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1 HCV virology and diagnosis

2 Steven Roger¹, Alexandra Ducancelle¹, H el ene Le Guillou-Guillemette¹, Catherine Gaudy^{2,3} and

3 Fran oise Lunel¹

4 ¹*Laboratoire de Virologie, CHU Angers et Universit  d'Angers, Laboratoire HIFIH UFR Sant *

5 *d partement M decine, SFR 4208-UPRES EA3859, BAT IBS - 4 rue Larrey- 49000 Angers, France*

6 ²*Service de Bact riologie-Virologie-Hygi ne, CHRU de Tours, 37000 Tours, France, ³INSERM*

7 *U1259, Universit  de Tours, 37000 Tours, France*

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9 INTRODUCTION

10 Hepatitis C virus (HCV) infection is a major cause of severe liver disease including chronic hepatitis,
11 cirrhosis and hepatocellular carcinoma. The HCV burden in public health is estimated at about 71
12 million people worldwide by World Health Organization (WHO) with at least 400,000 people that
13 died every year from HCV disease [1]. New hepatitis C treatments with oral direct-acting antivirals
14 (DAAs) showing high rates of response, with short treatment duration [2] have been available. HCV
15 can now be eradicated with minimal side effects. Unfortunately, there is no vaccine yet available, but
16 the development of a safe prophylactic vaccine remains a medical priority [3]. For this purpose,
17 Hepatitis B-C subviral envelope particles can be produced by industrialized procedure. It seems to be
18 very promising as this HBV-HCV vaccine candidate has been shown to elicit a broadly cross
19 neutralizing activity against HCV [4]. Despite this revolution in the HCV-treatment, one of major
20 challenge to achieve a global eradication of HCV remains to reduce the under diagnosis. The low rate
21 of diagnosis is a major obstacle in resources limited countries and is mainly due to the cost of
22 molecular tools, that are essential to diagnose and follow chronic HCV infection. In another hand, the
23 mild clinical symptoms observed in HCV chronic disease, may explain that the majority of HCV

24 infected individuals are unaware of their infection, because HCV testing is not generalized, like it is
25 for HIV.

26 HCV was discovered in 1989 after many years of work, by several researchers, who recently obtained
27 the Nobel price [5–7]. This major discovery allowed the description of the HCV genome and later on
28 of the virus replication and cell cycle, and also, importantly, the development of diagnostic tests for
29 the detection of HCV antibodies (Ab) and RNA who were a priority in transfusion.

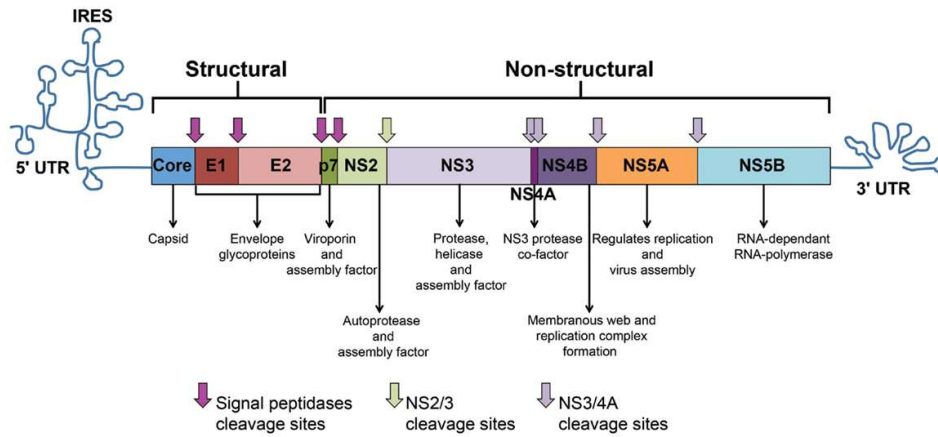
30 In this review, we will try to get into the virology and cell biology of HCV. Thereafter, we will discuss
31 the different categories of laboratory tests to diagnose/explore HCV infected subjects.

32 **1. HCV VIROLOGY**

33 **a. HCV genome**

34 HCV is a positive sense, single-stranded RNA virus, composed of approximately 9600 nucleotides
35 that belongs to the Flaviviridae family and is part of the Hepacivirus genus [8,9]. Figure 1 shows the
36 HCV genome organization. The viral genome encodes a single open reading frame (ORF) of 3010
37 amino acids flanked by 5' and 3' non-translated regions (NTRs) which are critical for viral replication
38 [10] and translation [11]. The 5' NTR contains an internal ribosome entry site (IRES).

39 Translation of the HCV protein starts from the 5'-UTR IRES [11] and generates a single polyprotein,
40 which is processed by cellular proteases to produce the structural proteins that form the viral particles
41 (core and envelope glycoproteins E1 and E2), or is processed by viral proteases to generate non-
42 structural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B) (Figure 1).



