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1 HCV virology and diagnosis

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9 INTRODUCTION

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

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

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

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

1. HCV VIROLOGY

a. HCV genome

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

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

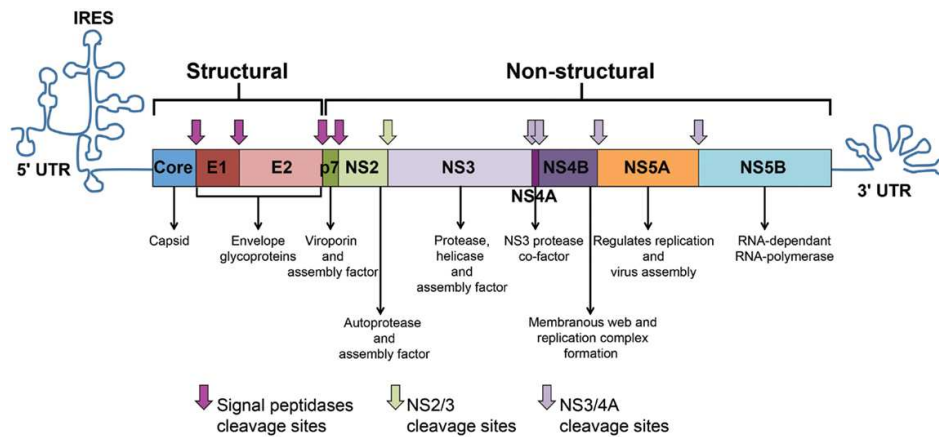


Figure 1 : HCV genome and viral particle structure. The HCV genome is composed of an open reading frame (ORF) flanked by 5' and 3' untranslated regions (UTRs). IRES-mediated translation of the ORF leads to the formation of a polyprotein that is processed into 10 viral proteins. Cleavage of the core protein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

b. Variability of HCV

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

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

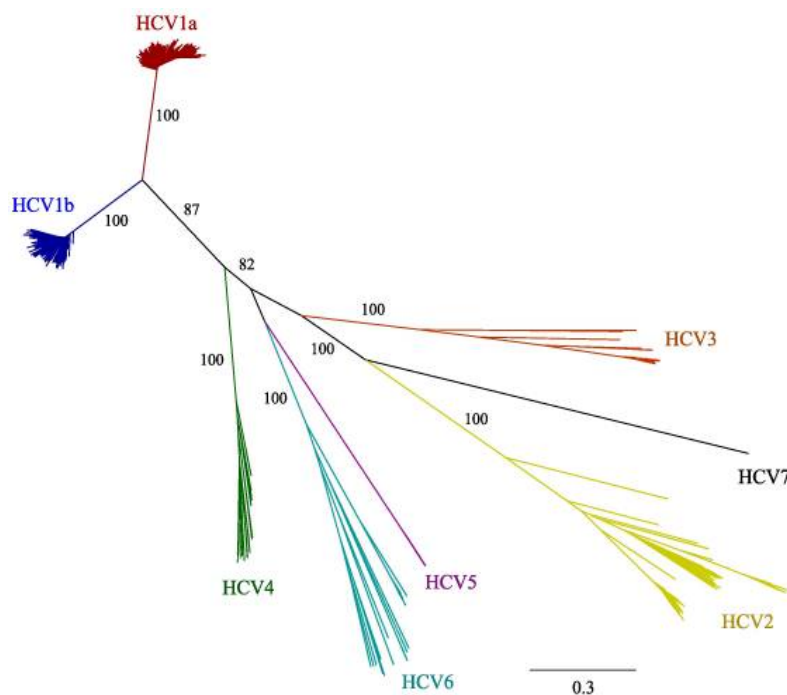
involved in translation or replication), or displaying major structural constraints (non-coding 5' and 3' ends) are the best conserved; indeed, the non-coding 5' region is the most highly conserved region of the genome, with more than 90% identity between the sequences of distantly related strains (4).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

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

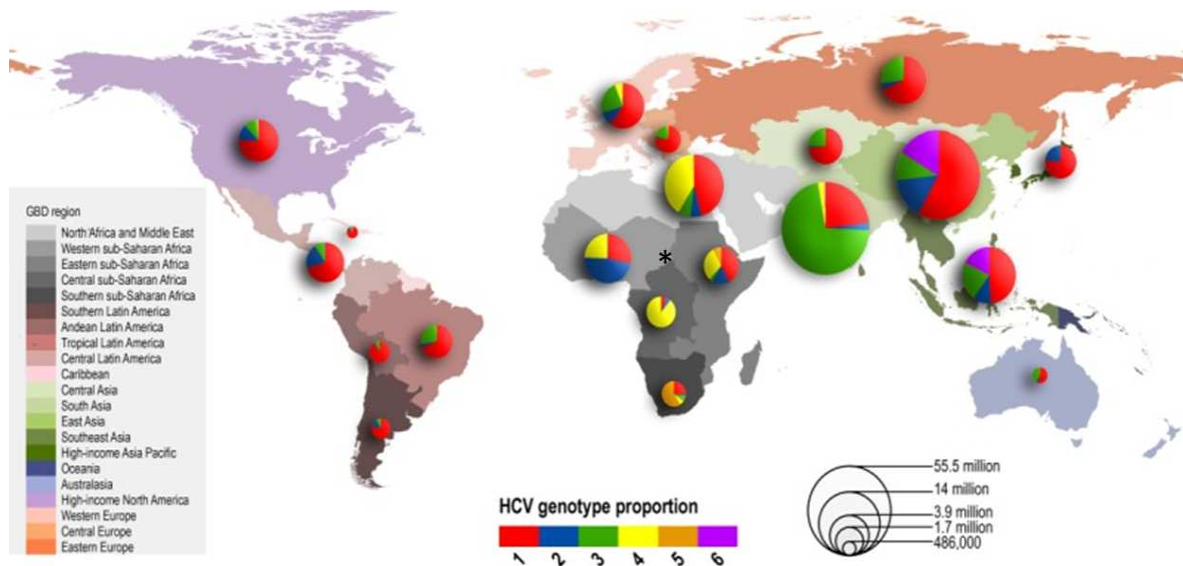


Figure 3 : Global distribution and prevalence of hepatitis C virus genotypes [38]. *Genotype 7 has only been found in few people from central Africa.

