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HEPATITIS C

Diagnostics Technology Landscape

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Abbreviations

AIDS	acquired immunodeficiency syndrome
Ag	antigen
ALT	alanine aminotransferase
APRI	AST-to-platelet ratio index
AST	aspartate aminotransferase
ºC	degree Celsius
cAg	core antigen
cDNA	complementary DNA
CIA	chemiluminescence assay
cm	centimetre
CMV	cytomegalovirus
CPA	Cross Priming Amplification
CT/NG	*C. trachomatis/N. gonorrhea*
DAA	direct acting antivirals drugs for HCV
DBS	dried blood spot
DNA	deoxyribonucleic acid
EDTA	ethylenediaminetetraacetic acid
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
EQA	external quality assurance
FDA	Food and Drug Administration (United States)
FIB-4	age (yr) x AST (IU/L)/platelet count (10^9/L x [ALT (IU/L)$^{1/2}$])
fmol	femtomoles
g	gram
GPRS	general packet radio service
GSM	global system for mobile communications
HBV	hepatitis B virus
HCV	hepatitis C virus
HCV cAg	hepatitis C virus core antigen
HIV	human immunodeficiency virus
HPV	human papillomavirus
IFN	interferon
in	inch
IU	Standardized International Unit
IVD	in vitro diagnostic
kg	kg
kPCR	kinetic polymerase chain reaction
L	litre
lb	pound
LED	light emitting diodes
LIMS	laboratory information management system
LiPA	line probe assay
LLOD	lower limit of detection
LLOQ	lower limit of quantification
ml	millilitre
mm	milimetre
MRSA	methicillin-resistant *Staphylococcus aureus*
MTB	*Mycobacterium tuberculosis*
NAAT	nucleic acid amplification test
NAT	nucleic acid-based test
NIBSC	National Institute for Biological Standards and Control

NS3	nonstructural 3 region of the HCV genome
NS4	nonstructural 4 region of the HCV genome
NS5	nonstructural 5 region of the HCV genome
PCR	polymerase chain reaction
peg-IFN-riba	pegylated-interferon-ribavarin
PEPFAR	United States President's Emergency Plan for AIDS Relief
pg	picogram
POC	point of care
QC	quality control
QS	quantitative standard
RDT	rapid diagnostic test
RIBA	recombinant immunoblot assay
RIF	rifampicin
RLU	relative light unit
RNA	ribonucleic acid
RT	reverse transcriptase
SVR	sustained virological response
TB	tuberculosis
TBD	to be determined
TE	transient elastography/ elastometry
TMA	transcription-mediated amplification
US$	United States dollar
UPS	uninterruptible power supply
USAID	United States Agency for International Development
USB	universal serial bus
UTR	untranslated region (of the HCV genome)
µL	microgram
WHO	World Health Organization

Introduction

Recent estimates indicate that between 130 and 150 million people worldwide have chronic hepatitis C virus (HCV) infection, and that between 350 000 and 500 000 of them die each year *(1,2)*. Because HIV and HCV share common routes of transmission, it is estimated that 4–5 million people are coinfected with both viruses *(3)*. Yet, most people infected with HCV are not aware they are infected; therefore, HCV has been called the "silent pandemic" *(4,5)*.

Due to the complexity and cost of testing and treatment for HCV, few individuals living in resource-limited settings have access to either. It is hoped that with the introduction of new treatments for HCV in the form of direct acting antivirals (DAAs), the cost, complexity and adverse effects of HCV treatment can be reduced and that patient outcomes can be improved (6).

It is furthermore expected that DAAs will not only transform treatment of HCV, but that they will also make it possible to simplify the current testing regimen required for the patients with active HCV infection – reducing the number, complexity and cost of required testing. In addition, as more diagnostic tests/platforms are introduced, it is hoped that access to testing for HCV patients, from screening through to cure, will be simplified and improved. In particular, molecular platforms that are being developed for a range of infectious diseases, including HIV, for use at or near the point of patient care, could be important.

This report describes the current continuum of testing for HCV, which is complex and expensive, which means that it is very challenging to implement in resource-limited settings. It examines the platforms/tests that are currently available across the range of required HCV testing from screening to confirmation and genotyping, fibrosis staging and treatment monitoring. The report also considers how the testing cascade for HCV may be simplified with the availability of DAAs, which would help make HCV testing attainable in resource-limited settings. Finally, it looks at the pipeline of tests/platforms for HCV that could be delivered at or near the point of patient care.

Methodology

The Hepatitis C Diagnostic Technology Landscape is compiled by Maurine M. Murtagh with support from UNITAID.

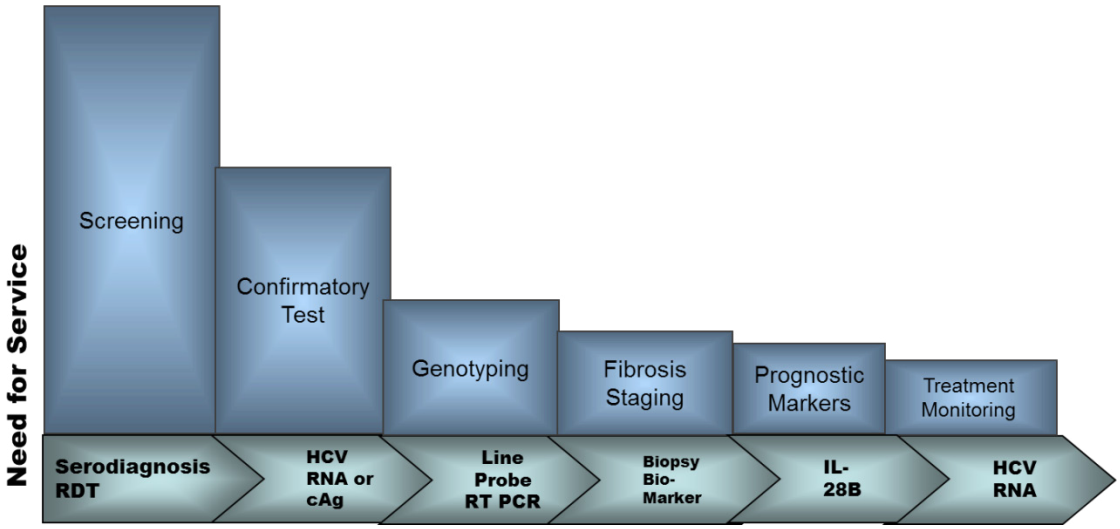
In addition to the report's author, UNITAID gratefully acknowledges the comments and suggestions from the following reviewers: Priya Abraham, Aghokeng Avelin, Peter Dailey, Claudia Denkinger, Jessica Markby, Teri Roberts and Stefan Wiktor.

The material in this landscape was gathered by the author from publicly available information, published and unpublished reports and prospectuses, and interviews with developers and manufacturers. Unless otherwise noted, the prices for diagnostic equipment and reagents cited in this report were obtained directly from manufacturers and are ex works prices, meaning that they are the prices at the manufacturer's factory, and do not include any delivery, distribution or commission charges. The material is current through October 2014.

Overview

Because of the nature of HCV and currently available treatment regimens, testing for HCV is a continuum, an illustrative example of which is presented in Figure 1.

Figure 1. HCV testing continuum of care with currently available treatment regimens



HCV screening

Detecting HCV infection generally begins with serological testing to detect anti-HCV antibodies. Antibodies to HCV can be detected in the blood, usually within two or three months after exposure to the virus. Antibody tests for HCV are enzyme immunoassays (EIAs), chemiluminescence assays (CIAs) or rapid diagnostic tests (RDTs). EIAs, CIAs and RDTs for HCV, as well as their advantages and disadvantages, are discussed more fully later in this report.

Antibody testing for HCV is an imperfect tool. Although a positive antibody test or tests mean that the individual has been exposed to HCV in the past, it does not mean that the person has active HCV infection because the individual may have spontaneously cleared the infection. Alternatively, if a person was infected with HCV in the recent past, the individual may not yet have produced anti-HCV antibodies, and an antibody test will be negative.

In many resource-limited settings, testing for HCV is "symptomatic" as HCV often remains undiagnosed until a patient presents at a health-care facility with cirrhosis or hepatocellular carcinoma (liver cancer). However, in its first guidelines for screening, care and treatment of persons with HCV (the 2014 Guidelines), the World Health Organization (WHO) recommends HCV screening for individuals who are either part of a population with high HCV seroprevalence or who have a history of HCV risk exposure and/or behaviour *(7)*.

There are a number of identifiable risk factors for HCV. These include persons who have injected illicit drugs (past or present) or engage in high-risk sexual behaviour, recipients of infected blood products, infants born to HCV-infected mothers and persons with HIV. In resource-limited settings, many HCV infections occur in the health-care setting due to bad injection safety.

HCV confirmatory testing

It is estimated that approximately 25% of people, who are initially infected with HCV are able to clear the virus from their bodies, usually within six months of exposure, while the remaining individuals will develop chronic HCV *(8)*. About 20–30% of those with persistent viraemia develop liver fibrosis, and are thereby at risk for cirrhosis, liver failure and hepatocellular carcinoma *(9)*. In HCV patients who are coinfected with HIV, these more serious conditions are accelerated, with progression to cirrhosis about threefold higher in coinfected patients *(10)*. Therefore, all persons with positive results from anti-HCV screening assays should additionally have a confirmatory HCV test in order to determine whether positive serology tests are due to the presence of active infection.

Either molecular or antigen assays are used for confirmatory HCV testing, with molecular tests most widely used today. In its 2014 Guidelines, WHO recommends that a nucleic acid-based test (NAT) for HCV ribonucleic acid (RNA) (either quantitative or qualitative) be performed "directly following an HCV seropositive test result to establish a definitive diagnosis of HCV infection in addition to the use of NAT as part of the evaluation for treatment eligibility" *(7)*. In addition, under interferon (IFN)-based HCV treatment regimens, a baseline quantitative measure of HCV RNA is required before treatment initiation *(11)*.[1] However, the requirement for a baseline quantitative HCV RNA viral load at treatment initiation will likely not be necessary with all DAA regimens, and qualitative molecular or antigen testing may be sufficient with these regimens.

The commercially available and pipeline NAT-based assays for detecting and quantifying HCV RNA, as well as assays based on HCV core antigen (cAg) detection, a newer technology, are described in detail later in this report.

Genotyping

There are at least six major genotypes of the HCV (genotypes 1 to 6), and there are geographical differences in the distribution of these genotypes. Genotype 1 (subtype 1a) is the most common in the United States and Europe. Genotype 1b is found worldwide and is estimated to account for about 70% of overall HCV infection. Genotypes 2a and 2b are found in the United States, Europe and Japan and account for between 10% and 30% of HCV worldwide. Genotype 3 is prominent in South-East Asia and Indonesia, whereas genotype 4 is found in the Middle East and North Africa. Finally, genotypes 5 and 6 are prevalent in South Africa and Hong Kong, respectively *(11,14)*.

Determining the genotype of a patient is particularly important given current treatment regimens for HCV. Certain antivirals for treating HCV (e.g. boceprevir; telaprevir; and simeprevir) are only effective against specific genotypes, while the duration of treatment and likely outcomes of such treatment with pegylated-interferon-ribavarin (peg-IFN-riba) depends on the HCV genotype of the patient *(7,15)*. Genotype 1

1 WHO established the first international standard for the HCV RNA NAT in 1997, and the IU rather than viral copies is now the preferred unit in which to report test results *(12,13)*.

patients require 48 weeks of treatment with full-dose ribavirin, whereas patients with genotypes 2 and 3 only require 24 weeks of dual therapy with a fixed dose of ribavirin *(16)*.[2]

Genotyping assays are based on four different technologies: real-time reverse transcriptase (RT) polymerase chain reaction (PCR); line probe assay; and DNA chip or sequencing; with the line probe assay being the most commonly used *(15)*. These tests are expensive, complex and require well-resourced laboratories with well-trained and qualified staff. Although genotyping assays are reviewed in more detail later in this report in the context of current HCV treatment regimens, they are difficult to perform and currently not recommended for use in most resource-limited settings.

Fibrosis staging

HCV is a leading cause of chronic liver disease and, as indicated earlier in this report, HCV-associated liver disease, including end-stage liver disease, is accelerated in HIV coinfected individuals *(10)*. In the 2014 Guidelines, WHO recommends that decisions regarding treatment initiation for HCV-infected individuals should be based on a patient's degree of fibrosis and the balance between the likelihood of cure versus the potentially serious side-effects from currently available treatment regimens *(7)*.[3] In general, patients with less advanced fibrosis can expect more favourable treatment outcomes, as measured by a higher sustained virological response (SVR), than those with more advanced fibrosis.[4]

Liver biopsy has long been considered the gold standard for staging liver disease, but it is an invasive procedure and has certain drawbacks that include: serious complications (pain, bleeding, possible perforation of other organs); sampling error; need for special expertise in interpreting results; and cost *(10,11)*. This has led to efforts to find alternative ways to determine the extent of liver fibrosis by non-invasive means, including blood marker panels *(18)*.

Currently, there are both serologic and imaging/radiologic tests for liver damage that are non-invasive and may be more suited to resource-limited settings than liver biopsy. Serologic tests can generally be classified into three groups: (i) indices from routine blood tests of liver function, e.g. APRI (aspartate aminotransferase [AST]-to-platelet ratio index) and FIB-4 (an index of age, AST, platelets, and alanine aminotransferase [ALT] level); (ii) markers of extracellular matrix metabolism (e.g. hyaluronic acid); and (iii) indices that combine markers of both types *(10,19)*. Of these, despite some reservations, in its 2014 Guidelines, WHO has recommended that for resource-limited settings the APRI or FIB-4 indices, which are based on commonly available laboratory tests, be used for the assessment of fibrosis resulting from HCV infection rather than other non-invasive tests.[5]

In addition, some studies have demonstrated the predictive value of two combinations of non-invasive, simple serum biochemical markers in patients with HCV: the FibroTest, which is known as FibroSure in the United States; and the Acti-Test *(20)*. The FibroTest measures liver scarring in HCV, while the Acti-Test measures liver inflammation. The FibroTest consists of an algorithm of five fibrosis markers and the Acti-Test requires the FibroTest scores plus an ALT score. Studies have validated the use of these two tests in combination as a non-invasive alternative to liver biopsy (*21,22*). However, these tests generally must be done in laboratories with sophisticated chemistry instruments and well-trained technicians.

Finally, imaging testing is also available for staging liver disease. One such technology is transient elastography/elastometry (TE), which uses ultrasound readings and low-frequency elastic waves to measure liver

2 Note, however, that for HCV/HIV coinfected patients, the current guidelines recommend a fixed course of 48 weeks with peg-IFN-riba, regardless of HCV genotype *(10)*.

3 Clinicians generally initiate treatment once significant fibrosis is demonstrated based on a liver biopsy-scoring system, of which the METAVIR system is most widely used. On the METAVIR scale, fibrosis is divided into four levels from F1 (mild) to F4 (cirrhosis) and must exceed F2 before treatment will be initiated *(15)*.

4 For patients treated with peg-IFN-riba, SVR is generally defined as undetectable HCV load 24 weeks after the completion of therapy (HCV level below the LLOD [<50 IU/mL] using first-generation viral load assays) *(16)*. For patients on triple-therapy regimens (peg-IFN-riba with either telaprevir or boceprevir), SVR is defined as an HCV RNA level of <25 IU/mL at 24 weeks after the end of treatment *(17)*.

5 Reservations include that FIB-4 has been evaluated only for the diagnosis of significant fibrosis (METAVIR ≥F2), while APRI has been validated for the diagnosis of both significant fibrosis and cirrhosis *(7)*. Generally speaking, FIB-4 and APRI assays identify both no fibrosis and advanced fibrosis well, but have difficulty in differentiating between F1, F2 and F3. Furthermore, for HIV/HCV coinfected patients, if their platelet or AST change, then the indirect serologic tests are less accurate, and it is possible that these patients would require transient elastrography or liver biopsy *(15)*.

elasticity or stiffness *(10,11)*. Because TE alone may not be sufficient to give an accurate diagnosis of significant fibrosis, a number of researchers have recommended using TE in combination with the FibroTest or other biomarker testing in a complementary way *(10,22–25)*. However, the use of TE, alone or with other biomarker testing, may not be suitable in resource-limited settings due to the high cost of equipment and the need for trained operators.

Because of the recommendations by WHO for the use of APRI or FIB-4 indices in resource-limited settings and because these indices can be calculated based on chemistry and haematology tests that are generally available both centrally, and in some cases at the point of care (POC), tests for fibrosis staging are not discussed further in this report.[6]

Prognostic markers

In high-income countries, once a patient has a confirmation of HCV infection and it has been determined that the patient is a suitable candidate for treatment, there are a number of tests that can be done to determine whether IFN-based treatment is likely to be successful. The predictors most often associated with IFN therapy are polymorphisms within the IL28B gene and levels of IP-10, which is a measure of inflammation and immune activation. Because individuals with certain HCV genotypes, namely genotype 1 and genotype 4, are not as responsive to IFN-based therapy, these tests are frequently done in high-income settings for patients with those particular genotypes in order to try and predict treatment response *(15)*.[7]

While prognostic marker testing is useful and has some predictive value, the tests are complex and expensive. And, in its 2014 Guidelines, WHO has concluded that "implementing treatment programmes for HCV infection in resource-limited settings does not require the use of prognostic marker tests (e.g. IL28B testing) for predicting treatment response" *(7)*. Therefore, such tests are not discussed further in this report.

Treatment and toxicity monitoring

Patients being treated for HCV should be monitored to assess their response to treatment/efficacy and for the occurrence of adverse side-effects/toxicity. During the course of its guideline development process, WHO developed a technical report on monitoring HCV patients during treatment. The results are summarized in Figure 2, which indicates the recommended tests and time points for both toxicity and efficacy testing under current treatment regimens, for which the latter are regimen dependent.

For required laboratory testing for potential toxicities, regardless of current treatment regimens, WHO recommends full blood count, serum creatinine and ALT levels at baseline and at Weeks 1, 2, 4, 12, 24 and at 12-week intervals thereafter. In addition, a thyroid function test, which measures thyroid stimulating hormone, should be monitored at baseline and every 12 weeks thereafter.

6 For further detail on assays available for fibrosis staging in chronic HCV infection, see Diagnosis and treatment of hepatitis C: a technical landscape *(15)*.
7 For a more detailed discussion of these tests, see MSF Access Campaign: Diagnosis and treatment of hepatitis C: a technical landscape *(15)*.

Figure 2. Suggested monitoring for the HCV patient under current treatment regimens

Monitoring time points recommended by the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of Liver Disease (EASL) and drug-registration literature

	TOXICITY			EFFICACY				
Time	FBC, creatinine, ALT	Thyroid function	Adherence, side effects	IFN/RBV	IFN/RBV TEL	IFN/RBV BOC	IFN/RBV SMV	IFN/RBV SOF
Week 0	X	X	X	X	X	X	X	X
Week 1[1]	X		X					
Week 2[1]	X		X					
Week 4	X		X	X	X	X	X	X
Week 8	X		X			X		
Week 12 EOT[2]	X	X	X	X	X	X	X	X
Week 24 EOT[3]	X	X	X	X	X	X	X	X
Week 36	X	X	X			X	X	
Week 48 EOT[4]	X	X	X	X	X	X	X	X[4]
Week 12 after EOT	X		X					
Week 24 after EOT	X		X	X	X	X	X	X

[1] Time points recommended in EASL, but not in AASLD guidelines. Additional monitoring is required 1 – 2 weekly in patients with moderate-to-severe anaemia, thrombocytopenia or neutropenia.
[2] EOT at 12 weeks is applicable only for patients treated with sofosbuvir.
[3] EOT: end of treatment depending on genotype, response to treatment, presence of cirrhosis or HIV coinfection
[4] Sofosbuvir EOT may be considered in patients with cirrhosis awaiting liver transplant.
ALT alanine aminotransferase; BOC boceprevir; FBC full blood count; IFN interferon; RBV ribavirin; TEL telaprevir; SMV simeprevir; SOF sofosbuvir; EOT end of treatment

Source: WHO (7).

Testing treatment response is regimen dependent, but for all current regimens requires HCV RNA testing by a sensitive assay (an assay with a lower limit of detection [LLOD] of ≤50 IU/mL) at baseline and at Weeks 4, 12, 24, the end of treatment, which may come at Week 12, 24 or 48 depending on the treatment regimen, and at 24 weeks after stopping treatment. In general, a 2 log IU/mL drop in a patient's viral load from baseline to Week 12 is required for a patient to continue treatment *(7,10)*. With all-DAA regimens, testing for treatment response may not be required.

Toxicity monitoring

Toxicity monitoring for HCV patients on treatment is challenging for resource-limited settings. Basic chemistry testing (ALT and creatinine) is generally available in mid-level laboratory facilities (Level II and Level III); however, haematology testing, other than haemoglobin (e.g. full blood count), can be difficult.

The technology options available for multi-parameter chemistry and haematology testing range from manual to semi-automated to fully automated low- and high-throughput laboratory-based instruments. A number of low-volume, robust, automated technology analysers designed for low-end laboratories are widely available and are becoming standard options. Similarly, semi-automated spectrophotometers for chemistry analysis have been traditionally placed in low-end laboratories and remain in widespread use today.

POC chemistry and haematology platforms/tests are not always available in resource-limited settings. Nevertheless, simple hand-held instruments exist for tests such as haemoglobin, as well as for fixed ranges from three to six chemistry parameters. Costs for instruments range between US$ 1000 and US$ 5000 for mobile units and between US$ 3000 and US$ 10 000 for larger, less mobile units. The average cost of the basic full blood count is approximately US$ 1.15 per test, while consumables average approximately US$ 2.00 per test. For chemistry testing, the costs vary per test run and on average range from US$ 0.10 to US$ 0.45 per test. Consumables average US$ 1.50 per test. If HCV treatment is to be effectively implemented in resource-limited settings, then chemistry and haematology testing capabilities will have to be expanded and further decentralized.

