DIAGNOSIS AND TREATMENT OF HEPATITIS C:

A technical landscape

Opportunities to Revolutionise Care in Developing Countries

This report provides an overview on the current state of play and a framework for action with regards to hepatitis C diagnostics and treatment in resource-poor settings.

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SUMMARY AND STATEMENT OF PRIORITY

Hepatitis C (HCV) has been a silent killer among people living in low- and middle-income countries. Factors including lack of epidemiologic data, poorly tolerated treatment with low success rates, and cost and complexity of care have all contributed to a vicious cycle of neglect that has allowed a growing epidemic of HCV to blossom unchecked.

But recent advances in both diagnosis and treatment, as well as new data on prevalence in low- and middle-income countries, provides an unprecedented opportunity to take the lead in turning back the growing tide of HCV and dramatically improve the wellbeing of people infected with HCV. New all-oral regimens offer the potential of being robust, well-tolerated and pan-genotypic. Thus, not only improving cure rates, but also simplifying diagnosis and management requirements. Advances in and scale up of molecular testing in low-resource environments facilitates diagnosis and monitoring of HCV. Taken together, these new tools open the door to managing this deadly co-infection in low- and middle-income countries. The simplified package of care may also enable decentralization of diagnosis and treatment as well as pave the way for eventual task-shifting to less specialized cadres of health workers.

However, several key interventions are required in order to spark this revolution in HCV care:

- Proactive normative guidelines at the WHO and at country level are needed
- Regular screening of patients at high risk for HCV, including those infected with HIV, is critical
- Access to appropriate diagnostics, including molecular tests, is of utmost importance and can be facilitated by utilizing the same platforms currently being rolled out for HIV
- Prices of both interferon-based therapy as well as new all-oral therapy must be appropriate to facilitate scale up in low- and middle-income countries, and biosimilar and generic competition is required in order to reach a fair price.
- Access to new oral therapies depends not only on price but also on registering of these new medications in key countries, as well as the WHO or other normative bodies signaling their importance by inclusion in the model Essential Medicines List.

There is no time like the present to rapidly address this hidden and ignored epidemic. The benefits of new tools and data will not be realized without key market interventions as well as prioritization of this disease at the WHO and at country level. But if the choice is made to invest now in the tools needed to fight HCV in low- and middle-income countries, the potential benefits are vast.

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1. INTRODUCTION

Worldwide, there are between 150 and 180 million people living with hepatitis C virus (HCV) infection, with the majority of affected individuals unaware of their infection. Referred to as the "silent epidemic"[1], a lack of reliable data means that the extent of the global epidemic remains unclear. Furthermore, it is estimated that between 6 and 10 million people living with HIV are currently co-infected with HCV or hepatitis B virus (HBV). They risk dying from HCV-related complications - such as liver cirrhosis or hepatocellular carcinoma - if they are not treated. Between 4 and 5 million people globally are estimated to be co-infected with HCV and HIV. Unfortunately, in resource-poor settings, very few HCV infected individuals have access to standard treatments and diagnostics, because treatment is administered only in specialised referral centres and remains extremely costly.

The HCV drug pipeline is robust, however, with many new drugs showing promising results and entering Phase II and III clinical trials. This means that we will soon be able to take advantage of the new HCV treatment opportunities for both mono-infected and HCV/HIV co-infected individuals. These drugs will ensure better, safer, and shorter treatments. Some of the most advanced once-daily oral regimens, which are interferon-sparing or interferon-free, offer treatment across all the HCV genotypes for 12 weeks with an improved drug side-effect profile and a sustained virological response of 90%-100%. This will allow for a simplification of both the diagnosis and monitoring of patients. The challenge now will be to ensure that infected individuals living in low and middle-income countries also have access to these new and improved diagnostic and treatment options.

In this report, Médecins Sans Frontières (MSF) has compiled an independent technical HCV landscape analysis to map the current and future trends in disease burden and product development, and we explore market evolution for diagnostics and medicines for HCV diagnosis and treatment. The aim of this report is to establish a prioritisation framework for researchers, civil society, and policy makers so that steps can now be taken to address gaps in research and development, as well as outlining a strategy to ensure that patients in resource-poor settings gain access to promising new products.

[1]Economist Intelligence Unit. The silent pandemic. Tackling hepatitis C with policy innovation. Economist: London, UK; 2012.

2.GLOBAL BURDEN OF HCV

2.1 Overview

Between 150 and 180 million people live with HCV infection globally and - together with HBV infection – these infections cause 1 million deaths each year. The infection is often asymptomatic, but when the virus persists in the liver (so-called chronic HCV infection) it can cause long-term damage that may only manifest after several years. Indeed, HCV and HBV infections account for 57% of all cases of liver cirrhosis and 78% of hepatocellular carcinoma, and remain a leading cause of liver transplantation[2].

Most people who develop acute hepatitis C have no symptoms. It is estimated that as many as 70%-90% of infected people fail to clear the virus during the acute phase of the disease and become chronic carriers. Of those with chronic liver disease, 5%-20% may develop cirrhosis. About 5% of infected persons may die from the consequences of long-term infection (liver cancer or cirrhosis)[3].

The transmission of HCV infection occurs via blood-to-blood contact. In resource-rich countries, over 90% of chronic HCV infections are attributed to contaminated blood, organs, tissues, and blood products, or via the sharing of unsterilised injection equipment among injecting drug users. Less commonly, HCV is transmitted sexually - particularly among HIV-infected men who have sex with men - or at birth via mother-to-child transmission. In resource-poor countries, the primary sources of HCV infection are unsterilised injection equipment and other medical and dental equipment, extra-medical transmission (barbers and traditional circumcision practices), and via infusion of inadequately screened blood or blood products.

There are eleven major genotypes of HCV – genotype 1 [GT1] to genotype 11 [GT11] – and many subtypes (designated a, b, c, etc). Genotypes 1-6 are the most common. Genotypes 1-3 have a worldwide distribution. Types 1a and 1b are the most common, accounting for about 60% of global infections. They predominate in Northern Europe and North America, and in Southern and Eastern Europe and Japan, respectively. In the United States, most cases are caused by GT1. GT2 is less frequently represented than GT1. GT3 is endemic in south-east Asia and is variably distributed in different countries. GT4 is principally found in the Middle East, Egypt, and central Africa. GT5 is almost exclusively found in South Africa, and GT6-11 are distributed in Asia[3].

The HCV epidemic is now described as a dual epidemic, which researchers and policy makers have referred to as “the silent pandemic”[1]. First, there is a concentrated epidemic affecting high-risk vulnerable groups situated predominantly in Eastern Europe and Asia. Second, there is a generalised epidemic in areas where HIV is highly prevalent, including countries in Africa such as Egypt, Nigeria, and Cameroon. In many countries globally, however, no reliable epidemiological data are available, and so the extent of the HCV epidemic remains unclear. Health authorities, particularly in resource-poor settings, need urgent access to reliable data in order to measure the magnitude of their domestic and regional disease burden and to prioritise the response accordingly.

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[2]Brown RS. Hepatitis C and liver transplantation. *Nature* 2005;36:973-978.
[3]WHO. Hepatitis C. (Available at: http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html [page 13])

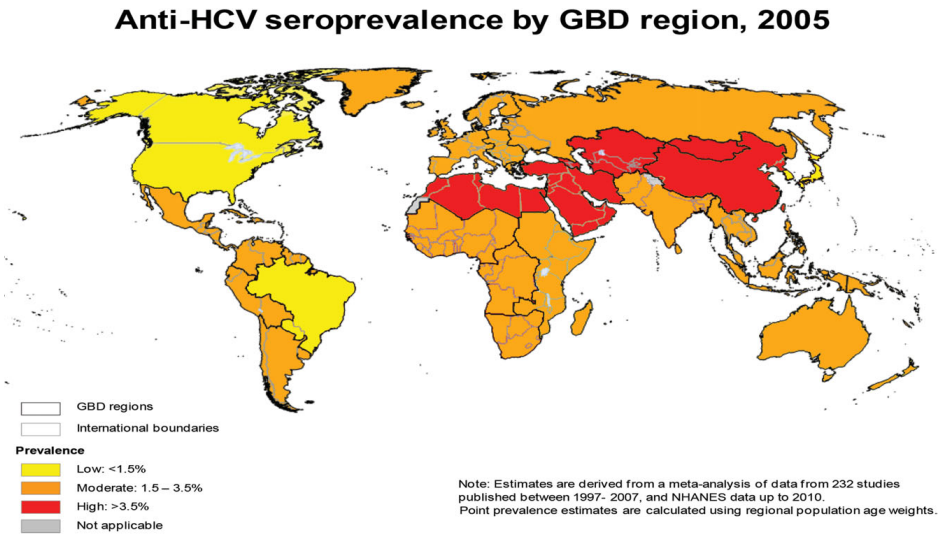

Figure 1: The global prevalence of HCV infection. Reproduced from Mohd Hanafiah K, et al [4]. GBD=global burden of disease.

2.2 HCV and HIV co-infection

Although their remains a paucity of data on the prevalence of HIV and HCV co-infection, it is estimated that between 4 and 5 million people are currently co-infected with HCV and HIV (5-15% of 33 million HIV infected people are HCV co-infected)[5]. Little is yet known about the contribution of HCV infection to morbidity and mortality in the antiretroviral (ART) era[6].

HIV has a strong impact on HCV progression, causing higher rates of chronicity and accelerated disease progression and mortality. In the era of ART, HIV/HCV co-infected patients are at increased risk of morbidity and mortality compared with patients with HIV infection alone and with chronic HCV alone[7]. Without highly active antiretroviral therapies (HAART), HIV accelerates HCV disease progression, resulting in death, histological fibrosis/cirrhosis, and decompensated liver disease.[8]

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[4]Mohd Hanafiah K, Groeger J, Flaxman AD, et al. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. *Hepatol* 2012;Epub ahead of print

[5]Easterbrook P, Sands A, Harmanci H. Challenges and priorities in the management of HIV/HBV and HIV/HCV coinfection in resource-limited settings. *Sem Liver Dis* 2012;32(2):147-157.

[6]Nelson P, Mathers B, et al. Global epidemiology of viral hepatitis among people who inject drugs: results of global systematic reviews. *Lancet* 2011;378:571-583.

[7]Lo Re V, et al. Increased risk of hepatic decompensation and hepatocellular carcinoma in HIV/HCV-co-infected patients compared to HCV-mono-infected patients despite combination antiretroviral therapy [Oral Abstract number 17867]. 19th International AIDS Conference. Washington DC, USA: July 22-27, 2012.

[8]Deng LP. Impact of human immunodeficiency virus infection on the course of hepatitis C virus infection: A meta-analysis. *World J Gastroenterol* 2009;15(8):996-1003.

A recent cohort study analysis among 4286 ART-treated HIV/HCV coinfected and 6639 HCV-monoinfected patients in the veterans Aging Cohort Study showed that compared to HCV mono-infected patients, ART-treated HIV/HCV coinfected patients had a higher cumulative incidence and risk of hepatic decompensation (303 of 4286 [7.1%] versus 370 of 6639 [5.7%] adjusted odds ratio: 1.76 [95% CI=1.50-2.06]) and hepatocellular carcinoma (50 of 4286 [1.2%] versus 60 of 6639 [0.9%], adjusted odds ratio: 1.69 [95% CI= 1.14-2.49]). After decompensation, mortality was higher in coinfected people (228 of 303) [75.2%] vs 210 of 370 [56.8%]; p <0.001).

In HCV mono-infected people, a systematic review and meta-analysis showed that duration of HCV infection was the most consistent factor significantly associated with progression of fibrosis.[9] The stage of hepatic fibrosis in co-infected patients is also independently associated with composite outcome of end-stage liver disease, hepatocellular carcinoma, and death[10]. In co-infected people, a low CD4 count is associated with higher liver fibrosis progression rate[11].

Increasing, morbidity is observed from chronic liver disease as patients survive longer on ART. HIV patients continue to have five-fold greater mortality than non-HIV-positive patients; it has been shown that chronic HCV infection is independently associated with a 50% increase in mortality among patients with a diagnosis of AIDS[12].

Consequently, experts recommended treating HIV early in HCV/HIV co-infected people. These patients are 66% less likely to experience end-stage liver disease, hepatocellular carcinoma, or liver-related deaths[13].

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[9]Thein HH. Yi Q, Dore GJ, Krahn MD. Estimation of stage –specific fibrosis progression rates in chronic hepatitis C virus infection: a meta-analysis and meta-regression. *Hepatology* 2008;48(2):418-431.

[10]Berkeley N, Limketkai MD, Shruti H Mehta, et al. Relationship of liver disease stage and antiviral therapy with liver-related events and death in 638 adults coinfected with HIV/HCV. *JAMA* 2012;308(4):370-378.

[11]Benhamou Y. Liver fibrosis progression in human immunodeficiency virus and hepatitis C virus coinfected patients. *Hepatology* 1999;30(4):1054-1058.

[12]Branch AD, Van Natta ML, Vachin ML, et al. Mortality in HCV-infected patients with a diagnosis of AIDS in the era of combination anti-retroviral therapy. *Clin Infect Dis* 2012;55:137-144.

[13]EASL. EASL Clinical Practice Guidelines: Management of hepatitis C virus infection. *Hepatology* 2011;55:245-264.

3. GUIDELINES

Although there are currently no WHO clinical guidelines for the management of HCV (or HBV) infection for resource-limited settings, WHO are in the process of drafting the 2013 consolidated HIV guidelines that should include an update for the management of co-infected patients being treated on ART. In parallel, WHO has started the process of drafting clinical guidelines for HCV mono-infected individuals.

Diagnosis involves a serologic test, confirmation with a viral load test, and subsequent staging to identify candidates for treatment who have evidence of significant liver fibrosis. Liver fibrosis stage is assessed by calculating what is called a METAVIR score. To be able to calculate this score, a liver biopsy or transient elastography ultrasound is performed, or a biomarker score is used. More details are available in the diagnosis section.

A number of other expert bodies have developed evidence-based guidelines on the diagnosis and treatment of HCV-infected individuals, based on the GRADE methodology, including the European Association for the Study of the Liver (EASL)[13] and the American Association for the Study of Liver Disease (AASLD)[14].

The primary goal of HCV therapy is to cure the infection, which results in eliminating detectable HCV after cessation of treatment. Sustained virological failure (SVR) is defined as an undetectable HCV RNA level (<50 IU/mL) 24 weeks after treatment withdrawal. SVR is generally associated with resolution of liver disease in patients without cirrhosis. Patients with cirrhosis remain at risk of life-threatening conditions like hepatocellular carcinoma. Treatment should be initiated in patients with advanced fibrosis (METAVIR score F3- F4 compensated), and strongly considered in patients with moderate fibrosis (F2)[13].

The current standard of care for chronic HCV infection is to treat with pegylated-interferon alpha plus ribavirin (peg-IFN-riba) for a period of 24-48 weeks according to genotypes. According to these guidelines standard of care for chronic GT1 HCV infection includes the addition of a direct acting first generation anti-HCV protease inhibitor antiviral (telapravir or boceprevir). However, because of the high cost of these drugs and the monitoring required because of their toxicity, the use of these drugs is not feasible in resource-poor settings.

According to these guidelines[13,14], for HIV/HCV co-infected individuals, earlier treatment is recommended due to rapid disease progression. In case of HIV/HCV co-infection, it is recommended that HIV ART be initiated early: at CD4 counts ≤500cells/μL. Indications for HCV treatment are identical to those for patients with HCV mono-infection.

A recent survey has highlighted the lack of country specific guidance for management of HIV/HCV co-infection in resource-poor settings, and lack of consensus, on how to test for and treat HCV infection. Only 32 (34%) of 93 resource-poor countries surveyed by WHO in 2012 provided guidance on various aspects of HIV/HCV co-infection management. The major shortfalls identified were: staging of liver disease, HCV diagnosis and timing of the initiation of HIV treatment, and choice of HCV treatment. The major area of discordance identified was: timing of ART in

[14]Ghany MG, et al. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology* 2011;54(4):1433-1444.

HIV/HCV co-infection (4 of 8 countries recommended early ART, and 4 of 8 recommended deferral of treatment)[15].

[15]Heenan RC, Wiersma ST, Vitoria M. Significant variation in recommendations for management of HIV-hepatitis B and C (HIV-HBV and HIV-HCV) co-infection: a survey of national guidelines from resource-limited settings (RLS). [Abstract number THPE682]. 19th International AIDS Conference. Washington DC, USA: July 22-27, 2012.

4. CURRENT AND PIPELINE TREATMENTS

4.1 Current Treatment

The goal of the treatment is to eradicate HCV infection in order to prevent the complications of HCV-related liver disease, including necro-inflammation, fibrosis, cirrhosis, hepatocellular carcinoma, and death[13].