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

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

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

d. Clinical Significance of Quasispecies and Genotypes

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

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

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

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

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

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

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

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

e. HCV particle

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

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

f. HCV life cycle

HCV entry

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

HCV RNA translation and replication

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

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

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

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

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

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

HCV assembly and release

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

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

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

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

2. DIAGNOSIS OF HCV INFECTION

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

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

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

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

Indirect Tests

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

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

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

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

Supplemental tests

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

Direct classical tests

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

Qualitative or quantitative detection of HCV RNA

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

300 Results are expressed in IU/ml.

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

New virological tools for indirect/direct diagnosis

Point of care tests (POC):

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

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

Rapid diagnostic tests (RDT)

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

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

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

	Manufacturer	Sample Type	Volume needed (μL)	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

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

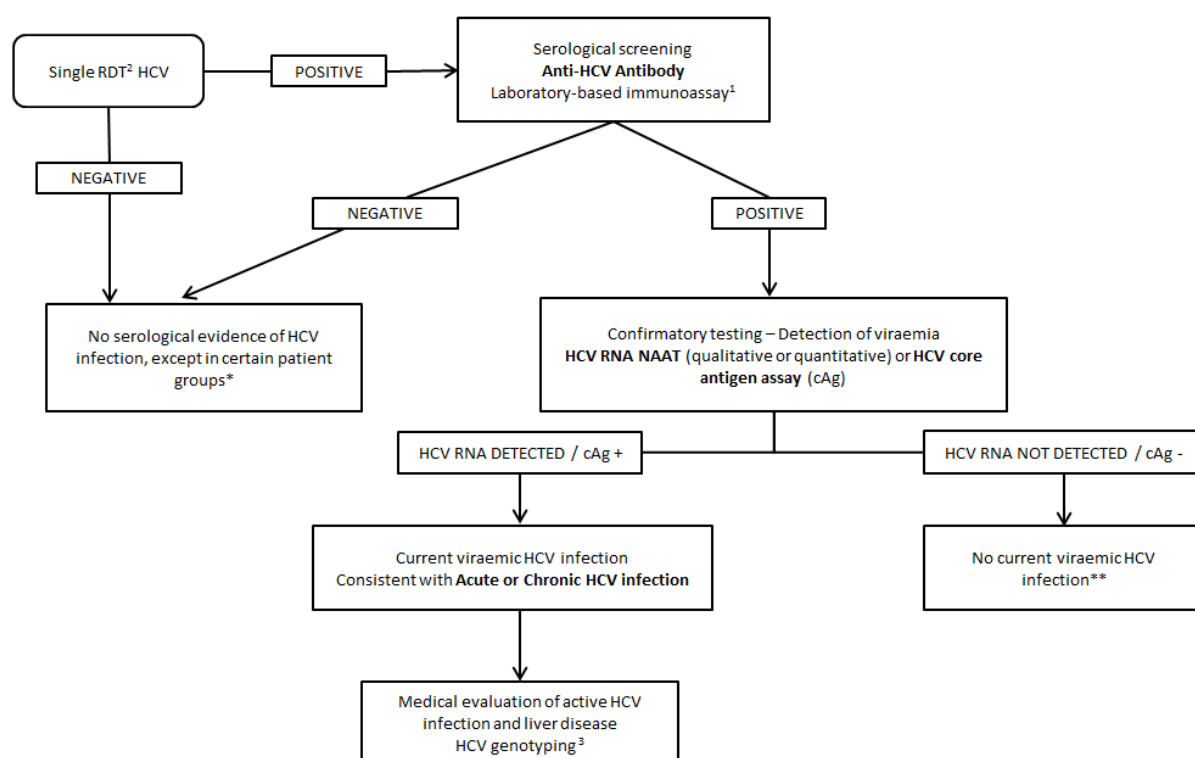


Figure 4: Algorithm for hepatitis virus C testing. *Initial testing for HCV RNA or HCV core antigen is required for immunocompromised patients, patients previously tested for HCV infection and those at risk of recent infection in the window period (Ab response is not always developed and anti-HCV antibodies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

HCV genotyping methods

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

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

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

CONCLUSION

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

REFERENCES

- [1] World Health Organization, Global Hepatitis Programme. Global hepatitis report, 2017. 2017.
- [2] Pawlotsky J. New Hepatitis C Therapies: The Toolbox, Strategies, and Challenges 2014;146:17.
- [3] Roingear P, Beaumont E. Hepatitis C Vaccine: 10 Good Reasons for Continuing. Hepatology 2020;71:1845–50. <https://doi.org/10.1002/hep.31182>.