43

44 Figure 1 : HCV genome and viral particle structure. The HCV genome is composed of an open reading
 45 frame (ORF) flanked by 5' and 3' untranslated regions (UTRs). IRES-mediated translation of the ORF
 46 leads to the formation of a polyprotein that is processed into 10 viral proteins. Cleavage of the core
 47 protein from E1 involves cellular signal peptidases, which also cleave E1, E2, and p7 from the
 48 polyprotein (pink arrows). The NS2–NS3 protease auto-cleaves itself (green arrow). The NS3 protease
 49 located in the first one-third of NS3, assisted by its membrane-bound cofactor, NS4A, cleaves the
 50 remaining proteins NS3, NS4A, NS4B, NS5A, and NS5B (violet arrows) [12].

51 **b. Variability of HCV**

52 HCV displays a high genetic diversity which results from defects in the repair activity of the RNA-
 53 dependent RNA polymerase (resulting in nucleotide substitutions) and from the absence of 5' to 3'
 54 exonuclease activity (lack of error correction) [13]. The mean frequency of nucleotide mutations
 55 varies from 1.4×10^3 to 1.9×10^3 substitutions per nucleotide and per year. This estimate is based on
 56 comparisons of the major sequences of complete genomes obtained after eight years of evolution in a
 57 chimpanzee and 13 years in a human [14],[15].

58 Some of the mutations accumulating during replication are silent or synonymous. These mutations
 59 have no impact on the amino-acid sequence of the viral protein, but may affect the secondary structure
 60 of the genomic RNA. Other so-called non-synonymous mutations lead to changes in protein sequence
 61 and the emergence of variants. Other mutations lead to the production of defective viral particles and
 62 are therefore lethal. The regions of the genome corresponding to essential viral functions (domains

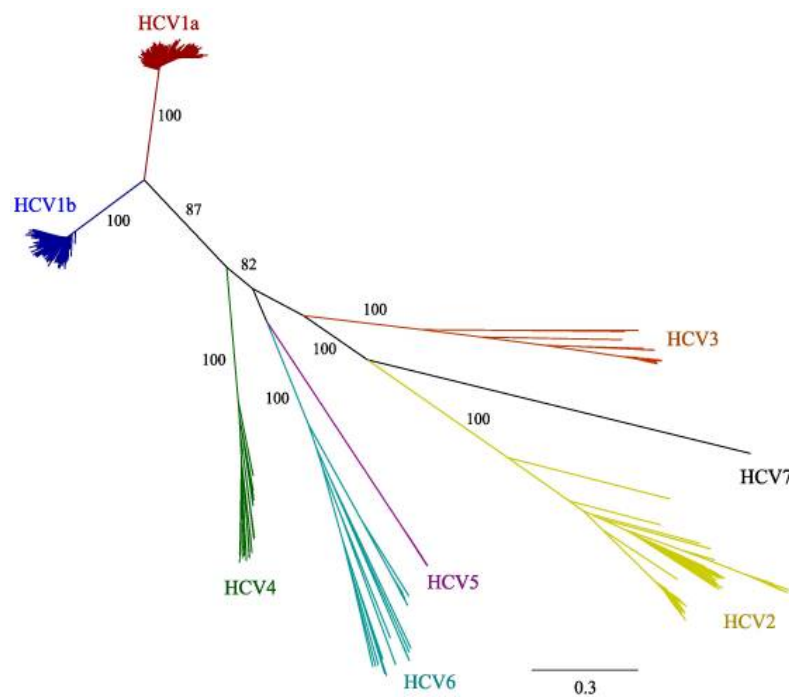
63 involved in translation or replication), or displaying major structural constraints (non-coding 5' and 3'
64 ends) are the best conserved; indeed, the non-coding 5' region is the most highly conserved region of
65 the genome, with more than 90% identity between the sequences of distantly related strains (4). The
66 region encoding the capsid is also well conserved, with around 80% sequence identity between
67 isolates (5). The most variable region of the genome is that encoding the envelope glycoproteins, E1
68 and E2. The sequences of hypervariable regions (HVR1 and HVR2) of E2 in strains isolated from
69 different patients may differ by more than 50%. The polypeptides encoded by these hypervariable
70 regions are therefore very tolerant to amino-acid substitutions [16,17]. HVR1 contains a central highly
71 variable domain (residues 8 to 18), flanked by six conserved positions (positions 2, 6, 7, 20, 23, and
72 26) that are assumed to be important for the structure of the whole HVR1 domain [18,19]. HVR1 was
73 shown to be a decoy antigen that diverts the host immune response away from more conserved
74 neutralizing epitopes [20]. The classification of HCV, is based on the topology of the trees obtained by
75 phylogenetic relationships between viral variants. HCV variants can be classified into seven clades,
76 and then into subtypes corresponding to subgroups of the most closely related viruses within a clade
77 [21]. The virus genotype is indicated by an Arabic number (from 1 to 7), associated with a lower-case
78 letter to indicate the subtype. Genotypes differ from each other by around 25–35% and subtypes differ
79 from each other by around 15–25% at the nucleotide level, according to the rules defined by the
80 International Committee on Taxonomy of Viruses) [22]. In a single-host, HCV exists as pools of
81 related genetic variants, referred to as quasispecies [23,24]. The genome instability of HCV was
82 reported as viral quasispecies [25]. The RNA-dependent RNA polymerase of HCV does not have
83 proofreading activity, and HCV has a high error rate, [26], leading to genetic heterogeneity and
84 quasispecies which were first identified via the hypervariable region (HVR) in the envelope E2
85 protein. HVR-1 and HVR-2 are located in E2 region (Figure 1). HVR-1 mutations can facilitate
86 escape from host immune responses. HVR-1 and HVR-2 mutations are generated very rapidly and
87 confer adaptive advantages for the virus i.e. tropism, virulence and drug resistance [27,28]. A high rate
88 of amino acid substitutions per site per year have been described in acute and chronic cases of HCV
89 infections [29]. The sequence variations in HVR-1 may reflect the existence of various quasispecies in
90 the acute phase, the persistence of which could explain chronic infection [30].