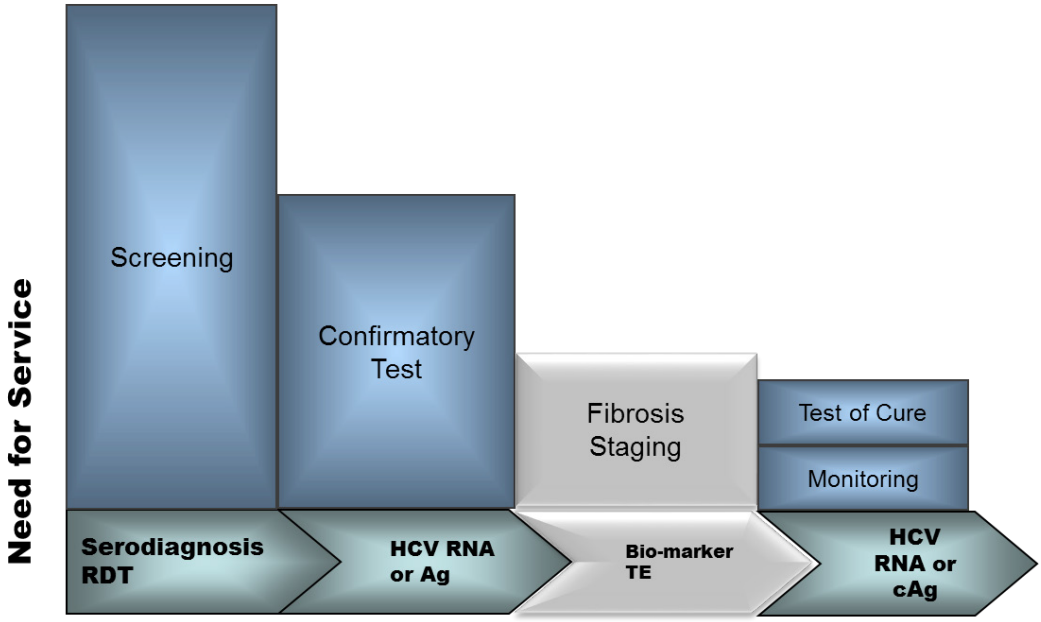
Treatment monitoring

NAT for the detection of HCV RNA is currently the gold standard for diagnosing active HCV infection and for monitoring treatment efficacy and cure. As discussed earlier in this report, HCV RNA testing is problematic in resource-limited settings given the infrastructure, level of platform sophistication and trained personnel required to perform these tests on laboratory-based instruments. In addition, the frequent visits to health facilities required by HCV patients on current treatment regimens will be difficult in such settings, especially if testing cannot be substantially decentralized.

Potential impact of DAAs on the HCV testing continuum

It is expected that the introduction of DAAs will make it possible to simplify the current testing regimen required for patients with active HCV infection who are either being considered for treatment or are on treatment. If all-DAA regimens are successfully introduced, affordable and as effective as anticipated, then the testing continuum for HCV might look like the one shown in the illustrative example in Figure 3.

Figure 3. Potential HCV testing continuum with DAA regimens



While screening and confirmatory testing will still be needed, it is possible these tests could be combined into a single qualitative assay to detect the presence of HCV RNA or HCV cAg. Treatment monitoring for purposes of determining HCV clearance and cure could also be performed using those same assays.

Unless and until tests for the definitive diagnosis of HCV are much more affordable, it is likely that in resource-limited settings RDTs will be used for HCV screening, while molecular or antigen assays will be used for diagnosis. However, because a number of the new DAAs are expected to be pan-genotypic, genotype testing may no longer be required to determine the required length of treatment or its expected outcome. Staging of liver disease before putting patients on treatment will likely also continue to be necessary; but non-invasive techniques using biomarkers or TE, are already available. Toxicity monitoring of patients on treatment will also likely continue to be required, but for all oral regimens, such monitoring may consist of ALT, creatinine and haemoglobin testing, all of which are generally available in resource-limited settings. Finally, although quantitative HCV RNA testing at baseline and during treatment to monitor efficacy may not be necessary with all-DAA regimens, molecular or antigen testing (qualitative or quantitative) will be necessary at the end of treatment to test for HCV clearance and, at a later point in time, to test for cure and clearance of the virus. As indicated earlier, molecular or antigen platforms currently available or under development for use at the POC, can perform this testing. In addition, where POC

testing is not available, dried blood spots (DBS) may be able to be used in order to expand decentralized access to HCV viral load testing performed on centralized molecular platforms *(15,26,27)*.

Factors to consider in diagnostic platform selection

Although rapid POC assays for detecting anti-HCV antibodies perform reasonably well when used correctly and are low cost, many of the available tests considered in this report are either too complex, expensive, have cold chain requirements or are not of high quality – based on lack of good manufacturing practice and lack of prequalification by WHO or approval by the United States Food and Drug Administration (FDA), or European Union CE marking or equivalent *(15,28)*. An additional and important issue with current RDTs for HCV is that their diagnostic accuracy is not as good in the presence of HIV infection *(29)*. Therefore, additional tests may be needed for use in resource-limited settings.

Because chemistry and haematology testing is generally regimen-based in HCV care, and because there are already a number of technologies available for use at the POC, these tests should not represent a significant barrier to accessing HCV care and treatment going forward and are not considered in detail in this report. Similarly, both biomarker testing and non-invasive TE are available for fibrosis staging.

Of the remaining tests currently required in the continuum of testing for HCV, the tests that are likely to present the most persistent access challenges today are genotyping tests (to the extent they continue to be necessary under available treatment regimens), confirmatory testing and monitoring for HCV treatment efficacy and cure. Increasing the availability of high-quality POC technologies for these tests as well as screening tests has the greatest potential to improve HCV diagnosis and treatment monitoring.

This report focuses on RDTs for screening of HCV, genotyping platforms/assays and molecular and antigen testing for both confirmation of HCV and treatment monitoring. The report examines: (i) the laboratory-based and/or POC or near-POC platforms currently available; and (ii) the POC technologies needed or in the pipeline for each test category.

Tiered laboratory system

The laboratory system in resource-limited settings importantly influences appropriate diagnostic platform selection and placement. The laboratory system is typically characterized as a tiered system as follows *(29)*.[8]

Photo 1.

Laalissa Health Post (Ethiopia)

Wamena Kota Health Center (Indonesia)

Kandangan Hospital (Indonesia)

Level I – Primary:[9] Health post and health centre laboratories that primarily serve outpatients. Often, health posts (Photo 1, left) have no laboratory capability, but are able to perform some non-equipment-based POC testing. Generally, no clinicians are onsite at a health post. Health centers (Photo 1, centre), however, usually have a simple laboratory, where basic testing can be performed – e.g. POC assays and some microscopy, if a microscopist is available – and clinicians are generally onsite.

Level II – District: Laboratories in intermediate referral facilities – e.g. district hospitals (Photo 1, right). These facilities can perform all of the services provided at Level I and additionally provide a broader menu of tests. They usually have automated equipment for tests such as CD4 count, bacterial culture and blood chemistries. Physicians and other clinicians (e.g. nurses) are commonly available onsite.

Level III – Regional and provincial: Laboratories in a regional or provincial referral hospital that may be part of a regional or provincial health bureau. These facilities will have still more expansive test menus than those found at Level II facilities. In addition to performing all of the tests and services provided at Levels I/II, regional and provincial facilities can usually provide additional testing capabilities such as blood cultures, full chemistry testing, acid-fast bacilli (AFB) solid culture and smear. Molecular testing may also be available.

Level IV – National and multi-country reference laboratory: The national reference laboratories are specialized facilities charged with strengthening laboratory capacity for diseases of public health concern. They often provide linkages with research laboratories, academic institutions and other public health laboratories, forming integrated laboratory networks that can provide assistance in clinical trials, evaluation of new technologies and surveillance. In addition, national reference laboratories perform molecular and other sophisticated testing beyond the capabilities of Level III facilities – e.g. nucleic acid assays, HIV drug resistance studies and *Mycobacterium tuberculosis* (MTB) drug susceptibility testing.

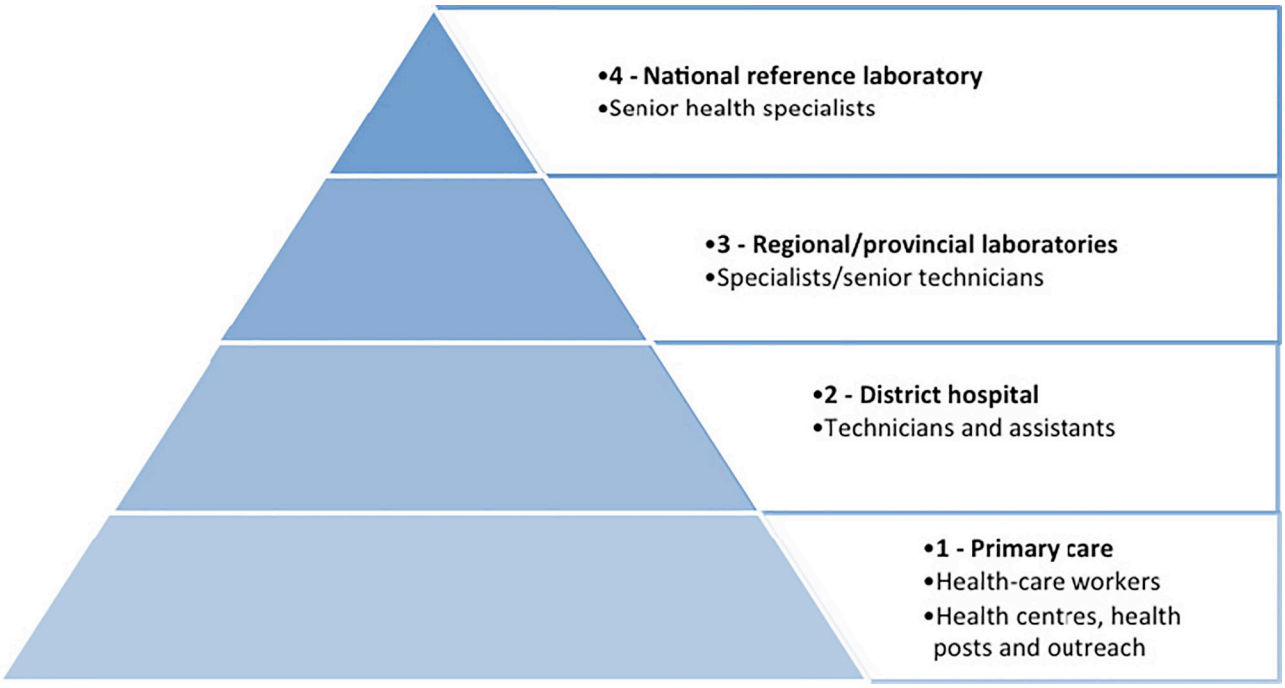
The laboratory system is often depicted as a pyramid, which illustrates that there are generally a large number of Level I facilities in-country. As one migrates up the levels of the system, there are a smaller number of more centralized facilities. In the case of national reference laboratories and some provincial laboratories, they may not serve patients with a broad set of consultative services, but rather are referral centres for quality assurance and training or for conducting complex tests (either using samples drawn at

8 The Maputo Meeting Report notes that the tiered levels of a laboratory system and the testing performed at each level may vary depending on the population served (e.g. infants; adults), level of service available, physical infrastructure, electricity, water, road conditions and the availability of trained technical personnel in-country.

9 The Maputo Meeting Report does not specifically place outreach services in Level I of the tiered laboratory system. Although some experts place outreach activities in Level I, some consider patient outreach to be at a level below Level I and add a fifth tier to the system, referred to as subprimary care or Level 0. Health posts would also generally be included in Level 0.

facilities lower in the system and transported or by receiving patients referred directly from other facilities) (Figure 4).

Figure 4. Diagram of tiered laboratory system



Laboratory-based testing in resource-limited settings

The great majority of tests available today in resource-limited settings, other than disposable rapid tests, were created for developed country settings, where laboratory-based diagnostics are operated by well-trained technicians on sophisticated instrumentation that is costly, often run in standard 96-well formats (high patient loads) and require dedicated laboratory infrastructure. Also, these instruments rely on a complex medical infrastructure that uses extensive sample transport networks to collect samples from multiple hospitals and clinics, and uses sophisticated patient tracking mechanisms that enable doctors and hospitals to return results to patients over weeks. These systems are not easily adapted for use in most regions of developing countries or low-resource settings, where access, cost, infrastructure and patient loss are significant barriers to increasing case detection rates. In addition, there are many settings where high-throughput platforms will not be required.

Laboratory infrastructure

Most of the HCV testing platforms described in this report are laboratory based and many require significant infrastructure, including continuous power and climate control/air-conditioning. For example, some laboratory-based HCV viral load platforms based on nucleic acid technology may require two or three dedicated rooms in a laboratory.[10] Each room should have minimal dust and preferably will be temperature controlled (air-conditioned in hot climates). The rooms are needed to accommodate the different stages of the testing process: Room 1 would be dedicated to receipt of the patient sample and sample extraction (most of which is done in a biosafety cabinet). Room 2 (which could be reduced to a Clean-Air Box in Room 1 if space is limited) would be used to prepare the reagents, which are prone to contamination. Finally, Room 3, which will become highly contaminated through the test process, would be dedicated to amplification and detection of the virus and results processing. In order to avoid contamination, workflow must proceed from Room 1 to Room 2 to Room 3. Each room needs to have 3–4 metres (approximately 10–

10 The Roche COBAS® AmpliPrep/COBAS® TaqMan® platform requires only one room; the Abbott m2000 requires two rooms.

13 feet) of bench space. Furthermore, test reagents generally will have to be stored at between 4 and 8 °C. And, as mentioned above, steady current is required so that the electrical test equipment is not damaged.

Sample transport

Many HCV tests described in this report require venous blood collection, processing (centrifuging) of that blood to obtain plasma or serum within a certain timeframe, cold chain and storage of specimens by trained personnel. Given the complexity of many HCV test platforms, especially those used for genotyping and for qualitative and quantitative detection of HCV RNA, such testing will only be done at Level III and Level IV laboratories in resource-limited settings. This means that patient samples will have to be transported from urban, peri-urban and rural settings to the laboratory for processing. This is done using sample transport networks in-country, taking advantage of courier or similar services to take samples to the laboratory and to return results at a later date. But, frequently, these services are not well developed, leading to long delays in returning sample results to patients and loss to follow-up.

Therefore, the ability to use DBS samples for HCV testing performed at central laboratories is an important consideration in the implementation of the testing because it greatly simplifies the transport of samples, providing enhanced stability and ease of use for health-care workers. The use of DBS is also cost effective. Studies to date have indicated good correlation between plasma samples and DBS samples for both detection and quantification of HCV viral load *(30–33)*.

Testing at the POC

Given that testing access, cost, infrastructure and patient loss are significant barriers to increasing case detection rates and care and treatment in resource-limited settings, it is generally believed that the introduction of appropriate, robust, POC diagnostics for HCV can improve access to testing in developing countries.

Given the robust pipeline of POC diagnostics for HCV RNA measurement and at least one HCV cAg assay, it is important to consider what is meant by testing at or near the point of patient care. Currently, there is no universally accepted definition of POC testing *(34)*. The College of American Pathologists defines POC tests as "tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space, and that are performed outside the physical facilities of clinical laboratories" *(35)*. But, in resource-limited settings, in particular, there are small, bench-top instruments being used in basic laboratories at Level I facilities, and POC devices being used in Level II and Level III facilities. These testing distinctions and boundaries are somewhat blurred.

It is often suggested that diagnostic tests for use at the point of patient care should meet the ASSURED criteria for the ideal rapid test, which was developed by WHO *(36)*. The ASSURED criteria are as follows:

A = Affordable
S = Sensitive
S = Specific
U = User-friendly (simple to perform in a few steps with minimal training)
R = Robust and rapid (results available in less than 30 minutes)
E = Equipment-free
D = Deliverable to those who need the test

While the ASSURED criteria provide a useful framework, it is somewhat restrictive in that it demands that tests are disposable, which is often not possible, and must provide results in less than 30 minutes. As Pai et al. suggested: "the technology as such does not define a POC test nor determine its use at the POC. Rather it is the successful use at the POC that defines a diagnostic process as POC testing" *(35)*. In fact, whatever definition is chosen for POC testing, there are critical features of testing that take place at or near the site of patient care that will determine its effectiveness in resource-limited settings. These include providing both the test and test result to the patient on the same day at a site where linkage to care is also available so that a clear, actionable decision can be made with respect to the test result. In other words, it is not enough to simply offer testing where patients present; rather, it is critically important that test results can

be linked to clinical decision-making at the same patient visit. This will have important implications for improving the loss of patients from the care and treatment cascade for HCV.

In this report, the appropriate target use setting for each of the technologies, both laboratory based and those intended for use at or near the point of patient care, is considered. There are a number of laboratory-based technologies for HCV that are suited only for Level III and Level IV facilities; on the other hand, there are a number of POC technologies that are targeted at Level I and Level II facilities.

It is important that countries review the operational characteristics of diagnostic platforms/devices when selecting which platforms to implement and at which level of the laboratory system to implement them. These characteristics include the following:

- type of technology (including whether for laboratory or POC) and output (test parameters measured);
- throughput and turnaround time;
- sample needed and sample stability (e.g. venous blood; plasma; capillary blood);
- protocol complexity;
- reagent stability;
- cost of instrumentation and cost per test for reagents;
- environmental requirements of the instrumentation, including power supply, ability to withstand heat and humidity, and tolerance of altitude;
- if instrument based, the size and weight of the instrument and associated devices (e.g. data station; printer);
- supplies (and cost thereof) required from parties other than the manufacturer of the instrument/test (e.g. vortex; pipettes;);
- recommended or required instrumentation beyond the analyser itself (e.g. data station; printer; barcode scanner);
- connectivity options;
- training required;
- availability of quality control (QC) reagents and compatibility with external quality assurance (EQA) programmes;
- recommended location for use (e.g. hospitals; clinics).

These operational characteristics are set out in Appendix 1 for each of the platforms currently available for HCV viral load testing, and where sufficient information is available from the developer, for each such platform in the pipeline.

In addition to the operational characteristics of the various platforms/devices, it is also important to consider the performance of the platform, i.e. the ability of the technology to give accurate and reproducible results. Both the accuracy and precision of a quantitative test should be evaluated.[11]

The accuracy of a technology is a measure of the degree of closeness of the reported value to the true value, and is evaluated by comparing results obtained by the test under evaluation with those obtained for the same samples using a reference technology. Although correlation of those results is one measure of accuracy, it is generally not a sufficient measure. It is important to measure bias and misclassification of the test results as well. Bias, which may be reported using Bland-Altman analysis, reflects the average/mean difference between the results of the technology under evaluation and the comparator or reference technology *(37)*. Misclassification probabilities, which may be upward misclassification probability or downward misclassification probability, describe the likelihood that a test will incorrectly categorize a result as higher or lower than a given cut-off value, respectively.

11 Note, however, that for a qualitative test, e.g. RDTs for HCV, accuracy and precision are not the relevant measures. Rather, sensitivity and specificity, as well as negative/positive predictive values, are needed.

The precision or reproducibility of a test is determined by the closeness of results when testing is repeated using a single technology. It is a particularly important measure when used in the context of following a patient's serial measurements using the same technology – e.g. the level of a patient's viral load from test to test. Data on precision are often reported as modified Bland-Altman plots or the coefficient of variation, which is a measure of dispersion. A lower coefficient of variation indicates less variation and greater assay reproducibility.

Screening tests for HCV

Serological assays

Serology-based assays for screening of persons at risk of HCV infection are able to detect the presence of anti-HCV antibodies, HCV antigen, or both simultaneously. The testing platforms can be EIAs, CIAs or rapid tests. The test principle in EIAs and CIAs is the same, but end-point detection in EIAs is measured as colour change and in CIAs as luminescence. Both EIAs and CIAs can detect HCV antibody (anti-HCV immunoglobulin G) in serum or plasma.

Over the years, improvements in assay performance, particularly of EIAs, have been delineated as "generations" of the assays. First-generation assays were based on a yeast-expressed recombinant protein containing an epitope from the NS4 region of the HCV genome and detected antibodies about 12–26 weeks following exposure, creating a long window period of infectivity. First-generation EIAs lacked both sensitivity and specificity *(38)*. Second-generation EIAs incorporated two more epitopes, one each from the core antigen and a nonstructural HCV antigen (NS3). These assays reduced the window period observed in first-generation assays and permitted anti-HCV to be detected between 10 and 24 weeks after exposure *(39)*. Finally, third-generation EIAs incorporated an additional antigen from the nonstructural HCV antigen (NS5) and further reduced the window period by about one week *(40)*.

Available EIA assays for use as in vitro diagnostics (IVDs) for HCV screening include: AxSYM HCV 3.0 (Abbott Diagnostics [hereafter Abbott]) and Innotest® HCV AB IV (Fujeribio, formerly, Innogenetics).[12] There are also at least two enhanced CIAs: VITROS Anti-HCV assay (Ortho-Clinical Diagnostics); and ARCHITECT Anti-HCV test (Abbott) for HCV screening. The CIA assays have been reported to have slightly higher sensitivity than the EIAs *(42)*.

