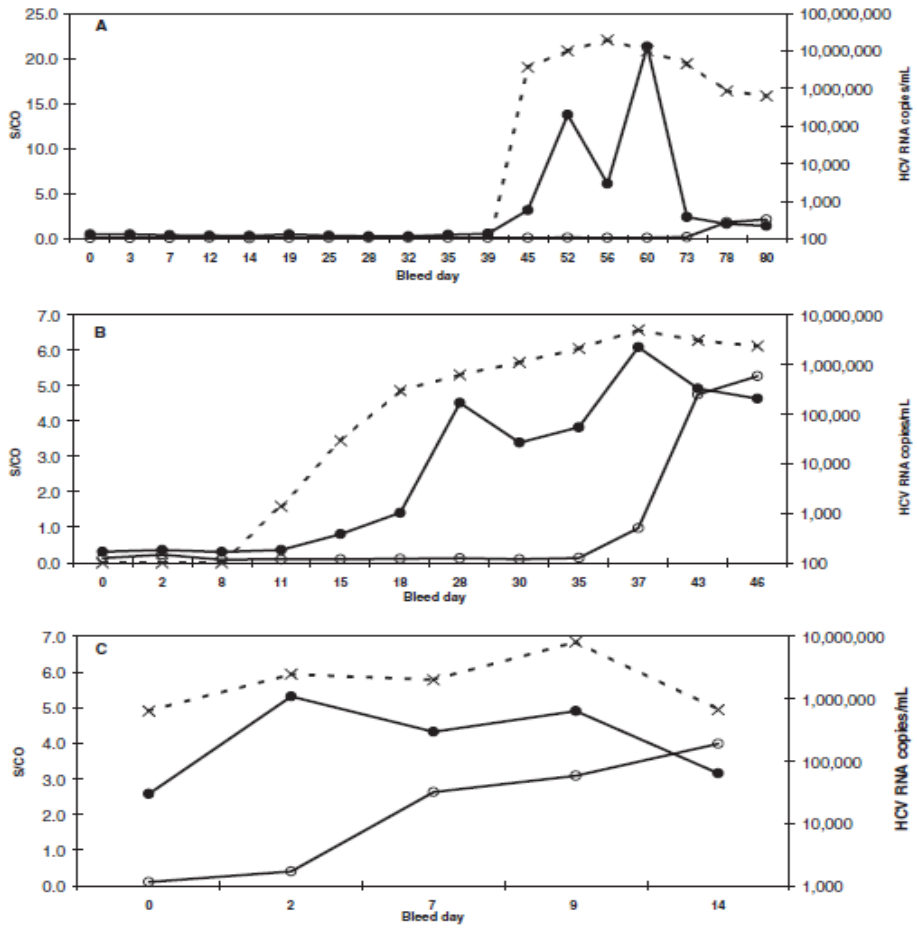