91

92 **c. HCV Genotypes**

93 The HCVDB (<http://hepatitis.ibcp.fr>), DDJB (<http://www.ddbj.nig.ac.jp>) and Los Alamos
94 (<http://hcv.lanl.gov>) databases currently include a large number of HCV sequences, making it possible
95 to compare an isolate with a large number of reference sequences [31]. Although currently restricted to
96 specialist laboratories, the gold standard method for HCV genotyping is sequencing of the NS5B
97 region followed by phylogenetic analysis including comparison to reference sequences [32,33].

98 Phylogenetic analysis permitted to classify HCV into 7 major genotypes and 67 subtypes [21] (Figure
99 2). Genotypes 1 and 3 are the most prevalent, accounting for more than 30% of all infections [34].
100 Genotypes 2, 4, 5, and 6 account for less than 10% of infections, respectively. Genotype 7 has been
101 found in few people from Central Africa [35,36].

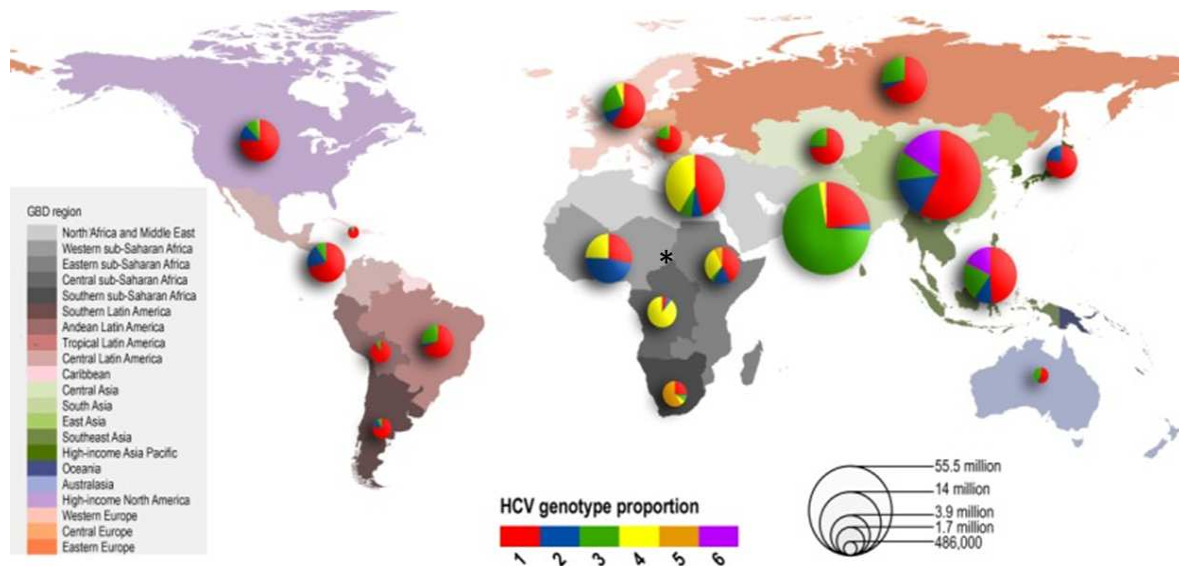


102

103 Figure 2 : Phylogenetic analysis of complete HCV genome [37].

104 As shown in figure 3, in Europe and North America, genotype 1 is the most prevalent in most
105 countries, followed by genotypes 2 and 3. Genotype 2 shows the highest prevalence in Central Africa,
106 and genotype 3 accounts for most infections in India, Pakistan, Thailand, and other Asian countries

107 and in Northern European countries. Genotypes 4 and 5 have probably recently increased in
 108 prevalence due to emigration from the Middle East and Africa, and transmission by intravenous drug
 109 users. Genotype 4 is mostly found in Central Africa and the Middle East, and genotype 5 frequency is
 110 higher in Southern Africa. Prevalence of genotype 6 is high in East and Southeast Asia, and is mostly
 111 found in Laos and Vietnam (Figure 3).



112
 113 Figure 3 : Global distribution and prevalence of hepatitis C virus genotypes [38]. *Genotype 7 has
 114 only been found in few people from central Africa.

115 Molecular epidemiological analyses using Bayesian evolutionary reconstruction have suggested the
 116 epidemiological history of HCV genotype.