In addition to the antibody-only tests, two combination fourth-generation antigen-antibody EIAs for detection of HCV have also been introduced: the Monolisa® HCV Ag-Ab ULTRA (Bio-Rad Laboratories); and the Murex™ Ag/Ab Combination (DiaSorin). Both assays are based on the simultaneous detection of HCV core antigen or nucleocapsid protein of HCV and anti-HCV. Although neither of these assays is as sensitive as HCV antigen-specific assays, both assays have demonstrated improved sensitivity over HCV Ab-only assays for the detection of HCV infection, especially in the window period, when antibodies are undetectable *(43–45)*. Although these combination assays are laboratory based and not ideally suited to resource-limited settings, it has been suggested that they could be a reasonable alternative for blood screening or detection of HCV when NAT cannot be used for reasons such as cost, affordability, feasibility, emergency or logistical challenges *(43,45)*.

Third-generation EIAs and CIAs are now the dominant HCV screening tools, and the specificity of EIA assays is reported to be greater than 99% in high-risk populations *(46)*. However, both false-positive and false-negative results are possible. False-positives are more likely to occur when testing in low-prevalence HCV settings; false-negatives are more likely to occur in the presence of HCV-HIV coinfection *(46,47)*.[13]

12 Fujeribio asserts that the Innotest® is a fourth-generation EIA that is able to offer high sensitivity and specificity for all genotypes of HCV as it utilizes unique antigens derived from different genotypes. The antigens utilized are derived from the core (two different epitope clusters), NS3, NSFA, NS4B as well as the NS5A regions; NS3 and NS4 antigens are derived from genotypes 1, 2, 1b, 2 and 3 *(41)*.

13 In addition to EIAs, more specific supplemental tests for anti-HCV, namely recombinant immunoblot assays (RIBA), were developed to deal with false-positives, particularly in low-prevalence settings. However, in resource-limited settings, reflex testing using RIBA has not been widely implemented due to assay complexity, long turnaround time of test results and cost. In addition, because the specificity of third-generation EIAs is quite high, and given the availability of very sensitive and specific NAT, the role of such assays has virtually disappeared *(11)*.

Rapid tests

Despite the accuracy and availability of anti-HCV tests, EIAs and CIAs generally require specialized instrumentation (e.g. incubators; mechanical washing; optical reading devices) and well-trained laboratory technicians. These tests may be implemented at Level II and above facilities in-country, but the tests are relatively expensive and do not provide same-day results. Given the limitations of EIAs and CIAs, and the need for more timely testing and provision of HCV results, rapid HCV tests have been developed.

There are a number of rapid HCV RDTs available, which use immunochromatography, immunofiltration or agglutination principles to detect the presence of anti-HCV in the test specimen. A specimen (whole blood, plasma, serum or oral fluid) is collected and mixed with a developing solution such as colloidal gold labelled with protein-A *(48)*. This mixture is then applied to the absorbent membrane of the test device, which contains immobilized HCV antigens (such as core, NS3 and NS4 antigens) in the test area of the assay. As the sample fluid moves through the test area, a coloured line or dot appears if anti-HCV antibodies from the sample react in the test area. Tests also have an internal control area, which contains a monoclonal antibody and confirms that the test sample has adequately passed through the test area by showing another coloured line or dot *(49)*. If there is no coloured line or dot, then the test is invalid. If there is only a control line or dot, then the test is valid and negative.

Some HCV RDTs require serum or plasma samples. These include: Advanced Quality One Step HCV Test (Bionike), Diagnos HCV Bi-Dot (J. Mitra), HCV-Tri-Dot (J. Mitra) and HCV Spot (MP Biomedicals). This means that a centrifuge is required to separate plasma or serum from the patient blood specimen before testing. Some of the RDTs also require cold-chain storage of test kits. However, equipment for plasma separation, refrigerators and stable electricity are often not found in resource-limited settings, particularly at Level I laboratories.

What is most needed in such settings are RDTs that use capillary blood or oral fluid specimens and require no electricity or cold chain. There are a number of these assays for HCV screening available in the market. Figure 5 lists some of these HCV RDTs and their basic specifications.

Figure 5. Selected RDTs for HCV screening

Product manufacturer	Specimen	Volume of oral fluid or whole blood required	Time to result (minutes)	Storage temperature (°C)	Test shelf-life (months)	Test type
OraQuick® HCV Rapid Antibody Test (OraSure Technologies, UnitedStates)	Oral fluid, whole blood, serum or plasma	1 drop of blood or oral swab	20–40	2–30	18; controls 12 at 2–8	Cassette-enclosed test strip
SM-HCV Rapid Test (SERO-Med, Germany)	Whole blood or serum	30–40 µL (~2–3 drops)	3	2–8 prior to opening package; <30 after opening	N/A	Cassette enclosed test card
Advanced Quality™ Rapid Anti-HCV Test* (InTec, China)	Whole blood, serum or plasma	1 drop	1	2–20	N/A	Cassette enclosed test card
Hexagon HCV (Human Diagnostics, Germany)	Whole blood, serum or plasma	10 µL (~1 drop)	5–20	2–30	N/A	Immuno-chromatographic
Multiplo Rapid HBV/ HIV/HCV Antibody test (MedMira, Canada)	Whole blood, serum or plasma	1 drop	3	2–30	N/A	Cartridge with test membrane
Multisure HCV Antibody Assay* (MP Biomedicals Asia Pacific, Singapore)	Whole blood, serum or plasma	25 µL (~2 drops)	15	2–28	N/A	Cassette enclosed test card
Genedia® HCV Rapid LF (Green Cross Medical Science, Korea)	Whole blood, serum or plasma	20 µL (~2 drops)	20–30	2–30	18	Cassette enclosed test card
Diagnostic Kit for Hepatitis C Virus Antibody (Colloidal Gold)* (NewScen Coast Bio-Pharmaceutical Co. Ltd, China)	Whole blood, serum or plasma	1 drop	5–30	N/A	N/A	Cassette enclosed test card or test card only
DIAQUICK HCV Ab Cassette (DIALAB, Austria)	Whole blood, serum or plasma	1 drop	2–30	10–20	[24]	Cassette enclosed test card

*Product is going through the WHO Prequalification Process.
N/A = Not available.

Of the RDTs listed above, none has yet been prequalified by WHO, and only three of these assays are currently going through the process: Advanced Quality™ Rapid Anti-HCV Test (InTec); Diagnostic Kit for Hepatitis C Virus Antibody (Colloidal Gold) (NewScen Coast Bio-Pharmaceutical Co. Ltd); and Multisure HCV Antibody Assay (MP Biomedicals). One test, the OraQuick® HCV Rapid Antibody Test, has been FDA approved.

While most of the RDTs above are easy to use, some are more complex than others. Some tests require cold chain (OraQuick® HCV Rapid Antibody Test for controls; SM-HCV Rapid Test after kit package is opened). Some tests are not tolerant of humidity (e.g. Genedia® HCV Rapid LF). And, while most of the tests are relatively inexpensive, with a price range of US$ ~1.25–~2.50, the OraQuick® HCV Rapid Antibody Test, is more expensive with a price of at least US$ 5.00 per test.

A relatively recent meta-analysis of HCV RDTs using serum and plasma samples, as well as blood (whole blood or capillary blood) or oral fluid specimens, which are best suited to use in resource-limited settings, found such tests to be accurate and appropriate for HCV screening initiatives *(28)*. However, it should be noted that a patient's HIV coinfection status can influence the diagnostic accuracy of screening tests. Because data are limited on this issue, it has been suggested that research studies stratified by HIV coinfection are needed to determine test accuracy in the presence of such coinfection *(28)*.

In general, more affordable, quality-assured RDTs for HCV screening are needed for use in resource-limited settings. These tests should be easy to use and robust, with high temperature/humidity tolerance and no cold chain requirements. Furthermore, their performance in the presence of HIV coinfection should be proven.

HCV screening tests in the pipeline

There are at least two HCV rapid screening tests in the pipeline. They are described below.

DPP® HCV Assay (*Chembio Diagnostic Systems Inc.*)

Chembio Diagnostic Systems Inc. (hereafter Chembio) is developing the DPP® HCV assay (Figure 6), which is expected to be launched in 2015 for use in resource-limited settings. It is a single-use immunochromatographic, rapid screening test for the detection of antibodies to HCV in oral fluid, fingerstick whole blood, venous whole blood, serum or plasma samples. The test includes the Chembio SampleTainer™ specimen collection bottle, which is a safe, closed system for handling potentially infectious blood samples. Time to result is between 10 and 25 minutes using oral fluid samples and between 10 and 15 minutes using fingerstick whole blood, venous whole blood, serum or plasma.

Figure 6. DPP® HCV test cartridge

In addition to this single-use POC test for HCV detection, Chembio is also developing two additional assays: one for combined detection of HCV and HIV and one for the combined detection of HCV, HIV and syphilis. Each of these POC tests uses a similar test procedure to the HCV assay.

MBio System (*MBio Diagnostics® Inc.*)

MBio Diagnostics® Inc. (hereafter MBio) intends to develop an infectious disease panel that will include an assay for the detection of HCV. Each product being developed by MBio is a diagnostic system combining an easy-to-use, software-driven portable reader with single-use disposable cartridges (Figure 7) that can deliver "laboratory-quality" results within the timeframe of a patient visit. The MBio System (the System) supports multiple assay formats, including whole blood cellular analysis and multiplexed immunoassays, providing flexibility, a product pipeline and cost profile that the company believes is unique in the POC market segment. The System has been designed specifically for applications in resource-limited settings.

Figure 7. MBio System

System features include:

- Product pipeline: the first product will deliver absolute CD4 count, with following releases delivering cartridges for immunoassays such as HCV, HIV, syphilis, viral hepatitis and tuberculosis (TB).
- Sample throughput: cartridges can be processed in parallel (batch mode) using a separate cartridge rack with automatic timing. One operator with one system can process 10–15 samples per hour, or approximately 80 samples per day.
- Time to result: turnaround time for a single sample is ~20 minutes, with a >1-hour read window.
- Connectivity: the System includes integrated Ethernet and wireless connectivity, which can facilitate QC, supply chain and post-market surveillance.
- Sample-to-answer: capillary or venous whole blood, serum or plasma are loaded directly into the cartridge. After a timed incubation in the MBio Rack, the cartridge is inserted into the Reader for automated analysis. There are no additional assay steps or user interactions.
- Cartridge: all assay reagents are integrated into the cartridge; there are no separate buffers or peripheral bottles to be managed. The disposable cartridge is a simple, robust design with no pumps, valves or complex fluidic features.
- Storage stability: integrated, dried assay reagents and device packaging have been designed to ensure environmental stability (heat and humidity) during transport and storage in resource-limited settings. There is no cold-chain storage requirement.
- Internal QC: every cartridge incorporates multiple internal QC features for every sample run, including sample type, sample volume, reagent quality, reader function and cartridge lot expiration.
- Biohazard and safety: blood and assay fluids stay on the sealed device, minimizing biohazard handling.

- Technology: the Reader is a proprietary two-colour fluorescence imaging device with results based on immunostaining and image analysis. The novel design capitalized on the robustness and low cost of modern consumer electronic components such as cell phone cameras and DVD lasers. The peripheral MBio Rack provides incubation timing and visual indicators to guide users running multiple cartridges in batch mode.

The System includes an onboard computer for sample analysis, results management, internal QC and event logs that can be exported in common and viewable file formats for data review. The user interface is an intuitive touchscreen with administrator-configurable settings such as user lockout/validation and QC scheduling. Cartridge barcodes are read automatically and the instrument will have multiple USB ports to support printers, external barcode readers and other peripherals. The System will include integrated GSM/GPRS connectivity.

The MBio product launch schedule will be determined by the company's commercialization priorities and funding availability.

Confirmatory testing for HCV

All positive HCV serologic tests require confirmation of active HCV infection. Either HCV cAg or HCV RNA molecular tests can be used for this purpose as these assays not only verify the result of the serological test, but also establish the presence of chronic HCV infection. HCV RNA molecular tests are either qualitative or quantitative assays. Qualitative tests can only determine the presence or absence of HCV RNA, whereas a quantitative NAT can generate an HCV RNA level.

In addition to using an HCV RNA qualitative assay to confirm chronic infection, it is also possible to use assays that measure the HCV cAg in serum. A study in 1992 found that circulating HCV cAg could be detected via an enzyme-linked immunosorbent assay (ELISA) sandwich antigen test, but, until relatively recently, the test procedures were complex *(50)*. However, test methodologies have evolved and there is one fully automated HCV cAg test on the market, the ARCHITECT HCV cAg Test from Abbott. Because under current treatment regimens the assay can be used both for confirmatory testing as well as monitoring of HCV antiviral therapy as a complement to NAT-based testing, it is discussed later in this report.

Qualitative HCV RNA tests

Since current HCV treatment regimens require a quantitative HCV viral load at treatment initiation, there is a general preference for the use of quantitative HCV RNA assays for confirmatory testing, and there is currently only one commercially available laboratory-based qualitative HCV RNA assay, which is described below.

Roche COBAS® AmpliPrep/COBAS® TaqMan® System (*Roche Molecular Systems*)

Roche Molecular Systems (hereafter Roche) currently manufactures a single real-time PCR assay, the COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test version 2 (hereafter CAP/CTM Qual Test). The assay uses the AmpliPrep instrument for automated viral nucleic acid extraction and the COBAS® TaqMan® 48 analyser, which is described below, for automated amplification and detection of the viral nucleic acid target.

The CAP/CTM Qual Test is a qualitative in vitro nucleic acid amplification test (NAAT) for the detection of HCV RNA genotypes 1 to 6 in human serum or ethylenediaminetetraacetic acid (EDTA) plasma. The company reports a lower limit of detection (LLOD) of 15 IU/mL.[14] The assay is based on three major processes: (i) specimen preparation to isolate HCV RNA; (ii) reverse transcription of the target RNA to generate complementary DNA (cDNA); and (iii) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labelled oligonucleotide detection probes specific to the target.

14 The LLOD is defined as the lowest amount of HCV RNA concentration that can be detected with 95% probability to determine presence or absence.

More specifically, the CAP/CTM Qual Test utilizes automated specimen preparation on the COBAS® AmpliPrep instrument by a silica-based capture technique. The sample input volume is 650 µL of serum or plasma. The test then uses reverse transcription of HCV RNA to cDNA and PCR amplification of cDNA using primers that define a sequence within the highly conserved region of the 5' untranslated region (UTR) of the HCV genome. Furthermore, the use of dual-labelled fluorescent probes (HCV RNA and HCV internal control RNA) allows for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. This increases the emission intensity of the individual reporter dyes and permits independent identification of HCV RNA and HCV internal control RNA. The HCV internal control serves as an extraction and amplification control for each independently processed specimen.

The CAP/CTM Qual Test is CE-IVD marked. At least one peer-reviewed evaluation of the assay has been published *(51)*. The evaluation demonstrated that the CAP/CTM Qual Test performs significantly better than its predecessor (the version 1 test).

The cost per test for the least developed countries and certain high-burden middle-income countries is about €34–40 (US$ 43–51).[15] Actual pricing is dependent on variables such as outright instrument purchase, reagent rental and volume-based, tiered pricing arrangements.

The COBAS® AmpliPrep System – The COBAS® AmpliPrep instrument is an automated sample preparation technology (Figure 8) for use in conjunction with the Roche COBAS® TaqMan® analysers discussed below. The company considers the AmpliPrep to provide "walk-away" sample preparation/extraction capability, which can significantly reduce hands-on time of laboratory technicians.

Figure 8. COBAS® AmpliPrep system

The instrument is large, weighing over 680 lbs. The run size for the instrument is 24 specimens, but it can process up to 72 samples at any given time. The first 24 samples take two hours to process. However, because the instrument allows for parallel processing, subsequent batches of 24 can be completed every hour as one rack of specimens will begin processing before the previous rack processing has been completed. The system is closed and requires the use of test-specific, barcoded, ready-to-use COBAS® AmpliPrep kits. The cost of the instrument is approximately US$ 80 000–100 000 (with the lowest pricing reserved for lower-income countries).

15 These are average prices to the end user, including distribution and other fees.

Roche TaqMan® 48 Analyser – The COBAS® TaqMan® 48 Analyser is a fully automated, closed-tube system. The TaqMan® 48 (Figure 9) is relatively compact and can run from 6 to 48 samples at a time. The instrument is equipped with two thermal cyclers that operate independently and provide run times of three hours.

Figure 9. COBAS® TaqMan® 48

The cost of the COBAS® TaqMan® 48 Analyser is approximately US$ 40 000–50 000.

VERSANT® HCV RNA Qualitative Assay (*Siemens Healthcare Diagnostics Inc.*)

The VERSANT® HCV RNA Qualitative Assay is manufactured by Hologic Inc. and licensed to Siemens Healthcare Diagnostics Inc. (hereafter Siemens). Siemens has decided to discontinue this product effective December 2014.

HCV RNA qualitative assays in the pipeline

Genedrive® (*Epistem Ltd*)

Epistem Ltd, a biotechnology company headquartered in the United Kingdom, has developed a new molecular diagnostic platform called Genedrive® (Figure 10), which uses end-point PCR-based detection. Genedrive® is a highly portable, POC platform weighing about 550 grams (1.2 lbs) and is approximately the size of an iPad mini. The platform accommodates both electric (110–240 V AC) and battery (12 V DC) power.

Figure 10. Genedrive® platform

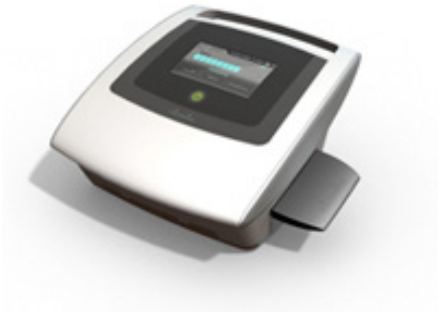
The first major test developed for the Genedrive® platform is for MTB and mutations associated with resistance to the front-line antibiotic rifampicin (RIF). Test results are available in less than 60 minutes. The Genedrive® platform is integrated with a simple extraction process based on an advanced composite paper technology that allows extraction and decontamination in a single step and is suitable for use in low-resource settings. The sample is manually transferred with one pipetting step into the Genedrive® reaction cartridge. Epistem Ltd expects to launch the MTB assay in India in 2015.

Epistem Ltd is also developing diagnostic tests for a number of additional infectious diseases, including the RNA viruses HCV and HIV from plasma and whole blood using the same integrated process. With respect to HCV, Epistem Ltd is involved in collaborations with the Pasteur Institute and is developing an HCV assay primarily for viral detection/diagnosis, but with the capability to perform viral load quantitation. It will be benchmarked against CE-marked/National Institute for Biological Standards and Control (NIBSC) reference panels (Hepatitis C Virus for Nucleic Acid Amplification Techniques) (the fourth WHO International Standard). The HCV viral detection and load, which will be pan-genotypic, is expected to be available as a research use only product in the second quarter of 2015, with user studies conducted towards the end of that period. Data from these studies (and from CE-marked/ NIBSC reference panels) will be used to support submission of the assay for certification towards the end of the third quarter of 2015.

PanNAT® Platform – *(Micronics Inc.)*

Micronics Inc., a subsidiary of Sony Corporation of America, has developed the PanNAT® system (Figure 11), which is a small, portable microfluidic platform for use near patient for in vitro molecular diagnosis of infectious diseases in resource-limited settings. It is a fluorescent-based reader capable of processing individual, disposable, assay-specific cartridges, each of which is designed to perform a single and/or multiplexed nucleic acid assay. The cartridge includes all necessary reagents on board. The system is lightweight, mains-powered, can store up to 350 test results before prompting the user to download or delete results, and can provide results within 30–40 minutes, depending upon assay parameters. A battery-operated/WiFi-enabled option is planned.

Figure 11. Micronics Inc. PanNAT® platform



The cartridge incorporates probes, primers, enzymes, buffers and controls for sample purification, amplification and detection, and because it is a closed-cartridge system, there is no PCR product cross-contamination. Cartridge design permits storage at ambient temperatures for prolonged periods. All waste is captured in the cartridge for safe disposal.

Micronics Inc. has been funded to develop a qualitative RNA-based assay for HCV, as well as for hepatitis B virus (HBV) and HIV. Timing for development of the HCV assay is not known.

Genotyping

Because current HCV antiviral treatment regimens are only effective against specific genotypes and because the duration of peg-IFN-riba therapy and its effectiveness depend on the HCV genotype of the patient, genotyping is currently recommended before starting patients on HCV therapy. It is expected, however, that most DAA regimens will be pan-genotypic, in which case, genotyping patients prior to starting them on such regimens will not be required.

There are several laboratory-based genotyping assays on the market. These are based on one of four technologies: RT-PCR; line probe assay; DNA chip; or DNA sequencing. They are described below.

RealTi*me* HCV Genotype II (*Abbott*)

The Abbott RealTi*m*e Genotype II is an RT-PCR assay for the identification of the genotype(s) of HCV in plasma or serum from individuals with chronic HCV infection. The Abbott RealTi*m*e HCV Genotype II detects genotypes 1, 1a, 1b, and 2–5 through the use of genotype-specific fluorescent-labelled oligonucleotide probes. It targets the 5'UTR for the classification of HCV genotypes 1, 2, 3, 4, 5 and 6, and the NS5b region to accurately subtype HCV genotypes 1a and 1b. Its performance, based on internal verification, is summarized in Table 1.