- [4] Beaumont E, Joël Clément B, Guérin V, Chopin L, Roch E, Gomez-Escobar E, et al. Mixing particles from various HCV genotypes increases the HBV-HCV vaccine ability to elicit broadly cross-neutralizing antibodies. *Liver Int* 2020;40:1865–71. <https://doi.org/10.1111/liv.14541>.
- [5] Feinstone SM, Kapikian AZ, Purcell RH, Alter HJ, Holland PV. Transfusion-Associated Hepatitis Not Due to Viral Hepatitis Type A or B. *N Engl J Med* 1975;292:767–70. <https://doi.org/10.1056/NEJM197504102921502>.
- [6] Kolykhalov AA. Transmission of Hepatitis C by Intrahepatic Inoculation with Transcribed RNA. *Science* 1997;277:570–4. <https://doi.org/10.1126/science.277.5325.570>.
- [7] Choo Q, Kuo G, Weiner A, Overby L, Bradley D, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;244:359–62. <https://doi.org/10.1126/science.2523562>.
- [8] Kapoor A, Simmonds P, Gerold G, Qaisar N, Jain K, Henriquez JA, et al. Characterization of a canine homolog of hepatitis C virus. *Proc Natl Acad Sci* 2011;108:11608–13. <https://doi.org/10.1073/pnas.1101794108>.
- [9] Drexler JF, Corman VM, Müller MA, Lukashev AN, Gmyl A, Coutard B, et al. Evidence for Novel Hepaciviruses in Rodents. *PLoS Pathog* 2013;9:e1003438. <https://doi.org/10.1371/journal.ppat.1003438>.
- [10] Tanaka T, Kato N, Cho MJ, Shimotohno K. A Novel Sequence Found at the 3'-Terminus of Hepatitis C Virus Genome. *Biochem Biophys Res Commun* 1995;215:744–9. <https://doi.org/10.1006/bbrc.1995.2526>.
- [11] Tsukiyama-Kohara K, Iizuka N, Kohara M, Nomoto A. Internal ribosome entry site within hepatitis C virus RNA. *J Virol* 1992;66:1476–83. <https://doi.org/10.1128/JVI.66.3.1476-1483.1992>.
- [12] Abdel-Hakeem MS, Shoukry NH. Protective Immunity Against Hepatitis C: Many Shades of Gray. *Front Immunol* 2014;5. <https://doi.org/10.3389/fimmu.2014.00274>.
- [13] Steinhauer DA, Domingo E, Holland JJ. Lack of evidence for proofreading mechanisms associated with an RNA virus polymerase. *Gene* 1992;122:281–8. [https://doi.org/10.1016/0378-1119\(92\)90216-C](https://doi.org/10.1016/0378-1119(92)90216-C).
- [14] Ogata N, Alter HJ, Miller RH, Purcell RH. Nucleotide sequence and mutation rate of the H strain of hepatitis C virus. *Proc Natl Acad Sci* 1991;88:3392–6. <https://doi.org/10.1073/pnas.88.8.3392>.
- [15] Okamoto H, Kojima M, Okada S-I, Yoshizawa H, Iizuka H, Tanaka T, et al. Genetic drift of hepatitis C virus during an 8.2-year infection in a chimpanzee: Variability and stability. *Virology* 1992;190:894–9. [https://doi.org/10.1016/0042-6822\(92\)90933-G](https://doi.org/10.1016/0042-6822(92)90933-G).
- [16] Smith DB. Evolution of the hypervariable region of hepatitis C virus. *J Viral Hepat* 1999;6:41–6. <https://doi.org/10.1046/j.1365-2893.1999.00010.x>.
- [17] Labonté P, Axelrod V, Agarwal A, Aulabaugh A, Amin A, Mak P. Modulation of Hepatitis C Virus RNA-dependent RNA Polymerase Activity by Structure-based Site-directed Mutagenesis. *J Biol Chem* 2002;277:38838–46. <https://doi.org/10.1074/jbc.M204657200>.
- [18] Gaudy C, Moreau A, Veillon P, Temoin S, Lunel F, Goudeau A. Significance of Pretreatment Analysis of Hepatitis C Virus Genotype 1b Hypervariable Region 1 Sequences To Predict Antiviral Outcome. *J Clin Microbiol* 2003;41:3615–22. <https://doi.org/10.1128/JCM.41.8.3615-3622.2003>.
- [19] Puntoriero G. Towards a solution for hepatitis C virus hypervariability: mimotopes of the hypervariable region 1 can induce antibodies cross-reacting with a large number of viral variants. *EMBO J* 1998;17:3521–33. <https://doi.org/10.1093/emboj/17.13.3521>.
- [20] Ray SC, Wang YM, Laeyendecker O, Ticehurst JR, Villano SA, Thomas DL. Acute hepatitis C virus structural gene sequences as predictors of persistent viremia: hypervariable region 1 as a decoy. *J Virol* 1999;73:2938–46. <https://doi.org/10.1128/JVI.73.4.2938-2946.1999>.
- [21] Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource. *Hepatology* 2014;59:318–27. <https://doi.org/10.1002/hep.26744>.