117 Indeed, the global distribution of HCV genotypes and analyses of their phylogenetic relationships
 118 (Figure 2) have provided insight into their emergence and diversification over the centuries. The
 119 simultaneous presence of a large number of subtypes of a viral genotype in a limited geographic
 120 region is indicative of the long-standing endemic presence of the virus in the population studied. The
 121 worldwide distribution of genotype 1 subtype 1b began 60 to 70 years ago, genotype 2 began some 90
 122 to 150 years ago. Many subtypes have been identified (2c to 2l) among genotype 2 strains, mainly
 123 found in West Africa [39]. The spread of genotype 3 began about 40 years ago. Infection of HCV
 124 genotype 3a was increased in India from 1940s to 1990s, followed a decrease after 21th century

125 [40,41]. Genotypes 4 and 6 emerged much earlier: 350 and 700 years ago, respectively [42].
126 Genotype 4 may be originated in central Africa and multiple strains have been exported to north
127 Africa since ~mid 19th century, explaining the high prevalence in Egypt [43]. Spread of genotype 4
128 was probably due to emigration during the 2nd World War. Multiple HCV genotype 6 (6a, 6e, 6l etc.)
129 and 1(1a,1b) subtypes are found in Vietnam and probably increased following Vietnam War [44].
130 Conversely, the limited number of subtypes in Europe (where genotype 1, 2, 3 are the more frequently
131 found), North America and Japan is consistent with the more recent introduction of HCV into these
132 populations.

133 **d. Clinical Significance of Quasispecies and Genotypes**

134 The main difference between HCV genotypes 1 and 2 were in their responsiveness to interferon (IFN)
135 treatment [45]. That's why, at the past area of IFN based regimens, genotype determination played a
136 crucial role for prediction of treatment responsiveness. Compared with patients infected with HCV
137 genotype 1b , a higher proportion of patients with genotype 2a showed sustained responses to IFN- α
138 (34,35). These results confirm the previous recommendation of HCV genotyping before starting IFN- α
139 treatment [47]. However, in the context of pangenotypic DAAs genotype determination is not essential
140 to determine the type of treatment.

141 The quasispecies diversity of HCV was also suspected to be involved in the responsiveness to IFN.
142 Nonstructural (NS) NS5A is a phosphoprotein found in a basally phosphorylated form. The amino
143 acid sequence is also varying depending on the genotype. Mutations in NS5A HCV were found to
144 confer sensitivity to IFN (30–32).

145 A region, called the IFN-sensitivity-determining region (ISDR) located in the C-terminal portion of
146 NS5A) (Figure 1) was initially described by japanese authors [50]. Contradictory data about ISDR
147 were reported from other countries than Japan, especially from Europe and USA (32–34).

148 In HCV-1b infected patients, IFN therapy response seems to be mostly influenced by the mutation
149 within the ISDR region in NS5A gene [53]. Inshauspe *et al* [54] identified another domain localised in
150 the C-terminal region of NS5A and termed the V3 domain. It is length of 27 amino-acid and harbours

151 a high variability level. Duverlie *et al* [55] demonstrated a relationship between the mutation level in
152 V3 and the response to interferon therapy. Our lab and others confirmed this correlation [33]. HCV
153 quasispecies diversity at baseline and under interferon-ribavirin combined treatment was studied in the
154 entire NS5A gene and in each region of interest (PKR-bd, ISDR and V3). The V3 domain showed a
155 higher quasispecies diversity in responder patients samples; confirming the potential role of the
156 genetic diversity in the response to the interferon-based therapy [33,56,57]

157 Liver steatosis is frequently observed in Chronic HCV disease [58], and genotype 3 seems to be
158 associated with non-alcoholic fatty liver disease (NAFLD) [59]. Insulin resistance (IR) was also
159 reported in patients infected with genotype 1 and 4 [60,61].

160 Studies have also suggested a potential involvement of the HCV proteins and Viral quasispecies
161 heterogeneity in the establishment of a chronic hepatitis and in the occurrence and outcome of
162 Hepatocellular carcinoma (HCC) [62,63].

163 HCV infects and replicates in human hepatocytes. Animal model is the chimpanzee, which played a
164 major role in the discovery of HCV [64]. Other animal models are HCV-permissive mouse models
165 [65]. Important advances have been achieved with the replicon system [66,67] and the identification of
166 a full-length isolate of HCV (JFH1) that could recapitulate the full viral life cycle in the human
167 hepatoma cell line Huh7 (cell culture HCV, HCVcc) [68]. The viral life cycle was studied using such
168 viral systems [69,70] and more recently primary human hepatocytes [71].

169 **e. HCV particle**

170 HCV virion structure is still poorly known. Its association with lipoproteins is one of its major
171 characteristic [72]. HCV particles are 50–80 nm in diameter [73] and contain the single-stranded RNA
172 genome associated with the core and the envelope glycoproteins, E1 and E2 [74]. The HCV genome
173 interacts with the core protein to form the nucleocapsid, and is surrounded by a lipid membrane, in
174 which are anchored the envelope glycoproteins. Apolipoproteins can also be found in association with
175 HCV particles [75,76]. The lipid composition of cell culture-HCV produced particles looks like very-

176 low density lipoproteins (VLDL) and low-density lipoproteins (LDL) with cholesteryl esters [77].
177 HCV envelope glycoproteins play a role in receptor binding and mediate the fusion process between
178 the viral envelope and an endosomal host cell membrane [78]. Crystal structures of the E2
179 glycoprotein was recently published [79,80]. Papers have suggested that E1 should be the fusion
180 protein or a fusion partner of an E1E2 fusion complex [81,82].