Table 1. Abbott RealTi*m*e Genotype II performance indicators

Sensitivity	500 IU/mL (0.5 mL, prep)
Specificity	100%
Target region	5'UTR and NS5b
Genotype detection	1a (NS5b), 1b (NS5b), 1, 2, 3, 4, 5, 6 (5'UTR)
Standardization	Second WHO International Standard for Hepatitis C Virus RNA
Internal Control	Yes; processed through sample preparation with each sample
External control	Yes; negative, positive
Results reported	Qualitative – genotype call

The Abbott RealTi*m*e HCV Genotype II assay is CE-IVD marked. Only two independent, peer-reviewed performance evaluations of the assay were found, and they demonstrated somewhat mixed results *(52,53)*. Sohn et al. concluded that the assay needs improvement to decrease cross reactivity among genotypes and to improve the ability to detect minor genotypes in mixed infections *(52)*, while Yang et al. found that the assay failed to identify some genotype 6 subtypes *(53)*.

Price information on the assay is available from Abbott.

Sample Preparation with the *m*2000 System – The Abbott RealTi*m*e Genotype II assay is designed to be used with the *m*2000*rt* amplification and detection instrument as well as with one of three methods of sample preparation: (i) manual (for laboratories with low throughput requirements); (ii) the *m*24*sp* instrument (for laboratories with low to medium throughput requirements); or (iii) the *m*2000*sp* instrument (for laboratories with medium to high throughput requirements). It should be noted that in addition to the HCV Genotype II assay, the Abbott *m*2000 System can also be used to perform both qualitative and quantitative HCV viral load assays, whereas the Roche and Siemens genotype assays must be performed on instruments that are different from those used for their respective viral load assays.

The *m*24*sp* (Figure 12) is a bench-top sample preparation and extraction device with a small footprint that is generally appropriate for facilities with medium throughput requirements. It provides a variable extraction system (extraction output can be stored either in deepwell trays or 1.5 mL tubes) with ready-to-use and reusable reagents as well as flexible batch size capabilities.

Figure 12. *m*24*sp* instrument

The cost of the *m*24*sp* is approximately US$ 80 000.

The *m*2000*sp* by Abbott (pictured in the centre of the image in Figure 13) is a larger and more automated sample preparation device than its sibling, the *m*24*sp*. With complete automation, comes increased walk-away time for the operator. It is a high-throughput system with a maximum batch size of 96 samples per run. When combined with the Abbott *m*2000*rt*, an amplification and detection instrument, the system can provide automation from barcoded laboratory tube through patient result.

The cost of the *m*2000*sp* is approximately US$ 162 000.

Figure 13. *m*2000*sp* instrument

The Abbott *m*2000*rt* is the amplification and detection platform for use with manual extraction, the *m*24*sp* and the *m*2000*sp* instruments, as described above. It is a high-performance system, but is relatively compact, weighing in at just over 75 lbs. The *m*2000*rt* (Figure 14) can run 96 samples at a time in about three hours of cycling time (not including time for sample preparation). The system will run both quantitative and qualitative analyses and offers key validity parameters such as maxRatio. Like other laboratory-based molecular systems, the operator must have a thorough knowledge of the applications run on the instrument (and on the sample preparation instrument) and must follow good laboratory practices when operating them.

Figure 14. *m*2000*rt* instrument

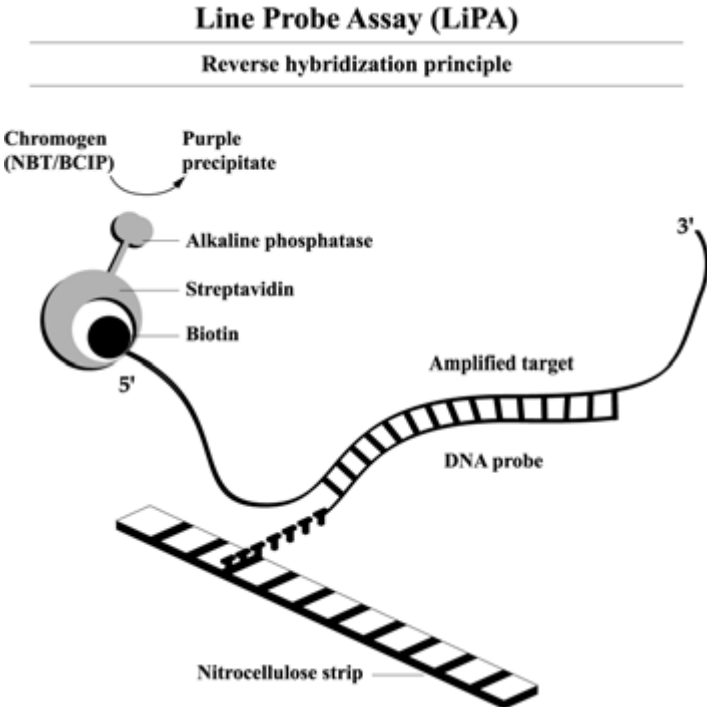
The cost of the *m*2000*rt* is approximately US$ 45 000.

VERSANT® HCV Genotype 2.0 LiPA (*Siemens*)

The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a line probe assay, for IVD use, which identifies HCV genotypes 1 to 6 and subtypes a and b of genotype 1 in human serum or EDTA plasma samples. Additional subtype information is available in a majority of cases. The VERSANT® HCV Genotype 2.0 Assay uses the 5'UTR region of the genotype, which contains multiple genotype-specific motifs distributed over seven small variable regions, to provide accurate genotyping information for genotypes 1–5 and genotype 6, subtypes a and b. However, the assay uses sequence motifs from the core region in addition to the 5'UTR to identify genotype 6, subtypes c to l. Additional core motifs are included to improve the accuracy of the identification of 1a and 1b.

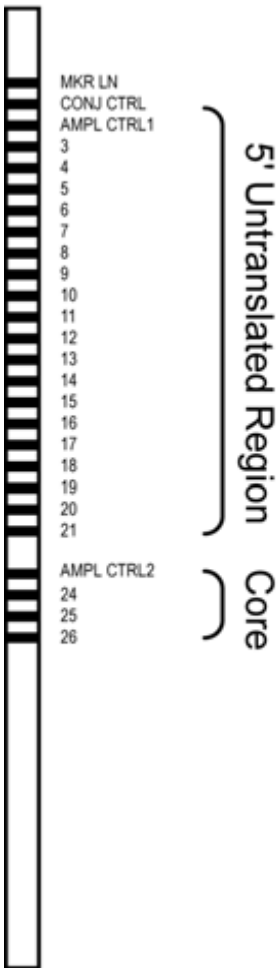
The VERSANT® HCV Genotype 2.0 Assay (LiPA) utilizes reverse hybridization (Figure 15). Biotinylated DNA PCR product, generated by RT-PCR amplification of the 5'UTR and core regions of HCV RNA, is hybridized to immobilized oligonucleotide probes. The probes, which are bound to a nitrocellulose strip by a poly(dT) tail, are specific for the 5'UTR and core region of different HCV genotypes. After the hybridization step, unhybridized PCR product is washed from the strip, and alkaline phosphatase labelled streptavidin (conjugate) is bound to the biotinylated hybrid. BCIP (5-bromo-4-chloro-3-indolu-phosphate)/NBT (nitro blue tetrazolium) chromogen (substrate) reacts with the streptavidin-alkaline phosphatase complex forming a purple/brown precipitate, which results in a visible banding pattern on the strip.

Figure 15. Reverse hybridization LiPA



The VERSANT® HCV Genotype 2.0 Assay (LiPA) strips have 3 control lines and 22 parallel DNA probe lines (Figure 16) containing sequences specific for HCV genotypes 1 to 6. The conjugate control (CONJ CTRL) line monitors the colour development reaction. The amplification control (AMPL CTRL 1) at line 2 contains universal probes that hybridize to the PCR product from the 5'UTR. The amplification control (AMPL CTRL 2) at line 23 contains universal probes that hybridize to the PCR product from the core region. HCV genotypes are determined by aligning the assay strips with the VERSANT® HCV Genotype 2.0 Assay (LiPA) Reading Card and comparing the line patterns from the assay strips with the patterns shown on the VERSANT® HCV Genotype 2.0 Assay (LiPA) Interpretation Chart.

Figure 16. VERSANT® HCV Genotype 2.0 assay strips



The assay must be used with the VERSANT® HCV Amplification 2.0 Kit (LiPA), which provides all reagents for reverse transcription and amplification of the 5'UTR and core region of the HCV genome. The HCV amplification procedure begins with viral RNA extracted from human serum or plasma. The QIAamp DSP Virus Kit (from QIAGEN) has been evaluated for purification of viral nucleic acids from plasma/serum for use in conjunction with the LiPA. In addition, the assay must be performed on an Applied Biosystems thermal cycler model GeneAmp PCR System 9700 or equivalent, which is not provided by Siemens.

The VERSANT® Genotype 2.0 Assay (LiPA) is CE-IVD marked. There are several published performance evaluations of the assay *(53,54)*. While Yang et al. concluded that the LiPA 2.0 cannot distinguish genotype 3b samples from genotype 6 samples *(53)*, Verbeeck et al. found the assay to be sensitive, accurate and reliable for HCV genotyping *(54)*.

HCV Genotype Plus Real-TM *(Sacace Biotechnologies)*

Sacace Biotechnologies (hereafter Sacace) manufactures the HCV Genotype Plus Real-TM assay for the determination of HCV-RNA genotypes 1a, 1b, 2, 3, 4, 5a and 6 in human plasma. The assay targets the most conserved area of the 5'UTR region of the genotype.

The assay is based on three major processes:

- isolation of HCV RNA from specimens;
- reverse transcription of RNA;
- real-time PCR.

An internal control serves as an amplification control for each individually processed specimen and to identify possible inhibition. The internal control is detected in a channel other than the HCV RNA.

Per the company, the analytical sensitivity of the HCV Genotype Plus Real-TM is about 1000 IU/mL, while the diagnostic specificity of the assay is 100% and the diagnostic sensitivity is also 100%. No published, peer-reviewed evaluations of the assay were found.

The HCV Genotype Plus Real-TM assay costs about €31.60 per test (~US$ 41 per test), but is subject to substantial discounts (up to 50%) from the company. Like all assays from Sacace, the test is platform independent, or open system, meaning that the assay can be used on a variety of platforms, either manual or automated, including the Rotor-Gene™ (QIAGEN), LineGeneK™ (Bioer Technologies) and the SmartCycler™ (Cepheid). In addition, Sacace also manufactures an RT-PCR platform, the SaCycler-96™.

SaCycler-96™

The SaCycler-96™ (Figure 17) is a new instrument for real-time amplification and melting analysis suitable for diagnostics applications. Two thermoelectric Peltier elements are designed to ensure high accuracy of temperature regulation and quiet operation. The design of the thermal block allows loading and unloading of test samples in a completely automated software-controlled way.

Figure 17. SaCycler-96™

Like most RT-PCR platforms, the SaCycler-96™ is a laboratory-based instrument. It is in a 96-well format that is suited to standard PCR microplates, test tubes and strips, and contains 4 or 5 channel multiplexing for discrimination of up to five targets in a single reaction well. There is a separate light emitting diode

(LED) light source for each channel and a matrix charge-coupled device (CCD) camera. The platform has a wide dynamic range of detection using a multiple exposure method, which greatly simplifies or even eliminates the need for fluorescence settings. The main applications of the platform are for RT quantitation, single nucleotide polymorphisms genotyping, melting curve and gene expression analysis.

The SA Cycler-96™ comes installed with the most frequently used Sacace protocols already installed in the software, which helps to minimize errors during programming. In addition, the platform offers ease of integration with any laboratory information system as the software can save all data in standard graphic or text formats ready to be loaded into databases.

The cost of the closed IVD version of the SA Cycler 96™, which works only with the company's test kits, is €14 400 (~US$ 18 645), including a notebook computer. There is also an open system version of the instrument that costs €16 800 (~US$ 21 750), not including a notebook computer. Discounted pricing on these instruments (up to 15%) is available from the company.

HCV treatment monitoring

As discussed earlier in this report, quantitative HCV RNA testing may be used to confirm active HCV infection and has the added benefit of generating an HCV RNA level (or HCV viral load), which is generally required with current HCV treatment regimens. Such tests are also used to monitor patients on HCV treatment and to test for HCV clearance and cure post-treatment.

It has been suggested that platforms that quantify HCV cAg may be a complement to or, with new DAA treatment regimens, an alternative to HCV viral load testing for HCV treatment monitoring. In the past, manual HCV-Ag ELISA assays have been available, but were not deemed to be sufficiently sensitive, with analytical sensitivity being significantly lower than that of HCV RNA assays and with a significant number of manual sample preparation steps, which limited throughput *(55)*. In recent years, Abbott developed a fully automated assay for HCV cAg on its ARCHITECT platform. It is described later in this report.

Existing HCV viral load platforms

Today, the most widely used quantitative HCV viral load assays measure HCV RNA using NAT technologies. NAT-based RNA assays have become the core viral load monitoring technology used in developed countries and resource-limited settings. All such technologies incorporate amplification techniques because levels of nucleic acids are otherwise too low to be detected directly. Amplification methods are either aimed at increasing the number of target molecules (viral nucleic acids) to a level that permits detection (target amplification methods) or are aimed at increasing the signal generated by the method (signal amplification methods) *(56)*. Currently, the bulk of commercially available HCV viral load assays are based on target amplification.

Whether an assay is based on target amplification or signal amplification, the assay will consist of the following common steps: (i) sample preparation and/or viral nucleic acid extraction; (ii) the actual amplification step that is either target amplification based or signal amplification based; and (iii) detection and/or quantification of the amplified viral nucleic acids.

Pre-amplification methods (sample preparation and/or viral nucleic acid extraction) are critical to the viral load testing process. For each sample to be analysed correctly and to achieve an accurate result, the nucleic acid must be both available for the reaction and purified. Protocols for the pre-amplification steps include the use of purification methods for cells, and virion centrifugation or a capture step for RNA in plasma, followed by an extraction step to free the target viral nucleic acid *(56)*. Molecular detection methods require prompt processing of samples (generally within six hours of collection), a rapid extraction method and appropriate storage of plasma or cells prior to assessing.

There are several *amplification methods* used to detect viral RNA after preparation of samples. In target amplification, many copies of a portion of the viral nucleic acid are synthesized via an amplification reaction; in effect, this method enhances the ability to detect very low levels of nucleic acids that occur

naturally in the blood. These techniques include RT-PCR used in the Roche, Abbott and QIAGEN assays. In signal and probe amplification methods, a probe or a reporter molecule attached to a probe is detected and the signal generated by this reaction is amplified/increased; in effect, these methods increase the "marker" that shows that the target is present. Signal amplification techniques include branched DNA (bDNA), which is used in the VERSANT® HCV 3.0 Assay by Siemens.[16]

Finally, *post-amplification methods* require the detection and/or quantification of either the amplification products (in target amplification methods) or the increased detection of signals that have been amplified (in signal amplification methods) *(56)*. Detection can be achieved using any one of a number of reagents – e.g. colourimetric; radioactive; fluorescence. Detection can either be done at the endpoint of the process (completion of the run) or in "real time" (during the production of results as they occur). Real-time techniques, in which amplification and detection occur simultaneously, are now commonly used. For example, the Roche TaqMan® platform uses real-time detection, which is achieved via specific, fluorescently labelled probes that bind to the DNA that is generated via the amplification process (called amplicons).

In general, in addition to their excellent sensitivity, the advantages of NAT-based approaches include that many of the assays using these approaches have been evaluated and are well validated, the assays are available in quality-assured kits and clinicians are comfortable interpreting the results. The assays vary in terms of sample preparation and amplification/detection methodologies, among other things.

Currently, there are six commercially available HCV RNA viral load assays, all of which are based on RT-PCR: (i) COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0 (Roche), (ii) RealTi*m*e HCV (Abbott), (iii) VERSANT® HCV 1.0 (kPCR) (Siemens), (iv) artus™ HCV QS-RGQ (QIAGEN); (v) careHCV RT-PCR assay v2 (QIAGEN); and (vi) Real-TM Quantitative Assay (Sacace Biotechnologies). These are described below.

COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0 (*Roche*)

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0 (hereafter CAP/CTM HCV test) is a NAAT for the quantitation of HCV RNA in human serum or EDTA plasma. The test is based on three major processes: (i) specimen preparation to isolate HCV RNA; (ii) reverse transcription of the target RNA to generate cDNA; and (iii) simultaneous PCR amplification of target cDNA and detection of cleaved dual-labelled oligonucleotide detection probes specific to the target.

The CAP/CTM HCV test uses magnetic silica bead-based nucleic acid extraction on the COBAS® AmpliPrep platform, followed by amplification with primers specific to the 5'UTR of the HCV genome and detection with a fluorescently labelled hydrolysis probe performed on the COBAS® TaqMan® thermal cycler to detect the target and a quantitative standard (QS) *(57)*. The COBAS® AmpliPrep and COBAS® TaqMan® 48 instruments are described earlier in this report.

The CAP/CTM HCV test is a second-generation assay that uses a dual-labelled fluorescent probe, which allows for real-time detection of PCR product accumulation by monitoring the emission intensity of fluorescent reporter dyes released during the amplification process. The test also uses an additional reverse primer to improve genotype 4 inclusivity and performance with known samples that are difficult to quantify *(57,58)*. Additional assay features include: (i) changes in the elution and lysis buffers that improve sample preparation; (ii) higher temperature RT step and shorter overall PCR cycles; and (iii) reduced sample input volume (650 µL sample requirement with 500 µL processed by the instrument).

The CAP/CTM HCV test has a lower limit of quantification (LLOQ) of at least 15 IU/mL to an upper limit of quantitation of at least 100 million IU/mL (linear range). The assay reports HCV RNA levels of 1–14 IU/mL as "HCV RNA detected, below the LLOQ" *(57)*. Performance of the assay has been evaluated and the assay is considered to exhibit very good sensitivity, reproducibility and dynamic range *(58,59)*.

16 The VERSANT® HCV 3.0 Assay, which is a bDNA sandwich nucleic acid hybridization method, will be discontinued by Siemens effective December 2014. Therefore, the bDNA HCV assay is not discussed in this report.

The CAP/CTM HCV test is CE-IVD marked and FDA approved. The cost per test for the least developed countries is about €28–30 (~US$ 3638).17

COBAS® TaqMan® HCV Quantitative Test v2.0 for use with the High Pure System (*Roche*)

The COBAS® TaqMan® HCV Quantitative Test v2.0 for use with the High Pure System (hereafter CTM HCV test) is a second-generation assay that uses a dual-labelled fluorescent probe and is substantially similar to the CAP/CTM HCV test described above. However, rather than automated sample preparation, the High Pure System Viral Nucleic Acid Kit allows manual specimen preparation followed by automated amplification and detection on the COBAS® TaqMan® 48 Analyser (described earlier in this report).

The CTM HCV test has a sensitivity (LLOD across all genotypes) of 20 IU/mL across all genotypes and a linear range from 25 IU/mL to 390 million IU/mL. Specimens containing HCV genotypes 1 to 6 have been validated for quantitation in the assay. The assay's performance characteristics have been established for individuals treated with peg-IFN-riba; no information is available on the assay's predictive value with other regimens.

The CTM HCV test is CE-IVD marked and FDA approved. The cost per test for the least developed countries is about €2830 (~US$ 3638).[17]

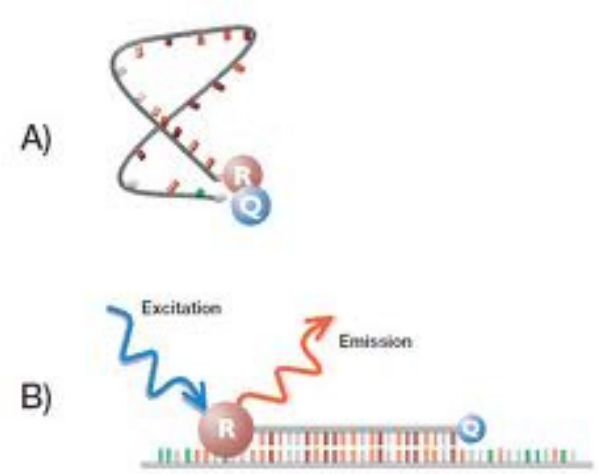
Abbott RealTi*m*e HCV Assay (*Abbott*)

The Abbott RealTi*m*e HCV assay is an in vitro RT-PCR assay for use with the Abbott *m*Sample Preparation System reagents and with the Abbott *m*2000*sp* and *m*2000*rt* instruments (described earlier in this report) for the quantitation of HCV RNA in human serum or EDTA plasma. The assay can be used to predict SVR response to HCV therapy. The assay's performance characteristics have been established for individuals treated with pegylated interferon alfa-2a or 2b and ribavirin; no information is available on the assay's predictive value with other regimens.

The Abbott RealTi*m*e HCV assay uses RT-PCR technology combined with homogeneous real-time fluorescent detection for the quantitation of HCV RNA. The selection of a conserved region of the HCV genome, the 5'UTR region, and the primers are designed to hybridize to the 5'UTR region with the fewest possible mismatches among HCV genotypes 1 to 6. The assay has a unique probe design, illustrated in Figure 18, such that in the absence of the target, the probe achieves quenching through random coiling: A). In the presence of target, the probe instead hybridizes to the target sequence, allowing fluorescent detection: B); cleavage is not required.

17 These are average prices to the end user, inclusive of distribution and other fees.

Figure 18. Probe action in Abbott RealTi*me* HCV assay



The company states that the external calibration curve is a key design feature of the Abbott RealTi*me* HCV assay that enables it to achieve high precision. The use of different primers for the HCV target and the internal control minimizes competitive effects in the PCR reaction. The stored calibration curve reduces the variability of the viral load calculation compared to an internal calibration design.