- 435 [22] Simmonds P, Becher P, Bukh J, Gould EA, Meyers G, Monath T, et al. ICTV Virus Taxonomy
436 Profile: Flaviviridae. *J Gen Virol* 2017;98:2–3. <https://doi.org/10.1099/jgv.0.000672>.
- 437 [23] Farci P, Bukh J, Purcell RH. The quasispecies of hepatitis C virus and the host immune
438 response. *Springer Semin Immunopathol* 1997;19:5–26. <https://doi.org/10.1007/BF00945022>.
- 439 [24] Tsukiyama-Kohara K, Kohara M. Hepatitis C Virus: Viral Quasispecies and Genotypes. *Int J Mol*
440 *Sci* 2017;19. <https://doi.org/10.3390/ijms19010023>.
- 441 [25] Farci P, Bukh J, Purcell RH. The quasispecies of hepatitis C virus and the host immune
442 response. *Springer Semin Immunopathol* 1997;19:5–26. <https://doi.org/10.1007/BF00945022>.
- 443 [26] Powdrill MH, Tchesnokov EP, Kozak RA, Russell RS, Martin R, Svarovskaia ES, et al.
444 Contribution of a mutational bias in hepatitis C virus replication to the genetic barrier in the
445 development of drug resistance. *Proc Natl Acad Sci* 2011;108:20509–13.
446 <https://doi.org/10.1073/pnas.1105797108>.
- 447 [27] Ootsuyama Y, Hijikata M, Ohkoshi S, SHIMOTOHNOI K. Humoral Immune Response to
448 Hypervariable Region 1 of the Putative Envelope Glycoprotein (gp70) of Hepatitis C Virus. *J*
449 *VIROL* n.d.:8.
- 450 [28] Cerny A, Chisari FV. Immunological Aspects of HCV Infection. *Intervirology* 1994;37:119–25.
451 <https://doi.org/10.1159/000150366>.
- 452 [29] Higashi K, Tsukiyama-Kohara K, Tanaka T, Tanaka E, Kiyosawa K, Kohara M. Characterization of
453 hypervariable region in hepatitis C virus envelope protein during acute and chronic infection.
454 *Arch Virol* 2005;150:883–98. <https://doi.org/10.1007/s00705-004-0470-0>.
- 455 [30] Gaudy C, Moreau A, Veillon P, Temoin S, Lunel F, Goudeau A. Significance of Pretreatment
456 Analysis of Hepatitis C Virus Genotype 1b Hypervariable Region 1 Sequences To Predict
457 Antiviral Outcome. *J Clin Microbiol* 2003;41:3615–22. [https://doi.org/10.1128/JCM.41.8.3615-](https://doi.org/10.1128/JCM.41.8.3615-3622.2003)
458 [3622.2003](https://doi.org/10.1128/JCM.41.8.3615-3622.2003).
- 459 [31] Tulio de Oliveira, Marco Salemi, Oliver Pybus, Sabrina Almeida, Andrew Rambaut.
460 BioAfrica.net. BioAfrica - Oxf HCV Subtyping Tool n.d.
461 <http://www.bioafrica.net/subtypetool/html/subtypeprocesshcv.html> (accessed November 20,
462 2020).
- 463 [32] Laperche S, Saune K, Deny P, Duverlie G, Alain S, Chaix M-L, et al. Unique NS5b Hepatitis C
464 Virus Gene Sequence Consensus Database Is Essential for Standardization of Genotype
465 Determinations in Multicenter Epidemiological Studies. *J Clin Microbiol* 2006;44:614–6.
466 <https://doi.org/10.1128/JCM.44.2.614-616.2006>.
- 467 [33] Le Guillou-Guillemette H. Genetic diversity of the hepatitis C virus: Impact and issues in the
468 antiviral therapy. *World J Gastroenterol* 2007;13:2416.
469 <https://doi.org/10.3748/wjg.v13.i17.2416>.
- 470 [34] Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution
471 and prevalence of hepatitis C virus genotypes. *Hepatology* 2015;61:77–87.
472 <https://doi.org/10.1002/hep.27259>.
- 473 [35] Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C Virus
474 Genotype 7, a New Genotype Originating from Central Africa. *J Clin Microbiol* 2015;53:967–
475 72. <https://doi.org/10.1128/JCM.02831-14>.
- 476 [36] Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, et al. Expanded classification
477 of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype
478 assignment web resource. *Hepatology* 2014;59:318–27. <https://doi.org/10.1002/hep.26744>.
- 479 [37] Cuypers L, Li G, Libin P, Piampongsant S, Vandamme A-M, Theys K. Genetic Diversity and
480 Selective Pressure in Hepatitis C Virus Genotypes 1–6: Significance for Direct-Acting Antiviral
481 Treatment and Drug Resistance. *Viruses* 2015;7:5018–39. <https://doi.org/10.3390/v7092857>.
- 482 [38] Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, et al. Global distribution
483 and prevalence of hepatitis C virus genotypes. *Hepatology* 2015;61:77–87.
484 <https://doi.org/10.1002/hep.27259>.
- 485 [39] Pybus OG. The Epidemic Behavior of the Hepatitis C Virus. *Science* 2001;292:2323–5.
486 <https://doi.org/10.1126/science.1058321>.

487 [40] Bressanelli S, Tomei L, Roussel A, Incitti I, Vitale RL, Mathieu M, et al. Crystal structure of the
488 RNA-dependent RNA polymerase of hepatitis C virus. *Proc Natl Acad Sci* 1999;96:13034–9.
489 <https://doi.org/10.1073/pnas.96.23.13034>.

490 [41] Akkarathamrongsin S, Hacharoen P, Tangkijvanich P, Theamboonlers A, Tanaka Y, Mizokami
491 M, et al. Molecular Epidemiology and Genetic History of Hepatitis C Virus Subtype 3a Infection
492 in Thailand. *Intervirology* 2013;56:284–94. <https://doi.org/10.1159/000351621>.

493 [42] Simmonds P. 2000 Fleming Lecture. The origin and evolution of hepatitis viruses in humans. *J*
494 *Gen Virol* 2001;82:693–712. <https://doi.org/10.1099/0022-1317-82-4-693>.

495 [43] Iles JC, Raghwan J, Harrison GLA, Pepin J, Djoko CF, Tamoufe U, et al. Phylogeography and
496 epidemic history of hepatitis C virus genotype 4 in Africa. *Virology* 2014;464–465:233–43.
497 <https://doi.org/10.1016/j.virol.2014.07.006>.

498 [44] Li C, Yuan M, Lu L, Lu T, Xia W, Pham VH, et al. The genetic diversity and evolutionary history
499 of hepatitis C virus in Vietnam. *Virology* 2014;468–470:197–206.
500 <https://doi.org/10.1016/j.virol.2014.07.026>.

501 [45] Orito E, Mizokami M, Mizoguchi N, Yamanaka-’ H, Oguri T, Hirashima N, et al. Hepatitis C virus
502 serotype II responds more favorably to interferon-a therapy n.d.:3.