181 **f. HCV life cycle**

182 **HCV entry**

183 The HCV virion is associated with lipoproteins to form a complex particle called lipoviroparticle [83].
184 The life cycle began by binding to glycosaminoglycans (GAGs) and SRB1. Then, the virus follows a
185 multi-steps process. A series of specific cellular entry factors are involved, including SRB1, CD81,
186 tight-junction proteins, CLDN1 and OCLN, EGFR, transferrin receptor (TfR), and NPC1L1 and
187 signaling proteins. Entry of HCV is probably restricted to hepatocytes. After binding to several
188 components of the host cell, HCV particle is internalized by clathrin-mediated endocytosis and fusion
189 takes place in endosomes [82,83].

190 **HCV RNA translation and replication**

191 IRES initiates translation of the HCV genome into a single polyprotein (Figure 1). Viral and host
192 encoded proteases process the viral polyprotein into the mature proteins, core, E1, E2, p7, NS2, NS3,
193 NS4A, NS4B, NS5A, and NS5B [84]. Peptidases mediate cleavage of the structural proteins, core, E1,
194 E2, and the p7/NS2 junction. NS3 mediates cleavage of NS4A and NS4B, and then NS4A associates
195 with the N-terminus of NS3. The resulting NS3/4A protease complex can then cleave at the NS4B/5A
196 and NS5A/5B junctions. The cleavage between NS2 and NS3 is mediated by the NS2 cysteine
197 protease.

198 After translation, the HCV proteins are associated with membranes derived from the endoplasmic
199 reticulum (ER). NS3/4A, NS4B, NS5A, and NS5B constitute the viral proteins of the replication

200 process, which replicates the positive sense RNA genome through a negative strand intermediate [85].
201 The viral RNA-dependent RNA polymerase NS5B is the key enzyme of RNA synthesis. RNA
202 genomes are translated to produce new HCV proteins, and are RNA templates for further RNA
203 replication and they are assembled to form infectious virions.

204 HCV, to replicate its genome, induces massive rearrangements of intracellular membranes to create in
205 the cytoplasm a micro-environment, called the “membranous web” [85]. Analyses with electron
206 microscopy and 3D reconstructions show double-membrane vesicles accumulating during the RNA
207 replication peak. NS5A is the only protein capable to induce double-membrane vesicles [83].

208 A large number of host factors influence translation and replication of HCV which were identified
209 using different approaches [86].

210 Lipid droplets (LDs) are also found in ultrastructural studies of the membranous web. It is suggested
211 that they play a role in the coordination of viral RNA synthesis and virion morphogenesis [83]. By
212 interacting with different cellular partners, NS5A plays a central role in the formation of the
213 membranous web and the recruitment of the replicase around LDs.

214 **HCV assembly and release**

215 The implication of the viral non-structural proteins, like in other members of Flaviviridae family, is
216 important in the HCV particle assembly. The core protein interacts with the genomic RNA to form the
217 nucleocapsid. After cleavage of its C-terminus, core protein is loaded onto LDs. The junction between
218 core-loaded LDs and the replication-complex-rich ER membranes is the site of virion assembly.

219 HCV glycoproteins E1 and E2 form a complex retained in the ER which migrate close to LDs where
220 assembly takes place [87]. Interactions between NS2 and E1, E2, and p7 and are essential for the
221 migration of E1E2 heterodimer at the virion assembly site [88]. P7 is also necessary for the final steps
222 of capsid assembly and envelopment [89].

223 The other non-structural proteins are also involved in the assembly process. The C-terminal domain of
224 NS5A is essential for NS5A interaction with the LD-bound core protein, and HCV assembly. Within
225 the replicase, the NS3/4A And NS4B and NS5B, have been implicated in virus assembly. Thus, newly
226 replicated viral genomes are transferred to the assembly sites via NS3/4A or NS5A, and NS2, and p7
227 connect replication complexes and core proteins to the glycoproteins [88].

228 The next steps in HCV virion morphogenesis are linked to the metabolism of VLDL assembly but is
229 poorly understood. In patients, HCV particles circulate as hybrid particles, known as lipoviroparticles
230 (LVPs), combining components of both HCV and very low-density lipoprotein (VLDL). The
231 importance of ApoE for the life cycle of HCV has been confirmed by studies showing ApoE to be
232 required for LVP production [90]. LVPs circulate in the serum of patients as part of a mixed
233 population, including lipoprotein-like particles and complete viral particles [91].

234 **2. DIAGNOSIS OF HCV INFECTION**

235 As already mentioned in the introduction, the development of oral very effective, direct acting
236 antivirals agents (DAAS), with very low toxicity has completely changed the management of HCV
237 infection, with the opportunity to eradicate HCV and cure the estimated 71 million people living with
238 HCV around the world. However, while DAAS are now available, the access to treatment is still
239 difficult.

240 Moreover, very few people living with HCV are aware of their infection. The challenge is to offer
241 access to affordable and simple diagnostic tools, especially in low and middle-income countries
242 (LMICs) where HCV is frequent and the diagnostic step essential to eradicate HCV worldwide.