The Abbott RealTi*me* HCV assay has an LLOD of 12 IU/mL, for a 0.5 mL sample volume and an LLOD of 30 IU/mL for a 0.2 mL sample volume. The linear range is from 12 IU/mL to 100 million IU/mL. Performance evaluations of the assay demonstrating its good correlation with the CTM HCV test and other HCV RNA quantitative assays have been published *(59,60)*.

The assay is CE-IVD marked. The price of the assay is available from Abbott.

VERSANT® kPCR Molecular System *(Siemens)*

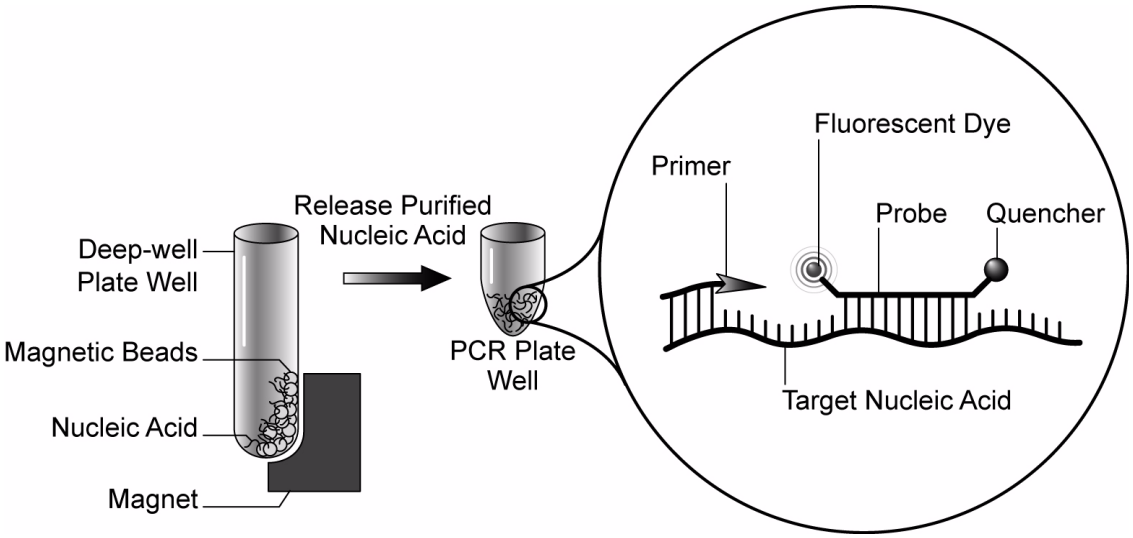
The VERSANT® kPCR Molecular System and the VERSANT® HCV 1.0 Assay (kPCR) are manufactured by Siemens. Because they are CE-IVD marked, but not FDA approved, they are only available outside of the United States. The Siemens HCV assay is a real-time kinetic polymerase chain reaction (kPCR) assay for quantitative detection of HCV RNA in plasma or serum of infected individuals. The system (Figure 19) is an automated amplification method based on reverse transcription and real-time PCR technology and consists of two modules: the Sample Preparation Module used to extract nucleic acids from plasma, as well as a wide variety of other samples; and the Amplification Detection Module, along with VERSANT kPCR software. The system is a "one-room" technology with no need for clean room operations due to closed-tube processing and other physical and chemical contamination controls.

Figure 19. VERSANT® kPCR Molecular System

Photo courtesy of Siemens Healthcare Diagnostics.

The VERSANT® kPCR Sample Preparation module along with the VERSANT® Sample Preparation 1.0 Reagents Kit are used to extract RNA from plasma. The reagents kit includes proprietary magnetic silica beads that provide for efficient and high-quality extraction of nucleic acids. Extraction consists of a lysis step that utilizes proteinase K and a chaotropic buffer, and several washes to remove non-nucleic acid components of the sample and elution. The VERSANT® kPCR Sample Preparation module also pipettes the purified RNA to a PCR plate containing HCV primer/probe mix and HCV enzyme mix. The wells are then sealed and transferred to the Amplification Detection module where the HIV and internal control RNA molecules are reverse transcribed to make cDNA and then simultaneously amplified and detected using the kPCR technique. The RT-PCR step uses primers and probes that target the highly conserved HCV 5'UTR region of the gene and detects all 6 HCV genotypes within +/- 0.5 log IU/mL. A schematic representation of the assay principle is shown in Figure 20.

Figure 20. VERSANT® HCV 1.0 Assay (kPCR) principle



Schematic courtesy of Siemens Healthcare Diagnostics.

The VERSANT® kPCR Molecular System provides the flexibility to process samples in batch sizes of 1–96 tests per run. The HCV assay provides patient results for up to 89 samples per run with a total time to result of less than six hours. The assay has an LLOD of 15 IU/mL (64.5 copies/mL) and a linear range between 15 IU/mL and 100 million IU/mL (64.5–430 million copies/mL).

The VERSANT® HCV 1.0 assay is CE-IVD marked. A peer-reviewed publication on the performance of the assay finds that it demonstrates good correlation with the CTM HCV assay *(61)*. Pricing for the assay and instrument is available from Siemens.

The artus™ HCV RG/QS-RGQ RT-PCR System *(QIAGEN N.V.)*

QIAGEN N.V. (hereafter QIAGEN) manufactures an RT-PCR-based assay for HCV, the artus™ HCV RG/QS-RGQ kit. The assay is CE-IVD marked and targets the conserved 5´UTR and core regions of the genome. The kits can be used in combination with an automated extraction and sample preparation system (QIAsymphony® SP/AS). The assay must then be run on one of the QIAGEN Rotor-Gene Q thermocyclers for amplification and detection. An example of a complete QIAsymphony® RGQ system is pictured in Figure 21.

Figure 21. QIAsymphony® RGQ System

The artus™ HCV RG/QS-RGQ assay has a linear range from 35 IU/mL to 17.7 million IU/mL (using automated extraction), and in combination with the Rotor-Gene Q can detect HCV down to an LLOD of 21 IU/mL (95% confidence interval of 16–33 IU/mL). The time to result is about five–six hours for 24 samples. Performance of the artus™ assay has been evaluated against the CTM HCV assay from Roche and good correlation was found between the two assays, although sensitivity of the artus™ assay was lower than that of the CTM HCV assay *(62)*.

QIAsymphony® SP/AS

Sample preparation for the artus™ HCV assay may be conducted on the fully integrated automated sample preparation, and assay setup is also available using the QIAsymphony® SP/AS instruments. The QIAsymphony® SP can process from 1 to 96 samples (in batches of 24) with sample volumes up to 1 mL. It is a ready-to-run instrument that requires minimal installation. The SP can be combined with the QIAsymphony® AS device in a fully integrated system that can automate the entire workflow. To reduce manual handling and minimize the risk of sample contamination, samples processed on the SP can be transferred automatically to the AS, or the two instruments can be operated independently.

The SP/AS system includes touchscreen controls, barcode-labelled sample tubes containing prefilled reagents, and allows for continuous loading in batches of up to 24 samples plus internal controls. The QIAsymphony® SP/AS instruments can also be integrated in laboratory information management systems. In addition to HCV, the artus™ panels for QIAsymphony® Rotor-Gene Q (RGQ) include assays for the HBV and HIV virus, plus a transplantation/immunosuppressed panel, with assays for detection and quantification of cytomegalovirus (CMV), Epstein-Barr virus, herpes simplex virus 1 and 2, varicella-zoster virus and BK virus.

Rotor-Gene thermocycler

The artus™ HCV assay can be run on the real-time PCR thermocycler RGQ. The RGQ has a unique centrifugal rotary design in which each sample tube spins in a chamber of moving air, which keeps all samples at precisely the same temperature. As each tube aligns with the detection optics in the device, the sample is illuminated and a fluorescent signal is quickly collected. QIAGEN indicates that this results in sensitive, precise and fast real-time PCR analysis and eliminates sample-to-sample variations and edge effects, which are unavoidable in traditional block-based instruments. The Rotor-Gene Q can be ordered with the Rotor-Gene AssayManager software for molecular diagnostics that automatically analyses real-time PCR data of artus™ assays.

careHCV RT-PCR Assay v2 (*QIAGEN*)

In addition to the artus™ HCV RG/QS-RGQ assay described above, QIAGEN's Shenzhen subsidiary in China manufactures the careHCV RT-PCR Assay v2. The assay, which was approved by the State Food and Drug Administration of China in 2011, is for quantitative detection of HCV and viral load monitoring. The assay uses membrane technology for the binding and purification of RNA. It is an RT-PCR assay that uses fluorescent probes for the detection and quantification of HCV RNA; it also includes an internal control for result validation and detection of all key HCV subtypes.

The LLOD of the assay is 500 IU/mL, and the linear dynamic range is from 1000 IU/mL to 50 million IU/mL. The cost of the assay is ~US$ 22 per test in China.

The careHCV RT-PCR Assay v2 can be used in conjunction with automated sample technology products, such as QIAGEN's QIAcube, but can also accommodate manual sample preparation with the QIAGEN viral RNA mini-kit. The QIAcube enables automated mid- to high-throughput nucleic acid purification using silica membrane technology.

The assay can be run on the RGQ thermocycler from QIAGEN, described above, or can be run on other instruments, including the 7000/7300 or 7500 (ABI), the iCycler iQ™ (Bio-Rad Laboratories), the LC480 (Roche), the Opticon Real Time Cycler (Bio-Rad Laboratories) or the LineGeneK™ (Bioer Technologies).

HCV Real-TM Quant Dx Assay (*Sacace*)

Sacace manufactures the HCV Real-TM Quant Dx Assay for the quantitative detection of HCV in human plasma and the simultaneous detection of an HCV-specific internal control, by dual colour detection. The assay extracts HCV RNA from plasma, amplifies it using real-time amplification and detects it using fluorescent reporter dye probes specific for HCV or HCV internal control. Amplification of both targets takes place simultaneously in the same reaction. Monitoring the fluorescence intensities in real time allows for the detection and quantification of the accumulating product without having to reopen the reaction tube after amplification.

The HCV-specific internal control, which represents a recombinant RNA-containing-structure, is carried through all steps of the analysis from nucleic acid extraction to PCR amplification and detection. The presence of this quantitative internal control allows the operator not only to monitor the extraction procedure and to check possible PCR inhibition, but also to verify possible losses of RNA during the extraction procedure. This enables precise calculation of the HCV viral load.

The target sequence for the HCV Real-TM Quant Dx assay is the 5'UTR region of the HCV genome, which is highly conserved. The LLOD of the assay is 13 IU/mL with a 1.0 mL sample preparation procedure. The linear range of the assay is from 13 IU/mL with a 1.0 mL sample preparation procedures to 100 million IU/mL.

The HCV Real-TM Quant Dx assay costs €13.13 per test (or ~US$ 17 per test), but substantial discounts (up to 50%) are available from the company. The assay can be run on the SaCycler-96™ manufactured by Sacace and described earlier in this report. But the test is platform independent and can also be run on the Rotor-Gene® 6000/Q (QIAGEN) as well.

No published, peer-reviewed evaluations of the assay were found.

Laboratory-based HCV RNA assays in the pipeline

VERIS MDx (*Beckman Coulter*)

Beckman Coulter has introduced its new, fully automated random access molecular diagnostics system, the VERIS MDx, pictured in Figure 22. This laboratory-based platform is a sample-to-answer system for the quantitative/qualitative analysis of molecular targets. The VERIS MDx system integrates the extraction, purification, quantification and results interpretation of infectious disease nucleic acid targets using PCR. This includes one-step sample introduction, proprietary bead extraction/purification, eluate transfer and reaction set-up, industry-standard RT qPCR amplification and detection, and results calculation and reporting.

Figure 22. VERIS MDx system

The VERIS MDx accepts several sample containers for plasma, serum and culture tubes; 48 samples can be lined up on 12 racks of 4 samples each. The time to result for DNA tests is approximately 70 minutes and for RNA tests is approximately 110 minutes. For multiplex analysis, five different detection colours are available with a bandwidth of 505 to 720 mm. The onboard capacity consists of 96 extraction and purification cartridges, and reagents are covered for 20 assays with 48 tests per assay. Reagents are stable in the machine for up to 14 days.

Depending on the assay, the VERIS MDx can process up to 450 samples in 24 hours. The system features walk-away time of at least two hours. The system also includes intuitive graphical touchscreen user interface and has laboratory information system interface capabilities.

The first assay for the VERIS MDx is the CMV, which is CE-IVD marked. Other assays in development for the VERIS MDx system include quantitative HIV viral load, quantitative HCV viral load and qualitative *C.*

trachomatis/N. gonorrhea (CT/NG), methicillin-resistant *Staphylococcus aureus* (MRSA), *C. difficile*, and human papillomavirus (HPV).

RT-TMA Technology (Real-Time Transcription Mediated Amplification) for the Panther® System (*Hologic Inc.*)

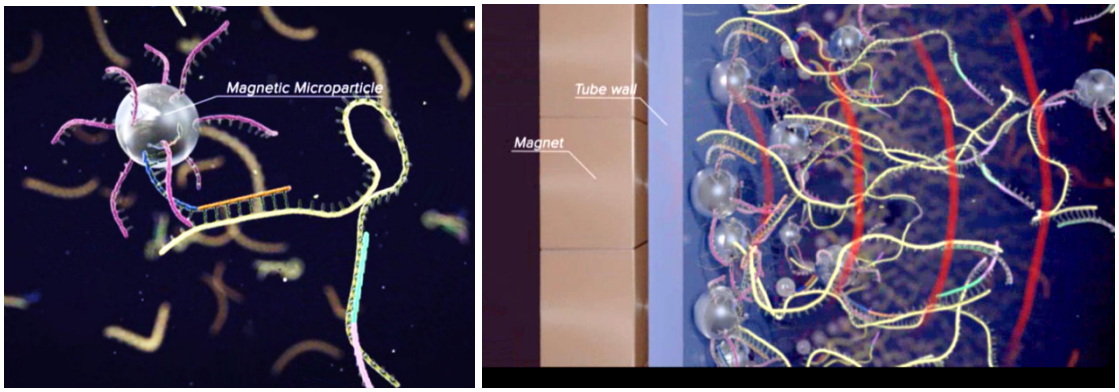
Hologic Inc. now offers the Panther® System, a fully integrated and automated molecular diagnostic platform with true random access testing capability. The platform brings the flexibility of clinical chemistry instrumentation to molecular diagnostics (Figure 23).

Figure 23. Panther® System

As part of its growing virology offering, the company is developing a quantitative HCV viral load assay, the Aptima® HCV Quant Dx assay for the Panther® System. The Aptima® assay requires three main processing steps, all of which take place in a single tube on the Panther® System: (i) target capture; (ii) target amplification by transcription-mediated amplification (TMA); and (iii) detection of the amplification products (amplicon) by fluorescent-labelled probes (torches).

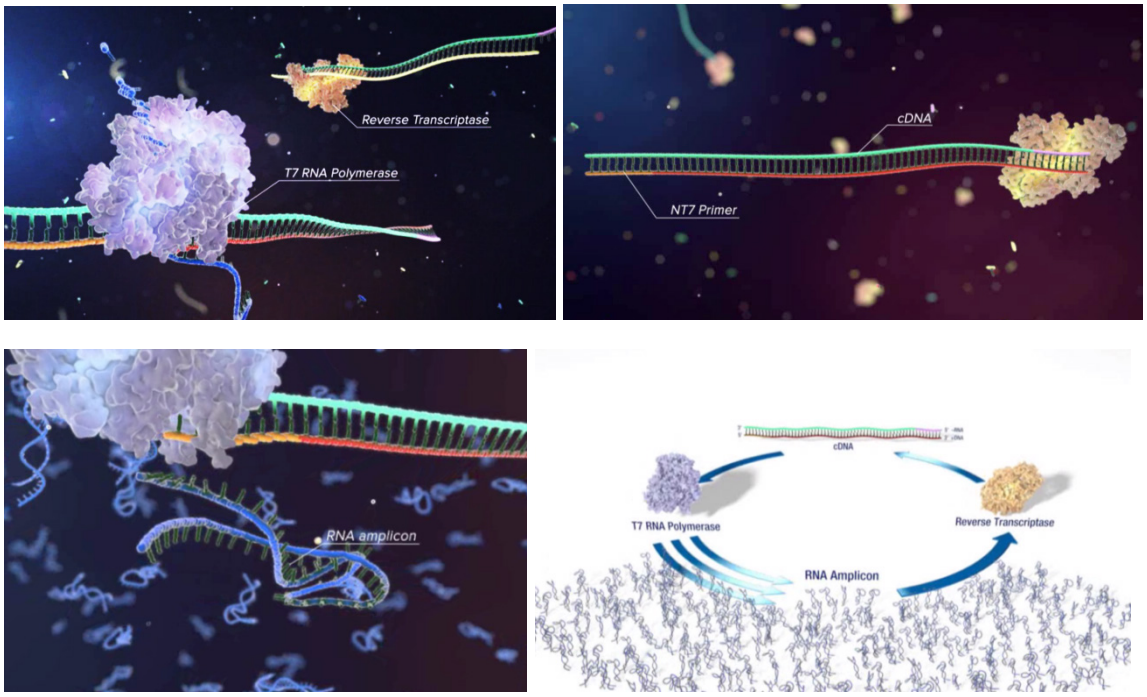
Target capture – During target capture, viral RNA is isolated from samples. The sample is treated with a detergent to release viral genomic RNA. Oligonucleotides hybridize to highly conserved regions of HCV RNA, if present, in the sample. As illustrated in Figure 24, the hybridized target is then captured on magnetic microparticles that are separated from the sample in a magnetic field. Finally, wash steps remove extraneous components from the reaction tube.

Figure 24. Target capture



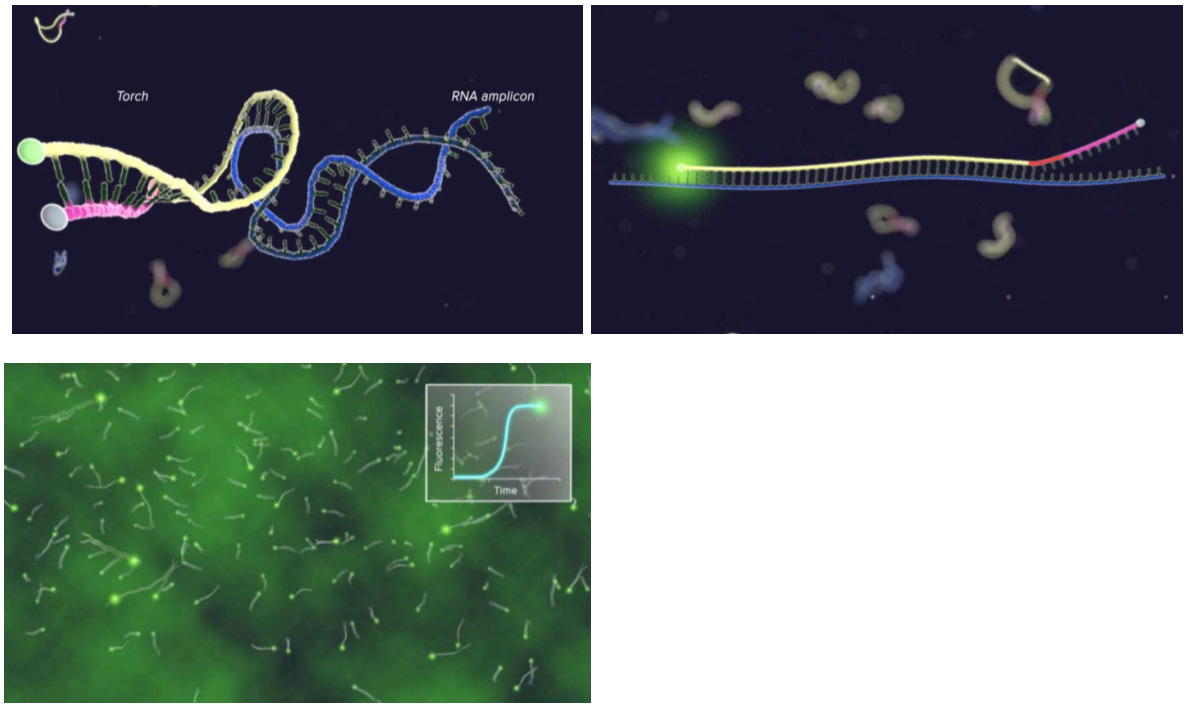
Target amplification – TMA is a transcription-based nucleic acid amplification method that utilizes two enzymes, RT and T7 RNA polymerase. The RT generates a DNA copy of the target sequence (containing a promoter sequence for T7 RNA polymerase). T7 RNA polymerase then produces multiple copies of RNA amplicon from the DNA copy template. The Aptima® assay utilizes TMA to amplify the 5'-UTR region of the HCV genome. The primer design ensures accurate detection and quantitation of HCV (Figure 25).

Figure 25. Target amplification



Detection of amplicon – Detection is achieved using single-stranded fluorescent probes (torches) that are present during the amplification and hybridize specifically to the amplicon in real time. The torches consist of a fluorophore and a quencher. When the torch binds to the amplicon, the fluorophore is separated from the quencher and will then emit fluorescence at a specific wavelength. As more torches hybridize to more amplicon, the fluorescent signal increases. The time taken for the fluorescent signal to reach a defined threshold is proportional to the starting HCV concentration. Each reaction also has an internal calibrator/internal control which controls for variations in sample processing, amplification and detection. The concentration of HCV in the sample is determined automatically by the Panther® System software using the HCV and internal control signals for each reaction and comparing them to stored calibration information (Figure 26).