The standard of care for HCV treatment is injectable pegylated-interferon-alpha (peg-IFN-alpha) 2a or 2b combined with ribavirin oral therapy – so-called peg-IFN-riba. Treatment duration varies according to genotype: GT1 and GT4 require up to 48 weeks of treatment, whereas GT2 and GT3 require 24 weeks of treatment. Treatment outcomes in HCV mono-infected individuals and HCV/HIV co-infected individuals vary considerably.

Treatment efficacy data are as follows:

- GT1 (treated with peg-IFN-riba, boceprevir, or telaprevir): ~70%
- GT1 HCV/HIV co-infected (treated with peg-IFN-riba): ~15-35%
- GT2 and GT3 (treated with peg-IFN-riba): ~80%
- GT2 and GT3 HCV/HIV co-infected (treated with peg-IFN-riba): ~55-73%
- GT4 (treated with peg-IFN-riba): 60-70%

Treatment failure occurs most often in those patients who are most in need of treatment, such as those with liver cirrhosis and HCV/HIV co-infection, or in post-transplant patients. Treatment with peg-IFN-riba is long and complicated. Adverse events are common and not always easy to manage, especially in resource-poor settings.

Current shortfalls of existing drugs include:

1. Treatment availability: drugs are only in tertiary specialised referral centres.
2. Length of treatment: 24 to 48 weeks. One injection of peg-IFN-alpha per week plus daily doses of oral ribavirin. Weekly medical consultation by trained personal is needed to check that treatment is well enough tolerated and to detect and treat complications as they arise.
3. Regular HCV RNA PCR is needed to calculate SVR and therefore check treatment efficacy at weeks 4, 12, 24, 48, and 12 weeks post treatment, rarely available in resource-poor settings.
4. Management of peg-IFN-riba related side-effects is needed, especially acute anaemia, low neutrophil count, low platelets count, psychiatric disorders (especially depression) and co-morbidities like thyroid disorders, hypertension, diabetes, HIV and ART, excessive alcohol consumption. Such management can be difficult in resource-poor settings[13].

A systematic review and meta-analysis on treatment outcomes in chronically infected HCV patients in low and middle-income countries[16], which included 12213 patients from 93 studies across 17 countries, showed a pooled SVR of 49% for GT1 and GT4, and 59% for GT2 and GT3. Factors associated with successful outcomes included treatment with peg-IFN-riba, infection with HCV GT2, GT3, the absence of

[16]Ford N, Kirby C, Singh K, et al. Chronic hepatitis C treatment outcomes in low and middle income countries: a systematic review and meta-analysis. *Bull World Health Organ* 2012;90:540-550.

liver damage, and HIV infection at baseline. However, treatment side-effects were a limiting factor, with 17% of adverse events resulting in a treatment interruption or dose modification and 4% of adverse events resulting in treatment discontinuation. The authors concluded that treatment outcomes in low and middle-income countries were similar to those reported in high-income countries. These findings show that it is feasible to successfully treat HCV-infected individuals in resource-poor settings with peg-IFN-riba.

Treatment outcomes in HCV/HIV co-infected people are less promising, however. A recent systematic review and meta-analysis of observational cohorts of treatment outcomes of treatment naïve hepatitis C patients co-infected with HIV showed that the pooled proportion of patients achieving SVR was 38%. Significantly poorer outcomes were observed for patients infected with GT1 or GT4 (pooled SVR 24.5%), compared to GT2 and GT3 (pooled SVR 59.8%).[17]

Increasing access to peg-IFN-riba in resource-poor settings would be a game-changer in our ability to treat patients with HCV infection

Very few people have access to peg-IFN-riba in resource-poor settings. The treatment is administered only in specialised referral centres and remains extremely costly.

The burden of HCV infection is a sensitive issue for many resource-poor settings. A strong civil society and considerable political will are needed to confront the reality of this "silent pandemic" and take the necessary steps towards universal access for all to treatment and diagnostics.

Adding boceprevir and telaprevir to peg-IFN-riba can improve treatment outcomes for patients with HCV/HIV co-infection: approximately 30% improvement of SVR with HCV protease inhibitor regimen in naïve GT1 patients[18]. However, treatment management is highly complex, requires frequent and expensive monitoring, and the management of adverse events is so difficult that this treatment option is not an option as yet for resource-poor settings[19].

[17] Davies A. Singh KP, Shubber Z, et al. Treatment outcomes of treatment–naïve hepatitis C patients co-infected with HIV: A systematic review and meta analysis of observational cohorts. *PloS One* 2013;8(2):e55373.
[18]Poordad F, McCone J, Bacon BR, et al. Boceprevir for Untreated Chronic HCV Genotype 1 Infection. *N Engl J Med* 2011;364:1195-1206.
[19]JM Pawlovsky. (National Center for Viral Hepatitis B, C, delta. Department of Virology and INSERM U 955. Henri Mondor Hospital. University Paris-Est)."Current standard of care and a 5–year perspective". Personal communication. MSF-TAG-OSI HCV Meeting: Paris, France, September 24-25, 2012.

Important steps that must now be taken by WHO

No aims and objectives have yet been established by the global health community to improve access to HCV diagnostics and treatments for resource-poor settings. The current drafting of the WHO guidelines represents a unique opportunity to set a new agenda for the diagnosis and treatment of HCV and HCV/HIV co-infection in resource-poor settings. We recommend that the target population for improved access to treatment include all patients with chronic HCV infection, regardless of genotype, transmission mode, or stage of fibrosis. Patients with more unfavourable prognostic factors (for example, GT1 HCV disease) and little evidence of liver damage, however, may elect to defer therapy while awaiting newer drugs.

Similarly, the upcoming HIV consolidated guidelines offer an opportunity to highlight this important co-infection, so that screening and care may be integrated.

WHO has not yet pre-qualified any HCV rapid diagnostic tests but the prequalification of a range of tests is expected shortly. It is important that WHO do this as efficiently and as quickly as possible.

Peg-IFN alpha does not yet appear on WHO's Essential Medicines List. MSF submitted an application in December 2012 (http://www.who.int/selection_medicines/committees/expert/19/applications/peginterferon/en/). For many civil societies, this will be the first step for political action on this issue. If peg-IFN-alpha is made part of WHO's Essential Medicines List then civil society groups and health authorities will be able to lobby their governments to ensure its inclusion in the countries' health care system.

Surveys carried out by MSF and other non-governmental organisations have highlighted that the price of peg-IFN-alpha-2a and 2b are often listed as between USD 200 and USD 400 per vial, in countries in which generic or biosimilar competition does not exist. The price structures offered by Roche and Merck, the two existing originator manufacturers, are aligned in the countries in which the two products are registered. Egypt is a notable exception, because the national Hepatitis C programme has reached an agreement to procure peg-IFN-alpha-2a (Pegasys) and peg-IFNalpha-2b (PEG-Intron) at USD 41 per vial, including a weekly supply of ribavirin[20],[21]. In Egypt, competition through manufacturers offering biosimilars or originator peg-IFN products has reduced the price of both originator products for a

[20]Esmat G, Fattah S. Evaluation of a novel pegylated interferon alpha-2a (Reiferon Retard) in Egyptian patients with chronic hepatitis C-genotype 4. Dig Liver Dis 2009 (Suppl 2009) 3:1.
[21]El Sayed N, Kandeel A, Genedy M, et al. Progress towards prevention and control of hepatitis C virus infection – Egypt, 2001-2012. Centers for Disease Control and Prevention.*MMWR* 2012 (July 27); 61: 29..

48-week treatment course of peg-IFN-riba from USD 10,000-20,000 to less than USD 2000[22].

Table 1: Average price of current treatments

Treatment regimen	Average price per course
Peg-IFN-riba	USD 10-20,000
Peg-IFN-riba + boceprevirortelaprevir	USD 60-90,000

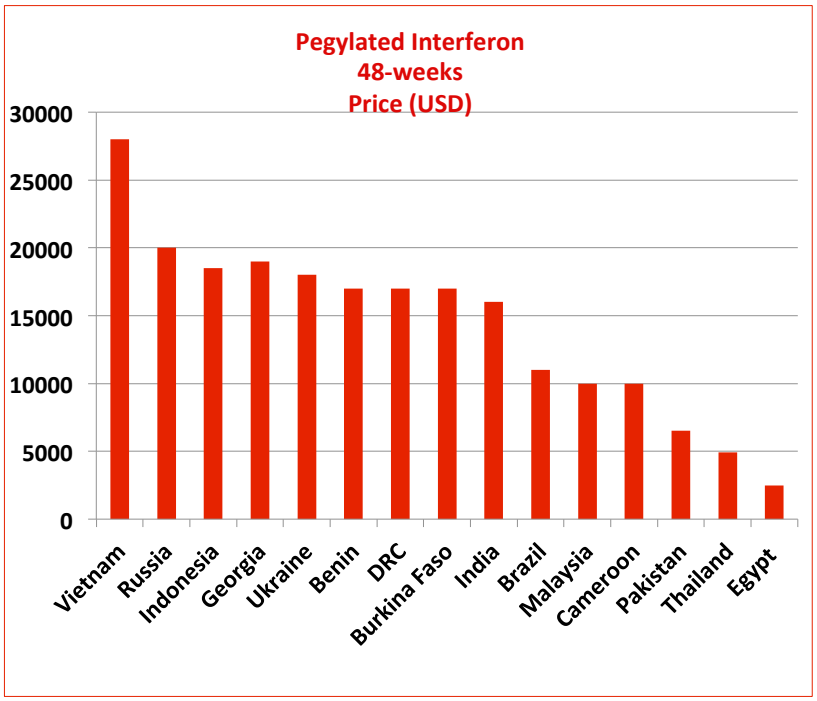

Figure 2. Average price of peg-IFN-riba in resource-poor settings.(Source: courtesy of Azadeh Momenghalibaf [Program Officer, IHRD & AEMI] and Paul Cawthorne [MSF Access Campaign Coordinator – Asia];, "Global Snapshot: HCV epidemiology & response", Paris MSF-OSF-TAG Meeting, September 24-25, 2012; Reproduced with permission from the authors)

4.2 Complications in the use of biosimilars

Interferons are biological substances (ie, large and complex proteins produced through a biological process in specially engineered living organisms). Both branded and generic biological products (called biosimilars, bio-generics, follow-on biologics, or follow-on-protein products) have a different regulatory pathway than small molecules made through a chemical process.

In order for biosimilars to gain approval from the regulators, they must be satisfied that these products are comparable to the reference product in terms of quality, safety, and efficacy. They must undergo an extensive (although less so than the

[22]National Institute for Health and Clinical Excellence. National Institute for Health and Clinical Excellence Technology Appraisal Guidance 106. Peginterferonalfa and ribavirin for the treatment of mild chronic hepatitis C. NICE: London, UK; 2006.

originator product) development process, including comparative pre-clinical and comparative clinical trials to establish safety and efficacy. These studies are not required for approval of generic drugs (small molecules), in which the process is based on demonstrating bioequivalence.

WHO has a limited set of standards for biosimilars, called the "Guidelines on Evaluation of Similar Biotherapeutic Products", which were only issued in 2009. Furthermore, WHO has not established any prequalification system for biological products beyond the category of vaccines.

One problem associated with the development of a biosimilar is that it requires a large investment of time and money as opposed to generic small molecules. It takes approximately 8 years to develop a biosimilar, far longer than the development of a generic small molecule product (approximately 2 years). Development is also extremely expensive, requiring an investment of USD 10–140 million compared with USD 1–5 million for a generic product.

In the short-term, MSF has called for Merck and Roche to increase access to the preferential prices that have been available in Egypt, yet in the long-term, it calls for alternative sources to the originator drugs. Even if Roche and Merck agree to decrease their prices to the level negotiated in Egypt (USD 41 per weekly treatment of peg-IFN-alpha), scale up will only be possible with safe and quality-assured alternative sources of this treatment. *It is therefore crucial that a system is created that enables proper evaluation of the quality, safety, and efficacy of the existing alternative peg-IFN-alpha products to the originator products*.

4.3 The lack of competition from biosimilars

Peg-IFN-alpha-2a and 2b are being developed and marketed in a number of low and middle-income countries, including Egypt, Vietnam, Iran, Cuba, and India. Some of these products include a different size and form of peg (polyethylene glycol) branches and linker, and so do not fall under the category of biosimilars to the originator products. Others have been developed as biosimilars. However, none of these products have gained approval from a stringent regulatory authority either as a new biologic or a biosimilar because competition is currently blocked by the existence and the enforcement of patents in these countries. On the other hand, there is no WHO prequalification scheme that can ensure quality, and safe and effective alternative products to the innovators, in order to foster competition and obtain decreases in prices that allow for affordable treatment in developing countries.

Governments need to play a greater role in ensuring a lowering of the price of peg-IFN-alpha. It is crucial to have quality assured alternatives to the originator products available to foster competition and decrease prices, and governments must play a role in registering and using originator products and biosimilars. Compulsory licences and patent oppositions may also be necessary to ensure production or importation and use of a biosimilar.

4.4 Current market trends

Access to testing
59% of the world's population has no access to HCV diagnostics. This finding correlates to the wealth of the country: serological diagnostic testing for the presence of primary infection is available to more than half of the population in 93% of high-income countries, 77% of upper-middle-income countries, 53% of lower-middle-income countries, and 11% of low-income countries. 84% of the population in lower-middle-income countries and 96% of the population in low-income-countries live in areas where testing is not widely accessible[23]. In this report, although the full diagnostic package is discussed in section 5, this market analysis refers only to serological screening tests.

Access to treatment
Standard treatment for HCV infection is extremely expensive, severely limiting its use in low and middle-income countries. The availability of full or part government funding for treatment of HCV infection depends heavily on the income status of that country. According to the latest World Hepatitis Alliance report[23], such funding is available in 83% of high-income countries, 77% of middle-income countries, and 33% of low-income countries. This report highlights that in all WHO regions, except for the African region, the majority of countries provide domestic funds that partially or completely fund HCV care and treatment programmes.

Global sales

- Peg-IFN-alpha-2a (Pegasys):
  - 1.655 billion Swiss Francs (CHF) in 2009, an increase of 5% from 2008[24]
  - 441 million CHF in the first quarter of 2010, a 15% increase from the first quarter of 2009[25]
  - Pegasys, for HCV and HBV infection, fell 11% to 695 million CHF for the first 6 months of 2011[26]
  - Pegasys sales growth: + 11% in 2012[27]

- Peg-IFN-alpha-2b (Pegintron):
  - 918 million USD in 2007[28]
  - 198 million USD in the third quarter of 2009[29]
  - 149 million USD fourth quarter of 2009 (post-merger with Merck)[30]

[23]World Hepatitis Alliance. Viral hepatitis: global policy. World Hepatitis Alliance: London, UK; 2011. Available from:http://www.worldhepatitisalliance.org/Policy/2010PolicyReport.aspx
[24]Roche. Strong operating performance for Roche. Roche: Basel, Switzerland; 2009.
[25]Roche. Excellent growth in first quarter of 2010. Roche: Basel, Switzerland; 2010.
[26]Roche posts healthy financials for first half of 2011". World news. July 21, 2011. Pharmatimes.
[27]ZacksEquityResearch. Roche grows in 2012. Zacks: Chicago, USA; 2012 (www.zacks.com/stock/news/91818/roche-grows-in-2012).
[28]Pan D, Bronshtein S, Shikani W. Schering-Plough (SGP). 2010; http://www.wikinvest.com/stock/Schering-Plough.
[29]Schering-Plough Corporation. Schering-Plough announces 2009 third quarter financial results. Medical News: Macclesfield, UK; 2009. (http://www.news-medical.net/news/20091022/Schering-Plough-announces-2009-third-quarter-financial-results.aspx?page=2).
[30]Merck. Merck Announces Fourth-Quarter and Full-Year 2009 Financial Results [Media Release]. Merck: NJ, USA; 2010 (http://www.merck.com/newsroom/news-release-archive/financial/2010_0216.html).

- Worldwide sales of Pegintron for the treatment of chronic HCV infection in 2010 were USD 274 million; worldwide sales of Pegintron were USD 319 million for the first 6 months of 2011, a decrease of 14% compared with the same period in 2010. According to Merck, these declines were attributable, in part, to patient treatment being delayed by health-care providers in anticipation of new therapeutic options becoming available.[31]
- Worldwide sales of Pegintron declined 1% in 2012 to USD 653 million.[32]

"The hepatitis C market is expected to become one of the fastest growing markets in Pharma over the next decade, given a sizable 170 million patient population, a significant unmet need, and rapid advancements in the clinic. The management of this infectious disease is on the verge of a revolution that will bring considerable changes to the treatment algorithm."