243 From a public health perspective, HCV diagnosis should be as more simple as possible and should be
244 linked to the HCV cure process. It also has been underlined that liver assessment is very important for
245 choosing treatment duration and particularly for monitoring patients with cirrhosis [92,93].

246

247 In this chapter, we will discuss the different categories of laboratory tests: classical serologic tests able
248 to detect anti HCV antibodies (Ab), named indirect tests, and assays that can detect and quantify HCV
249 particle components such as HCV RNA or HCV Core Antigen (cAg) named direct tests. We will also
250 discuss new virological tools for screening and monitoring HCV infection. All these tests are
251 complementary for the diagnosis of infection, therapeutic decisions and to assess treatment efficacy
252 (cure or eradication of infection) after the end of treatment.

253 Indirect Tests

254 Viral antigens used to develop serological assays were obtained by cloning HCV genome soon after
255 the discovery of HCV. Serological tests were first developed for blood donors screening and prevent
256 blood transmission.

257 Anti HCV antibodies (Ab) are generally identified using ELISA (enzyme linked immunosorbent
258 assay) or chemiluminescence immuno-assay (CLIA) tests. We are now using third generation assays
259 which incorporate several HCV epitopes from the different structural and nonstructural regions of
260 HCV (C100-3, NS4 region in the first generation assays and C22 (Capsid region), C33 (NS3 region)
261 and NS5 antigen in the third generation assay).

262 During acute infection, as in many other viral infections, there is a window period where specific anti-
263 HCV Ab are not detectable. With the third generation assays, the window period can vary from patient
264 to patient and from test to test: the seroconversion generally occurs after 4 to 8 weeks after the onset of
265 infection [94–97]. Fourth-generation assays are also known as “antigen-antibody combo” tests, the
266 principle of these assays being the detection of both anti- HCV Ab and HCV Ag at the same time [98].
267 These assays are positive earlier in acute infections and thus reduce the window period [99].
268 Sensitivity and specificity of third generation assays are high in our countries, when tests are approved
269 by European or American authorities (FDA) (nearly 99%). Some false positive results are described
270 in various pathological conditions and in blood donors. It has been shown that high titres of anti HCV
271 antibodies (high ratio) are more often true positive than low titres which are more often false positive
272 (88-89). The sensitivity was sometimes in deficit with first generation assays especially in

273 immunocompromised patients, however the third generation tests generally are able to detect anti-
274 HCV anti-Ab in these patients except if immunosuppression is serious. In this case, serological tests
275 may be negative despite active infection [99].

276 Supplemental tests

277 Recombinant immunoblot assays (RIBA) were used in the past to confirm the ELISA tests. They were
278 also useful to determinate false positivity in case of a weakly EIA signal in the absence of viremia
279 [100]. The results indicated the presence of specific anti-HCV antibodies, meaning that tested
280 individuals have currently an active infection or have come into contact with HCV at some point. But
281 they are now no more used.

282 Direct classical tests

283 HCV core Antigen detection was developed in the early 2000 [101]. At the beginning, they were
284 commercialized as shaper alternatives to nucleic acid test (NAT) i.e. to screen blood donors or to be
285 used in low- and middle-income countries (LMICS), where laboratories are not well equipped. These
286 assays are either ELISA or CLIA and require less expertise and are less expensive compared to NAT.
287 A lot of teams have evaluated HCV core Ag in transfusion or for diagnosis of infection and for
288 monitoring treatments like interferon [102–106] and more recently DAAS [107]. New generation
289 CLIA quantitative test (Architect, Abbott laboratories) has good sensitivity but less than that of real
290 time PCR (limit of detection equivalent to 500-3000 HCV RNA IU/ml) [108,109].

291 Qualitative or quantitative detection of HCV RNA

292 Molecular tests are used to confirm HCV infection. They used either RT PCR or transcription
293 mediated amplification (TMA). These tests are indicated to confirm replication in subjects found anti
294 HCV Ab positive or to detect viremia in acute infection, during the window period (when Ab assays
295 are still negative). Commercialized HCV RNA tests are very sensitive and able to detect and quantify
296 less than 30 copies/ml (reference) or <15 IU/ml of HCV RNA or less [110]. Quantification of HCV
297 RNA is more often used.

298 The following table 1 summarizes the available, approved tests for HCV RNA detection and/or
 299 quantification[110,111].

	Assay principle	Sample type (mL)	Automation level	Range of quantification (IU/mL)	Lower limit of detection (qualitative, IU/mL) (plasma/serum)	Lower limit of quantification (IU/mL)	Analysis time (hours)	Direct sample processing
RealTime HCV (Abbott)	RT-PCR	0.5 mL plasma/serum	Automated assay	12-10 ⁸	10.5/7.2 IU/mL	12	4.6	No
CAP/CTM HCV 2.0 (Roche)		0.650 mL plasma/serum		15-10 ⁸	9.3/8.8 IU/mL	15	5.7	No
Xpert® HCV (Cepheid)		1 mL plasma/serum		10-10 ⁸	4.0/6.1 IU/mL	10	1.75	Yes
VERIS HCV (Beckman)		1 mL plasma		12-10 ⁹	12 IU/mL	12	1.75	Yes
Aptima® HCV Quant Dx (Hologic)		TMA		0.5 mL plasma/serum	10-10 ⁸	4.3/3.9 IU/mL	10	2.5

300 Results are expressed in IU/ml.

301 Before DAAS availability, the follow up of patients treated with Pegylated interferon and ribavirin
302 was based on HCV RNA decline which was a good predictor of response. With DAAs, HCV RNA
303 measurements are no longer required/necessary during treatment but they are recommended 3 months
304 after the end of therapy, to confirm HCV eradication, or in case of reinfection suspicion [47].