503 [46] Kohara M, Tanaka T, Tsukiyama-Kohara K, Tanaka S, Mizokami M, Lau JYN, et al. Hepatitis C
504 Virus Genotypes 1 and 2 Respond to Interferon-a with Different Virologic Kinetics n.d.:5.

505 [47] Pawlotsky J-M, Negro F, Aghemo A, Berenguer M, Dalgard O, Dusheiko G, et al. EASL
506 Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018;69:461–511.
507 <https://doi.org/10.1016/j.jhep.2018.03.026>.

508 [48] Nobuyuki E, Ikuo S, Yasuhiro A, Masayuki K, Takeshi M, Chikara Y, et al. Mutations in the
509 Nonstructural Protein 5a Gene and Response to Interferon in Patients with Chronic Hepatitis C
510 Virus 1b Infection. *N Engl J Med* 1996:5.

511 [49] Castelain S, Khorsi H, Roussel J, François C, Jaillon O, Capron D, et al. Variability of the
512 Nonstructural 5A Protein of Hepatitis C Virus Type 3a Isolates and Relation to Interferon
513 Sensitivity. *J Infect Dis* 2002;185:573–83. <https://doi.org/10.1086/339051>.

514 [50] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in
515 the Nonstructural Protein 5a Gene and Response to Interferon in Patients with Chronic
516 Hepatitis C Virus 1b Infection. *N Engl J Med* 1996;334:77–82.
517 <https://doi.org/10.1056/NEJM199601113340203>.

518 [51] Squadrito G, Raffa G, Restuccia T, Pollicino T, Brancatelli S, Raimondo G. Is investigation of
519 hepatitis C virus NS5A gene heterogeneity a tool for predicting long-lasting response to
520 interferon therapy in patients with HCV-1b chronic hepatitis? *J Viral Hepat* 2002;9:360–9.
521 <https://doi.org/10.1046/j.1365-2893.2002.00379.x>.

522 [52] Chung RT, Monto A, Dienstag JL, Kaplan LM. Mutations in the NS5A region do not predict
523 interferon-responsiveness in American patients infected with genotype 1b hepatitis C virus
524 n.d.:6.

525 [53] Shen C, Hu T, Shen L, Gao L, Xie W, Zhang J. Mutations in ISDR of NS5A gene influence
526 interferon efficacy in Chinese patients with chronic hepatitis C virus genotype 1b infection. *J*
527 *Gastroenterol Hepatol* 2007;22:1898–903. <https://doi.org/10.1111/j.1440-1746.2006.04566.x>.

528 [54] Inchauspe G, Prince AM. Genomic structure of the human prototype strain H of hepatitis C
529 virus: Comparison with American and Japanese isolates. *Proc Natl Acad Sci USA* 1991:5.

530 [55] Duverlie G, Khorsi H, Castelain S, Jaillon O, Izopet J, Lunel F, et al. Sequence analysis of the
531 NS5A protein of European hepatitis C virus 1b isolates and relation to interferon sensitivity
532 n.d.:9.

533 [56] Puig-Basagoiti F, Forn S, Furčić I, Ampurdanés S, Giménez-Barcons M, Franco S, et al.
534 Dynamics of hepatitis C virus NS5A quasispecies during interferon and ribavirin therapy in
535 responder and non-responder patients with genotype 1b chronic hepatitis C. *J Gen Virol*
536 2005;86:1067–75. <https://doi.org/10.1099/vir.0.80526-0>.

- [57] Veillon P, Payan C, Gaudy C, Goudeau A, Lunel F. Analyse des mutations des domaines ISDR et V3 de la protéine NS5A du virus de l'hépatite C avant le traitement par l'interféron avec ou sans ribavirine. *Pathol Biol* 2004;52:505–10. <https://doi.org/10.1016/j.patbio.2004.07.011>.
- [58] Lonardo A. Pathogenesis and significance of hepatitis C virus steatosis: An update on survival strategy of a successful pathogen. *World J Gastroenterol* 2014;20:7089. <https://doi.org/10.3748/wjg.v20.i23.7089>.
- [59] Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay A. The interaction of hepatic lipid and glucose metabolism in liver diseases. *J Hepatol* 2012;56:952–64. <https://doi.org/10.1016/j.jhep.2011.08.025>.
- [60] Moucari R, Asselah T, Cazals-Hatem D, Voitot H, Boyer N, Ripault M, et al. Insulin Resistance in Chronic Hepatitis C: Association With Genotypes 1 and 4, Serum HCV RNA Level, and Liver Fibrosis. *Gastroenterology* 2008;134:416–23. <https://doi.org/10.1053/j.gastro.2007.11.010>.
- [61] Thompson AJ, Patel K, Chuang W-L, Lawitz EJ, Rodriguez-Torres M, Rustgi VK, et al. Viral clearance is associated with improved insulin resistance in genotype 1 chronic hepatitis C but not genotype 2/3. *Gut* 2012;61:128–34. <https://doi.org/10.1136/gut.2010.236158>.
- [62] El-Shamy A, Pendleton M, Eng FJ, Doyle EH, Bashir A, Branch AD. Impact of HCV core gene quasiespecies on hepatocellular carcinoma risk among HALT-C trial patients. *Sci Rep* 2016;6:27025. <https://doi.org/10.1038/srep27025>.
- [63] Amougou-Atsama M, Jean Adrien Atangana P, Noah Noah D, Fewou Moundipa P, Pineau P, Njouom R. The role of hepatitis C virus genotypes and core mutations in hepatocellular carcinoma in Cameroon. *J Viral Hepat* 2020;27:880–5. <https://doi.org/10.1111/jvh.13303>.
- [64] Bukh J. Animal Models for the Study of Hepatitis C Virus Infection and Related Liver Disease. *Gastroenterology* 2012;142:1279–1287.e3. <https://doi.org/10.1053/j.gastro.2012.02.016>.
- [65] Billerbeck E, de Jong Y, Dorner M, de la Fuente C, Ploss A. Animal Models for Hepatitis C. In: Bartenschlager R, editor. *Hepat. C Virus Mol. Virol. Antivir. Ther.*, vol. 369, Berlin, Heidelberg: Springer Berlin Heidelberg; 2013, p. 49–86. https://doi.org/10.1007/978-3-642-27340-7_3.
- [66] Manns MP. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006;55:1350–9. <https://doi.org/10.1136/gut.2005.076646>.
- [67] McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon Alfa-2b Alone or in Combination with Ribavirin as Initial Treatment for Chronic Hepatitis C. *N Engl J Med* 1998;339:1485–92. <https://doi.org/10.1056/NEJM199811193392101>.
- [68] Lohmann V. Hepatitis C Virus RNA Replication. In: Bartenschlager R, editor. *Hepat. C Virus Mol. Virol. Antivir. Ther.*, vol. 369, Berlin, Heidelberg: Springer Berlin Heidelberg; 2013, p. 167–98. https://doi.org/10.1007/978-3-642-27340-7_7.
- [69] Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, et al. Complete Replication of Hepatitis C Virus in Cell Culture 2005;309:5.
- [70] Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005;11:791–6. <https://doi.org/10.1038/nm1268>.
- [71] Lagaye S, Shen H, Saunier B, Nascimbeni M, Gaston J, Bourdoncle P, et al. Efficient replication of primary or culture hepatitis C virus isolates in human liver slices: A relevant *ex vivo* model of liver infection. *Hepatology* 2012;56:861–72. <https://doi.org/10.1002/hep.25738>.
- [72] Thomssen R, Bonk S, Propfe C. Association of hepatitis C virus in human sera with I - lipoprotein n.d.:8.
- [73] Catanese MT, Uryu K, Kopp M, Edwards TJ, Andrus L, Rice WJ, et al. Ultrastructural analysis of hepatitis C virus particles. *Proc Natl Acad Sci* 2013;110:9505–10. <https://doi.org/10.1073/pnas.1307527110>.
- [74] Vieyres G, Dubuisson J, Pietschmann T. Incorporation of Hepatitis C Virus E1 and E2 Glycoproteins: The Keystones on a Peculiar Virion. *Viruses* 2014;6:1149–87. <https://doi.org/10.3390/v6031149>.