- Global Hepatitis C Market Analyzed& Forecast in New FirstWord Report available at MarketPublishers.com
(http://marketpublishers.com/report/pharmaceuticals/drugs/kol-insight-hepatitis-c-race-4-first-interferon-free-regimen.html)

In 2011, the HCV therapeutics market was estimated at USD 2.6 billion. From 2004 to 2011, the market grew at the compound annual growth rate of 2.7%, with the launch of Incivek and Victrelisin 2011 (mainly for the treatment of HCV, HBV is a minor market), boosting positive growth[33]. Since then the market has seen a significant decline in value due to the low uptake of peg-IFN therapies and subsequent reluctance to put patients on peg-IFN-riba in anticipation of more effective drugs being launched. The launch of the two direct acting antiviral (DAA) protease inhibitors – telapravir and bocepravir - added a billion dollars to the market in 2011 and resulted in a positive compound annual growth rate.

Global BusinessIntelligence (GBI) Research has estimated that the *hepatitis C therapeutics market is in the top seven markets and will be valued at USD 14.9 billion by 2018*, with a compound annual growth rate of 28.3% over the forecast period. These estimates are based on the continued adoption of the protease inhibitors as the gold standard treatment option for HCV, and the imminent launch of all-oral DAA molecules, the first of which should be in the market in 2014-2015.(GBI. Expected Launch of GS-7977 in 2015 will Pave the Way for an Oral Interferon-free Combination Therapy Hepatitis C Therapeutics Market to 2018. October 15, 2012).

[31]Tickerpot.com. tickerpot.com/symbol/mrk/310158/topic/pegintron/2011
[32]Pegintron mentioned by Merck and Co (MRK) in 2011. Tickerpot.com; 2011 (tickerpot.com/symbol/mrk/310158/topic/pegintron/2011).
[33]Hepatitis C Therapeutics Market to 2018.
(http://www.researchandmarkets.com/research/ngbvll/hepatitis_c)

4.5 HCV treatment pipeline

The HCV drug pipeline is extremely rich. It is now clear that we can anticipate the availability of all-oral DAA regimens by 2014-2015. DAAs act at various levels of viral replication to stop the virus from reproducing with the aim of cure e.g. SVR. These regimens are expected to be potent and simple to administer, have improved safety, tolerability, and efficacy. Some of them are expected to cover genotypes 1-6, and may be compatible with ART. Data have also shown that several of these pipeline regimens may have efficacy in the setting of advanced liver disease or in treatment-experienced patients. Data from Phase IIb trials and now preliminary data from phase III trials of sofosbuvir+ribavirin and sofosbuvir-GS5885+ribavirin - as well as several other all-oral combinations - shows SVR rates of 90-100% in mono-infected patients with only 12 weeks of therapy in GT2 and GT3 and SVR rates of 84% in GT1 treatment naïve patients. For GT1, 4, 5 and 6 in treatment naïve patients receiving sofosbuvir in combination with peg-IFN-riba SVRs are also 90-100%[34].This dramatic change in drug safety and efficacy will likely allow for simplified monitoring and treatment strategies, which will greatly enhance our ability to successfully treat patients with HCV infection in resource-poor settings (See diagnostics Section 5 for further details).

There are 5 drug classes of oral regimens; we will present only the most promising pipeline drugs (Table 2). These drugs were selected for inclusion in this report according to a specific target product profile adapted for resource-poor settings and according to the latest available information (as of March 1, 2013). For a complete overview of pipeline drugs see the Annex.

It is important to note that these are pipeline drugs and, as such, Phase III trials are not yet finished. Any results or data presented here must be analysed with caution. Large phase III trials are currently ongoing that will improve data regarding the safety and efficacy of these new drugs. More complete information is available in the Annex and the Pipeline Report from Treatment Action Group[35].

[34]Gilead 's Sofosbuvir for Hepatitis C Meets Primary Endpoint in Fourth Pivotal Phase 3 Study (www.gilead.com/pr_1786260; Accessed Feb 18, 2012)
[35]Swan T. HIV HCV TB 2012 Pipeline Report. Treatment Action Group: New York, USA; July 2012 (www.pipelinereport.org).

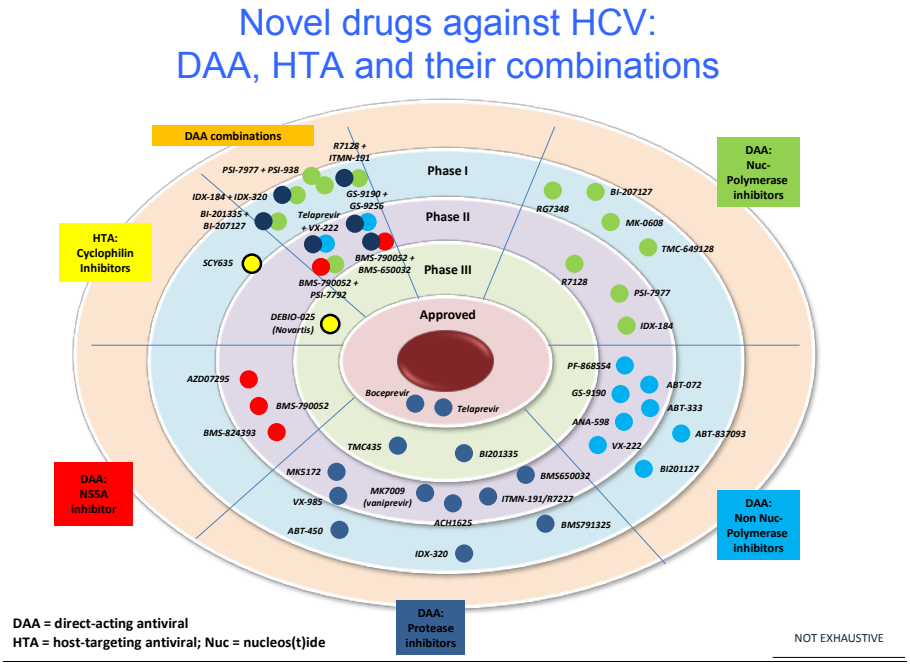

Figure 3: The HCV drug pipeline (source: Merck).

Based on MSF's assessment, we believe that if Phase III trials of these pipeline drugs bear fruit, these all-oral treatment regimens represent appropriate products for resource-poor settings, according to the following criteria: (a) safety; (b) efficacy; (c) simplicity; (d) potential combinations; (e) dose of drug; (f) potency; (g) pan-genotypic coverage; (h) tolerability; (i) robustness; (j) compatibility with ART; (k) compatibility and efficacy with advanced liver disease; (l) absence of interactions with Opioid Substitution Therapy, TB treatment, and other diseases; (m), and performance in treatment-experienced patients. MSF believes the potential utility of new DAA regimens may be judged by these criteria and phase III studies will provide further supporting data.

The question now, therefore, is not if these drugs are a breakthrough for treatment of HCV, but whether HCV infected individuals in low and middle-income countries will be able to access these game-changing regimens at an affordable price.

TABLE 2: THE TOP 6 MOST PROMISING PIPELINE REGIMENS FOR HCV INFECTION

This table reviews five of the major direct acting antivirals (DAAs) in later-stage trials and reviews only the all-oral combinations that are being trialled. This table is not exhaustive, but is included as a brief reference for select drugs that are furthest down the pipeline. For further information, please see the Annex table.

Drugs name (drug class); manufacturer; combinations being studied	**Geno-types studied**	**Major adverse events/safety issues; drug-drug interactions**	**Treatment duration (weeks), efficacy, and robustness**	**Simplicity**	**HCV/HIV, treatment experienced, and advanced liver disease**	**Barrier to resistance (If high barrier to resistance, low likelihood of failing with resistance mutations)**	**Trial status and timeline**
Sofosbuvir (Nucleotide NS5B polymerase inhibitor) Manufacturer: Gilead Sofosbuvir+RBV Sofosbuvir+Ledipisvir+/-RBV	1-6	Well tolerated, no need of food intake, no rash, no pancytopenia, improved anaemia compared with interferon-based therapy but is still an issue due to ribavirin. No significant	GT2/3, phase III 12 weeks Sofosbuvir+RBV :SVR12 97% and 56% for GT2 and 3, respectively GT1 phase II 12 weeks sofosbuvir+RBV SVR12 84% GT1 phase II 12 weeks	Once daily Fixed dose combination of sofosbuvir and GS 5885	GT1 treatment-experienced, phase II 12 weeks Sofosbuvir+ledipisvir+ribavirin: SVR12 100% GT2/3 treatment-experienced Sofosbuvir+ribaviri n: - 12 week treatment: GT2 SVR12=86% GT3 SVR12=30% .	High	Sofosbuvir registration expected mid-2013; Sofosbuvir-GS 5885 expected 2014 Phase III with sofosbuvir+RBV and sofosbuvir+ledipisvir+ RBV currently underway, including HIV co-infected patients

Sofosbuvir+daclatasvir+/-RBV		drug-drug interactions related to sofobuvir	sofosbuvir+ledipisvir+RBV: SVR12 100% 12weeks GT 1,2,4,5,6 12 weeks In treatment-naïve patients: SVR =90-100% when used in combinatn with peg-IFN-riba		- 16 week treatment: GT2 SVR12 94%; GT3 SVR 62%		
Ledipisvir (NS5A inhibitor) Manufacturer: Gilead Ledipisvir+Sofosbuvir+/-RBV	1	Well tolerated, no need of food intake, no rash, no pancytopenia, improved anaemia compared with interferon-based therapy but is still an issue due to ribavirin.	GT1 phase II 12 weeks sofosbuvir+ledipisvir+RBV: SVR12 100%	Once daily Fixed dose combination of sofosbuvir and GS 5885	GT1 treatment-experienced, phase II 12 weeks Sofosbuvir+ledipisvir+ribavirin:SVR12 100%	Low	Sofosbuvir-GS 5885 registration expected 2014 Phase III sofosbuvir+ledipisvir+RBV currently underway, including HIV co-infected patients
Daclatasvir (NS5A inhibitor) Manufacturer: BMS	1,2,3,4	Safe and well tolerated Fatigue, headache, nausea Significantly	GT1 phase II daclatasvir+sofosbuvir+/-RBV: -12 week treatment SVR12=95%	Once daily	Limited data available in advanced liver disease and HCV/HIV co-infected individuals	Medium-high	Phase III trials of daclatasvir+asunepravir+/-BMS 791325 expected 2014 Manufacturerer

Daclatasvir+sofosbuvir+/-ribavirin Daclatasvir+Asunapravir+/-BMS 791325		less adverse events without ribavirin	-24 week treatment SVR=93% GT1 phase II daclatasvir+asunepravir+BMS 791325 -12 or 24 weeks treatment SVR12 94% GT2/3 phase II 24 weeks daclatasvir+sofosbuvir+/-RBV: SVR12 88-100%		and treatment-experienced individuals		elected to stop studies of daclatasvir+sofosbuvir
ABT 450 / ritonavir (Protease inhibitor) Manufacturer: Abbott ABT 450/r+/-ABT267+/-ABT 333+/-ribavirin	1, 2, 3	1% discontinuation. Common adverse events: fatigue, headache, pain, vomiting, hyperbilirubinaemia Ritonavir=high potential for drug-drug interactions	GT1 phase II 12 weeks 450/r+ABT267+ ABT 333+/-ribavirin: SVR12 87-97%	Twice daily ABT/r+ABT267 formulated in a fixed dose combination	GT1 null-responders phase II 12 weeks 450/r+ABT267+/-ABT 333+ribavirin: 87-93%	Low	Phase III underway (started Oct 2012)

Simepravir (Protease inhibitor) Manufacturer: Janssen/Tibotec/ Medivir Simeprevir + TMV 647055/ritonavir Simeprevir + sofosbuvir +/- ribavirin Simeprevir+Daclatasvir Simeprevir+ VX135	1, 4	Flu-like symptoms, rash, neutropenia, anaemia, transient elevation of bilirubin Co-administration with efavirenz is not recommended	Awaiting results of all-oral regimens using this drug	Once a day	Awaiting results of all-oral regimens using this drug	Limited information	Phase II with simepravir-containing all oral-therapy underway

Brief information on other DAAs:

Name	Class	Manufacturer
Asunapravir	Protease inhibitor	BMS
BMS 791325	Non-nucleoside NS5B polymerase inhibitor	BMS
ABT267	NS5A	Abbott
ABT333	Non-nucleoside polymerase inhibitor	Abbott
TMV 647055	Non-nucleoside NS5B polymerase inhibitor	Tibotec/Medivir
VX135	Nucleotide polymerase inhibitor	Vertex

BMS=Bristol-Myers Squibb. SVR= sustained virological response. SVR12=sustained virological response at 12 weeks. SVR24=sustained virological response at 24 weeks. RBV=ribavirin.

5. CURRENT AND PIPELINE DIAGNOSTICS

"Whenever easy oral treatments will be available, the key issue will be screening. Governments will hate it, because it will mean they will have to pay for it". JM Pawlotsky, Professor of Medicine at the University of Paris, Agence Nationale de Recherche sur le Sida (Paris MSF-OSF-TAG Meeting, September 24-25)

At present, HCV diagnosis and monitoring is complex, involving multiple steps and resulting in an expensive and resource-demanding process. *New treatments that require a much more simplified monitoring algorithm will have a hugely positive impact on the minimum access requirements for HCV monitoring tests.*

5.1 Serological rapid diagnostic tests

The first step in the diagnostic algorithm is to screen for HCV by measuring whether the person has any antibodies to the virus. However, because cured patients, and patients who are exposed to the virus but clear it naturally, retain antibodies to HCV, an additional molecular test to measure the actual virus must be done to confirm active infection. The easiest way to perform a non-laboratory-based serological testing is by a point-of-care rapid diagnostic test (RDT), a similar diagnostic test kit to a pregnancy test.

MSF has spent considerable resources in recent years searching for a good point-of-care RDT, yet we have not found anything suitable for resource-poor settings that meets all basic requirements. Requirements for such a test are summarised in the Panel below.

Panel 1: Requirements for a point-of-care RDT for HCV infection in resource-poor settings

1. Close to 100% sensitivity and a high negative predictive value
2. Simple procedure
3. No cold chain requirement
4. No additional equipment
5. WHO prequalified, CE marked, or FDA approved (as class I/A product)
6. Good manufacturing practice
7. Low cost
8. No interaction with other co-morbidities (especially HIV/AIDS)

Available products are currently too complex and expensive, have cold chain requirements, do not have good manufacturing practice, are not CE marked, and/or are not approved by the Food and Drug Administration (FDA) (as a class I/A (or high

risk) products). In order to ensure quality, it is important that products are quality approved by a strict regulatory authority. Often there are no good performance evaluation reports or published data, or the evaluation is relatively old (>10 years). The OraQuick test (OraSure, USA) would be the preferred RDT for procurement for resource-poor settings if it was affordable but, at EUR 14 per test, it is 4-12 times more expensive that other HCV RDTs. The test has a good performance (over 99% accurate), especially in patients who have seroconverted (recently become anti-HCV positive), and is made using good manufacturing practice. Unlike other tests that must be done on serum or plasma, the OraQuick RDT can also be done on whole blood (for example, finger-prick) or oral fluid, which is much more convenient for decentralised testing where there is no option of processing blood samples. This test also has no cold chain requirements (although must be kept below 30°C). Table 3 provides an overview of currently available tests; Table 4 provides an overview of pipeline tests.

A recent systematic review and meta-analysis of rapid and point-of-care screening tests for HCV revealed that tests done on blood samples have a higher accuracy that those done on oral fluid[36], which is to be expected. The review also provides test specifications of products included.

One major gap in the evidence base is that there is limited evidence on the accuracy of HCV RDTs in HCV/HIV co-infection. We do know, however, that several HCV screening tests can show false-negative or false-positive results in cases of HCV/HIV co-infection, and this remains a major concern[37]. Shivkumar S et al[36] showed that both HIV infection and ART initiation could influence the immune response sufficiently to alter HCV test accuracy. The researchers conclude that future implementation research studies stratify patients by HCV/HIV co-infection to resolve the issue of test accuracy.

5.2 Confirmation of active infection

Once serological positivity has been established, active infection must be confirmed by a nucleic acid amplification test – a molecular test that can measure the presence of the actual virus. This may be done by one of two tests: (1) a qualitative test that provides a “yes/no” answer – much like measuring HIV DNA for early infant diagnosis; or (2) a quantitative test that measures the viral load in international units per millilitre (IU/mL).

Qualitative tests are cheaper and easier to perform, however, a baseline viral load measurement is required before treatment initiation so that the log drop may be measured. Due to the fact that not all patients will respond to the current therapy with peg-IFN-riba, if a patient has not achieved a 2 log IU/mL drop in their viral load by 12 weeks of therapy (termed an early virological response), it is advised that they discontinue treatment as they have a negligible chance of achieving a SVR by the end of therapy (ie, patients will undergo a toxic treatment course without a chance of cure)[38]. For this reason, if there is a strong suspicion that the patient has an active

[36]Shivkumar S, et al. Accuracy of Rapid and Point-of-Care Screening Tests for Hepatitis C: A Systematic Review and Meta-analysis. *Ann Intern Med* 2012;157:558-566.
[37]Smith BD, Drobeniuc J, Jewett A, et al. Evaluation of three rapid screening assays for detection of antibodies to hepatitis C virus. *J Infect Dis* 2011;204(6):825–831. doi:10.1093/infdis/jir422
[38]P Deltenre, V Canva, M El Nady et al. A 2-log Drop in Viral Load at 1 Month is the Best Predictor of Sustained Response in HCV Patients with Normal ALT: A Kinetic Prospective Study. *J Viral Hepat* 2009;16(7):500-505.