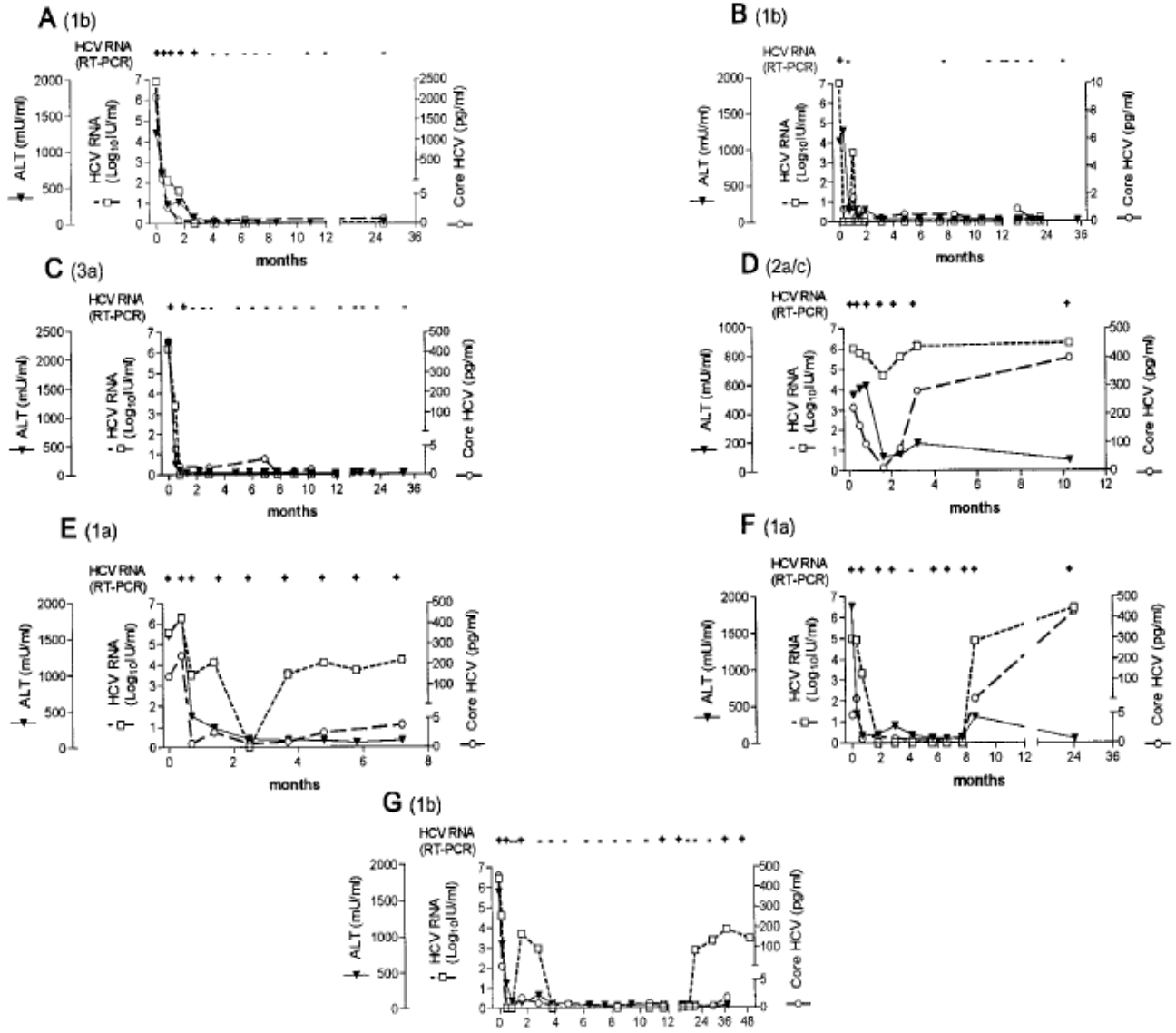