- [75] André P, Komurian-Pradel F, Deforges S, Perret M, Berland JL, Sodoyer M, et al. Characterization of Low- and Very-Low-Density Hepatitis C Virus RNA-Containing Particles. *J Virol* 2002;76:6919–28. <https://doi.org/10.1128/JVI.76.14.6919-6928.2002>.
- [76] Chang K-S, Jiang J, Cai Z, Luo G. Human Apolipoprotein E Is Required for Infectivity and Production of Hepatitis C Virus in Cell Culture. *J Virol* 2007;81:13783–93. <https://doi.org/10.1128/JVI.01091-07>.
- [77] Merz A, Long G, Hiet M-S, Brügger B, Chlanda P, Andre P, et al. Biochemical and Morphological Properties of Hepatitis C Virus Particles and Determination of Their Lipidome. *J Biol Chem* 2011;286:3018–32. <https://doi.org/10.1074/jbc.M110.175018>.
- [78] Krey T, d'Alayer J, Kikuti CM, Saulnier A, Damier-Piolle L, Petitpas I, et al. The Disulfide Bonds in Glycoprotein E2 of Hepatitis C Virus Reveal the Tertiary Organization of the Molecule. *PLoS Pathog* 2010;6:e1000762. <https://doi.org/10.1371/journal.ppat.1000762>.
- [79] Kong L, Giang E, Nieusma T, Kadam RU, Cogburn KE, Hua Y, et al. Hepatitis C Virus E2 Envelope Glycoprotein Core Structure. *Science* 2013;342:1090–4. <https://doi.org/10.1126/science.1243876>.
- [80] Khan AG, Whidby J, Miller MT, Scarborough H, Zatorski AV, Cygan A, et al. Structure of the core ectodomain of the hepatitis C virus envelope glycoprotein 2. *Nature* 2014;509:381–4. <https://doi.org/10.1038/nature13117>.
- [81] Lavillette D, Pécheur E-I, Donot P, Fresquet J, Molle J, Corbau R, et al. Characterization of Fusion Determinants Points to the Involvement of Three Discrete Regions of Both E1 and E2 Glycoproteins in the Membrane Fusion Process of Hepatitis C Virus. *J Virol* 2007;81:8752–65. <https://doi.org/10.1128/JVI.02642-06>.
- [82] Douam F, Dao Thi VL, Maurin G, Fresquet J, Mompelat D, Zeisel MB, et al. Critical interaction between E1 and E2 glycoproteins determines binding and fusion properties of hepatitis C virus during cell entry: *Hepatology*. *Hepatology* 2014;59:776–88. <https://doi.org/10.1002/hep.26733>.
- [83] Dubuisson J, Cosset F-L. Virology and cell biology of the hepatitis C virus life cycle – An update. *J Hepatol* 2014;61:S3–13. <https://doi.org/10.1016/j.jhep.2014.06.031>.
- [84] Moradpour D, Penin F. Hepatitis C Virus Proteins: From Structure to Function. In: Bartenschlager R, editor. *Hepat. C Virus Mol. Virol. Antivir. Ther.*, vol. 369, Berlin, Heidelberg: Springer Berlin Heidelberg; 2013, p. 113–42. https://doi.org/10.1007/978-3-642-27340-7_5.
- [85] Lohmann V. Hepatitis C Virus RNA Replication. In: Bartenschlager R, editor. *Hepat. C Virus Mol. Virol. Antivir. Ther.*, vol. 369, Berlin, Heidelberg: Springer Berlin Heidelberg; 2013, p. 167–98. https://doi.org/10.1007/978-3-642-27340-7_7.
- [86] Shulla A, Randall G. Hepatitis C virus—host interactions, replication, and viral assembly. *Curr Opin Virol* 2012;2:725–32. <https://doi.org/10.1016/j.coviro.2012.09.013>.
- [87] Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, et al. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007;9:1089–97. <https://doi.org/10.1038/ncb1631>.
- [88] Dubuisson J, Cosset F-L. Virology and cell biology of the hepatitis C virus life cycle – An update. *J Hepatol* 2014;61:S3–13. <https://doi.org/10.1016/j.jhep.2014.06.031>.
- [89] Gentzsch J, Brohm C, Steinmann E, Friesland M, Menzel N, Vieyres G, et al. Hepatitis C Virus p7 is Critical for Capsid Assembly and Envelopment. *PLoS Pathog* 2013;9:e1003355. <https://doi.org/10.1371/journal.ppat.1003355>.
- [90] Boyer A, Dumans A, Beaumont E, Etienne L, Roingeard P, Meunier J-C. The Association of Hepatitis C Virus Glycoproteins with Apolipoproteins E and B Early in Assembly Is Conserved in Lipoviral Particles. *J Biol Chem* 2014;289:18904–13. <https://doi.org/10.1074/jbc.M113.538256>.
- [91] Piver E, Boyer A, Gaillard J, Bull A, Beaumont E, Roingeard P, et al. Ultrastructural organisation of HCV from the bloodstream of infected patients revealed by electron microscopy after specific immunocapture. *Gut* 2017;66:1487–95. <https://doi.org/10.1136/gutjnl-2016-311726>.