Figure 26. Detection of amplicon

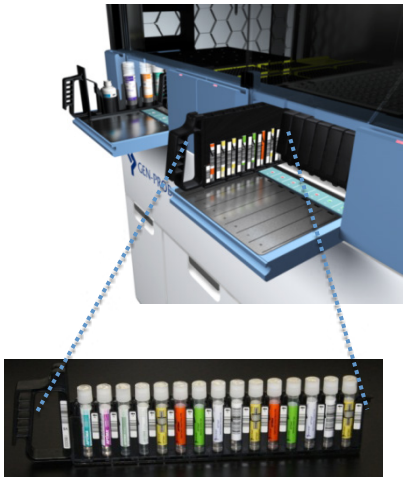


All nucleic acid testing steps, from primary sample tube to final results, are fully automated within the Panther® System, with the first reportable results available within three hours after loading samples and five results after every five minutes thereafter. Samples can be continuously loaded, with up to 120 samples on the Panther® System at a time. Reagent controls and calibration are valid for 24 hours. More than 300 samples can be run during an 8-hour shift, or 550 in a 12-hour period (resulting in an additional 225 samples that can be run without operator attendance) (Figure 27).

Four reagent lanes allow up to four Aptima® reagent kits to be onboard and randomly accessed at any time (e.g. four kits of the Aptima® HCV Quant Dx assay or any combination of the other molecular diagnostic assays available on the Panther® System including: HIV-1 Quant Dx, CT/NG; *Trichomonas vaginalis*; HPV; HPV genotyping; HBV Quant; and herpes simplex virus 1/2 assays).[18]

18 The HCV Quant Dx, HBV Quant and herpes simplex virus1/2* assays are in development.

Figure 27. Panther® continuous loading system



The Panther® System intuitive, task-driven software with touchscreen interface (Figure 28) simplifies set-up, adding or removing reagents or samples, and onboard inventory management of reagents and consumables. The bi-directional laboratory information system interface capability can automate requests for samples placed on the instrument and manage the release of results as configured by the operator. A low-volume dilution option allows quantitative results to be obtained from as little as 240 µL of specimen, with the software automatically adjusting for the dilution to report the actual concentration. The system can be programmed to perform automated maintenance outside of laboratory hours. Reagent management is simplified with 72-hour onboard stability and 30 days of refrigerated storage. Up to 2000 tests of common system fluids are managed by radio frequency identification (RFID) tags and the Panther® software.

Figure 28. Panther® touchscreen

Hologic Inc. will pursue CE-IVD, FDA and WHO prequalification for the Aptima® HCV Quant Dx Assay on the Panther® System. DBS is also being validated for use with the system. The assay is expected to be available with CE-IVD approval in 2015. Pricing will be variable and dependent on a variety of elements including instrument purchase, reagent rental and volume-based pricing.

POC HCV RNA platforms in the pipeline

Each of the NAT-based HCV RNA viral load systems described above requires testing to be done in a laboratory setting, generally speaking at a central or national reference laboratory (Level IV), by well-trained technicians. Each requires dedicated space, a clean room or rooms and other specialized and sophisticated infrastructure to diminish contamination and assure accurate testing. HCV viral load testing that could be conducted at or near the point of patient care would reduce the need for such infrastructure and would reduce the level of training required. In addition, the availability of quality POC HCV viral load testing would ensure that patients on treatment in remote areas would have access to appropriate diagnostic and monitoring tools with same-day test results, which can minimize loss to follow-up.

There are currently no POC HCV RNA viral load assays on the market. However, there are a number of platforms/assays in development, one or more of which will likely be launched in 2015. Described below are new quantitative HCV RNA assays in the pipeline.

The current viral load POC pipeline is presented in Appendix 2.

Alere™ q (*Alere Inc.*)

The Alere™ q (Figure 29), is a generic platform for the implementation of nucleic acid testing. One of the tests in the pipeline for the Alere™ q is a quantitative HCV viral load plasma assay. Other assays targeted for the platform include an integrated test for the qualitative detection of HIV-1 and HIV-2 simultaneously from 25 ml of whole blood as well as the release of additional tests for the quantitative measurement of HIV-1 and HIV-2 viral load from 500 µL of plasma and a test for the quantitative detection of HIV-1 and HIV-2 simultaneously from 25 ml of whole blood. Additional tests in development include those for the detection of MTB in sputum as well as a series of drug susceptibility tests for MTB. The device on which the assay is run has a small footprint, is portable, contains an integrated uninterrupted power supply (UPS), can be run either on mains power or from a dedicated battery pack, and is ruggedized to withstand harsh environments.

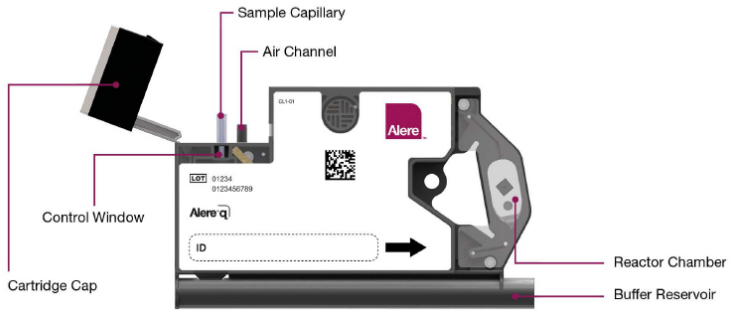
Figure 29. Alere™ q

The Alere™ q utilizes a single test cartridge that contains all reagents required for the assay in a stabilized form. The Alere™ q HCV viral load plasma test requires preparation of a plasma sample, which is then directly applied to the test cartridge. Once inserted into the Alere™ q analyser the cartridge is processed

performing cell lysis, target capture, reverse transcription, RT-PCR amplification and real-time fluorescence detection based on competitive reporter probe hybridization on an integrated micro array (Figure 30).

The disposable test cartridge is fully self-contained, and once capped, cannot be reopened; the cartridge remains completely sealed. At no time does the sample or the reagent actually come into contact with the analyser, thus greatly reducing any possibility for cross-contamination. The actual hands-on time for the device is expected to be less than three minutes (i.e. sample collection and loading of the cartridge onto the analyser).

Figure 30. Alere™ q test cartridge



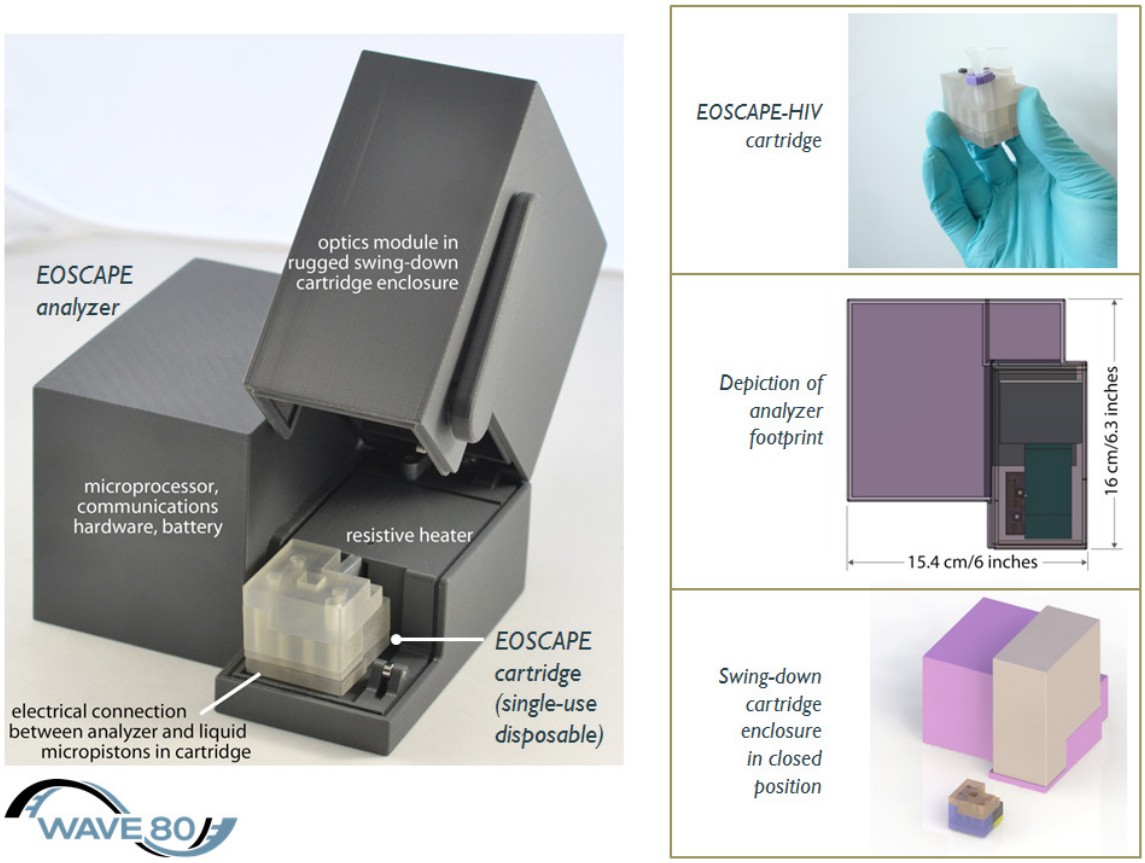
Test workflow for the operator is straightforward and consists of: (i) collecting blood via venipuncture, preparation of plasma sample and transferring the plasma directly into the cartridge sample collection chamber; (ii) manually capping the cartridge; (iii) inserting the cartridge into the analyser; and (iv) entering the operator and sample IDs on the analyser. When the assay is complete, audible and visual prompts alert the operator to remove the cartridge from the instrument and the results are displayed on a built-in screen. The result can be printed immediately, but results are also stored in an onboard archive and can be viewed and printed at a later date, exported to a USB memory stick or exported to a remote server via the use of an optional USB connectivity package that makes use of GSM mobile telephone network infrastructure.

The Alere™ q is CE-IVD marked (July 2014) and HCV, HIV as well as MTB assays are currently in development/regulatory approval with first commercial availability of the Alere™ q HCV viral load plasma assay expected in 2017. Pricing for the instrument and disposable test cartridges has not yet been determined.

EOSCAPE-HCV Rapid RNA Assay System *(Wave 80 Biosciences)*

Based on its liquid micropiston technology, Wave 80 Biosciences is developing a quantitative HCV viral load test designed for use in resource-limited settings. The EOSCAPE system (Figure 31) consists of: (i) a single-use, prefilled, disposable cartridge; and (ii) a small, bench-top analyser that runs on mains power with 4-hour battery backup if needed. The company is also developing a 16-cartridge unit. A tablet-format touchscreen user interface, which is separate from the device, is used for input of patient and other data. The system is easy to use and will require about one day of training for operators.

Figure 31. EOSCAPE System



In addition to an HCV assay, assays in development for the EOSCAPE system include single-plex assays for HIV viral load and for diagnosing active MTB infection, a 2-plex assay for CT/NG, and a 2-plex assay for acute respiratory infection. A variant of the system with enhanced multiplexing capability is also in development, with assays including a MTB drug resistance test and a 20-plex acute respiratory infection test, as well as other infectious and non-infectious diseases.

The EOSCAPE system is designed for health centre laboratories (Level I) in the tiered laboratory system.

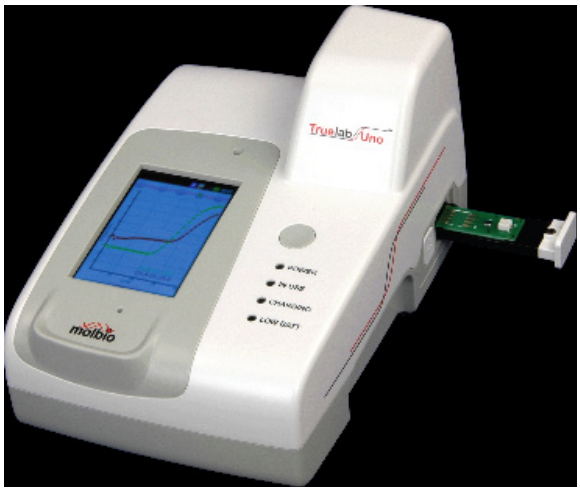
Truelab™ Real Time micro PCR System *(Molbio Diagnostics Pvt Ltd [A Tulip Group – Bigtec labs partnership])*

Molbio Diagnostics Pvt Ltd has developed a comprehensive, rapid, near-patient RT-PCR platform called the Truelab™ Real Time micro PCR System. The system is portable and includes all instrumentation, reagents and essential accessories that are required for the operator to conduct a real-time, quantitative PCR assay, from sample preparation through to final result reporting, all within one hour. A Truelab™ micro PCR printer is also available. The system works on ready-to-use Truenat™ disease-specific assays that are stable at room temperature. Assays for MTB, HBV, dengue fever, Chikungunya, H1N1 and malaria (both *P. falciparum* and *P. vivax*) are currently available, and quantitative assays for HIV viral load and HCV viral load, among others, are in development.

The testing process begins with sample collection (blood, serum or plasma) followed by extraction, which uses the Trueprep™ MAG Sample Prep Device and Trueprep™ MAG sample prep kits. The extraction process takes about 20–25 minutes per sample. From there, 6 µL of the extracted nucleic acid is dispensed into the reaction well of the disease-specific Truenat™ micro PCR chip. The chip, which contains all of the chemistry required to complete an assay, is then inserted into the Truelab™ Uno Real Time micro PCR Analyser, pictured below. Thermal cycling takes place automatically within the analyser (Figure 32).

During amplification, the Truenat™ micro PCR chip exponentially releases flurophores. These signals are captured by sensors and are displayed as an amplification curve on the Truelab™ screen. Test results are compared to lot-specific standard values preset into the Truenat™ chip, which enables quantitative estimation of the test analyte and displays as RT-PCR results in approximately 30 minutes. An internal control is provided from the extraction stage for a complete validation of the test results.

Figure 32. Truelab™ Uno Real Time micro PCR System



Test results are automatically stored in the analyser memory (up to 5000 results) and can be printed and transported wirelessly to any server/compatible device by Wi-Fi, GPRS, Bluetooth or even short message service (SMS).

The HCV viral load assay is currently undergoing laboratory-based trials in India. The assay is expected to be available in 2015.

GeneXpert® System (*Cepheid*)

The Cepheid GeneXpert® System, which is a fully automated and integrated system for PCR-based nucleic acid testing, currently has 14 FDA-cleared and 14 CE-IVD assays, including tests for enteroviral meningitis, MRSA, *C. difficile*, influenzas A and B, MTB detection and with simultaneous detection of resistance to rifampicin (MTB/RIF), CT/NG and group B Strep, among others. In addition to the tests listed, Cepheid has 14 tests in active development, including tests for HPV (simultaneous detection and typing), Qualitative and Quantitative HIV, Quantitative HCV, TV, and Carba-R (Carbapenemase Resistance). Any of these tests can be run on virtually all of the more than 5500 GeneXperts placed worldwide.

The GeneXpert® HCV viral load assay is on track to be launched commercially in 2015. The quantitative HCV assay will use 1 mL of plasma or serum. The assay targets the 5'UTR region of the HCV genome. The LLOD of the assay is 5 IU/mL for all HCV genotypes and has a linear range from 10 IU/mL to 100 million IU/mL.

The workflow for the quantitative assay is as easy as: (i) collecting 5 mL of whole blood in an EDTA plasma (K2EDTA or plasma preparation tube) or serum tube; (ii) separating plasma or serum from whole blood; (iii) transferring 1 mL of plasma or serum into the cartridge via transfer pipette; (iv) scanning the cartridge barcode; and (v) loading the cartridge into the GeneXpert® module and closing the door. The time to result is ~105 minutes.

Although it is not currently known what the price per cartridge will be for the HCV viral load assay, the FIND-negotiated price of the GeneXpert® System (with four modules, shown in Figure 33 on the left) for high-burden developing countries is approximately US$ 17 000; and, as a result of an agreement between the United States President's Emergency Plan for AIDS Relief (PEPFAR), the United States Agency for International Development (USAID), UNITAID and the Bill & Melinda Gates Foundation, the current price per cartridge for MTB/RIF is about US$ 9.98 in these countries. Uptake of the programme via USAID, PEPFAR and other agencies has been escalating rapidly; as of 30 June 2014, a total of 3269 GeneXpert instruments (comprising almost 16 000 modules) and more than 7.5 million Xpert MTB/RIF cartridges have been procured in the public sector in 108 of the 145 countries eligible for concessional pricing. All GeneXpert tests, including the proposed HCV RNA assay, can be run on the systems placed initially for TB testing.

Figure 33. GeneXpert® System (left) and cartridge (right)

The GeneXpert® System integrates and automates sample preparation, amplification and detection in a single-use, self-contained cartridge (shown in Figure 33 on the right). Most liquids and dry reagents along with enzymes are prefilled so that pre-analytical steps are minimized, greatly reducing opportunities for sample mix-ups and operational errors. GeneXpert cartridges can handle a variety of sample volumes (millilitre range) within macrofluidic chambers and then concentrate the target material down to microfluidic volumes, which can increase the sensitivity of the assays, if needed.

Furthermore, the GeneXpert® System is modular. Individual modules contain solid state circuitry that control temperature, pressure, rotation of the valve that moves the liquid between reservoirs, and the detection software. These individual modules are packaged in units of 1, 2, 4, 16, 48 or 80, and the latter two systems are fully automated, walk-away robotic instruments developed for high-throughput laboratory applications. Additionally, the modules can be removed and replaced individually so that the entire system is not incapacitated if one module fails.

The GeneXpert® System is sufficiently simple that training can usually be completed within half a day. Furthermore, although the system was designed to use AC power, its low wattage requirements allow it to be powered by a 12 VDC/120 VAC voltage converter in mobile laboratories, and it has also been installed in remote clinic sites powered by solar panels. The GeneXpert® software comes pre-installed on a desktop or laptop computer and results can be displayed for each module in real time or uploaded via an Internet connection to a central database. Wireless data connections via satellite phone networks are in development, as is a cloud-based system for remote access, online system calibration, and interfacing with the laboratory information system.

RT CPA HCV Viral Load Test (*Ustar Biotechnologies*)

Ustar Biotechnologies (hereafter Ustar) has developed Cross Priming Amplification (CPA), a novel isothermal nucleic acid amplification technology with multiple iterative designs that can address a wide variety of key obstacles to traditional amplification technologies such as PCR. By using multiple crossing primers and probes, target DNA sequences can be rapidly and precisely amplified at a uniform temperature (typically 63 °C) in an easy-to-use protocol with high sensitivity and specificity. By utilizing its CPA technology, Ustar is now developing assays for HIV, HCV, CT/NG and polio virus (the latter two together with PATH).

Recent work at Ustar and the University of Victoria has shown that RT-CPA can effectively amplify an RNA template with similar performance to existing DNA-based assays. After extensive testing, results indicate that the use of an RNA template does not alter the overall performance in CPA (e.g. sensitivity or specificity) compared to the use of a DNA template. Additionally, using novel enzymes together with inherent RT activity, as little as 0.1 picogram of RNA can be detected in less than 30 minutes. Therefore, the company believes that RT-CPA is an excellent candidate for the development of a new HCV viral load diagnostic test. Finally, Ustar also possesses a proprietary glassification process that stabilizes enzymes for ambient temperature transport and storage.[19]

The goal of Ustar is to develop a quantitative RT-CPA HCV viral load assay and test cartridge in conjunction with a robust and user-friendly portable instrument that will provide viral load measurements from fingerstick whole blood. For this purpose, Ustar plans to modify the commercially available Genie®, a portable instrument developed by OptiGene Ltd. In addition, Ustar will: (i) develop an automated sample preparation instrument and method for the extraction of viral RNA from whole blood; (ii) develop an RT-CPA assay for the detection and quantitation of all major HCV genotypes; and (iii) integrate the automated sample preparation instrument with the existing Genie® instrument for a fully automated sample-in, answer-out system.

The final Ustar diagnostic test kit is expected to be comprised of a reagent-containing cartridge and a portable device for sample preparation, amplification and detection. Reagents will consist of glassified enzymes for ambient temperature transport and storage, a reconstitution buffer and sample preparation buffers, all housed in the cartridge.

The testing process will require the user to: (i) take a fingerprick or heelprick and place a drop (100 µL) of blood directly the cartridge; and (ii) close the cartridge and place into the instrument for automated sample preparation, amplification and detection.

19 Glassification, or glass transition, refers to the transformation of a glass-forming liquid into a glass, which usually occurs as a result of rapid cooling. In dehydrated glass states, enzymes and antibodies have almost no biochemical activity, and as a result, are stable at a wide range of temperatures. Ustar has developed a simplified glass transition process for stabilizing certain DNA polymerase solutions.

A fully quantitative HCV viral load measure will be available in as little as 20 minutes (depending on the limit of detection required), and the sample can be run for 45 minutes to ensure a viral load measure of < 5000 IU/mL. The linear range is expected to be from < 10 000 IU/mL to 1 million IU/mL. Onboard software will calculate an offset value based on any delay in the amplification of the internal control caused by inhibition and a simple readout – "number of IU/mL", "not detectable" or "invalid" – will be available to the user and will be automatically uploaded to an external server (e.g. a national HCV programme), along with detailed information regarding each run.

Ustar is now actively working on the development of its HCV viral load assay with completion and launch expected in 2017.

Existing HCV cAg tests

As stated earlier in this report, there is currently only one fully automated HCV cAg assay, the ARCHITECT. It is described below.