Fig. 3. Comparison of HCV RNA concentrations and assay responses of HCV core antigen and antibody combination and anti-HCV assays in three HCV seroconversion panels: BCP6225 (A), BCP6213 (B), and SC0040 (C). HCV RNA (×) is expressed in copies per mL on a logarithmic scale (at the right); HCV core antigen and antibody (●) and anti-HCV (○) are shown in S/CO value in linear scale (at the left).

Antigénémie HCV

- Hépatite C aigue
  - Profil comparable entre Ag et ARN
  - Négativation plus précoce des Ag (Trac-C)
  - La biologie moléculaire reste préférable pour s'assurer de la négativation de la virémie



Antigénémie HCV

- Hépatite C chronique
  - Stabilité du profil en l'absence de traitement
  - Alternative satisfaisante à la charge virale
    - Coût
    - Sensibilité (nombre élevé de copies/mL)

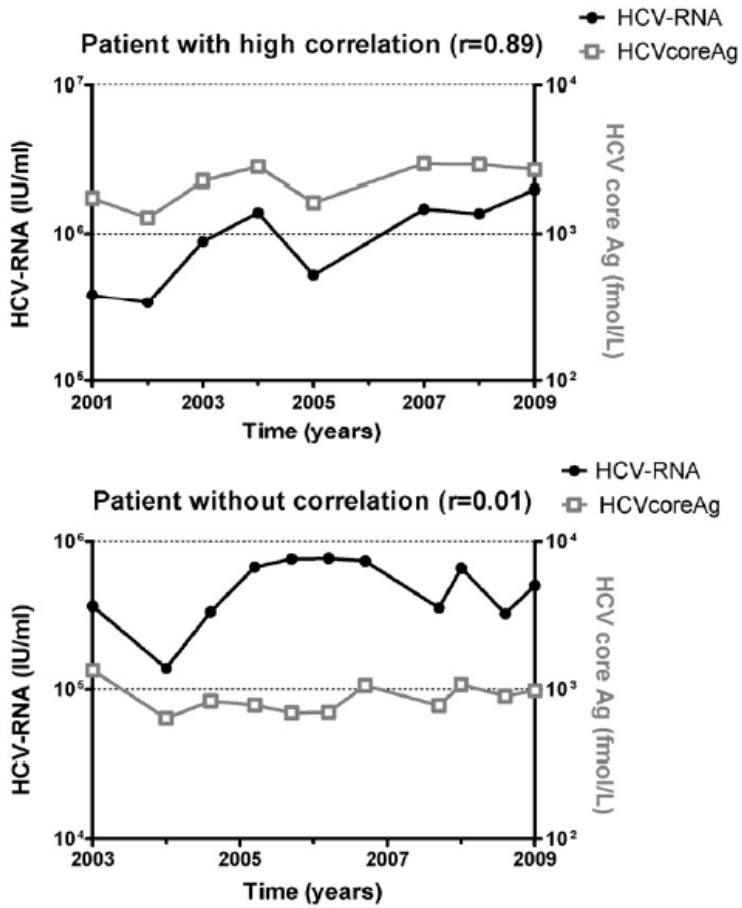


Fig. 2. Correlation of HCVcoreAg and HCV-RNA (longitudinal). Samples of patients who did not receive antiviral treatment were studied over a period of up to 8 years. Two respective patients are presented: one patient with high correlation and one without mathematical correlation between HCVcoreAg and HCV-RNA over time.

Antigénémie HCV

- Réponse au traitement antiviral
  - Diminution de l'Ag parallèle à celle de l'ARN
  - Sensibilité acceptable pour évaluer la réponse rapide à la thérapie mais pas en fin de traitement