- 638 [92] Calès P, Boursier J, de Ledinghen V, Halfon P, Bacq Y, Leroy V, et al. Evaluation and
639 improvement of a reliable diagnosis of cirrhosis by blood tests Évaluation et amélioration du
640 diagnostic fiable de cirrhose par les tests sanguins 2020;11.
- 641 [93] Cale P, de Le V. Improved fibrosis staging by elastometry and blood test in chronic hepatitis C.
642 Liver Int 2013;11.
- 643 [94] Blach S, Zeuzem S, Manns M, Altraif I, Duberg A-S, Muljono DH, et al. Global prevalence and
644 genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet
645 Gastroenterol Hepatol 2017;2:161–76. [https://doi.org/10.1016/S2468-1253\(16\)30181-9](https://doi.org/10.1016/S2468-1253(16)30181-9).
- 646 [95] Razavi H, Robbins S, Zeuzem S, Negro F, Buti M, Duberg A-S, et al. Hepatitis C virus prevalence
647 and level of intervention required to achieve the WHO targets for elimination in the European
648 Union by 2030: a modelling study. Lancet Gastroenterol Hepatol 2017;2:325–36.
649 [https://doi.org/10.1016/S2468-1253\(17\)30045-6](https://doi.org/10.1016/S2468-1253(17)30045-6).
- 650 [96] Chevaliez S, Pawlotsky J-M. Diagnosis and management of chronic viral hepatitis: Antigens,
651 antibodies and viral genomes. Best Pract Res Clin Gastroenterol 2008;22:1031–48.
652 <https://doi.org/10.1016/j.bpg.2008.11.004>.
- 653 [97] Kamili S, Drobeniuc J, Araujo AC, Hayden TM. Laboratory Diagnostics for Hepatitis C Virus
654 Infection. Clin Infect Dis 2012;55:S43–8. <https://doi.org/10.1093/cid/cis368>.
- 655 [98] Larrat S, Bourdon C, Baccard M, Garnaud C, Mathieu S, Quesada J-L, et al. Performance of an
656 antigen–antibody combined assay for hepatitis C virus testing without venipuncture. J Clin
657 Virol 2012;55:220–5. <https://doi.org/10.1016/j.jcv.2012.07.016>.
- 658 [99] Marwaha N. Current testing strategies for hepatitis C virus infection in blood donors and the
659 way forward. World J Gastroenterol 2014;20:2948. <https://doi.org/10.3748/wjg.v20.i11.2948>.
- 660 [100] Lunel-Fabiani F, Payan C. [Virological tools for the diagnosis and follow up of hepatitis C: use
661 and role of new tests]. Gastroenterol Clin Biol 2003;27:718–26.
- 662 [101] Lunel F, Veillon P. Antigène et charge virale. Transfus Clin Biol 2003;10:74–7.
663 [https://doi.org/10.1016/S1246-7820\(03\)00028-4](https://doi.org/10.1016/S1246-7820(03)00028-4).
- 664 [102] Gaudy C, Thevenas C, Tichet J, Mariotte N, Goudeau A, Dubois F. Usefulness of the Hepatitis C
665 Virus Core Antigen Assay for Screening of a Population Undergoing Routine Medical Checkup.
666 J Clin Microbiol 2005;43:1722–6. <https://doi.org/10.1128/JCM.43.4.1722-1726.2005>.
- 667 [103] Veillon P, Payan C, Picchio G, Maniez-Montreuil M, Guntz P, Lunel F. Comparative evaluation
668 of the total hepatitis C virus core antigen, branched-DNA, and amplicor monitor assays in
669 determining viremia for patients with chronic hepatitis C during interferon plus ribavirin
670 combination therapy. J Clin Microbiol 2003;41:3212–20.
671 <https://doi.org/10.1128/jcm.41.7.3212-3220.2003>.
- 672 [104] Pivert A, Payan C, Lunel F. Comparaison des cinétiques de l'ARN et de l'antigène de capside du
673 virus de l'hépatite C dans le suivi thérapeutique des patients co-infectés par le virus de
674 l'hépatite C et le virus de l'immunodéficience humaine, traités par bithérapie interféron–
675 ribavirine, dans le cadre du protocole RIBAVIC. Pathol Biol 2004;52:522–8.
676 <https://doi.org/10.1016/j.patbio.2004.07.036>.
- 677 [105] Chevaliez S, Soulier A, Poiteau L, Bouvier-Alias M, Pawlotsky J-M. Clinical utility of hepatitis C
678 virus core antigen quantification in patients with chronic hepatitis C. J Clin Virol 2014;61:145–
679 8. <https://doi.org/10.1016/j.jcv.2014.05.014>.
- 680 [106] Rockstroh JK, Feld JJ, Chevaliez S, Cheng K, Wedemeyer H, Sarrazin C, et al. HCV core antigen
681 as an alternate test to HCV RNA for assessment of virologic responses to all-oral, interferon-
682 free treatment in HCV genotype 1 infected patients. J Virol Methods 2017;245:14–8.
683 <https://doi.org/10.1016/j.jviromet.2017.03.002>.
- 684 [107] Lamoury FMJ, Soker A, Martinez D, Hajarizadeh B, Cunningham EB, Cunningham P, et al.
685 Hepatitis C virus core antigen: A simplified treatment monitoring tool, including for post-
686 treatment relapse. J Clin Virol 2017;92:32–8. <https://doi.org/10.1016/j.jcv.2017.05.007>.
- 687 [108] Chevaliez S, Feld J, Cheng K, Wedemeyer H, Sarrazin C, Maasoumy B, et al. Clinical utility of
688 HCV core antigen detection and quantification in the diagnosis and management of patients