ARCHITECT HCV cAg Assay (*Abbott*)

Abbott Diagnostics has developed the ARCHITECT HCV cAg assay, which is a CIA using microparticles coated with monoclonal anti-HCV for the detection of HCV cAg. The assay can be used as a reflex test to definitively diagnose individuals with active infection following a positive screening test for HCV; but it can also be used to detect HCV during the early window period of the disease. Moreover, because the assay is quantitative, it can be used to monitor the effectiveness of antiviral therapy as a complement to NAT-based testing, or in the light of all-DAA regimens, as a standalone assay for monitoring the clearance and cure of antiviral therapy.[20]

The ARCHITECT HCV cAg assay is a two-step immunoassay using CIA technology, with flexible assay protocols referred to as CHEMIFLEX, for the quantitative determination of core antigen of HCV. The assay includes a sample pretreatment step followed by combining the pretreated sample, an assay-specific diluent and anti-HCV microparticles. The objectives of the pretreatment step (which is automated) are to: (i) dissociate antibody-bound core antigen; (ii) lyse viral particles and expose core antigen; and (iii) inactivate antibody. The HCV cAg present in the pretreated sample binds to the anti-HCV coated microparticles in the first step. After washing, acridinium-labelled anti-HCV conjugate is added in the second step. Following another wash cycle, with Pre-Trigger (containing 1.32% hydrogen peroxide) and Trigger (containing 0.35N sodium hydroxide) solutions, the resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HCV cAg in the sample and the RLUs detected by the ARCHITECT optical system.

The concentration of hepatitis cAg in the specimen is determined using a previously generated ARCHITECT HCV cAg calibration curve. If the concentration of the specimen is ≥3.00 fmol/L, the specimen is considered reactive for HCV cAg.

There are several performance studies of the ARCHITECT HCV cAg assay *(63–65)*, which found the assay to be highly specific and easy to perform *(63)*, and to have utility for reflex testing on anti-HCV positive individual to confirm or exclude an active HCV infection *(64)* and for screening for acute HCV infection *(65)*.

The question with respect to cAg assays is whether they are sensitive enough to be used for HCV treatment monitoring. Although the ARCHITECT assay is not as sensitive as HCV RNA assays, it has demonstrated good correlation with such assays regardless of the HCV genotype *(66–67)*. Chevaliez et al. concluded that the ARCHITECT HCV cAg assay is «a valuable screening, diagnostic and monitoring tool, especially in the era of new all-oral IFN-free antivral strategies that do not require high analytical sensitivity» *(63)*.

20 The concentration of cAg of a patient specimen is expressed in fmol/L, which has a non-linear correlation with HCV RNA. The level of HCV cAg can be calculated as an RNA-equivalent viral load. For example, 3 fmol/L of cAg corresponds to between 700 and 1100 IU/mL of HCV RNA.

The price of the ARCHITECT HCV cAg assay, which is not for sale in the United States, is available from Abbott.

i2000SR Platforms (*Abbott*)

The ARCHITECT HCV cAg assay can be performed on the i2000SR (Figure 34), a laboratory-based analyser from Abbott.

Figure 34. ARCHITECT i2000SR

The i2000SR analyser uses CHEMIFLEX, and includes a robotic sample handler and immediate "STAT" processing. The i2000SR accommodates 200 tests per hour and can be integrated with a chemistry analyser to consolidate clinical chemistry and immunoassays on a single platform.

The price of the analyser is available from Abbott.

HCV cAg assays in the pipeline

Daktari™ System (*Daktari Diagnostics Inc.*)

Daktari Diagnostics Inc. (hereafter Daktari) has developed a portable and robust diagnostic device that will be capable of a number of assays. Currently, the platform performs an absolute CD4 assay, which was launched in 2014. Additional assays for the system include an HCV cAg assay for diagnosis of HCV as well as for quantitative determination of HCV cAg. The Daktari™ system will be capable of other assays, which may include full blood counts, CD4 percentages, and bacterial and HIV viral load diagnostics (Figure 35). The HCV assay is expected to be launched in 2016.

Figure 35. Daktari™ System

Intended for use at the point of patient care, the Daktari™ system eliminates sample preparation through the use of a technology known as "microfluidic immunochromatography", which isolates cells (or viruses) in a miniature sensing chamber. No pipetting, labels or reagents are required; the only user step is to apply a drop of whole blood to the cartridge. Similarly, the Daktari™ device does not require fragile and expensive optical sensors, but rather uses a second group of innovations in electrochemical spectroscopy, which provide 10^{10}-fold signal amplification, on a par with PCR-based detection methods, and require only a simple sensor to interpret the electrical signal, and quantify cell counts or viral load. The Daktari™ instrument reports results in 15 minutes for cell counts, and 30 minutes for viral load (cAg) assays.

The Daktari™ system includes an integrated data management system with a keypad user interface, wireless data transmission and a back-end data package that can stand alone or can be integrated with customer databases.

The anticipated cost of the Daktari™ Instrument is less than US$ 8000 for the device. Per test cost of the HCV assay is anticipated to be approximately US$ 15–20, but volume discounts may drive the price lower. If the instrument is damaged, then the low cost and portability would allow it to be swapped out with a replacement device rather than being repaired onsite.

Future directions for HCV testing

This report has detailed the current continuum of testing for HCV, which with existing HCV treatment regimens, includes screening and confirmatory testing, genotyping, fibrosis staging, prognostic marker testing and treatment monitoring. The continuum is complex and expensive. As a result, in many resource-limited settings, few have access to testing, and HCV often remains undiagnosed until patients present at healthcare facilities with symptoms that may be the result of serious liver disease, including liver cancer.

Many of the tests in the existing continuum of testing for HCV, including genotyping, prognostic marker testing and fully quantitative HCV viral load, are dictated by existing treatment regimens that consist of peg-IFN-riba with or without oral drugs like boceprevir and sofosbuvir. For reasons that include cost, infrastructure requirements and the need for trained laboratory technicians, many of these tests are not available in resource-limited settings or are only available at national reference laboratories. In order to reach patients in peri-urban and rural settings with laboratory-based HCV testing, it would be necessary to set up sample transport networks to transfer patient blood samples to the reference laboratory for testing and to return results to the patient. These demands put pressure on the sample transport system and add

costs to the process. The use of DBS with some of the laboratory-based HCV testing platforms could help to make the sample transport process more manageable, removing some of the time pressure.

The introduction of all-DAA regimens will make it possible to simplify the current continuum of testing for HCV, likely eliminating the need for genotyping and prognostic marker testing. While screening and confirmatory testing will still be required, it is anticipated that baseline quantification of HCV may not be necessary for patients with chronic HCV. Rather, it is expected that with these shorter and more effective regimens, quantitative HCV RNA testing for HCV treatment monitoring can be eliminated. Instead, it would be possible to use a highly sensitive qualitative HCV RNA assay or HCV cAg assay to: (i) confirm the presence of the virus for diagnosis of HCV; (ii) test for HCV clearance at the end of treatment; and (iii) test to confirm cure at a defined point post-treatment.

Other required testing for the HCV patient can also be simplified with all-DAA regimens, especially in resource-limited settings. Staging of liver disease before putting HCV patients on treatment will likely also continue to be necessary, with the possible exception of HIV coinfected patients; but non-invasive techniques using biomarkers or TE are already available. Toxicity monitoring of patients on treatment will also likely continue to be required, but for all oral regimens such monitoring may consist of ALT, creatinine and haemoglobin testing, all of which are generally available in resource-limited settings.

The advent of all-DAA treatment regimens for HCV offers great promise in resource-limited settings. However, in order to improve access to HCV testing and bring down the cost, more and better RDTs for screening HCV are needed. Two such platforms are in the pipeline, although their performance is not yet known. RDTs that can reliably detect HCV in HIV coinfected patients are a particular need. In addition to being robust and quality assured, all HCV RDTs should be easy to use and robust, with high temperature/humidity tolerance and no cold chain requirements.

The development and market introduction of sensitive HCV RNA and/or HCV cAg tests for use at or near the point of patient care are also needed. There is a reasonably robust pipeline of HCV such assays, one of which is an HCV cAg assay. With respect to HCV cAg assays, more studies are needed to demonstrate that these assays are sufficiently sensitive for use in diagnosis and testing for HCV clearance/cure in all-DAA regimens.

The remaining products in the pipeline are expected to be fully quantitative HCV viral load platforms. An open question is whether such quantitative HCV viral load tests will be required in the market going forward, or whether in light of all-oral HCV treatment regimens, highly sensitive qualitative HCV RNA or cAg assays would be sufficient and could be provided at lower cost. Developers of new POC HCV assays need guidance from the stakeholder community with respect to the key market requirements for HCV screening/diagnosis as well as clearance/cure testing. Stakeholder-vetted target product profiles are being developed by FIND and will be useful in this respect.

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Appendix 1: Operational characteristics of diagnostic platforms

A. HCV diagnostic platforms on the market

HCV Assays: Roche Molecular Systems	
Roche COBAS®AmpliPrep/COBAS® TaqMan® HCV Qualitative Test version 2	
Type of assay	Qualitative in vitro NAAT for the detection of HCV genotypes
Target region of HCV genome	Conserved 5'UTR
Genotype and subtype	1 to 6
Sensitivity (LLOD)	15 IU/mL
Specificity	99.8%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/798)
Regulatory approval	CE-IVD marked
Cost per test	€34–40 (US$ 43–51)
Equipment	COBAS® AmpliPrep System for sample preparation; COBAS® TaqMan® 48 for amplification and detection
Roche COBAS®AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0	
Type of assay	NAAT for the quantitation of HCV RNA in human serum or EDTA plasma using dual-labelled fluorescent probe
Target region of HCV genome	Conserved 5'UTR
Genotype and subtype	1a, 1b, 2a, 2b, 3, 4, 5, 6
Sensitivity (LLOD)	15 IU/mL (assay reports levels of 1 to 14 IU/mL as HCV RNA detected below the LLOQ)
Linear range	15 IU/mL–100 million IU/mL
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/798)
Regulatory approval	CE-IVD marked; FDA approved
Cost per test	€28–30 (US$ 36–38)
Equipment	COBAS® AmpliPrep System for sample preparation; COBAS® TaqMan® 48 for amplification and detection
COBAS® TaqMan® HCV Quantitative Test v2.0 for use with the High Pure System	
Type of assay	NAAT for the quantitation of HCV RNA in human serum or EDTA plasma using dual-labelled fluorescent probe
Target region of HCV genome	Conserved 5'UTR
Genotype and subtype	1a, 1b, 2a, 2b, 3, 4, 5, 6
Sensitivity (LLOD)	20 IU/mL across all genotypes
Linear range	25 IU/mL–390 million IU/mL
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/798)
Regulatory approval	CE-IVD marked; FDA approved
Cost per test	€28–30 (US$ 36–38)
Equipment	Manual sample preparation using High Pure System Viral Nucleic Acid Kit; COBAS® TaqMan® 48 for amplification and detection

RT-PCR: Roche COBAS® AmpliPrep System automated extraction instrument	
Type of technology	Automated extraction and sample preparation
Output	Samples ready for amplification and detection on COBAS® TaqMan® Analyser
Turnaround time	Three racks of 24 specimens in approximately 5 hours; with 216 seconds processing time per specimen
Capacity (per run)	72 samples per run (maximum) that can be analysed simultaneously Batch size is 24 specimens per run
Throughput per technician/per day	Up to 168 specimens per 8-hour shift, based on testing combinations and laboratory workflow
Sample needed and stability	Sample needed varies by assay (200 µL for qualitative assay; 650 µL for quantitative assay; 500 µL for quantitative assay with High Pure) Plasma can be transported/stored at 2–8 °C for five days or frozen at - 70 °C
Specimen preparation and protocol complexity	Plasma transferred to a properly identified, sterile screw-cap, polypropylene tube after centrifugation Requires test-specific, barcoded, ready-to-use COBAS® AmpliPrep Kits Reagents are all liquid and ready to use, but specimens require mixing to HCV RNA uniformity prior to testing
Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F); all reagents are stable until expiration date
Cost/instrument	Approximately US$ 80 000–100 000
Physical dimensions (cytometer only) (W x D x H)	Width: 165 cm (65 in) Depth: 75 cm (29.5 in) Height: 95 cm (37.4 in) Trolley table: 167 cm (65.7 in) x 76 cm (29.9 in) x 55 cm (21.7 in)
Weight	373 kg (822 lbs)
3rd party supplies	Pipettors, vortex mixer, refrigerator, gloves and other laboratory consumables
Electric power requirements	100–125 V AC mains and 200–240 V AC mains (+10, -15%) 50–60 Hz
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: <80% (for temperatures up to 32 °C) Maximum altitude: 2000 metres (6500 feet)
Data station	Custom-built PC (included) with Microsoft® Windows® XP and AMPLILINK® software to control COBAS® AmpliPrep System
Monitor	Monitor VGA 14 in
Printer	Printer HP 1320; printer interface: LPT interface via parallel port
Barcode scanner	Supplied with instrument COBAS® AmpliPrep: onboard barcode scanner for reagent racks, reagent cassettes and specimen clips AMPLILINK® data station: hand-held barcode scanner for original specimen/specimen clip
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Internal control/quantitation standard is incorporated into each individual sample and is carried through the sample preparation Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Roche COBAS® TaqMan® 48 Automated amplification/detection instrument	
Type of technology	Fully automated real-time amplification and detection
Output	HCV RNA quantification or qualitative detection of HCV genotypes, depending on assay
Turnaround time	Amplification and detection cycle takes 3 hours and 5 minutes
Capacity (per run)	Two independent segments of 24 samples each up to two different tests onboard simultaneously; each thermal cycler can run individual PCR profiles
Throughput per technician/per day	Including processing time on AmpliPrep, 48 samples (on an 8-hour shift)
Sample needed and stability	PCR-ready setup samples from AmpliPrep; processed specimens and controls should not be exposed to light after completion of specimen and control preparation
Sample preparation and protocol complexity	Once removed from the COBAS® AmpliPrep Instrument, processed specimens and processed controls can be stored in the output tubes at 2–8 °C for up to one day (24 hours) Preparation of reagent cassettes for amplification and extraction is moderately complex
Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F); all reagents are stable until expiration date
Cost/instrument	US$ 40 000–50 000
Physical dimensions (W x D x H)	Width: 50 cm (19.7 in) Depth: 79 cm (31.1 in) Height: 58 cm (22.8 in)
Weight	55 kg (121 lbs)
3rd party supplies	Microtiter plate centrifuge (not supplied by Roche) and other general supplies
Electric power requirements	120 or 240 V AC mains 50–60 Hz
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: <80% (for temperatures up to 32 °C) Maximum altitude: 2000 metres (6500 feet)
Peripherals/supporting instrumentation	Custom-built PC supplied with the analyser; data station runs Microsoft® Windows® XP Professional operating system and AMPLILINK® software AMPLILINK® software is a Windows®-based, laboratory information system-compatible user interface that manages up to three COBAS® TaqMan® 48 Analysers
Barcode scanner	AMPLILINK® hand-held barcode scanner for original specimen/specimen clip
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are included as part of reagent kit and required for each preparation run for FDA-approved assays
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

HCV Assays: Abbott Diagnostics	
ARCHITECT Anti-HCV cAg Assay	
Type of assay	Chemiluminescent microparticle immunoassay for the quantitative detection of HCV cAg for use in the diagnosis of HCV as well as monitoring of infected individuals
Target region of HCV genome	Core antigen
Genotype and subtype	1 to 6 (for samples yielding concentrations of ≥10 fmol/L)
Sensitivity (LLOD)	3 fmol/L (corresponds to 700–1100 IU/mL of HCV RNA)
Specificity	≥99.5%
Standardization	N/A1[21]
Regulatory Approval	CE-IVD marked
Cost per test	Available from Abbott
Equipment	Abbott i2000SR (described below)
Abbott RealTime HCV Genotype II Assay	
Type of assay	RT-PCR assay for the identification of HCV genotype 1-6 in human plasma or serum
Target region of HCV genome	5'UTR for classification of HCV genotypes 1 to 5; and NS5b region to subtype HCV sub-genotypes 1a and 1b
Genotype and subtype	1, 1a, 1b, and 2 to 6
Sensitivity (LLOD)	500 IU/mL with a 0.5 mL sample volume
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/798)
Regulatory approval	CE-IVD marked
Cost per test	Available from Abbott
Equipment	Abbott *m*24sp, *m*2000*sp* and *m*2000*rt* instruments
Abbott RealTime HCV Assay	
Type of assay	RT-PCR assay combined with homogeneous real time fluorescent detection for the quantitation of HCR RNA
Target region of HCV genome	5'UTR
Genotype and subtype	1 through 6
Sensitivity (LLOD)	12 IU/mL with a 0.5 mL sample volume and 30 IU/mL with a 0.2 mL sample volume
Specificity	99.5%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/790)
Regulatory approval	CE-IVD marked
Cost per test	Available from Abbott
Equipment	Abbott *m*24sp, *m*2000*sp* and *m*2000*rt* instruments
Abbott i2000SR	
Type of technology	Fully automated immunoassay analyser using CHEMIFLEX
Output	Quantitative detection of HCV-Ag
Turnaround time (full run)	Unknown
Capacity (per run)	Up to 200 tests per hour 130 sample load capacity
Throughput per technician/per day	Up to 1600 tests per 8-hour day

21 Throughout the tables, N/A means the information is not available.

Sample needed and stability	158 µL for the first HCV cAg test plus 108 µL for each additional HCV cAg test from same sample cup Human serum (included serum collected in serum separator tubes) Human plasma collected in Sodium EDTa, Potassium EDTA and equivalent Specimens may be stored on or off the clot, red blood cells, or separator gel for up to five days refrigerated at 2–8 °C
Sample preparation and protocol complexity	Moderately complex Steps include vortexing (internal control, calibrators, controls and specimens) pipetting, centrifuge, etc.
Reagent stability	Reagents must be stored at 2–8 °C in an upright position ARCHITECT HCV cAg Reagent Kit may be stored on board the ARCHITECT System (in a refrigerated reagent carousel) for a maximum of 30 days
Cost/test	N/A
Cost/instrument	US$
Physical dimensions (W x D x H)	Width: 61 in (154.9 cm) Depth: 49 in (124.5 cm) Height: 48 in (121.9 cm)
Weight	1081 lbs (490.3 kg)
3rd party supplies	Pipettes, vortex mixer and refrigerator; freezer
Electric power requirements	AC 180–264 V, 47–63 Hz
Environmental requirements	Temperature: Humidity: Maximum altitude:
Peripherals/supporting instrumentation	Data station, colour touchscreen monitor, keyboard and mouse; remote diagnostics through AbbottLink®
Barcode scanner	Yes, integral to instrument; sample result storage: 50 000
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	External controls are required, but must be purchased separately Internal control: is integral to the assay.
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories
RT-PCR: Abbott m2000sp Automated extraction instrument	
Type of technology	Automated extraction and sample preparation (magnetic particle technology)
Output	Automated preparation and extraction of samples, creation of PCR master mix and creation of PCR plate for use on the Abbott *m*2000*rt*
Turnaround time	HCV genotype: extractions: depends on number of samples from 2 hours and 39 minutes for 24 samples to 4 hours and 54 minutes for 96 samples Amplification and detection: 3 hours per run (up to 96 samples) HCV RNA: extraction inclusive of PCR plate preparation: depends on number of samples from 2 hours and 30 minutes for 24 samples to 4 hours and 45 minutes for 96 samples Amplification and detection: 3 hours per run (up to 96 samples)
Capacity (per run)	HCV genotype: 96 samples (1–94 patient samples + two controls) HCV RNA: 96 samples (1–93 patient samples + three controls)
Throughput per technician/per day	HCV genotype and HCV RNA 192 samples (two batches of 96 samples)

Sample needed and stability	HCV genotype and HCV RNA: 500 µL of plasma or serum HCV genotype and HCV RNA: freshly drawn whole blood can be held at 2–30 °C for up to 6 hours After centrifugation, serum or plasma can be stored at 15–30 °C for up to 24 hours, at 2–8 °C for up to three days, from -25 to -15 °C for up to 60 days, and at -70 °C for up to 60 days
Sample preparation and protocol complexity	Moderately complex Steps include vortexing (internal control, calibrators if applicable, controls and specimens), pipetting, centrifuge, etc. Once all required consumables, reagents and samples are placed in the *m*2000*sp*, each process is walk away (extraction and mastermix addition)
Reagent stability	HCV genotype: reagent packs, as well as controls (internal, negative and positive controls), must be stored from -25 °C to -15 °C or colder when not in use and must be shipped on dry ice *m*Plus (Amplification Reagent Extended Use) allows reuse of amplification reagent HCV RNA: amplification reagents, controls and calibrators must be stored at -10 °C or colder when not in use Reagents are shipped on dry ice Extraction reagents are ready to use and can be stored at 15–30 °C All reagents are stable until expiration date
Cost/test	N/A
Cost/instrument	US$ 162 000
Physical dimensions (W x D x H) - 70 °C	Width: 145 cm (57.1 in) Depth: 78 cm (30.7 in) Height: 174.5 cm (68.7 in)
Weight	211 kg (465 lbs)
3rd party supplies	Pipettes, vortex mixer and refrigerator, freezer
Electric power requirements	100–240 V
Environmental requirements	Temperature: 15–30 °C (59–86 °F) Humidity: 30–80% relative non-condensing at 30 °C (86 °F or below) Maximum altitude: up to 2000 metres (6600 feet)
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Barcode scanner	Supplied with instrument (integrated on work desk)
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are available and required for each preparation run, two (HCV genotype) or three (HCV VL) controls per run up to 96 batch size Internal control: a defined, consistent quantity of internal control is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity; the internal control is comprised of an RNA sequence unrelated to the HCV target sequence
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