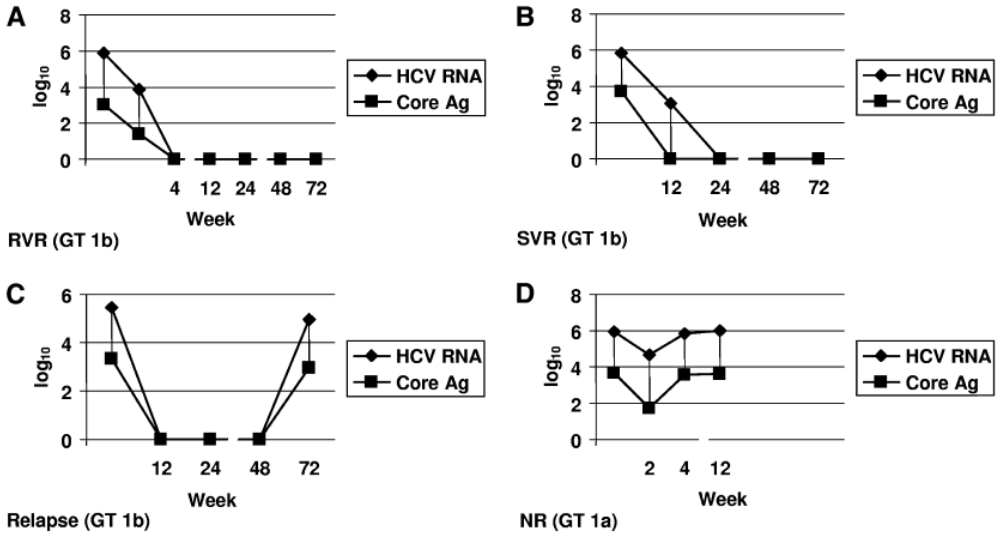


FIG. 3. Viral kinetics estimated by testing for HCV core antigen (■) and RNA (◆) in four individuals receiving antiviral combination therapy with pegylated alpha interferon and ribavirin. (A) Rapid virological response (RVR); (B) sustained virological response (SVR); (C) results for a patient who developed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

Antigénémie HCV

- Confirmation de la sérologie
  - Limitations des Ac anti-HCV
    - Délai d'apparition dans le sérum
    - Taux élevé de faux positifs malgré une bonne Se/Sp (faible prévalence)
    - Pas de distinction entre infection active ou résolue
    - Faux négatifs possible chez certains patients (immunodéprimés)

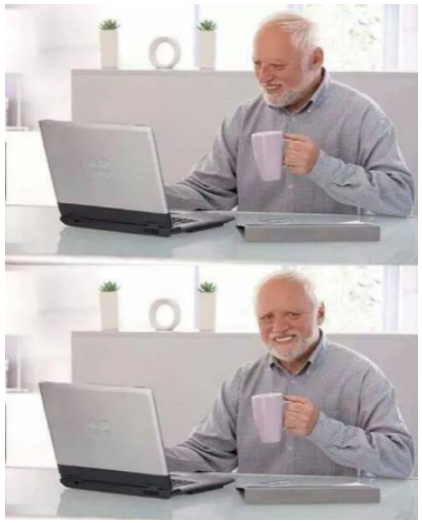
TABLE 2. Antibody to hepatitis C virus (anti-HCV) screening-test–positive average signal-to-cut–off (s/co) ratios by recombinant immunoblot assay (RIBA®) 3.0 and nucleic acid test (NAT) results in groups with different anti-HCV prevalences

Anti-HCV prevalence	Study group	Total tested*	Average s/co ratio	No. (%) screening-test–positive	RIBA results (%)			HCV RNA-positive (%)
					Negative	Ind†	Positive	
2% (average)	Multiple groups§ with prevalences ranging from 0.8% to 4.4%	24,012	Total	689 (100.0)	26.9	7.3	65.9	41.6¶
			<3.8	231 (33.5)	78.8	16.9	4.3	3.7
			≥3.8	458 (66.5)	0.7	2.4	96.9	80.2
9.5%	Hemodialysis patients	2,936	Total	351 (100.0)	9.7	7.3	83.0	80.3
			<3.8	45 (12.8)	64.4	33.3	2.2	4.4
			≥3.8	306 (87.2)	1.6	3.4	95.0	91.5
24.9%	STD clinic clients selected based on risk**	498	Total	124 (100.0)	1.6	4.0	94.4	NA††
			<3.8	3 (2.4)	66.7	33.3	0	NA
			≥3.8	121 (97.6)	0	3.3	96.7	NA

* HCV EIA 2.0 or HCV Version 3.0 ELISA.
† Indeterminate.
§ College students, general population, health-care workers, and sexually transmitted disease (STD) clinic clients not selected by risk factor.
¶ In the low (2%) prevalence group, only a sample of 214 were tested for HCV RNA.
** History of injection-drug use, blood transfusion before 1992, or incarceration.
†† Not available.

Antigénémie HCV

- Confirmation de la sérologie



5min plus tard

Antigénémie HCV

- Confirmation de la sérologie

Table 1
Positivity rates for HCV Ag according to anti-HCV antibody levels, expressed as sample/cutoff (S/CO) values, in 596 subjects positive for anti-HCV by Ortho Vitros Eci and in 600 subjects positive for anti-HCV by Abbott ARCHITECT. In the disaggregation used for S/CO ranges, samples with a S/CO from 1 to 1.99 are low-level reactives by both antibosy assays, while the other two groups are not directly comparable.