- p>with chronic hepatitis C receiving an all-oral, interferon-free regimen.
- Antivir Ther*
- 2016;23:211–7.
- <https://doi.org/10.3851/IMP3042>
- .
- [109] Chevaliez S, Pawlotsky J-M. New virological tools for screening, diagnosis and monitoring of hepatitis B and C in resource-limited settings. *J Hepatol* 2018;69:916–26. <https://doi.org/10.1016/j.jhep.2018.05.017>.
- [110] Guillou-Guillemette H, Lunel-Fabiani F. Detection and Quantification of Serum or Plasma HCV RNA: Mini Review of Commercially Available Assays. In: Tang H, editor. *Hepat. C Methods Protoc.*, vol. 510, Totowa, NJ: Humana Press; 2009, p. 3–14. https://doi.org/10.1007/978-1-59745-394-3_1.
- [111] Chevaliez S, Rodriguez C, Pawlotsky J. New Virologic Tools for Management of Chronic Hepatitis B and C. *Gastroenterology* 2012;142:1303-1313.e1. <https://doi.org/10.1053/j.gastro.2012.02.027>.
- [112] Gupta E, Agarwala P, Kumar G, Maiwall R, Sarin SK. Point -of -care testing (POCT) in molecular diagnostics: Performance evaluation of GeneXpert HCV RNA test in diagnosing and monitoring of HCV infection. *J Clin Virol* 2017;88:46–51. <https://doi.org/10.1016/j.jcv.2017.01.006>.
- [113] McHugh MP, Wu AHB, Chevaliez S, Pawlotsky JM, Hallin M, Templeton KE. Multicenter Evaluation of the Cepheid Xpert Hepatitis C Virus Viral Load Assay. *J Clin Microbiol* 2017;55:1550–6. <https://doi.org/10.1128/JCM.02460-16>.
- [114] Tuillon E, Mondain A-M, Meroueh F, Ottomani L, Picot M-C, Nagot N, et al. Dried blood spot for hepatitis C virus serology and molecular testing. *Hepatology* 2009;NA-NA. <https://doi.org/10.1002/hep.23407>.
- [115] Chevaliez S, Poiteau L, Rosa I, Soulier A, Roudot-Thoraval F, Laperche S, et al. Prospective assessment of rapid diagnostic tests for the detection of antibodies to hepatitis C virus, a tool for improving access to care. *Clin Microbiol Infect* 2016;22:459.e1-459.e6. <https://doi.org/10.1016/j.cmi.2016.01.009>.
- [116] Pawlotsky J-M, Negro F, Aghemo A, Berenguer M, Dalgard O, Dusheiko G, et al. EASL Recommendations on Treatment of Hepatitis C 2018. *J Hepatol* 2018;69:461–511. <https://doi.org/10.1016/j.jhep.2018.03.026>.
- [117] Kumar A, Rajput M, Paliwal D, Yadav A, Chhabra R, Singh S. Genotyping & diagnostic methods for hepatitis C virus: A need of low-resource countries. *Indian J Med Res* 2018;147:445. https://doi.org/10.4103/ijmr.IJMR_1850_16.
- [118] Lingala S, Ghany MG. Natural History of Hepatitis C. *Gastroenterol Clin North Am* 2015;44:717–34. <https://doi.org/10.1016/j.gtc.2015.07.003>.
- [119] AASLD/IDSA HCV Guidance Panel. Hepatitis C guidance: AASLD-IDSA recommendations for testing, managing, and treating adults infected with hepatitis C virus: HEPATITIS C VIRUS GUIDANCE PANEL. *Hepatology* 2015;62:932–54. <https://doi.org/10.1002/hep.27950>.
- [120] Kumar A, Rajput M, Paliwal D, Yadav A, Chhabra R, Singh S. Genotyping & diagnostic methods for hepatitis C virus: A need of low-resource countries. *Indian J Med Res* 2018;147:445. https://doi.org/10.4103/ijmr.IJMR_1850_16.
- [121] Stuyver L, Wyseur A, van Arnhem W, Hernandez F, Maertens G. Second-generation line probe assay for hepatitis C virus genotyping. *J Clin Microbiol* 1996;34:2259–66. <https://doi.org/10.1128/JCM.34.9.2259-2266.1996>.