HCV infection and could start treatment fairly soon, it is better to just do one test - the viral load test - and skip the qualitative test altogether. Furthermore, manufacturers are phasing out their supply of qualitative tests and focusing now on quantitative testing platforms (usually based on real-time PCR). This is evident by the fact that there are only two known suppliers of qualitative tests whereas there are five known suppliers of viral load tests. Table 5 provides an overview of commercially available qualitative HCV tests, and Table 6 an overview of pipeline tests. Viral load tests for the purposes of treatment monitoring will be reviewed later in this report.

With the introduction of the new pipeline drugs, it is possible that only qualitative testing may be needed. The use of a newer generation of DAA treatment may require only a binary +/- measurement (to confirm viral replication or suppression, respectively) both pre-treatment, at 4 weeks, and then post-treatment - depending on the lower limit of detection of the qualitative assay.

5.3 Genotyping

The required length of peg-IFN-riba treatment, the current standard of care, and the expected outcome from treatment, is dependent on the HCV genotype, of which there are 6 main genotypes (GT1-GT6):

- GT1, GT4, GT5, GT6: 48 weeks of treatment, less good outcomes
- GT2, GT3: 24 weeks of treatment, better outcomes

Current genotyping tests are based on four different types of technologies: real-time PCR, line probe assay, and DNA chip or sequencing. The most popular is the line probe assay. Table 7 provides an overview of available tests and Table 8 provides an overview of pipeline tests. When sending a sample to the laboratory for testing, costs can be well over USD 100. Tests are complicated and require well-resourced laboratories with highly qualified staff. The good news is that automated sample preparation platforms have greatly reduced hands-on time and the need for more highly qualified staff. Furthermore, generic options, like the HCV Genotype Plus Real-TM kit (Sacace, Italy) may be used with any real time PCR instrument, obviating the need to invest in additional instrumentation if the laboratory already has the basic devices installed. The same real time PCR machine can also be used for viral load testing. Importantly, because new HCV treatments will be pan-genotypic, genotypic testing may no longer be necessary.

5.4 Fibrosis staging and the need for non-invasive tests

As chronic HCV infection often takes years, if not decades, to progress to significant liver damage, and the currently available treatment is relatively toxic, staging should be performed before treatment decisions are made for patients living with chronic HCV[39]. Depending on HCV genotype and patient factors, including HCV/HIV co-infection and race/ethnicity, the SVR may range from 25%-80%. Thus, in particular for GT1 and GT4, which are associated with a worse treatment response, many clinicians will not initiate IFN-based antiviral therapy until significant fibrosis is demonstrated and the risk-benefit of treatment tips towards initiating treatment. Most clinicians will initiate treatment once significant liver scaring - or fibrosis - is demonstrated. For example, fibrosis - which is divided into 4 levels from F1 (mild) to F4 (severe) - must exceed F2 (METAVIR>/= F2). Importantly, as HCV treatments

[39]EASL. EASL clinical practice guidelines: management of hepatitis C virus infection. *J Hepatol* 2011;55:245–264.

improve in terms of safety and efficacy, these staging procedures may no longer be necessary.

Until recently, staging depended exclusively on a liver biopsy to examine the histology of the liver and diagnose the degree of inflammation and fibrosis in a given patient[40]. Liver biopsies are invasive and carry risks, including risk of internal haemorrhage and infection. Biopsies require a trained individual to perform then and a relatively experienced pathologist to interpret the biopsy. As liver damage does not occur in a uniform pattern throughout the liver, there is also the risk of inappropriate staging because of sampling error[41].

Thus, there is a need for non-invasive staging tests to help determine candidates for IFN-based HCV treatment. A non-invasive test would ideally be affordable, easy to perform (for example, based on phlebotomy), and accurate for a wide range of patients, including those with co-morbidities such as HIV. Currently, non-invasive tests of liver damage include radiologic and serologic measures. One of the most commonly used radiologic tests is based on transient elastography and measures the liver 'stiffness'. The more liver fibrosis there is, the stiffer the liver will be. Serologic tests are based on a combination of biomarkers that are correlated with liver disease. The biomarker measurements are put into an algorithm, the score of which may then be interpreted in a standardised way. The question for resource-poor settings is which tool is best in terms of performance and feasibility?

Given the expense, maintenance, and need for operator experience involved in transient elastography, this modality is certainly reasonable for resource-poor settings, however, it should likely be carried out at a higher level health centre or referral centre to ensure that the operators have sufficient experience to provide accurate and reliable results.

The other option is using biomarkers. Although many biomarker-based testing algorithms exist, most biomarkers are not able to be measured because the laboratory tests are unavailable, and the calculation of the algorithm is complex. Out of these, two serological tests may be considered feasible for resource-poor settings: APRI and FIB-4. These tests demonstrate acceptable accuracy in diagnosing no or minimal fibrosis or cirrhosis in mono-infected HCV patients. However, as their predictive value at intermediate ranges (F1-F3) is low, these patients may be considered for a confirmatory test, such as transient elastography. Biomarkers are still recommended for all settings where volumes are low and it does not make financial sense to purchase a FibroScan® (to perform transient elastography). While APRI may not be the best possible biomarker test, it is still suitable for use in resource-poor settings and the advantages include the use of a simple formula that is amenable to calculation by hand or with a simple calculator, and the use of laboratory test results that are likely to be routinely available (aspartate transaminase (AST) and platelets). For patients who are HCV/HIV co-infected, if levels of platelets or AST change, the indirect serologic tests are less accurate, and these patients may require transient elastography or liver biopsy.

We summarise the performance of the main indirect markers in Panel 2. Further information about the measurement of fibrosis can be found in reference 44.

[40]Bravo AA, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; 344:495–500.
[41]Regev A, Berho M, Jeffers LJ, et al. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002;97:2614-2618.

Panel 2: Summary of the performance of the main indirect markers

Fibrosis Marker	Performance
Aspartate transaminase (AST) / alanine transaminase (ALT) ratio	Not useful on its own[42]
AST to platelet ratio index (**APRI**): (AST/ULN (upper limit of normal)) / platelet count x 100	A recent meta-analysis of 40 studies revealed that APRI threshold of 0.7 for severe fibrosis showed a sensitivity of 77% and specificity of 72%; for cirrhosis, a cut-off of 1.0 showed a sensitivity of 76% and specificity of 72%; AUROC (area under the receiver operating curve) for significant fibrosis, severe fibrosis and cirrhosis was 0.77, 0.80, and 0.83, respectively[43]
PGA index: PT, GGT, Apolipoprotein A1	Accuracy in detecting cirrhosis: 66-72% in patients with alcoholic liver disease[44]
FibroIndex (platelets, AST, GGT)	Currently under investigation; prediction of significant fibrosis is 0.83[44]
FIB-4 Index (platelets, ALT, AST, age)	Tested in mono-infected HCV patients: AUROC = 0.85 for severe fibrosis; positive predictive value of 82.1% for significant fibrosis (at a cut off >3.25); values of 1.45-3.25 were not very predictive[45,46]

[42]Imperiale TF, Said AT, Cummings OW, et al. Need for validation of clinical decision aids: use of the AST/ALT ratio in predicting cirrhosis in chronic hepatitis C. *Am J Gastroenterol* 2000; 95(9):2328-2332.
[43]Lin ZH, Xin YN, Dong QJ, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. *Hepatol* 2011; 53:726-736.
[44]Ahmad W, Ijaz B, Gull S, et al. A brief review on molecular, genetic and imaging techniques for HCV fibrosis evaluation. *Virol J* 2011; 8;8:53.

Fibrometer: platelets, PT, AST, alpha-2 macroglobulin, hyaluronate, BUN, age	Can differentiate fibrosis progression[44]
FibroTest (FibroSure): alpha-2 macroglobulin, haptoglobin, gamma globulin, apolipoprotein A1, GGT, & total bilirubin. Determines fibrosis stage F0/1 vs F2/3/4	• Sensitivity 75% and specificity 85% for determining stage >F2 • FibroTest and FibroScan have been combined to improve correct diagnosis: in a recent study of 183 patients with chronic HCV infection, the AUROC was 0.88 for identifying fibrosis state >F2, 0.95 for >F3, and 0.95 for >F4[47,48]
ActiTest: FibroTest with ALT	• Improves identification of more advanced fibrosis and inflammation • May be used for monitoring[48]
Hepascore: total bilirubin, GGT, hyaluronic acid, alpha-2 macroglobin, age, & gender	AUROC of this test is 0.85 for significant fibrosis, 0.96 for advanced fibrosis and 0.94 for cirrhosis[44]

[45]Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. Comparison with liver biopsy and fibrotest. *Hepatol* 2007; 46:32-36.
[46]Sterling RK, Lissen E, Clumeck N, et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatol* 2006;43:1317-1325.
[47]Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection: comparison with liver biopsy and fibrotest. *Hepatol* 2007; 46:32-36
[48]Poynard T, et al. Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comparative *Hepatol* 2004;3:8.

5.5 Prognostic markers that predict response to HCV IFN-based treatment

Once a patient is identified as being a candidate for treatment, a variety of predictive tests may be done to determine their likelihood of responding to IFN-based treatment. The type of IL28B gene (for example, CC, TT, etc] and levels of IP-10 (a measure of inflammation and immune activation) are the most studied predictors of treatment response to IFN-based treatment. These tests are generally done for HCV genotypes that are not as responsive to IFN-based therapy (for example, GT1 and GT4). The likelihood of response for other genotypes is so high that these tests are not considered to be as valuable. For reasons stated below, these tests are not likely to be important in the era of DAAs.

IL-28B

Polymorphisms of IL28B can predict treatment response and have been shown to be the strongest baseline predictor of SVR in HCV infection with GT1[49]. More favourable genotypes are as follow: CC genotype at rs12979860 (Odds ratio [OR] of SVR 5 vs unfavourable genotypes); TT at rs8099917 (OR 3.7 vs unfavourable genotypes); AA at rs12980275 (OR 8.3 vs unfavourable genotypes). For those with unfavourable genotypes, SVR drops to between 30-35%[50].

The various polymorphisms of the IL28B gene are strongly associated with race/ethnicity and favourable genotypes are more likely to be found in Caucasian populations. For example the CC polymorphism is found in 30% of Caucasians compared with 15% of African Americans and 20-23% of Asians[51].

However, rapid virological response (RVR), which is the viral response at 4 weeks into treatment, is as good a predictor of success (OR 5) for GT1 as is the CC polymorphism[52]. In a study of telapravir, considering RVR after the lead in with peg-IFN-riba made the effect of CC non-significant[53]. Thus, while IL28B CC is the strongest baseline predictor of SVR, RVR is the strongest overall predictor of SVR (when considering CC, race, serum glucose, viral load, METAVIR, and fibrosis stage F0 or F1).

IP-10

The other most studied predictor of response to IFN-based therapy in HCV GT1 is IP-10.. As IFN-based treatment is based on activating the immune system to fight HCV, higher baseline levels of IP-10 suggest that additional immune stimulation with IFN might not be useful and in fact, higher levels of IP-10 predict a worse response.

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[49]Thompson AJ, Muir AJ, Sulkowski MS, et al. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterol* 2010; 139:120-129.

[50]Grebely J, Petoumenos K, Hellard M, et al. Potential role for interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. *Hepatol* 2010; 52(4):1216-1224.

[51]Gonzalez SA, Keeffe EB. IL-28B as a Predictor of Sustained Virologic Response in Patients with Chronic Hepatitis C Virus Infection. *Gastroenterol Hepatol* 2011; 7(6):366-373.

[52]Beinhardt S, Rutter K, Stättermayer AF, et al. Revisiting the predictors of a sustained virologic response in the era of direct-acting antiviral therapy for hepatitis C virus. *Clin Infect Dis* 2013; 56(1):118-122.

[53]Pol S, Aerssens J, Zeuzem S, et al. Limited impact of IL28B genotype on response rates in telaprevir-treated patients with prior treatment failure. *J Hepatol* 2013; Epub ahead of print.

IP-10 and unfavourable IL-28B genotypes are not directly related and, thus, these two tests can be considered complementary[54]. Levels >600 pg/mL (measured by ELISA) had negative predictive values for achieving SVR of 78%[55]. Thus, levels of >600 pg/mL are considered as a cut-off for predicting poor response. However, in one study, only 14% of patients had IP-10 >600 pg/mL. So, once again, IP-10 and RVR are highly correlated and - when taking into account the predictive value of RVR - IP-10 levels become non-significant and RVR remains the only independent predictor of SVR.

Although these markers may be useful for predicting response to IFN-based treatments for patients living in resource-rich countries, these tests are complex and expensive. They also only have moderate predictive value for treatment response. Considering a resource-poor setting, where the only options are peg-IFN-riba treatment or nothing, these markers may hold little added value. Furthermore, investing in infrastructure to test these markers may not produce long-term gains as these markers will no longer be important when we move to DAA, oral HCV drugs.

Implementing HCV treatment programmes in resource-poor settings does not require the use of prognostic marker tests for predicting treatment response; therefore the absence of these tests should not hinder the opening of treatment programmes, even for programmes treating only with peg-IFN-riba.

5.6 Safety and monitoring

Due to potential toxicities of peg-IFN-riba, regular laboratory monitoring is required. EASL recommends measuring ALT and complete blood count at baseline, weeks 1, 2, and 4, and then every 4-8 weeks during treatment. TSH (thyroid stimulating hormone) must also be monitored at baseline and every 12 weeks. As both ribavirin and peg-IFN-alpha are renally dosed (dosed according to renal function), most clinicians will also assess creatinine at baseline and during treatment. Due to the teratogenic effects of ribavirin, pregnancy testing is also recommended. A move to new, all-oral HCV treatments may offer an opportunity to also simplify laboratory monitoring. For example, the combination of sofosbuvir-ribavirin may only require ALT, creatinine, and haemoglobin testing at baseline and 3-4 times during treatment. Unlike TSH or even complete blood count, ALT, creatinine, and haemoglobin are regularly available in mid-level health facilities in low and middle-income countries. This decreased need for laboratory monitoring also reduces the laboratory related costs of treatment.

5.7 Viral load for treatment monitoring

Viral load testing is required to monitor the effectiveness of treatment. At a minimum the baseline, week 12, week 24, and end of treatment viral loads must be measured, with - where possible - an additional viral load 6 months post-treatment to confirm

[54]Darling JM, Aerssens J, Fanning G, et al. Quantitation of pretreatment serum interferon-γ-inducible protein-10 improves the predictive value of an IL28B gene polymorphism for hepatitis C treatment response. *Hepatol* 2011; 53:14–22.
[55]Lagging M, Romero AI, Westin J, et al. IP-10 predicts viral response and therapeutic outcome in difficult-to-treat patients with HCV genotype 1 infection. *Hepatol* 2006; 44:1617–1625.

that there has been no disease reactivation (or reinfection). The use of oral regimens in the future may obviate the need for tests at week 12 and week 24.

A 2 log IU/mL baseline to 12 week drop is required for a patient to continue treatment. A high viral load is considered to be above 800 000 IU/mL. There are currently 6 different commercially available viral loads tests for HCV, described in Table 9, including a cheaper generic version, the HCV Real-TM Quant (Sacace, Italy), which may be run on any real time PCR instrument. This therefore saves the laboratory having to purchase extra instrumentation if they already have a real-time PCR machine in place.

These laboratory-based tests are complex, requiring well-resource laboratories and skilled staff. Automated sample preparation instruments have greatly reduced the hands-on time and level of technical skill required, which may allow for greater decentralisation of testing. A number of studies have also looked at the use of dried blood spots for both genotyping and viral load assays. Although further research is required, preliminary results show that dried blood spots (DBS) can be used for transportation of blood samples to laboratories that may be far away from clinics[56,57,58,57]. DBS are easy to prepare from a finger-stick of blood and are stable at ambient temperatures for a few weeks. Furthermore, a number of point-of-care test manufacturers that are on the cusp of launching viral load tests for HIV (for example, IQuum, Alere, Cepheid, and Wave80) are also planning to release test cartridges for HCV in the more distant future (Table 10).

Together this means that, if prices are not too high, viral load testing in resource-poor settings will be possible. An HCV viral load test can cost over USD 100 so price-reducing mechanisms, such as competition and volume price discounts, will be necessary to drive costs down.