Assay	S/CO range	N. samples	HCVAg+	% HCV Ag+
Vitros	1.00–1.99	140	1	0.7%
	2.00–7.99	271	9	3.3%
	≥8	185	110	59.5%
ARCHITECT	1.00–1.99	170	1	0.6%
	2.00–9.99	217	64	29.5%
	≥10	213	178	83.6%

Antigénémie HCV

- Confirmation de la sérologie

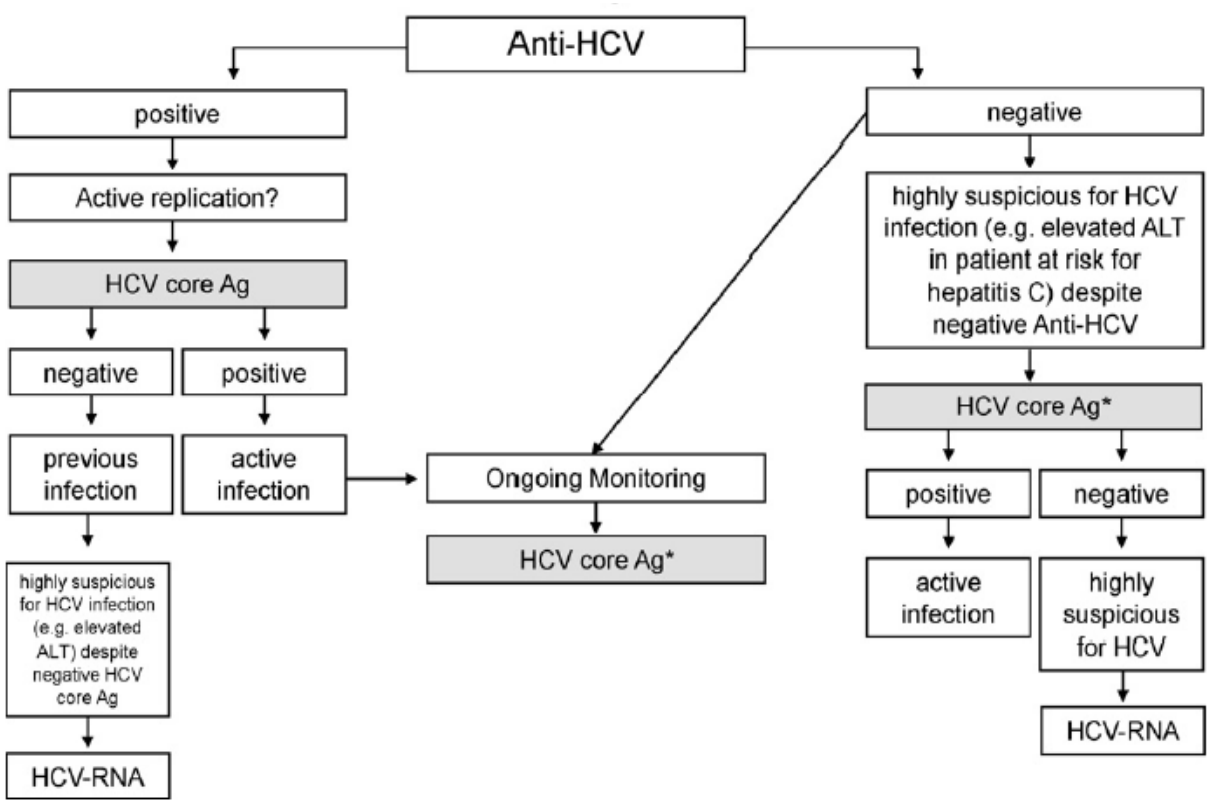


Fig. 6. Proposed diagnostic algorithm. *Based on viral load in anti-HCV positive samples, more than 86.5% of HCV-RNA positive samples have a viral load above 10,000 IU/mL[8] which is in a range, where they are also tested positive for HCVcoreAg.