305 New virological tools for indirect/direct diagnosis

306 Point of care tests (POC):

307 These tests are so called POC tests because they can be used close to the patient, locally or can be sent
308 by post without needing cold chain.

309 These tests use plasma [112,113], or whole blood drops by veno-puncture. Other matrices, like
310 capillary blood, using manual or automated device (lancette) which can be used in children (puncture
311 of the finger or the heel). Capillary blood can be collected in a tube, a pipette or paper (DBS, dried
312 blood spot). Oral fluid similarly can be also collected [98]. DBS can be used to collect whole blood
313 specimens for EIA detection of anti-HCV antibodies in a central laboratory. A second spot on the
314 same paper can be used to test for HCV RNA (to be performed in anti-HCV antibody-positive
315 samples) [114].

316 Rapid diagnostic tests (RDT)

317 Their principle is to capture antibodies on a solid surface where complementary antigens are attached.
318 Different technologies are used, like immune-chromatography, lateral flow tests, cassettes. There is a
319 control line which can confirm the correct liquid migration.

320 RDT have a lot of advantages but also disadvantages: they are easy to use, do not need special
321 equipment, have generally good sensitivity and specificity, when approved by international authorities
322 like WHO or FDA. The result can be given very fast, permitting early management /cure of patients
323 [115].

324 However, the interpretation of the result is subjective and the quality of the results may depend on the
325 quality of matrices (i.e patients with low Ab quantity can be falsely negative) [109].

	Manufacturer	Sample Type	Volume needed (μ L)	Reading time (min)	Sensitivity – Whole Blood (95%CI)	Specificity – Whole blood (95%CI)
Oraquick® HCV Rapid Antibody Test	Orasure Technologies, Bethlehem, PA	Oral fluid, whole blood, serum, plasma	5 or 40 (oral fluid)	20-40	99.4% (97.7-99.9) 97.6% (oral fluid)	100% (97.9-100.0) 100% (oral fluid)
Toyo® anti-HCV test	Turklab, Izmir, Turkey	Whole blood, serum, plasma	30 (serum, plasma) or 60 (whole blood)	5-15	96.2% (93.3-98.0)	98.2% (94.8-99.4)
Labmen® HCV test	Turklab, Izmir, Turkey	Whole blood, serum, plasma	10	15	62.7 % (54.8-69.5)	100% (94.4-100.0)
Signal HCV v2.0	Span Divergent, Udhna, India	Serum, plasma	100	10		
MultiSure HCV	MP Biomedicals, Singapore	Whole blood, serum, plasma	25	15		
Assure HCV Rapid test	MP Biomedicals, Singapore	Whole blood, serum, plasma	5 (serum, plasma) or 50 (whole	15		

			blood)			
First Response HCV Card Test	Premier Medical Corporation, Daman India	Whole blood, serum, plasma	35	20-30		
SD Bioline HCV	Standard Diagnostics, Yongin, Korea	Whole blood, serum, plasma	10	5-20		
Advanced Quality Rapid Anti-HCV Test	Intec Products Inc., China	Whole blood, serum, plasma	10	15-20		