Since diagnostic and treatment monitoring costs for HCV are expensive, bringing prices down will be a major step forward.

It is estimated that the screening test (1x serological RDT) plus viral diagnostic test (1x viral load + 1x genotype), plus treatment monitoring (2x viral load tests) currently cost in the region of EUR 400-500 per patient.

In summary, current diagnosis and monitoring algorithms - which include staging for patients with GT1 or GT4 HCV disease - are relatively complex and costly, but feasible even in resource-poor settings. With use of DAAs, the diagnostic and monitoring algorithm may be significantly simplified, reducing, or may even eliminate the need for genotyping, staging, and intensive monitoring for drug side-effects (for example, TSH).

[56]Tuaillon E, Mondain AM, Meroueh F, et al. Dried blood spot for hepatitis C virus serology and molecular testing. *Hepatol* 2010; 51(3):752–758.
[57]Solmone M, Girardi E, Pucillo CF, et al. Simple and Reliable Method for Detection and Genotyping of Hepatitis C Virus RNA in Dried Blood Spots Stored at Room Temperature. *J Clin Microbiol* 2002; 40(9):3512–3514.

6. HCV MARKET SHORTFALLS

Table 11: HCV commodity market shortfalls and potential solutions

Market shortcoming	Description of market Shortcoming	Reasons for market shortcoming	Solutions
Lack of epidemiological data	Unknown magnitude of the pandemic Different epidemiological profiles: highly vulnerable groups vs concentrated or generalised epidemics No sentinel surveillance system	Absence of public and medical knowledge and awareness Lack of civil society mobilisation and political will Fear of escalating treatment costs Diagnostics are largely available	Increase public and professional awareness Promote civil society mobilisation Create adequate funding mechanisms Ensure diagnostics available Put surveillance systems in place Develop guidelines for screening and design optimised tests for staging.
Marginalised affected groups	Injection drug users; men who have sex with men; prisoners; HCV/HIV co-infected people	Segregation issues; human rights issues, non inclusion in study design and in operational research .	Decriminalisation of drug use; universal access to HIV and HCV care and treatment
No access to screening, diagnostics, and monitoring tools	HCV treatment: complex, unreliable, unaffordable, a care package required (HCV antibody testing, viral load testing, genotyping, liver fibrosis evaluation, and treatment monitoring)	Low demand Little innovation Low availability Low accessibility Low utilisation Monopolies, with markets concentrated in resource-rich countries	Create the demand Stimulate innovation Simplify algorithms for screening, diagnostics, and monitoring Stimulate competition for new devices: point of care viral load tests, open

			polyvalent PCR platforms, robust and affordable rapid diagnostic tests, Increase use of pre-qualified devices
Lack of prevention activities	Safe injection package is currently unavailable Avoid recontamination Management of risk factors and co-morbidities Universal hygiene precautions Blood bank precautions	Lack of awareness about safe injections and safe medical and dental practices Medical negligence Absence of sterilisation of devices, waste management, safe injection package for injecting drug users Check blood donations for HBV, HCV, and HIV.	Safe injections and safe medical and dental practices and blood transfusions should be universal standard of care Improve access to needle exchange programmes Improve awareness of the management of risk factors and co-morbidities Educate population about good hygiene practices
Little access to peg-IFN-riba treatment	Unaffordable Complex Adverse events difficult to manage Low efficacy of peg-IFN-riba regimen in HCV/HIV co-infected people Delivery issues Low acceptability	High costs, low volumes Very specialised complex care No competition Monopoly by Roche&Merck Regulatory pathway for biosimilars unavailable Pre-qualification process for biosimilars does not exist	Access to all-oral HCV treatments will allow simplified and shorter treatment regimen

		Low uptake – particularly poor for peg-IFN-riba No WHO guidance as yet	
Access to all-oral HCV treatment regimen	A promising all oral pipeline Drugs not available before 2014	Pharma giants will compete to occupy the HCV market	Differential pricing strategy needed Patent oppositions to challenge patents given without true innovation Patent pool or other voluntary licensing strategy for HCV drugs needed Pooling strategies needed
No funding mechanism	Lack of domestic funding No international donor commitment No innovative funding mechanism	Market concentrated in resource-rich countries	UNITAID to kick start an innovative funding mechanism for resource-poor settings

7. EMERGING OPPORTUNITIES AND STEPS FORWARD

The burden of HCV and HCV/HIV co-infection in low and middle-income countries is significant and remains largely unaddressed to date. The unprecedented progress in the HCV drug pipeline – with new drugs likely coming onto the market in early 2014 – will radically modify the way HCV care will be provided. Major steps forward in drug development will now mean that we can move from complex treatment requiring specialised centres, to simplified protocols that will allow progressive decentralisation and scale-up in resource-limited settings.

At the same time, increased simplification and automation in terms of laboratory technologies will allow development of reliable and simple HCV viral load point-of-care devices and laboratory-based tests that will be suitable for district level laboratories. These new treatments will also simplify diagnostic and monitoring algorithms. We may need only one viral load test, or qualitative test, prior to treatment, and one after treatment termination. Genotyping and staging may no longer be necessary. Limited side-effects will allow for a simplified monitoring schedule.

In order to reduce the barriers to ensuring patients can access HCV diagnosis and care, the following factors have been identified as being in urgent need of addressing:

- Peg-IFN must be registered and included on the WHO's Essential Medicines List.
- WHO sentinel epidemiological surveys and data collection systems must be put in place in every country/or globally.
- WHO should ensure the pre-qualification of HCV RDTs and virological tests with urgency.
- Simple and reliable pre-qualification system for biosimilars needs to be developed by regulators.
- Competition between drugs must be encouraged to decrease the price of peg-IFN-riba globally.
- Governments and policy-makers should ensure aggressive access policies for the new DAAs, including addressing intellectual property and registration barriers that may exist to block potential for generic competition.
- Maintain appropriate and affordable pricing for new DAAs.
- Ensure that guidelines – at both WHO and country-level - are developed for diagnosis and treatment of HCV in resource-poor settings and include guidance on new DAAs as evidence becomes available.
- Encourage governments to offer increased access to freely available screening for HCV for key populations and in generalised epidemics.
- The research community must push for the implementation of real-life treatment feasibility trials in resource-poor settings and among vulnerable groups, including people living with HIV, injecting-drug users, pre-post transplant patients, cirrhotic and fibrosis stage F3-F4 patients, and people who do not tolerate IFN.
- Increase public, patient, and professional awareness.
- Improve the reliability of blood transfusion and ensure adherence to universal hygiene precautions for safe medical procedures.
- Service-providers should develop new models of care to suit everyone: from centralised to decentralised settings, to families, vulnerable groups, children, and HCV/HIV co-infected people.

- Create incentives for developers to include access to all-oral HCV drugs for people living in resource-countries in their market strategies, at affordable prices.
- Unitaid, the Global Fund, domestic funding etc should urgently set up new funding opportunities to secure treatment for resource limited settings, including middle income countries and emerging markets.
- Increased political will is a critical next step in this process.

There is now a unique window of opportunity for innovators and policy makers to ensure that people living with HCV in resource-poor settings gain access to new diagnostics and effective and affordable new treatments.

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ANNEX 1: THE MOST PROMISING PIPELINE HCV DRUGS

Definitions used for treatment outcomes

Sustained virological response (SVR) 24: HCV becomes undetectable during treatment, and remains undetectable for 24 weeks post-treatment completion. Considered as a cure.

SVR12: HCV becomes undetectable during treatment, and remains undetectable for 12 weeks post-treatment. Considered as highly predictive of treatment success. Accepted as co-primary endpoint with SVR24.

SVR4: HCV becomes undetectable during treatment, and remains undetectable for 4 weeks post-treatment.

SOFOSBUVIR (Ex GS-7977)		
Class	Nucleoside/nucleotide polymerase inhibitor	*COMMENTS*: Interest: HIGH Pan-genotypic All-oral HCV regimen: initial indication for GT2& GT3, not for GT1 yet. Better outcomes in GT2 than GT3 with sofosbuvir+ribavirin Sofosbuvir plus peg-IFN-riba for 3 months course: initial
Company	Gilead	
Daily dose	400mg once daily	
Development	Phase III	
Potent	YES	
Genotype	1, 2, 3, 4, 5, 6	
Universal	Adults	
Simple to administer	Yes	
Safety /Tolerability/adverse events	Well tolerated. Common adverse events: fatigue, headache, nausea, insomnia, dizziness. No need of food intake. No significant drug-drug interactions, no rash, less anaemia. Sofosbuvir-GS5885-ribavirin [Gane ref 3]: (n=34); serious adverse	

	events = 8%; 1 treatment interruption for severe adverse events; common adverse events - anaemia (20%), depression (8%), headache (4%). 44% laboratory abnormalities including anaemia.	indication for GT1,GT4,GT5, and GT6.
Combinations and SVR	GT1 treatment naïve: never received HCV treatment before It is important to note that the indication in GT1, GT4, GT5, and GT6 includes the addition of 12 weeks of peg-IFN-riba, so it is not IFN free ATOMIC: Sofosbuvir+Peg-IFN-riba for 12 or 24 weeks. SVR=90%[1] NEUTRINO: Sofosbuvir+ Peg-IFN+riba for 12 weeks. SVR12=89% [2] IFN-free: ELECTRON: Sofosbuvir+GS 5885 + ribavirin. GT1 naïve (n=25) and prior null responders (n=9) SVR12=100% (34/34).[3]Limited numbers but encouraging results. ION-1: Sofosbuvir+GS 5885+/-ribavirin.studyon going, includes cirrhotic patients.[4] Sofosbuvir+Daclatasvir +/- ribavirin 12 or 24 weeks. SVR=100%.[5] ELECTRON: Sofosbuvir+ribavirin 12 weeks. SVR4= 88%.[67] QUANTUM: Sofosbuvir+ribavirin comparing 12weeks treatment vs24 weeks treatment. ->12weeks results: 10/17: SVR=59%.[89] Sofosbuvir+BMS-791325 +ribavirin 12 weeks. SVR=100% (n=25/25).[10] SPARE: sofosbuvir+ ribavirin; weight based or 600mg for 12 weeks.	Market entry expected for 2013 or 2014 for GT2& GT3, 2015 for GT1with GS 5885 Sofosbuvir 400mg + GS5885 90mg single dose fixed dose combination under development (Phase III) More studies are needed for HCV/HIV co-infected individuals, those living with advanced liver disease, and treatment-experienced individuals.

	Non-cirrhotic group (n=10)24 weeks of weight based ribavirin SVR12=90%. [11] <u>GT2& GT3 treatment naïve</u> FISSION/NEUTRINO: Phase III trial, sofosbuvir+ peg-IFN-riba for 24 weeks (SVR=67% [n=162/243])vs sofosbuvir+ribavirin for 12 weeks (SVR=67% [n=170/253]). Break down by GT: 12 weeks sofosbuvir+ribavirin. SVR12 for GT2= 97% (vs SVR12= 78% for 24 weeks peg-IFN-riba). SVR12 for GT3=56% (vs SVR12=63% for 24 weeks peg-IFN-riba .[12] ELECTRON: sofosbuvir+ribavirin for 8 weeks. SVR12=64% (n=16/25).[13],[14] ELECTRON: sofosbuvir+/- peg-IFN + ribavirin. SVR24=100% (n=50). - Group 12 weeks, no peg-IFN. SVR24=100% (n=24) - Group 8 weeks, with peg-IFN. SVR24=100% (n=24) - Group 8 weeks, no peg-IFN. SVR12=64% (n=25)[15] AI 444-040: Daclatasvir+sofosbuvir+/-ribavirin. Treatment naïve, non cirrhotic, GT2& GT3.[16] -24 weeks, sofobuvir lead in, no ribavirin (n=16): SRV24=88% - 24 weeks, no ribavirin (n=14): SRV24=100 % - 24 weeks, with ribavirin (n=10): SRV24=93%	

	GT4, GT5, and GT6 NEUTRINO: 12 weeks sofosbuvir=-peg-IFN-riba. SVR 12=97% (n=35). Of the 35 patients with GT4, GT5, GT6, 97% achieved SVR12. Break down by GT: GT4: sofosbuvir+peg-IFN-riba : SRV24= Is 10/12 GT6: sofosbuvir+ peg-IFN-riba. SVR24= Is 5/5 No relapses at week 12 and week 24. (ref 12) Large Phase III trials ongoing.	
Resistances	High genetic barrier to resistance (means drug resistance is unlikely)	
HCV/HIV co-infection	Currently under study, similar HCV viral kinetic as mono-infected, well tolerated.[17],[18] Treatment outcomes of sofosbuvir-GS5885 without ribavirin are not yet known.	
Treatment experienced	GT1 treatment experienced (limited data available) ELECTRON: sofosbuvir +ribavirin for 12 weeks. GT1 null responders, SVR12=10% (n=1 of 10). ELECTRON: sofosbuvir+GS5885+ribavirin 12 weeks in 9 GT1 null responders. SVR12= [A: %?] (n=9/9) (ref 3) GT2& GT3 treatment experienced FUSION: 12 or 16 weeks sofobuvir+ribavirin.[19] - Group 12 weeks: SVR12= 50% (n=50/100) - Group 16 weeks:SVR12=73% (n=69/75) Break down by GT: - 12 weeksgroup;GT2 SVR12=86%; GT3 SVR12=30%.	

	- 16weeksgroup;GT2 SVR12= 94%; GT3 SVR12=62% POSITRON study in GT2 & GT3 intolerant to peg-IFN;sofosbuvir+weight- based ribavirin.[20] -GT2: SRV12=93% -GT3: SRV12=61%Studies so far show a better outcome in GT2 treatment- experienced patients than in GT3 treatment-experienced patients with sofosbuvir+ribavirin.	
Advanced liver disease	Still very few data for cirrhotic patients. Under study in cirrhotic pre-transplant patients. <u>GT1</u> NEUTRINO: sofosbuvir+Peg-IFN-riba among patients with cirrhosis, 80%achieved SVR12 SPARE: sofosbuvir+ribavirin : All stage *fibrosis* (n=50); *advanced fibrosis/compensated cirrhosis* (n=13/50). 2 sub-analysis: -24weeks, weight-based ribavirin (n=25): SVR12=72% - 24 weeks, low dose ribavirin (n=25): SVR12=56%. Note: relapse rates were 29% with weight-based ribavirin and 45% with low-dose ribavirin. (ref 11) <u>GT2& GT3</u> FISSION[21]: 20 of 100 participants (20%) had compensated cirrhosis. 72% had GT3. Among people with cirrhosis at baseline who received sofosbuvir+ribavirin, 47% achieved SRV12. 38% of cirrhotic patients who received peg-IFN+riba achieved SVR12. FUSION:34% (N=68)of participants had compensated cirrhosis at	

	baseline. 31%achieved SVR12 in the 12 weeks group, and 66% achieved SVR12 in the 16weekgroup.Break down data by GT not available yet.(ref 20) POSITRON (ref 20):207 patients in the sofosbuvir+ribavirin group. 31 of them had compensated cirrhosis, 108 of them had GT2.99 had GT3. In GT2: SVR12= 93% In GT3: SVR 12=61%	

DACLATASVIR (DCV) BMS-790052		
Class	NS5A Inhibitor	*COMMENTS:* INTEREST: High Pan-genotypic Dual DAA: DCV-sofosbuvir SRV=85-100% in GT1,GT2, GT3 treatment naive. Regardless of ribivarin use. Unfortunately DVC-sofosbuvir combination studies have been interrupted.
Company	Bristol Myers Squibb	
Daily dose	60 mg once daily	
Development	Phase III	
Potent	Yes	
Genotype	1,2,3,4	
Universal	Adults	
Simple to administer	Yes	
Safety/ Tolerable / AE	In study DCV+ asunaprevir in treatment-experienced patients: 5 serious adverse events reported: high fever, gastroenteritis, elevated bilirubin (unrelated study drugs), hypochondria. 2 discontinuations for liver-enzyme elevation, 1 for elevated bilirubin. Other adverse events: headache, cold, diarrhoea, fever, stomach pain, malaise, constipation, back pain, appetite loss. DCV+asunaprevir+ BMS-791325 study adverse events: headache,	

	diarrhoea, asthenia No safety signal	DCV-asunaprevir may be an interesting option in treatment-experienced patients too.
Combinations and SVR	<u>GT1 treatment naïve</u> AI444-040: DCV-GS-7977+/- ribavirin 24 weeks. SVR=100% in GT1, and > 85% in GT2 & GT3 regardless of ribavirin use.[22] AI444-040: DCV+ sofosbuvir+/- ribavirin. Population: GT1, non cirrhotic (n=126), 12 weeks (n= 82): SVR4= 95-98%, 24 weeks(n= 44) SVR12= 93-100%. AI443-014: DCV+ asunaprevir +BMS-791325,[23] GT1 treatment naive, non-cirrhotic (n=32), 24weeks (n=16): SVR4=94%. 12 weeks (n=16) SVR24=94%. D-LITE: Phase IIbpeg-IFN-lambda+ribavirin+ asunaprevir or DCV in treatment naïve, non-cirrhotic, early responders (n=69).[24] -24 weeks, 4drugs asunaprevir (n=32). SVR12=75% (GT1a: 67%, GT1b: 91%, IL28b CC: 90%, IL28b non-CC: 68%) - 24-weeks, 4 drugs, DCV (n=37). SVR12=100%, all GT1b, IL28b: no impact. <u>GT2& GT3</u> AI 444-040: DCV+sofosbuvir+/-ribavirin, in treatmentnaïvenon cirrhotic patients, GT2& GT3.[25] -24 weeks, sofobuvir lead in, no ribavirin, (n=16). SRV24=88% - 24 weeks, no RBV (n=14). SRV24=100 % - 24 weeks, with RBV (n=10). SRV24=93% <u>GT4</u>	

	DCV + Peg-IFN-ribaSVR12=100% in the 60mg group at week 12.[26]	
Resistances	Low genetic barrier to resistance (class effect)	
HIV-HCV	GT1: DCV-Peg-IFN-riba for 24 weeks followed by 24 weeks peg-IFN-riba "tail". Phase III, recruiting. No clinically significant pharmacokinetic interaction between DCV and tenofovir. DCV dose adjustment with efavirenz and atazanavir/ritonavir may be needed. Well tolerated.[27]	
Treatment experienced	HCV GT 1b Phase IIa, DCV-asunaprevir 24 weeks among a group of 21 null responders, and 23 IFN-ineligible/intolerant patients.[28],[29] SVR 24=77%. SVR24 was higher among null responders (91%) than among IFN-ineligible/intolerant people (64%). AI 447-011: Phase II. HCV GT1, peg-IFN-ribavirin+ DCV+ asunaprevir once or twice daily: null responders (n=41) mostly GT1a, mostly non-CC 2 groups: -24 weeks , 4drugs (asunaprevir once daily) (N=21): SVR24= 95% -24 weeks, 4-drugs (asunaprevir twice daily) (n=20): SRV24=90%	
Advanced liver disease	HCV1b, DCV+asunaprevir, null responders, 16% with advanced fibrosis (n=38): once daily (n=20) SVR12=62%; twice daily (n=18) SVR 12=78%.[30] Study AI444-040: GT1, GT2, GT3. 40% had absent to mild fibrosis (Metavir F0-F1), about half had moderate to advanced liver disease (F2-F3), and roughly 15% had cirrhosis (F4).	