Antigénémie HCV

- Sensibilité/Spécificité
  - Architect Ag assay
    - Se: 93,4% (90,1 – 96,4)
    - Sp: 98,8% (97,4 – 99,5)
  - Ortho Ag ELISA
    - Se: 93,2% (81,6 – 97,7)
    - Sp: 99,2% (87,9 – 100)
- Limitation dans les valeurs basses >< PCR

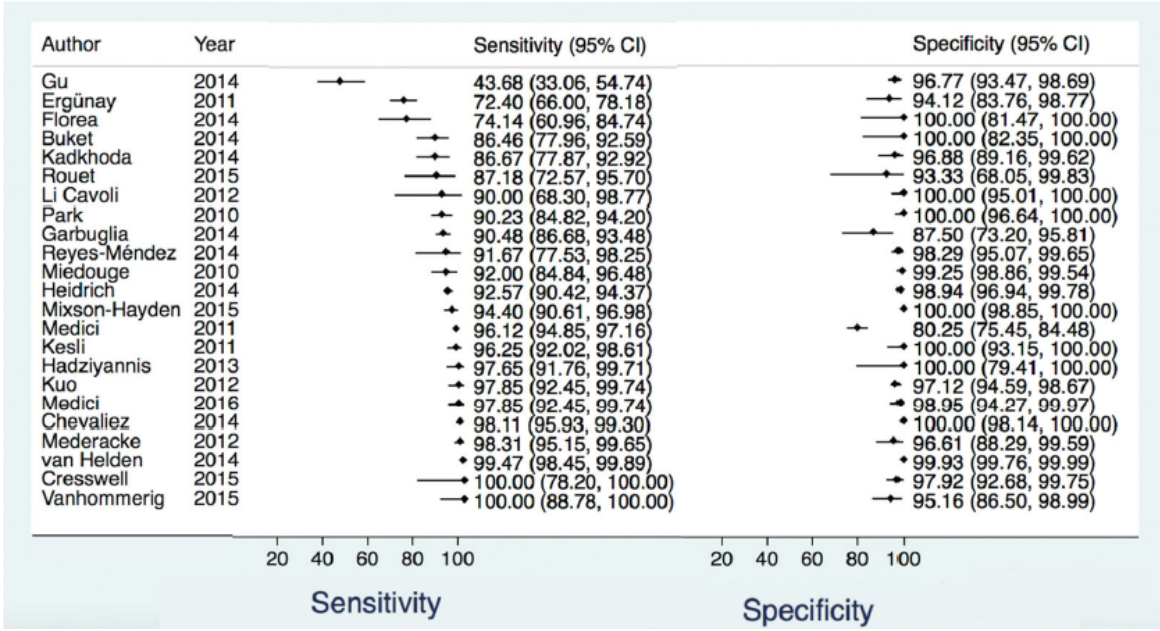


Figure 1.
Forest plot of Abbott ARCHITECT HCV Ag Assay sensitivity and specificity for the diagnosis of active HCV infection compared to NAT reference test for all samples regardless of HCV Ab status.

Antigénémie HCV

- Conclusion

  - L'Ag HCV est un test fiable et rapide
    - Stabilité
    - Automatisation : Possibilité de test « reflexe »

  - Bonne corrélation avec les techniques de biologie moléculaire
    - Bon marqueur de réplication virale
    - Alternative chez les patients HCV chronique
    - Intérêt dans la confirmation des sérologies « douteuse »

  - Sensibilité < biologie moléculaire
    - Mais > à la sérologie (Screening)
    - Ne remplace pas la recherche d'ARN viral (Traitement, haute suspicion…)

C.H.U. *de Charleroi*

Merci

Des questions ?