327

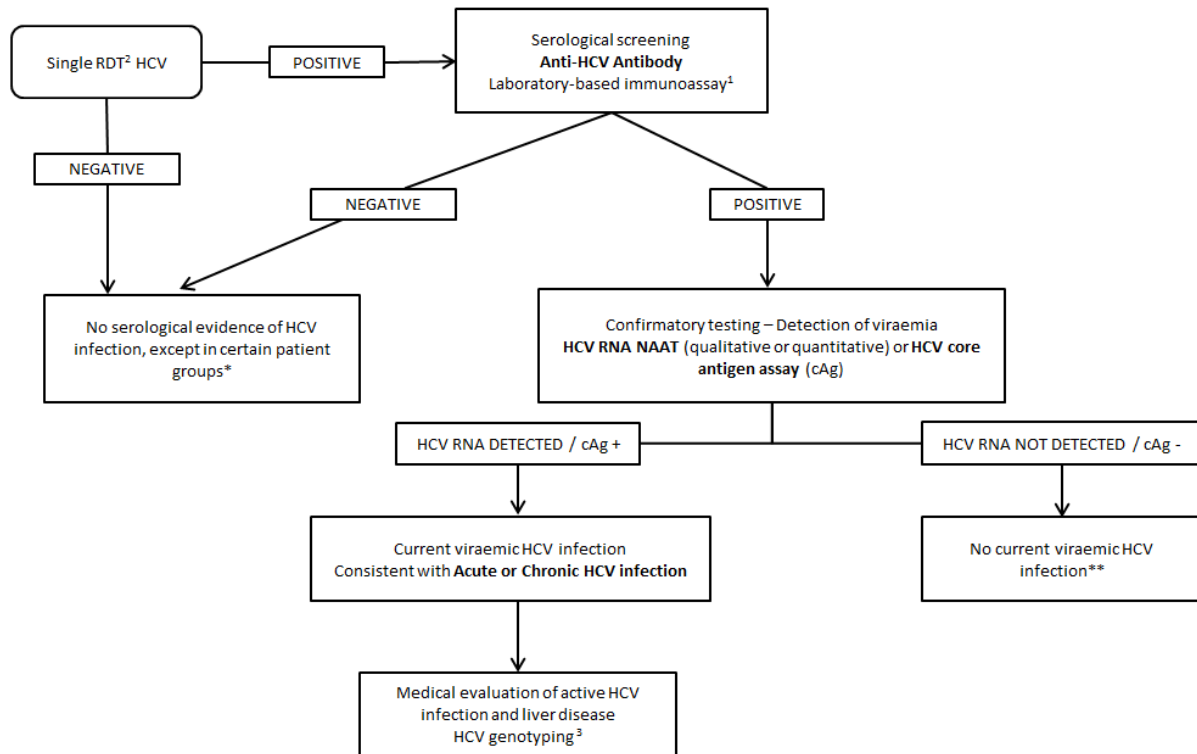
328 3. HCV diagnosis

329 HCV can be diagnosed by the detection of anti-HCV antibodies in the serum or plasma samples,
330 where a non-reactive result is considered as absence of HCV infection. To assess that HCV antibodies
331 implies active infection, it needs to be confirmed by a HCV RNA test.

332 In figure 4, we propose a simple algorithm for HCV testing applicable theoretically worldwide.
333 However, screening strategies for HCV infection should be defined according to the local
334 epidemiology of HCV infection, ideally within the framework of national plans [116]. If acute
335 infection is suspected, as previously mentioned, initial testing for HCV RNA or HCV core antigen
336 may be required in immunocompromised patients, patients previously tested for HCV infection and
337 those at risk of recent infection during the window period.

338 HCV infection can sometimes be cleared after the acute phase in a low percentage of cases [117,118]
339 and individuals may remain anti-HCV Ab positive without any detectable viremia. Thus, if anti-HCV

340 antibodies are detected, the presence of HCV RNA, or alternatively HCV core antigen (if HCV RNA
 341 assays are not available and/or not affordable) should be determined to identify patients with active
 342 infection [98,116,119]. Of course, clinical and biological evaluation is mandatory if acute or chronic
 343 infection is confirmed.



344
 345 Figure 4: Algorithm for hepatitis virus C testing. *Initial testing for HCV RNA or HCV core antigen is
 346 required for immunocompromised patients, patients previously tested for HCV infection and those at
 347 risk of recent infection in the window period (Ab response is not always developed and anti-HCV
 348 antibodies might not yet been detected). Moreover, HCV RNA or cAg may be tested in case of high
 349 suspicion of HCV infection. HCV RNA should be tested four to six weeks after the exposure. ** past
 350 infection or false positive Ab assay. ¹it includes chemiluminescence immunoassay (CLIA), enzyme
 351 immunoassay (EIA) or electrochemiluminescence assay (ECL). ²RDT : Rapid Diagnostic Test. NAAT
 352 : Nucleic Acid Amplification Test. ³ : HCV genotyping is only necessary in few cases to decide the
 353 treatment [47].

354 **HCV genotyping methods**

355 Generally, the most recommended methods to determine the HCV genotype are sequencing the highly
356 conserved regions of the HCV genome such as NS5, core, E1 and 5' UTR. There are also kit-based
357 assays using probes corresponding to genotype specific oligonucleotides and amplified products are
358 then hybridized with these probes [120]. The most widely used method is based on reverse
359 hybridization with the line probe assay [121]. Deep sequencing will soon be also useful for
360 genotyping.

361 Together with prior treatment failure and the presence of severe fibrosis, the HCV genotype, including
362 genotype 1 subtype, may still be useful in some cases to decide the treatment regimen and duration,
363 particularly in previous non responders or in reinfections [47].

364 However, it is possible to treat individuals with pan-genotypic HCV drugs without HCV
365 genotype/subtype, in particular in LMICs where molecular tests are not available or too costly, or to
366 simplify therapy to improve access to care in some populations i.e, IV drug addicts [116].

367

368 CONCLUSION

369

370 The identification of HCV in 1989, a few years after the blood scandal, was one of the major event in
371 the medical field, as recently recognized by the Medicine Nobel price. The main consequences of this
372 discovery were the development of screening tests first used for blood donors, to avoid post
373 transfusion hepatitis (which was observed in 5% of blood recipients, before the implementation of anti
374 HCV Ab assays). Almost thirty years later, the increasing knowledge of HCV biology was also at the
375 origin of the development of DAA able to achieve HCV cure. The last challenge will be to increase
376 access to diagnostic and care of HCV infection, especially in low income countries.

377

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