	Note:very limited data in treatment naïve or treatment-experienced patients with cirrhosis	

ABT-450/ritonavir (ABT 450/r)		
Class	Protease Inhibitor	*COMMENTS:* INTEREST: High Ritonavir booster required All-oral combination ABT450/ r is being studied in: GT1 treatment naïve, GT1 treatment experienced, GT1 non responders, GT2& GT3 treatment naïve, up to 48 weeks Results are available for GT1 only. Studies are needed for
Company	Abbott	
Daily dose	100/100mg, 200/100mg twice daily Boosting with ritonavir required	
Development	Phase IIb	
Potent	Yes	
Genotype	1,2,3 : under study, results pending	
Universal	Adults	
Simple to administer	Ok	
Safety/ Tolerability/adverse events	AVIATOR: 1% discontinuation 5 serious adverse events: 1 arthralgia possibly drug related Common adverse events: fatigue, headache, pain, vomiting, hyperbilirubinaemia No safety issues	
Combinations And SVR	AVIATOR: GT1 non cirrhotic naïve and prior peg-rbv null responders[31] ABT 450/r (dose100/100 mg to 200/100 mg) + ABT-267 (25mg daily) + ABT-333 (400mg twice daily) + ribavirin	

	SVR 12 in GT1 treatment naïve=97.5% (n=77/79) SVR12 in GT1 nullresponders=93.3% (n=42/45) SVR12 in GT1a treatment naïve=96% (n=52/54) SVR12 in GT1a nullresponders=89% (n=25/28) SVR12 in GT1b treatment naive=100% (n=25/25) SVR12 in GT1b nullresponders=100% (n=17/17) Results from the 12 week triple DAA groups without ribavirin SVR12 in GT1=87.3% (n=69/79) SVR12 in GT 1a=83% (n=43/52) SVR12 in GT1b=96%(n=24/25) ABT450/r + ABT 333+ weight-based ribavirin in GT1 and previous non responders to peg-IFN-riba[32] SVR12 RNA < 25 IU/mLat weeks 4-12 Treatmentnaïve ABT450/r 250mg+ABT-333 400mg(n= 19) SVR=95% Treatmentnaive ABT450/r 150mg+ ABT 333 400mg (n=14) SVR= 93% Previous non-responders ABT450/r 150mg+ ABT 333 400mg (n=17) SVR=47% ABT 450/r + ABT 333 + weight-based ribavirin: under study[33]	HCV/HIV co-infected individuals, those living with advanced liver disease, and treatment-experienced patients.
Resistances	Low barrier to resistance (class effect)	
HCV/HIV co-infection	No data yet Drug interactions are expected/Ritonavir	
Treatment experienced	Treatment experienced and IFN null responders currently under study	
Advanced liver	Under study	

disease	No data yet in treatment naïve or treatment-experienced cirrhotic patients	

Drug characteristics	Product **SIMEPREVIR**	Comments
Class	Protease Inhibitor	*COMMENTS:*
Company	Jansssen/Tibotec/Medivir	
Daily dose	150 mg once daily	INTEREST: Medium
Development	Phase III	
Potent	Yes	Some suggestion of improved outcomes for treatment experienced people and people with advanced liver fibrosis.
Genotype	1,4	
Universal	Adults	
Simple to administer	No	
Safety/Tolerable /adverse events	Simeprevir + Peg-IFN-riba:no increased serious adverse events compared to placebo. Adverse events: fatigue, flu-like symptoms, itching, headache, nausea.No difference in incidence of rash, anaemia, neutropenia by treatment group.Transient elevation in bilirubin levels in Simeprevir patients.	
Combinations & SVR	Simeprevir + peg-IFN-ribaSVR24=78% Simeprevir + TMV 647055 (non-nucleoside HCV polymerase inhibitor) boosted ritonavir Simeprevir + sofosbuvir +/- ribavirin Simeprevir-DCV Simeprevir+ VX [A: VX – a drug name?] 135 Phase III studies are needed	

Resistances Forgiving	Limited information available	
HCV/HIV co-infection	Co-administration with efavirenz is not recommended (efavirenz reduces the dose effect of simeprevir). Raltegravir, rilpivirine, tenofovir can be co-administered without dose adjustment. Co-administration of simeprevir with single dose tacrolimus or cyclosporine appears to be well tolerated with no serious adverse events.[34]	
Treatment experienced	Simeprevir + peg-IFN-riba in prior relapsers, partial responders, null responders. Compared simeprevir dose (100mg vs150mg) and duration (12 vs24 vs48 weeks). The best results were seen in the 150mg dosing groups: prior relapsers (SVR=85%), prior partial responders (SVR=75%) , and null responders achieved (SVR=51%).	
Advanced liver disease	Simeprevir + sofosbuvir +/- ribavirin: treatment naïve or null responders GT1, METAVIR score F3-F4, 12 or 24 weeks, recruiting December 2012. ASPIRE: Simeprevir+ peg-IFN-riba**:** 31% of prior null responders with cirrhosis were cured.[35]ASPIRE enrolled about 462 treatment-experienced GT1 HCV patients - prior relapsers, partial responders, and null responders - including people with advanced fibrosis or cirrhosis (METAVIR stage F4). They were randomly assigned to receive 100 or 150mg once-daily simeprevir or placebo with peg-IFN-riba for 12, 24 or 48 weeks; the 12 week and 24 week groups then continued on peg-IFN alone to week 48.[36]	

	PILLAR trial: 386 treatment-naive GT1 chronic HCV patients including people with advanced fibrosis (METAVIR stage F3). Participants were randomly assigned to receive 75mg or 150mg once-daily simeprevir or placebo in combination with peg-IFNalpha-2a and 1000-1200mg/day weight-adjusted ribavirin for 12 weeks or 24 weeks. ➔simeprevir 150mg in HCV GT1 treatment-experienced patients:33% response rate for prior null responders with advanced fibrosis/cirrhosis (very small number of patients) (linked to Aspire trial above?)	

FALDAPREVIR (BI 201335)		
Class	Protease Inhibitor- second generation HCV NS3/4a protease inhibitor	*COMMENTS:* INTEREST: Medium
Company	Boehringer Ingelheim	
Daily dose	Once daily, 120mg (treatment naïve), 240mg (treatment experienced)	
Development	Phase III	
Potent	Yes	
Genotype	1	
Universal	Adults	
Simple to administer	Yes	
Safety/Tolerable	With peg-IFN-riba:	

/adverse events	Severe adverse events were reported in 11.8% of people in the 120mg BI 201335 group, 12.8 to 15.9% in the 240mg group, and 4.2% in the placebo group. Discontinuation rates: 4.4% in the 120mg group, 5.4% to 11.6% in the 240mg group, 1.4% in the placebo group. One death was reported in the placebo group. Discontinuations for rash, jaundice, and photosensitivity occurred only in the 240mg dosing groups. SOUND 2: No serious adverse events Moderate adverse events lead to 9 treatment discontinuations (2 jaundice, 3 vomiting, 4 diarrhoea) in the 28 weeks treatment group. Laboratory abnormalities in the 28weekgroup: elevated bilirubin; 26% grade 3 ALT elevation and 10% grade 4; ALT elevation: 3% grade 3, and 2% anaemia.	
Combinations SVR	Peg-IFN-riba+ BI 201335 120mg or 240mg or placebo once daily.[37] BI 201335 OD + BI 207127 (non-nucleoside polymerase inhibitor)+/- ribavirin Peg-IFN-riba+ BI 201335 120mg or 240mg or placebo once daily. SVR in the Bi 201335 groups ranged from 71% to 83%. SOUND-C2: 5 group trial to identify a DAA regimen that is effective for treatment naïve people with HCV GT1b and GT1a who have IL28B CC genotype. Components: BI 201335 OD + twice vs thrice daily BI 207127 (non-nucleoside polymerase inhibitor) +/- ribavirin, 16 to 40 weeks treatment. - Non ribavirin group: SVR12=39% - BI 201335 + QD BI 207127 + ribavirin: SVR12=68%	

	. in GT1a: SVR12=43% . in GT1b: SVR 12=83% . in CC GT: SVR12=79% . in non-CC GT: SVR12=64% Overall: SVR12 among individuals with GT1a, non=CC=32% SVR12 among GT1a, CC=75% SVR12 among GT1b, non CC=82% SVR12 among GT1b, CC=84%.[38]	
Resistances	Low genetic barrier (class effect)	
HCV/HIV co-infection	No data available	
Treatment experienced	Under study GT1 partial or null responders: SILEN-C2: -240mg BI 201335 OD +/-3 days peg-IFN-riba lead-in, or -BI 201335 QD+ 3 days peg-IFN-riba lead-in.	
Advanced liver disease	*People with compensated cirrhosis:* SOUND-C2: n=32. 25 of them had HCV GT1b.Overall the SVR12 with thrice-daily BI 207127 was 57%, versus 54% for twice daily Bi 207127 (and 33% for the no ribavirin group). In the 28week group: - Cirrhosis HCV GT1a twice daily dosing BI207127, SVR28:=71% - Cirrhosis GT1b SVR28=33%. SVR ranged from 27% (once-daily, with lead-in) to 31% (twice daily, with lead-in), up to 41% (once-daily, no lead-in group). The highest SVR in partial responders was 50%; among null responders it was 35%.	

	SILEN-C3: treatment with 12 or 24 weeks of once daily 120mg BI 201335 and Peg/IFN, followed by response guided therapy peg-IFN-riba for 24 weeks). Was equally effective with SVR=65% vs SVR=73%.[39] It is not yet sure whether or not this drug will be further studied for treatment- experienced individuals.	

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TABLE 3: SEROLOGICAL POINT-OF-CARE AND RAPID DIAGNOSTIC TESTS (RDTs) FOR HCV

Test	Manufacturer	Time to result (minute)	Antigen used	Specimen required for testing	Volume required for testing	Storage temperature (°C)	Shelf life (months)	Test type
OraQuick HCV Antibody Test*	OraSure Technologies	20-40	Core, NS3, NS4	Oral fluid, whole blood, serum, plasma	1 drop	2-30	NA	Point-of-care; lateral flow casette
Dual Path Platform test	Chembio Diagnostic Systems	15-30	Core, NS3, NS4, NS5	Oral fluid, whole blood, serum, plasma	NA	NA	24	Point-of-care
Multiplo Rapid HIV/HCV Antibody Test	MedMira	3	Core, NS3	Whole blood, serum, plasma	1 drop	2-30	NA	Point-of-care
SD Bioline HCV	Standard Diagnostics	5-20	Core, NS3, NS4, NS5	Whole blood, serum, plasma	10-20µL	2-30	18	Point-of-care
Hexagon HCV	Human Diagnostics Worldwide	5-20	Core, NS3, NS4, NS5	Whole blood, serum, plasma	NA	15-30	NA	Point-of-care
Genedia HCV Rapid LF	Green Cross Medical Sciene	20-30	Core, NS3, NS4, NS5	Whole blood, serum, plasma	10-20µL	2-30	18	Point-of-care; immunofiltration
Anti-HCV Antibody rapid test	Tema Ricerca	3	NA	Whole blood	1 drop	NA	NA	Point-of-care
SM-HCV rapid test	SERO-MED Labor Spezialitaten	3	Core, NS3, NS4	Whole blood, serum	30-40µL	2-8; after opening: <30	NA	Point-of-care
Bioeasy HCV Test	Bioeasy Diagnostica	10	Core, NS3, NS4, NS5	Whole blood, serum, plasma	10µL	2-30	NA	Point-of-care
Advanced Quality One Step HCV Test	Bionike	6	NA	Serum, plasma	4µL	2-30	18	RDT
Serocard HCV	Trinity Biotech	19	NA	Whole blood, serum, plasma	80µL	2-8	16	RDT
Diagnos HCV Bi-Dot	J. Mitra	3	Core, NS3, NS4, NS5	Serum, plasma	NA	2-8	15	RDT
HCV-Tri-Dot	J. Mitra	5	Core, NS3, NS4, NS5	Serum, plasma	45µL	2-8	12	RDT
HCV Spot	MP Biomedicals	10	NA	Serum, plasma	45µL	2-25	6-8	RDT
Rapid Anti-HCV Test	InTec Products	15	Unknown	Whole blood, serum, plasma	10µL	2-30	Unknown	Lateral flow, casette
One Step Hepatitis C Virus	Guangzhou Wondfo Biotech	15	Unknown	Serum, plasma	80-100µL	4-30	24	Lateral flow, casette
Quickview HCV ANTIBODY TEST CARD	Lumiquick	20	Core, NS3, NS4, NS5	Whole blood, serum, plasma	10µL	4-30	18	Lateral flow, casette
HCV Ab	Dialab	10-20	structural & non-structural antigens	Whole blood, serum, plasma	4 drops serum, plasma; 1 drop whole blood	2-30	24	Lateral flow, casette
NanoSign HCV	Bioland Ltd	10-15	Core, NS3, NS4, NS5A	Whole blood, serum, plasma	10µL serum, plasma; 20µL whole blood	4-30	24	Lateral flow, casette

Compiled from the internal MSF laboratory working group review and from data from Shivkumar S, Peeling R, Jafari Y, et al. Accuracy of Rapid and Point-of-Care Screening Tests for Hepatitis C. Ann Intern Med 2012;157:558–566.
*Eur 14 per test = ~10x more expensive than other tests. Best test in terms of performance and good manufacturing practice.
RDT=Rapid diagnostic test; NA=Not available

TABLE 4: SEROLOGICAL PIPELINE POINT-OF-CARE TEST FOR HCV

	Mbio
	Prototype Hepatitis C Virus Antibody System
Assay type	HCV antibody reactivity; point-of-care immunoassay
Technological set-up	Single-use, disposable cartridges, simple, robust, low-cost reader
Extraction method / sample preparation	No extraction required; direct addition of whole blood (finger stick or venous), plasma or serum
Target region	Can be configured to detect antibodies against multiple HCV antigens (eg, core, NS3, NS4, etc)
Genotypes, subtypes	Not applicable
Linear range	Not applicable; dynamic range ~3.5 logs
Sensitivity / limit of detection	Limit of detection for analogous immunoassay (HIV p24 antigen) is ~20pg/mL
Specificity	For preliminary performance assessement see Lochhead, et al. J Clin Micro 2011; 49: 3584-3590.
Standardisation	Multiple internal quality controls, and compatibility with external quality assurance standards
Time to result	< 20 minutes
Throughput	80 - 100 tests per day (10 - 15 / hour)
Sample type	Whole blood (finger stick or venous), plasma, serum
Sample volume	~10 µL
Controls	Multiple in-cartridge controls (sample addition, non-specific binding); compatible with external quality assessment
Transport requirements	No cold chain required
Equipment required	MBio Reader is portable, robust, and can be run on an internal rechargeable battery for up to 8 hours
Indicative cost of equipment ($)	~$5000 (USD)
Indicative cost per test (for reagents/cartridge) ($)	Volume dependent; competitive with visual read rapid diagnostic tests
Technical skill	Minimally trained healthcare worker
Laboratory set-up	Access to power for battery recharge; battery provides 8 hours of operation
Storage conditions	target is 12 months at ≤40°C in original packaging
Applicable settings	Point-of-care, health post, district hospital, small and medium volume laboratories
Regulatory approval	MBio Diagnostics expects ISO 13485 certification in the second quarter of 2013; HCV product still in development
Reference	Manufacturer

TABLE 5: COMMERCIALLY AVAILABLE QUALITATIVE AMPLIFICATION TESTS FOR HCV

	Roche	Siemens	Hologic (Gen-Probe)
	COBAS® AmpliPrep/COBAS® AMPLICOR HCV Test v2.0 (being discontinued)	**VERSANT® HCV RNA Qualitative Assay**	**APTIMA HCV RNA Qualitative Assay**
Assay type	Qualitative PCR with colourimetric detection	Qualitative target amplification-based nucleic acid probe test using Transcription-Mediated Amplification and chemiluminiscent detection	In-vitro nucleic acid amplification assay for the detection of HCV RNA; Validated on DTS 400 System; The assay utilizes Transcription-Mediated Amplification (TMA) to amplify conserved regions within the HCV genome (TMA utilizes Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (RT) and T7 RNA polymerase to generate multiple RNA copies from viral nucleic acid template)
Technological set-up	Manual extraction, automated amplification and detection	Semi-automated, open system	Automated, closed system (the assay has 3 main steps performed in a single tube: sample preparation, target amplification and detection)
Extraction method / sample preparation	Uses the AMPLICOR® HCV Specimen Preparation Kit, version 2.0	Manual	Sample preparation involves detergent lysis of virus, followed by hybridization of free virus nucleic acid with capture oligonucleotides complementary to a conserved region of HCV genome (5'UTR); Hybridization steps take place in solution; Internal control or viral RNA hybridized targets are subsequently captured by magnetic microparticles and separated from remaining specimen components using a magnet; After wash steps to remove inhibitory substances, the target is ready for amplification
Target region	Conserved 5´UTR and core region	Conserved 5´UTR and core region	Conserved 5´UTR region
Genotypes, subtypes	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6; and subtypes 2a, 2b, 3a, 4a, 5a, and 6a have all been tested using clinical specimens and transcripts of the 5'UTR region
Sensitivity / limit of detection	50 IU/mL for EDTA plasma; 60 IU/mL for serum	5.3 IU/mL	5.3IU/mL (95% probability)
Specificity	99,99%	99,60%	0,996
Standardisation	WHO International Standard for HCV RNA, NIBSC code 96/790	WHO International Standard for HCV RNA	Not provided
Time to result	Approx 8 hours	5 hours	Not provided
Throughput	Approx 3 hours; 24 samples / day	10 - 94 samples / day (flexible)	DTS 400: 400 results in 8hrs (kit is 100 tests)
Sample type	Plasma, serum	Plasma, serum	Fresh or frozen plasma (EDTA, sodium citrate, and ACD) or serum
Sample volume	200µL	0.5 mL	0.5 mL
Controls	AMPLICOR® HCV (–) Control and the AMPLICOR® HCV (+) Control	Internal; external (optional): positive, negative	Assay performance is monitored using an internal nucleic acid control added to each specimen with the Target Capture reagent; Internal Control: RNA transcripts in HEPES Buffer with detergent to monitor whole procedure from sample preparation to detection; Negative Calibrator and HCV Positive Calibrator to determine run validity and analyte and Internal Control cutt-offs
Transport	Refrigerated item (4-8°C)	Requires refrigeration	Whole blood may be held and transported at room temperature for 24 hours prior to processing serum or plasma; Serum or plasma may be stored/transported at 2-8°C for 48 hours or frozen below -20°C for longer periods
Equipment required	COBAS® AMPLICOR Analyzer & 3rd party equipment as described on package insert	Luminometer (Siemens or any brand)	Hologic DTS 400 system (or DTS 800 or DTS 1600)
Indicative cost of equipment (USD $)	Cobas®Amplicor® 30,000	Please contact manufacturer to discuss project specific pricing	DTS 400: 63,000
Indicative cost per test (for reagents) (USD $)	49-56	Please contact manufacturer to discuss project specific pricing	80 (US Price Catalogue: $8000 for 100 test Kit)
Technical skill	Medium-highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes
Laboratory set-up	Specialised; 2-3 dedicated areas are required	Not provided	Not provided
Storage conditions	2-8°C	-15°C to -35°C: box 1; 2-8°C: box 2; 15-30°C: box 3	-15°C to -35°C: box 1; 2°C to 8°C upon receipt: box 2; 15°C to 30°C upon receipt: box 3
Applicable settings	Developed / highly resourced settings	Developing, low-medium resourced settings	Developed / highly resourced settings
Regulatory approval	CE-IVD	CE-IVD; US-FDA-IVD	CE-IVD; US-FDA-IVD
Advantages		Extremely high sensitivity, superior to RT-PCR-based qualitative HCV RNA detection assays	
Disadvantages	Not for monitoring of patients; phasing out plan fourth quarter of 2013	Not for monitoring of patients	Not for monitoring of patients
Reference	http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASAMPLICORHCVTestv20.aspx; package insert; manufacturer	http://www.medical.siemens.com/webapp/wcs/stores/servlet/ProductDisplay~q_catalogId~e_-101~a_catTree~e_100001,1023815,1023070,1015865~a_langId~e_-101~a_productId~e_172984~a_storeId~e_10001.html; package insert; manufacturer	http://www.gen-probe.com/products-services/aptima-hcv-qualitative-kit; Manufacturer

Disclaimer: price is subject to region and can differ from one market to another; in addition, final pricing within a region is also subject to training, installation, technical support and other requirements.

TABLE 6: PIPELINE QUALITATIVE AMPLIFICATION TESTS FOR HCV

	WAVE80
	EOSCAPE-HCV-D
Assay type	Isothermal target amplification with bipartite photonic signaling
Technological set-up	Disposable cartridge, processing unit and analyzer
Extraction method / sample preparation	Integrated sample preparation
Target region	Genotype: NS5b and E1 regions; detection: 3'-X-tail (highly conserved >99%)
Genotypes, subtypes	1, 2, 3, 4, 5 and 6
Sensitivity / limit of detection	<10-15 IU/mL
Specificity	>98%
Standardisation	External calibration for linear range (>100,000 IU/mL)
Time to result	<1 hour
Throughput	Parallel processing with multiple processing units (up to 10 processing units per analyzer)
Sample type	Whole blood (capiliary), plasma
Sample volume	100µL, acquired through fingerstick using standard lancets through protocol developed by Wave 80
Controls	IAC on-board
Transport	No cold chain requirement
Equipment required	EOSCAPE analyzer
Indicative cost of equipment ($)	<10,000
Indicative cost per test (for reagents) ($)	20
Technical skill	CLIA moderate complexity
Laboratory set-up	Not specialised, laboratory or point-of-care
Storage conditions	Refrigeration may be required for external calibrants
Applicable settings	Resource-poor, low-medium resourced settings
Regulatory approval	CE-IVD; US-FDA-IVD to be sought
Advantages	Low cost, point-of-care, easy to use, same device for all their tests
Disadvantages	
Reference	Manufacturer

TABLE 7: COMMERCIALLY AVAILABLE GENOTYPING TESTS FOR HCV

	Abbott	Roche	Siemens		Sacace
	RealTi*me* HCV Genotype II	LINEAR ARRAY HCV Genotyping Test (being discontinued)	VERSANT® HCV Genotype 2.0 Products (LiPA)	TRUGENE® HCV Genotyping Assay	HCV Genotype Plus Real-TM
Assay type	Qualitative real-time PCR using fluorescently-labelled probes; 3 separate reactions required: A (GT 1a, C), B (GT 1, 1b, 2), and C (GT 4, 5, 6)	Linear array	Line probe assay (LiPA) uses reverse hybridization. Biotinylated DNA PCR product, generated by RT-PCR amplification of the 5'UTR and core region of HCV RNA, is hybridized to immobilized oligonucleotide probes.	CLIP Sequencing; produces bi-directional sequences using two fluorescently-labeled DNA primers	Real time PCR with 2-channel fluorescent detection
Technological set-up	Fully automated or manual, closed system	Manual, closed system	Automated, closed system	Semi-automated, closed system	Automated or manual, open system
Extraction method / sample preparation	Manual or automated (m24sp or m2000sp)	Manual using the AMPLICOR® HCV Specimen Preparation Kit, version 2.0	Automated (using the strip processors Auto LiPA 48 or AutoBlot 3000H); PCR product is produced using the VERSANT HCV Amplification 2.0 Kit (LiPA) after extraction of viral RNA	Previously amplified product used with TRUGENE HCV 5'NC Genotyping Kit and OpenGene® DNA Sequencing System	Manual (any nucleic acid extraction kit; Sacace recommend their own one) or automated (e.g. NucliSens® easyMAG™ (bioMérieux))
Target region	Conserved 5´UTR, core region and NS5b	Conserved 5'-UTR and core region	Conserved 5´UTR, core regions and NS5b	5'UTR	5'UTR
Genotypes, subtypes	1a, 1b (NS5b), 1, 2, 3, 4, 5, 6 (5'UTR)	1, 2, 3, 4, 5 and 6	1a, 1b, 2, 3, 4, 5 and 6 (subtypes c-l)	6 major hepatitis C genotypes and 41 sub-types	1a, 1b, 2, 3, 4, 5a, 6
Sensitivity / limit of detection	≥500 IU/mL, 0.5 mL prep; ≥1250 IU/mL, 0.2 ml prep	≤500IU/mL	96%	NA	1000 IU/mL; 100%
Specificity	≥ 97.0%	99.99%	99.4%	NA	100%
Standardisation	Second WHO international standard for HCV RNA (NIBSC 96/798)	unknown	NA	NA	4th WHO International Standard for HCV RNA
Time to result	Manual 10.5 hr; m2000 6 hours	Approx 3 hours	Approx 8 hours for 48 samples: ~2.7 hours for amplification and ~3.3 hours for strip processing with AutoLiPA 48 (both fully hands-off processes)	30 minutes	Dependent on the extraction method used (~120 min)
Hands-on time per patient sample	Manual 13 min; m2000 4 min	Approx 10 hours	30 minutes	NA	Dependent on the extraction method used (~40 min using Ribo-Virus Sacace kit)
Throughput	48 samples / day	24 samples / day	96 samples / day with VERSANT kPCR SP module and 2x Auto-LiPA 48. Scalable strip processing automation: up to 48 tests with the AutoLiPA 48; up to 20 tests with the AutoBlot 3000H	16 samples / day	50 samples / day
Sample type	Plasma, serum	plasma, serum	plasma, serum	plasma, serum	Plasma
Sample volume	0.2 mL or 0.5 mL	100µL of denatured amplicon (using COBAS AMPLICOR detection material)	Depends on extraction method used; 10µL of amplicon	4µL of amplicon	0.1-1.0 mL
Controls	Internal; external: positive (5000 IU/mL of GT1 and GT4 of RNAs diluted in HCV negative plasma) and negative	AMPLICOR® HCV (–) Control and the AMPLICOR® HCV (+) Control	VERSANT HCV Control 2.0 Kit (LiPA): 3 control lines: conjugate control, 2 amplification controls (5'UTR and core), negative control	external: positive, negative	internal; external: positive, negative
Transport	Requires refrigeration (ship on dry ice)	Requires refrigeration (4-8°C)	Requires refrigeration and freezing (amplification kit only)	requires refrigeration	2-8°C (max 3-4 days)
Equipment required	m24sp or m2000sp (sample preparation) plus m2000rt (amplification + detection)	COBAS® AMPLICOR Analyzer & 3rd party equipment (as described on package insert)	Auto LiPA 48 or AutoBlot 3000H; VERSANT® HCV Genotype 2.0 Assay (LiPA); Amplification 2.0 Kit; Control 2.0 Kit; LiPA Scan HCV Interpretation Software	OpenGene DNA sequencing system	Sacace (or any) real time PCR instrument with 2 fluorescent channels; optional automated sample prep instrument
Indicative cost of equipment (USD $)	Please contact manufacturer to discuss project specific pricing	COBAS® AMPLICOR® 30,000	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	SaCycler real time PCR instrument, 20,000
Indicative cost per test (for reagents) (USD $)	Please contact manufacturer to discuss project specific pricing	Including extraction, detection and genotyping 94-111	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	20 (without extraction)
Technical skill	Medium-highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes
Laboratory set-up	Specialised; 1 dedicated area is required	Specialised; 3 dedicated areas are required	Specialised; 2-3 dedicated areas are required	Specialised; 2-3 dedicated areas are required	Specialised; 2-3 dedicated are required
Storage conditions	-10°C: reagens kits A, B and C; internal control; + and -B3 controls	2-8°C	2-8ºC for Genotype kit; -25ºC to -15ºC for Amplification kit	-20°C	2-8°C: Part N°1; -20°C: Part N°2 and N°3

Applicable settings	Developed / highly resourced settings	Developed / highly resourced settings	Both developed and developing settings, highly resourced	Both developed and developing settings, highly resourced	Resource-poor, low-medium resourced settings
Regulatory approval	CE-IVD	CE-IVD	CE-IVD; RUO in the USA	no, RUO	None
Advantages	HCV viral load and HCV genotyping on one automated platform; results obtained more rapidly compared to sequencing-based assays (1.5 days)	Fast turn around time with minimal hands on time	It is the only commercially available assay able to detect 6 (c-l). Only 4% of clinical specimens are mistyped with the VERSANT genotype assay relative to the reference method (direct sequence analysis of the NS5B region), which makes it the most accurate method for use in clinical practice.	Compared to other methodologies that require hybridization of the HCV virus, the TRUGENE® HCV Genotyping Assay directly amplifies and sequences the virus allowing direct examination of the viral RNA.	Low cost, flexible
Disadvantages	Does not bind to unusual subtypes	phasing out plan fourth quarter of 2013			Not regulatory approved
Reference	http://www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/hepatitis-hcv-genotype-II-assay.html; Abbott broshure (package insert not found online); manufacturer	http://molecular.roche.com/ASSAYS/Pages/LINEARARRAYHepatitisCVirusGenotypingTestv2.aspx (package insert not found on line); manufacturer	http://healthcare.siemens.com/molecular-diagnostics/molecular-diagnostics-systems/versant-hcv-genotype-2-products; package insert; manufacturer	http://healthcare.siemens.com/molecular-diagnostics/molecular-diagnostics-research-use-only/trugene-hcv-genotyping-assay; package insert; manufacturer	http://www.sacycler.com; package insert; manufacturer

NA=Not available; RUO=research use only

Disclaimer: price is subject to region and can differ from one market to another; in addition, final pricing within a region is also subject to training, installation, technical support and other requirements.

TABLE 8: PIPELINE GENOTYPING TEST FOR HCV

	WAVE80	Epistem
	EOSCAPE-HCV-G	**HCV genotype, VL and IL-28B (multiplex)**
Assay type	Isothermal target amplification with bipartite photonic signaling	See info under viral load pipeline tests
Technological set-up	Disposable cartridge, processing unit and analyzer	
Extraction method / sample preparation	Integrated sample preparation	
Target region	Genotype: NS5b and E1 regions; detection: 3'-X-tail (highly conserved >99%)	
Genotypes, subtypes	1, 2, 3, 4, 5 and 6	
Sensitivity / limit of detection	<10-15 IU/mL	
Specificity	>98%	
Standardisation	External calibration for linear range (>100,000 IU/mL)	
Time to result	<1 hour	
Hands-on time per patient sample	Blood draw	
Throughput	Parallel processing with multiple processing units (up to 10 processing units per analyzer)	
Sample type	Whole blood (capiliary), plasma	
Sample volume	100µL, acquired through fingerstick using standard lancets through protocol developed by Wave 80	
Controls	IAC on-board	
Transport	No cold chain requirement	
Equipment required	EOSCAPE analyzer	
Indicative cost of equipment (USD $)	<10,000	
Indicative cost per test (for reagents) (USD $)	50	
Technical skill	CLIA moderate complexity	
Laboratory set-up	Not specialised, laboratory, or point-of-care	
Storage conditions	Refrigeration may be required for external calibrants	
Applicable settings	Resource-poor, low-medium resourced settings	
Regulatory approval	CE-IVD; US-FDA-IVD to be sought	
Advantages	low cost, point-of-care, easy to use, same device for all their tests	
Disadvantages		
Reference	Manufacturer	

TABLE 9: COMMERCIALLY AVAILABLE VIRAL LOAD TESTS FOR HCV

	Abbott	Roche		Siemens		QIAGEN		Sacace
	RealTime HCV assay	**COBAS® Ampliprep®/COBAS® TaqMan® HCV Test, v2.0**	**COBAS® TaqMan® HCV Test, v2.0 with the use of Highpure**	VERSANT® HCV RNA 3.0 Assay (bDNA)	VERSANT® HCV RNA 1.0 Assay (kPCR)	**careHCV RT-PCR Kit V2 (from the Shenzhen subsidiary)**	**artus HCV QS-RGQ PCR Kit**	**HCV Real-TM Quant**
Assay type	quantitative real time PCR using fluorescently-labeled probes	quantitative real time PCR using fluorescently-labeled probes; automated specimen preparation on the COBAS® AmpliPrep Instrument by a generic silica-based capture technique with magnetic glass particles (MGP)	quantitative real time PCR using fluorescently-labeled probes	bDNA signal amplification using ELISA with chemiluminescent detection	Reverse transcription (RT) kinetic polymerase chain reaction (kPCR) procedure for quantifying HCV RNA in human serum and plasma	quantitative real time PCR assay incorporating QIAGEN silica membrane column purification technology for sample preparation and one-step RT-PCR enzyme which enables reverse transcription and real-time PCR reactions in a single tube	quantitative real time PCR assay incorporating QIAGEN magnetic particle technology for sample preparation and one-step RT-PCR enzyme which enables reverse transcription and real-time PCR reactions in a single tube	quantitative real time PCR using fluorescently-labeled probes and dual-colour detection
Technological set-up	fully automated or manual, closed system	fully automated, closed system	manual extraction automated amplification and detection	automated, closed system	automated, closed system	automated (QIAcube) or manual, open system	automated (QIAsymphony SP/AS instrument), open system	automated or manual, open system
Extraction method / sample preparation	manual or automated (m24sp or m2000sp)	1 KIT CAP/CTM HCV QUANT 72T v2.0	manual extraction (High Pure System Viral Nucleic Acid Kit)	no sample extraction required	automated using VERSANT kPCR Molecular System	manual (Qiagen viral RNA mini kit) or automated (on QIACube); sample preparation reagents are included in the kit	sample prep and assay set-up can be run on QIAsymphony SP/AS instrument	manual (any nucleic acid extraction kit; Sacace recommend their own one) or automated (e.g. NucliSens® easyMAG™ (bioMérieux))
Target region	conserved 5´UTR and core region	conserved 5´UTR and core region	conserved 5´UTR and core region	conserved 5´UTR and core region	conserved 5´UTR	conserved 5´UTR and core region	conserved 5´UTR and core region	5´UTR
Genotypes, subtypes	1, 2, 3, 4, 5 and 6	1a, 1b, 2a,2b, 3, 4, 5 and 6	1a, 1b, 2a,2b, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5, 6	1, 2, 3, 4, 5, 6	1, 2, 3 ,4, 5, 6
Linear range	12 IU/mL (1.08 log IU/mL) to 100 million IU/mL (log 8.0 IU/mL)	15 to 100,000,000 IU/mL	25 to 390,000,000 IU/mL	615 to 7,690,000 IU/mL (2.79 to 6.89 log IU/mL)	15 IU/mL (64.5 copies/mL) to 1 x 108 IU/mL (4.3 x 108 copies/mL)	$1x10^3$ - $5x10^7$ IU/ml	35.0 to 1.77 x 107 IU/ml	20 -108 IU/ml
Sensitivity / limit of detection	12 IU/mL, 0.5 ml sample; 30 IU/mL, 0.2 ml sample	15 IU/ml	15 IU/ml	615 IU/mL	15 IU/mL (64.5 copies/mL) to 1 x 108 IU/mL (4.3 x 108 copies/mL)	500 IU/ml	21 IU/ml	10 IU/ml; 100% (new kit)
Specificity	≥ 99.5%	100%	100%	98.8%	100%	100% (tested against the national standard panel (China) for common infectious disease, which includes HAV, HBV, HIV, HDV, CT, NG, CMV, HPV, TB, and showed no cross reactivity)	100%	100%
Standardisation	second WHO international standard for HCV RNA (NIBSC 96/798)	First WHO International StandardHCV RNA (NIBSC code 96/790)	First WHO International Standard HCV RNA (NIBSC code 96/790)	WHO International Standard for HCV RNA	WHO 3rd Hepatitis C Virus (HCV) RNA International Standard (06/100)	2nd WHO International Standard for HCV RNA (NIBSC code 02/202)	2nd WHO International Standard for HCV RNA (NIBSC code 02/202)	4th WHO International Standard for HCV RNA (NIBSC code 96/798)
Time to result	manual: 10.5 hr; m2000: 6 hr	~ 5.5 hours (including setting-up the COBAS® AmpliPrep Instrument)	5.5 hr	22 hr	≤6 hrs	3 hr	unknown	it depends on the used extraction method (~120 min)
Throughput	96 samples / d	~ 30 minutes per 48 tests (0.6 minutes/patient sample); 72 samples/day up to 168 samples/day	2.15 hr; ≤48 samples / d	12 – 168 samples / run	89 samples / batch = 178 samples / d	72 samples / d	unknown	100 samples
Sample type	plasma, serum	plasma, serum	plasma, serum	plasma, serum	plasma, serum	plasma, serum	plasma	plasma
Sample volume	0.5 ml or 0.2 ml	650 µl	500 µl	50 µl	500 µl	200 µl	1000 µl	0.1-1.0 ml
Controls	internal; external: low positive, high positive, negative	internal (quantitation std); external: low positive, high positive, negative	internal (quantitation std); external: low positive, high positive, negative	external: low positive, high positive, negative	external: low positive, high positive, negative	internal; external: positive, negative	internal; external: positive, negative	internal; external: low positive, high positive, negative
Transport	refrigeration required (ship on dry ice)	refrigeration required (4-8°C)	refrigeration required (4-8°C)	refrigeration required (Box 1) and deep-freezing @ -60 to -80°C (Box 2)	Box 1 refrigerated; Box 2 deep frozen @ -60 to -80°C	dry ice or blue ice	dry ice or blue ice	2-8°C (max 3-4 days)
Equipment required	m24sp or m2000sp (sample prep) plus m2000rt (amplification + detection)	COBAS® AmpliPrep (sample prep) plus COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (amplification and detection)	COBAS® TaqMan® 48 Analyzer (amplification and detection) & 3rd party equipment such a centrifuge with a plate rotor as described on package insert	VERSANT 440 Molecular System	VERSANT kPCR Molecular System	Rotor-Gene Q real time PCR cycler plus QIAcube (optional); other real-time PCR instruments such as ABI7300/7500, LC480, and i-Cycler are also compatible and have been validated for use	Rotor-Gene Q real time PCR cycler plus QIAsymphony SP/AS instrument	Sacace (or any) real time PCR instrument with 2 fluorescent channels; optional automated sample prep instrument
Indicative cost of equipment ($)	Please contact manufacturer to discuss project specific pricing	Cobas® Ampliprep® 80-100,000; Cobas® TaqMan96 Analyzer with docking station 110,000 or Cobas® TaqMan® 48 Analyzer 40-50,000	Cobas® TaqMan® 48 Analyzer 40-50,000	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	SaCycler real time PCR instrument, 20,000
Indicative cost per test (for reagents) ($)	Please contact manufacturer to discuss project specific pricing	42-52	52-64	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	Please contact manufacturer to discuss project specific pricing	17 (without extraction)
Technical skill	Medium-highly trained, precision pipetting required at low volumes	Medium trained, basic precision pipetting	Medium-highly trained, precision pipetting required at low volumes	Medium-highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes	Highly trained, precision pipetting required at low volumes
Laboratory set-up	Specialised; 1-2 dedicated areas are required	Specialised; 1 dedicated area only	Specialised; 2-3 dedicated areas are required	Not specialised; single work area; deep-freezing required	Specialised; can be set up in one-room; deep-freezing (-20°C) required for kit storage	Specialised; 2-3 dedicated areas are required	Specialised; 2 dedicated areas are required	Specialised; 2-3 dedicated areas are required
Storage conditions	-10°C: amplification reagent pack and internal control, control kit, calibrator kit	2-8°C: COBAS AmpliPrep/COBAS TaqMan HCV v2.0 Test; 2-30°C: COBAS AmpliPrep/COBAS TaqMan Wash Reagent	2-8°C: COBAS TaqMan HCV v2.0 48 Tests 2-30°C Highpure Viral Nucleic Acid kit 48 tests	2-8°C: box 1; -60°C to -80°C: box 2	-20ºC for Boxes 1 & 2	-20ºC	-20ºC	2-8°C: Part N°1; -20°C: Part N°2
Applicable settings	Developed / highly resourced settings	Developed / highly resourced settings	Developed / highly resourced settings	Both developed and developing settings, highly resourced	Developed / highly resourced settings	developing, low-medium resourced settings	Developed / highly resourced settings	developing, low-medium resourced settings
Regulatory approval	CE-IVD; US-FDA-IVD	CE-IVD, US-FDA-IVD, Canada-IVD, Japan-IVD	CE-IVD, US-FDA-IVD, Canada-IVD	CE-IVD, US-FDA-IVD	CE-IVD; not available in the US	China (SFDA)	CE-IVD	CE certification on-going
Advantages				ELISA-like format; single-room technology		careHCV test designed for use in areas with limited resources or infrastructure; first locally-manufactured PCR test in the domestic market that can be used in conjunction with automated sample technology solutions, such as QIAGEN's QIAcube, which limits hands-on time		low cost, flexible
Disadvantages				Due to the potential for false-positive results, this assay should NOT be used for initial detection of hepatitis C virus (HCV) RNA in patients with suspected acute or chronic HCV infection. It should be used to quantify HCV RNA in patients who have been found to be positive by a more sensitive HCV RNA detection or quantification assay.				not yet regulatory approved
Reference	http://www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/hepatitis-hcv-assay.html; Abbott broshure; package insert; manufacturer	http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASTaqManHCVTest.aspx; http://molecular.roche.com/assays/Pages/COBASTaqManHCVTest.aspx; package insert; manufacturer	http://molecular.roche.com/assays/Pages/COBASAmpliPrepCOBASTaqManHCVTest.aspx; http://molecular.roche.com/assays/Pages/COBASTaqManHCVTest.aspx; package insert; manufacturer	http://healthcare.siemens.com/molecular-diagnostics/molecular-diagnostics-in-vitro-diagnostics/versant-hcv-rna-3-assay/features; http://www.mayomedicallaboratories.com/test-catalog/print/81130; package insert; manufacturer	http://www.medical.siemens.com/webapp/wcs/stores/servlet/PressReleaseView~q_catalogId~e_-1~a_catTree~e_100005~a_langId~e_-1~a_pageId~e_144756~a_storeId~e_10001.htm (package insert not found or provided); manufacturer	http://www.qiagen.com/about/pressreleases/pressreleaseview.aspx?PressReleaseID=337 (package insert not found online)	www.qiagen.com/Products/.../artus-HCV-RT-PCR-Kits-CE; package insert; manufacturer	http://www.sacycler.com; package insert; manufacturer
HBV test	yes	yes	yes	yes	yes	no	yes	yes

IU = 2-5 viruses (3 is generally used but no standard has been fixed); ≥ 800 000 - 1 million IU/mL is considered a high viral load

EASL recommends lower limit of detection for quantitative VL at 10-20 IU/ml

Disclaimer: price is subject to region and can differ from one market to another; in addition, final pricing within a region is also subject to training, installation, technical support and other requirements.

TABLE 10: PIPELINE VIRAL LOAD TESTS FOR HEPATITIS C*

	Wave80	Daktari	Epistem	Molbio	Cepheid
	EOSCAPE-HCV-Q	**HCV**	**HCV genotype, VL and IL-28B (multiplex)**	**Truenat HCV**	**Xpert HCV**
Assay type	Isothermal target amplification with bipartite photonic signaling	microfluidics	PCR	real-time quantitative PCR	Fully automated real time amplification and detection
Technological set-up	disposable cartridge, processing unit and analyzer	disposable cartridge, analyzer	disposable cartridge, analyzer; 3 capillaries therefore 3 different tests can be run in parallel (i.e. genotype, VL and IL-28B)	Truenat HCV: Ready to use, disposable micro chips; Truelab: Portable, automated, battery operated real-time micro PCR analyzer	fully automated, closed system
Extraction method / sample preparation	integrated sample preparation	integrated sample preparation	proprietary activated filter paper-based extraction	Trueprep Mag: Magnetic nano particles-based reagents using portable semi-automated battery-operated sample prep device	automated within system; no off-line sample prep steps
Target region	genotype: NS5b and E1 regions; detection: 3'-X-tail (highly conserved >99%)	core antigen	proprietary	core gene	conserved region of HCV genome
Genotypes, subtypes	1, 2, 3, 4, 5 and 6	not provided	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6	1, 2, 3, 4, 5 and 6
Linear range	50-1e6 IU/mL (LLOQ 50 IU/mL)	<10,000 - 10 million copies/mL	10 to 10^6 copies/mL	2 to 9 log (under evaluation)	10 – 1x108 IU/ml
Sensitivity / limit of detection	<10-15 IU/mL	<10,000	TBD	2 log range (being evaluated)	≤ 10 IU/ml
Specificity	>98%	TBD	TBD	100% (based on preliminary evaluation)	99.5% (in seronegative blood donor)
Standardisation	external calibration for linear range (>100,000 IU/mL)	TBD	not provided	WHO standard	WHO 3rd Hepatitis C Virus (HCV) RNA International Standard (06/100)
Time to result	<1 hr	30 min	< 60 min	<60 min	~90 min
Throughput	parallel processing with multiple processing units (up to 10 processing units per analyzer)	12-24 results/instrument/day	1 sample/hour/unit	12 samples/day	comparable to Xpert MTB/RIF, system dependent (device sizes: single, 2, 4, 16 modules)
Sample type	whole blood (capiliary), plasma	whole blood	whole blood	whole blood, plasma, serum	plasma, serum
Sample volume	100µL, acquired through fingerstick using standard lancets through protocol developed by Wave 80	100µl	20µl	100µl	≤ 1ml
Controls	IAC on-board	On board positive/negative controls	Internal inhibition control	full process Internal Positive Control	two Internal Quantification Standards (IQS) - high and low - used in quantification
Transport	no cold chain requirement	no cold chain requirement	no cold chain requirement	2 - 30°C with transportation stress up to 35°C	comparable to Xpert MTB/RIF
Equipment required	EOSCAPE analyzer	Daktari analyzer	Genedrive	Truelab micro PCR work station (no additional equipment required)	GeneXpert
Indicative cost of equipment ($)	<10,000	TBD	4,000	8,000	comparable to Xpert MTB/RIF
Indicative cost per test (for reagents) ($)	40	TBD	TBD	15	to be decided
Technical skill	CLIA moderate complexity; calibration required	Community health worker and above	very low	Minimally skilled operator with pipetting knowledge	low; comparable to Xpert MTB/RIF
Laboratory set-up	not specialised, laboratory or point-of-care	not specialised, laboratory or point-of-care	not specialised, laboratory or point-of-care	not specialised but precision pipetting required, laboratory or near patient	minimal; comparable to Xpert MTB/RIF
Storage conditions	refrigeration may be required for external calibrants	4 to 40°C	Ambient	Stable at 2-30°C for 1 year	to be decided
Applicable settings	developing, low-medium resourced settings	developing, low-medium resourced settings; health posts and above	developing, low-medium resourced setting; remote settings	developing, low-medium resourced setting; all levels of health care chain up to peripheral laboratories with minimal or no infrastructure support	to be decided
Regulatory approval	CE-IVD; US-FDA-IVD to be sought	CE-IVD, ISO 13485 certification, WHO PQ, local country approval to be sought	TBD	CE-IVD to be sought	CE-IVD; US-FDA-IVD to be sought
Advantages	low cost, point-of-care, easy to use, same device for all their tests	low cost, point-of-care, easy to use, same device for all their tests	Multiplex for genotype, viral load and IL-28B (but pan-genotypic treatment with DAAs may not need genotyping or IL-28B testing)	automated device, relatively easy to use, same device for all their tests	automated, modular device, easy to use, same device for all their tests
Disadvantages				not true POC (multiple steps and precision pipetting required)	not under preferential pricing, not true POC
Reference	Manufacturer	Manufacturer	Manufacturer	Manufacturer	Manufacturer

*Alere and Iquum also have pipeline HCV viral load tests but were not able to provide information at this time.