RT-PCR: Abbott m2000rt Automated amplification/detection instrument	
Type of technology	Fully automated real-time amplification and detection
Output	Objective qualitative results for HCV genotype determination or quantitative HCV viral load results
Turnaround time	Amplification and detection cycle takes 3 hours
Capacity (per run)	HCV genotype: 24 samples (1–22 patient samples + two controls) HCV RNA: 96 samples (1–93 patient samples + three controls)
Throughput per technician/per day	288 samples per day; sample preparation and extraction can be the limiting factor
Sample needed and stability	PCR-ready samples (nucleic acid from *m*2000*sp*, *m*24*sp* or manual sample preparation/ extraction protocol including mastermix addition)
Sample preparation and protocol complexity	Manual sample preparation: moderately complex Steps include vortexing (internal control, calibrators, controls and specimens), pipetting, centrifuging, etc. *m*2000*rt*: easy to use User-friendly software, multiple languages available, very limited hands on time needed Work list can be imported via network, CD-ROM or created manually Once 96-well plate is loaded in the *m*2000*rt*, process is walk away
Reagent stability	No onboard reagents are required on the instrument All reagent addition is performed during the sample preparation process
Cost/test	N/A
Cost/instrument	US$ 45 000
Physical dimensions (W x D x H)	Width: 34 cm (13.4 in) Depth: 48 cm (17.8 in) Height: 49 cm (19.3 in)
Weight	75.2 lbs (34.1 kg)
Electric power requirements	100–240 V
Environmental requirements	Temperature: 15–30 °C (59–86 °F) Humidity: 30–80% relative humidity, non-condensing Maximum altitude: not exceeding 3000 metres (9800 feet) above sea level
Peripherals/supporting instrumentation	Data station, monitor and printer are supplied with the instrument
Barcode scanner	Supplied with the instrument
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are available and required for each run, two (HCV genotype) or three (HCV RNA) controls per run up to 96 batch size Internal control: a defined, consistent quantity of internal control is introduced into each specimen and control at the beginning of sample preparation and detected on the Abbott *m*2000*rt* instrument to demonstrate proper specimen processing and assay validity The internal control is comprised of an RNA sequence unrelated to the HCV target sequence
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

HCV Assays: Siemens Healthcare Diagnostics Inc.	
VERSANT® HCV Genotype 2 LiPA Assay	
Type of assay	Line probe assay using reverse hybridization for the identification of the genotype(s) of HCV in plasma or serum
Target region of HCV genome	5'UTR plus core motifs to identify genotype 6, subtypes c to l, and to improve the accuracy of the identification of genotypes 1a and 1b
Genotype and subtype	1a, 1b, 2, 3, 4, 5 and 6 (subtypes c-l)
Sensitivity (LLOD)	96%
Specificity	99.4%
Standardization	N/A
Regulatory approval	CE-IVD marked
Cost per test	
Equipment	Requires QIAamp DSP Virus Kit (QIAGEN), VERSANT® HCV Amplification 2.0 Kit (LiPA) and VERSANT® HCV Control 2.0 Kit (LiPA); assay may be performed on an Applied BioSystems thermal cycler model GeneAmp PCR System 9700 or equivalent (not described in this report)
VERSANT® HCV RNA 1.0 Assay (kPCR)	
Type of assay	RT kinetic polymerase chain reaction (kPCR) assay for quantitative detection of HCV RNA in plasma or serum
Target region of HCV genome	5'UTR
Genotype and subtype	1 to 6
Sensitivity (LLOD)	15 IU/mL
Linear range	15 IU/mL–100 million IU/mL
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 06/100)
Regulatory approval	CE-IVD marked
Cost per test	
Equipment	VERSANT® kPCR Molecular System
Real-time PCR: VERSANT® kPCR Molecular System automated sample preparation and amplification/detection modules	
Type of technology	Automated real-time extraction, amplification and detection (kPCR technique)
Output	HCV RNA quantification
Turnaround time	Sample preparation system setup <10 minutes; sample extraction <3 hours; amplification detection <3 hours
Capacity (per run)	96 tests per run (91 clinical samples and two calibrators and three controls) run in less than 6 hours; flexible run sizes of 1–96 tests per batch
Throughput per technician/per day	Up to 182 patient results per shift
Sample needed and stability	Up to 500 µL input volume; whole blood collected in EDTA tubes can be stored for 6 hours at room temperature or for up to 24 hours at 2–8 °C before centrifugation; plasma can be stored for up to 24 hours at room temperature or for up to five days at 2–8 °C
Sample preparation and protocol complexity	Steps: (i) load the dedicated sample preparation reagents into a trough; (ii) place the reagents on the module; (iii) load plasma samples onto the sample carrier; and (iv) place the sample carriers on the auto load tray of the VERSANT® Sample Prep module – from that point on, sample preparation module is fully automated

Reagent stability	Reagents are stored frozen (from -30 °C to -10 °C); calibrators and controls are stored frozen (from -90 °C to -60 °C)
Cost/test	N/A
Cost/instrument	N/A
Physical dimensions: sample preparation module; application/detection module (W x D x H)	Width: 112.4 cm (44 in)/ 36.8 cm (14.5 in) Depth: 100.6 cm (39.5 in)/53.4 cm (21 in) Height: 90.5 cm (35.5 in)/45.7 cm (18 in)
Weight: sample preparation module; application/detection module	320 lbs (145 kg)/55 lbs (25 kg)
Electric power requirements	100–240 V; 50 or 60 Hz
Environmental requirements	Temperature: 18–30 °C Humidity: 30–80% non-condensing Maximum altitude: 0–2000 metres (6560 feet)
Peripherals/supporting instrumentation Physical dimensions Weight	Computer supplied 17 in screen and separate keyboard; printer options 38.1 cm × 14.0 cm × 33.0 cm (15 in × 5.5 in × 13 in) 12 kg (26 lbs)
Barcode scanner	Supplied with the instrument
Training	Fully trained laboratory technician required; dedicated training on instrument; electronic training for VERSANT kPCR is widely available using Siemens Personalized Education Program (PEP)
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are included as part of reagent kit and required for each preparation run
EQA	Amenable to EQA
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

HCV Assays: Sacace Biotechnologies	
HCV Genotype Plus Real-TM Assay	
Type of assay	RT-PCR assay using fluorescence detection for the identification of HCV genotypes
Target region of HCV genome	5'UTR
Genotype and subtype	1a, 1b, 2, 3, 4, 5a and 6
Sensitivity (LLOD)	1000 IU/mL; 100%
Specificity	100%
Regulatory approval	None
Cost per test	
Equipment	SaCycler-96™ and Rotor-Gene™ (QIAGEN), LineGeneK™ (Bioer Technologies), or SmartCycler™ (Cepheid); manual sample extraction (Sacace Biotechnologies or other) or automated sample extraction (e.g. NucliSens® easyMAG™ [bioMérieux])
HCV Real-TM Quant Dx Assay	
Type of assay	RT-PCR assay using fluorescence detection for the quantification of HCV RNA
Target region of HCV genome	5'UTR
Genotype and subtype	1 to 6
Sensitivity (LLOD)	13 IU/mL with 1.0 mL sample preparation procedure
Linear range	13 IU/mL–100 million IU/mL with 1.0 mL sample preparation procedure
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 06/102 15)
Regulatory approval	CE-IVD marked
Cost per test	
Equipment	SaCycler-96™ and Rotor-Gene™ (QIAGEN); manual sample extraction (Sacace Biotechnologies or other) or automated sample extraction (easyMAG [bioMérieux], SaMag [Sacace Biotechnologies])
bioMérieux NucliSens® easyMAG® Automated extraction instrument (for use with Sacace Biotechnologies HCV assays)	
Type of technology	Automated extraction instrument
Output	Purified nucleic acids (RNA and DNA)
Turnaround time	24 samples, lysis onboard: 60 minutes 24 samples, lysis offboard: 40 minutes
Capacity (per run)	1–24 patient samples per run
Throughput per technician/per day	Up to 168 extractions per shift – lysis onboard workflow Up to 240 extractions – lysis in tube workflow
Sample needed and stability	100–1000 µL plasma; whole blood collected in EDTA should be separated into plasma and cellular components by centrifugation within 6 hours Isolated plasma may be stored at 2-8 °C for an additional three days Alternatively, plasma may be stored at -18 °C for up to one month or for one year when stored at -70 °C
Specimen preparation and protocol complexity	Entire extraction process takes place in a single sample compartment, which minimizes potential sample loss and cross-contamination Reagents are ready to use

Reagent stability	HCV genotype assay: controls may be stored at 2–8 °C; "Reverta-L" and HCV genotype reagents must be stored at -20 °C HCV RNA Quant assay: all elements of kit must be stored at 2–8 °C All reagents are stable until expiration date
Cost/test	N/A
Cost/instrument	Approximately €72 000 (US$ 95 000)
Physical dimensions (cytometer only) (W x D x H)	Width: 100 cm (39.4 in) Depth: 65 cm (25.6 in) Height: 53 cm (20.9 in)
Weight	106 kg (233.7 lbs); PC monitor and keyboard: 8 kg (17.6 lbs)
3rd party supplies	Dedicated pipettes and filter tips, vortex mixer and refrigerator
Electric power requirements	100–240 V AC mains 50–60 Hz
Environmental requirements	Operating temperature: 15–30 °C Humidity: maximum relative humidity: 80%, non-condensing at 30 °C Maximum altitude: 2500 metres (8202 feet)
Monitor	Onboard monitor
Printer	None supplied
Barcode scanner	Supplied with the system
Training	Fully trained laboratory technician required; dedicated training on instrument that requires strong computer skills
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Yes; a synthetic calibrator added in a known concentration at the extraction stage, functions as an internal control for the isolation, amplification and detection procedure
Infrastructure requirements	Technology can be used at regional/central or national reference (or comparable) laboratories
SaCyler 96™ amplification/detection instrument	
Type of technology	Automated amplification and detection analysis using RT-PCR
Output	HCV genotypes or quantification of HCV RNA
Turnaround time	~2 hours, depending on extraction method used
Capacity (per run)	Up to 96 samples
Throughput per technician/per day	HCV genotype: ~50 samples per 8-hour day HCV RNA: ~100 samples per 8-hour day
Sample needed and stability	PCR-ready setup samples from manual sample preparation or appropriate automated sample preparation instrument (e.g. SaMag, easyMag, described above)
Specimen preparation and protocol complexity	Moderately complex; requires precision pipetting, vortexing, etc.
Reagent stability	HCV genotype assay: controls may be stored at 2–8 °C; "Reverta-L" and HCV genotype reagents must be stored at -20 °C HCV RNA Quant assay: all elements of kit must be stored at 2–8 °C
Cost/test	HCV genotype: €31.60 per test (~US$ 41 per test), but is subject to substantial discounts (up to 50%) from the company HCV RNA: €13.13 per test (or ~US$ 17 per test), but substantial discounts (up to 50%) are available from the company

Cost/instrument	Closed system that works with company assays only is €14 400 (~US$ 18 645), including a notebook computer Open system version of the instrument is €16 800 (~US$ 21 750), not including a notebook computer Discounted pricing on these instruments (up to 15%) is available from the company
Physical dimensions (cytometer only) (W x D x H)	Width: 21 cm (8.3 in) Depth: 54 cm (21.3 in) Height: 54 cm (21.3 in)
Weight	27 kg (59.4 lbs)
3rd party supplies	Centrifuge, refrigerator, laboratory freezer and various additional laboratory consumables
Electric power requirements	200–240 V AC mains, 550 W (peak)
Environmental requirements	Temperature: 15–30 °C Humidity: 30–80% Maximum altitude: 2000 metres (6500 feet)
Peripherals/supporting instrumentation	USB 2.0 for computer interface
Barcode scanner	
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are included as part of reagent kit and required for each preparation run
EQA	
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

HCV Assays: QIAGEN	
artus® HCV QS-RCQ Kit	
Type of assay	RT-PCR assay using fluorescent probes for the quantification of HCV RNA
Target region of HCV genome	5'UTR and core regions of the HCV genome
Genotype and subtype	1 to 6
Sensitivity (LLOD)	35 IU/mL using automated extraction; in combination with Rotor-Gene Q, can detect HCV down to 21 IU/mL
Linear range	35 IU/mL–17.7 million IU/mL using automated extraction
Specificity	100%
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 02/202)
Regulatory approval	CE-IVD marked
Cost per test	Available from QIAGEN
Equipment	Automated sample preparation with QIAsymphony® SP/AS; amplification and detection on RGQ Thermocycler
careHCV RT-PCR Assay v2	
Type of assay	RT-PCR assay using fluorescent probes for the detection and quantification of HCV RNA
Target region of HCV genome	5'UTR and core regions of the HCV genome
Genotype and subtype	1 to 6
Sensitivity (LLOD)	500 IU/mL
Linear range	1000 IU/mL–50 million IU/mL
Specificity	100% (tested against the national standard panel (China) for common infectious disease, which includes HAV, HBV, HIV, HDV, CT, NG, CMV, HPV and TB; showed no cross reactivity)
Standardization	WHO International Standard HCV RNA Technology Assays (NIBSC code 96/798)
Regulatory approval	State Food and Drug Administration of China
Cost per test	~US$ 22 (in China)
Equipment	Manual sample preparation or automated sample preparation with QIAcube; assay can be performed on the RGQ Thermocycler and on other instruments, including the ABI 7000/7300 or 7500, the iCycler, the LC480, the Opticon or the Line-Gene
QIAcube®: sample preparation and assay setup	
Type of technology	Automated sample preparation and assay setup
Output	Samples ready for amplification and detection on Rotor-Gene Q
Turnaround time	Unknown
Capacity (per run)	1–12 samples per run
Throughput per technician/per day	Unknown
Sample needed and stability	Up to 1000 µL of plasma Plasma can be stored at 2–8 °C for not more than 24 hours; or at -20 °C for not >3 months; or frozen at -70 °C for long-term storage; plasma should be transported at 2–8 °C or frozen at -20 °C
Specimen preparation and protocol complexity	Moderately complex; requires pipetting, centrifugation, incubation, addition of buffers, etc. Customized protocols are available on request

Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F); however, nucleic acid amplification reagents, controls and quantification standards must be stored at -20 °C Shelf-life of the purification kit is 12 months
Cost/test	N/A
Cost/instrument	N/A
Physical dimensions (W x D x H)	Width: 65 cm (25.6 in) Depth: 57 cm (22.4 in) Height: 62 cm (24.4 in)
Weight	QIAcube: 71.5 kg (157.6 lbs)
3rd party supplies	Vortex, refrigerator, gloves and other laboratory consumables
Electric power requirements	100–240 V AC mains, 50–60 Hz; mains supply voltage fluctuations not to exceed 10% of nominal supply voltages
Environmental requirements	Temperature: 18–28 °C (64.4–82.4 °F) Humidity: 15–75% (non-condensing) Maximum altitude: 2000 metres (6500 feet)
Data station	N/A
Monitor	N/A
Printer	N/A
Barcode scanner	N/A
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	N/A
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories
QIAsymphony® SP/AS: sample preparation and assay setup instruments	
Type of technology	Automated sample preparation and assay setup
Output	Samples ready for amplification and detection on Rotor-Gene Q
Turnaround time	Approximately 3 hours for extraction and assay setup
Capacity (per run)	1–96 samples per run with continuous loading in batches of 24 samples plus internal controls
Throughput per technician/per day	Up to three specimens per 8-hour shift, based on testing combinations and laboratory workflow
Sample needed and stability	Up to 1000 µL of plasma Plasma can be transported/stored at 2–8 °C for five days or frozen at -70 °C
Specimen preparation and protocol complexity	The QIAsymphony® SP accepts a wide variety of primary tubes for process safety and reduced hands-on time The instrument offers over 17 different sample purification kits with over 45 standard protocols Customized protocols are available on request
Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F) Shelf-life of the purification kit is 12 months
Cost/test	N/A
Cost/instrument	N/A
Regulatory status of assays	The entire automated workflow from sample to result, including the assay, is CE-IVD marked

Physical dimensions (W x D x H)	QIAsymphony® SP: 130 x 75 x 103 cm (51.2 x 29.5 x 40.6 in) QIAsymphony® AS: 59 x 103 x 73 cm (23.2 x 29.5 x 28.7 in) Integrated: 185 x 103 x 73 cm (72.8 x 29.5 x 28.7 in)
Weight	QIAsymphony® SP: 175 kg (385.8 lbs); QIAsymhony® AS: 90 kg (198.4 lbs); Integrated: 265 kg (584.2 lbs)
3rd party supplies	Vortex, refrigerator, gloves and other laboratory consumables
Electric power requirements	100–240 V AC mains, 50–60 Hz
Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: 15% (for temperatures up to 31 °C, decreasing linearly to 50% humidity at 32 °C) non-condensing Maximum altitude: 2000 metres (6500 feet)
Data station	N/A
Monitor	N/A
Printer	N/A
Barcode scanner	Supplied with instrument
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	N/A
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

Rotor-Gene Q automated amplification/detection instrument	
Type of technology	Automated amplification and detection
Output	HCV RNA quantification
Turnaround time	Including sample preparation, 5–6 hours per 24 reactions
Capacity (per run)	67 samples
Throughput per technician/per day	
Sample needed and stability	PCR-ready setup samples from QIAsymphony® AS or QIAamp DSP Virus Kit
Specimen preparation and protocol complexity	
Reagent stability	Varies by reagent, but most must be stored at 2–8 °C (36–46 °F)
Cost/test	N/A
Cost/instrument	N/A
Regulatory status of assays	CE-IVD marked
Physical dimensions (cytometer only) (W x D x H)	Width: 37 cm (14.6 in) Depth: 42 cm (16.5 in); door open: 56 cm (22.0 in) Height: 27.5 cm (41.0 in)
Weight	14 kg (31 lbs)
3rd party supplies	Centrifuge, refrigerator, laboratory freezer and various additional laboratory consumables
Electric power requirements	100–240 V AC mains and 200–240 V AC mains, 50–60 Hz; 560 V AC (peak)

Environmental requirements	Temperature: 15–32 °C (59–89 °F) Humidity: 15–75% (for temperatures up to 31 °C, decreasing linearly to 50% humidity at 32 °C) non-condensing Maximum altitude: 2000 metres (6500 feet)
Peripherals/supporting instrumentation	
Barcode scanner	
Training	Fully trained laboratory technician required; dedicated training on instrument
Maintenance	Routine preventative maintenance required In case of breakdown, vendor-trained technician required to repair
Internal QC	Controls are included as part of reagent kit and required for each preparation run
EQA	
Infrastructure requirements	Technology can be used at national reference (or comparable) laboratories

B. HCV diagnostic platforms in the pipeline

Hologic Inc.	
APTIMA® HCV Quantitative Assay	
Type of assay	RT TMA for the quantitation of HCV RNA
Equipment	Laboratory-based Panther® System
Aptima® HCV Quant Dx Assay on the Panther® System	
Type of technology	Real-time TMA
Output	HCV quantitation in plasma
Turnaround time	Time to first result: less than 3 hours (for first five results), with five additional results every 5 minutes thereafter
Capacity	Panther® can hold up to 120 patient specimens with continuous loading after first rack of 15 specimens is pipetted
Throughput per technician/per day	More than 300 samples per 8-hour day or 550 samples per 12-hour day (additional 225 samples processed without operator attendance) Random, continuous loading of samples eliminates the need for batching
Sample needed and stability	Minimum volume for primary collection tubes is 1200 µL and for secondary specimen aliquot tube (SAT) the minimum volume is 700 µL Dilution feature allows quantitation with 240 µL of plasma Whole blood, plasma or serum in primary collection tubes can be stored at 2–30 °C for up to 24 hours after specimen collection Plasma can be stored in the primary collection tube at 2–8 °C for up to three days; or in the SAT at 2 °C for up to five days; or in the SAT at -20 °C or -70 °C for up to 90 days Serum can be stored in the primary collection tube at 2–8 °C for up to three days; or in the SAT at 2–8 °C for up to five days; or in the SAT at -20 °C for up to seven days
Sample preparation and protocol complexity	Plasma in primary blood tubes or secondary tubes can be placed on Panther® after centrifugation to separate red blood cells Test protocol is fully automated following reagent reconstitution and instrument setup Reagents are shipped lyophilized with paired reconstitution solution and collar Once reconstituted, reagents are placed on Panther® and ready for use
Reagent stability	Reagents stable until expiration (typically >6 months shelf-life) Once reconstituted, reagents are stable for up to 48 hours on the Panther® and for up to 30 days refrigerated
Cost/test	TBD

Cost/instrument	TBD
Regulatory status	TBD
Physical dimensions (W x D x H)	Width: 122 cm (48 in) Depth: 81.5 cm (32 in) Height: 175 cm (69 in)
Weight	363 kg (~800 lbs)
3rd party supplies	Pipettors, vortex mixer, refrigerator, gloves and other laboratory consumables
Electric power requirements	100–230 V
Environmental requirements	Operating temperature: 15–30 °C (59–86 °F) Humidity: 20–85% Maximum altitude: 2000 metres (6000 feet) above sea level
Data station	Built-in Dell computer running Windows® Vista
Monitor	Touchscreen monitor attached to Panther® for easy access
Printer	Supplied: HP Office Jet Pro 8000
Barcode scanner	Built-in scanners automatically read reagents and samples on loading Hand-held scanner attached to Panther® for master lot input Both read Code 39, Code 93, Code 128 (isbt 128), Interleaved 2 of 5, and Codabar
Training	5-day operator training is provided at certified training facility or customer site
Maintenance	Panther® provides ability to schedule many routine maintenance procedures Routine preventative maintenance by authorized service representative every six months Hologic Secure PRO360° allows remote issue evaluation to either resolve or send out appropriate applications or engineering support
Internal QC	An internal calibrator/control is added to each sample at the beginning of the processing to control for nucleic acid capture, amplification and detection and is used to normalize target signals for quantitation Panther® also has multiple in process and validity checks to ensure proper performance of the system
EQA	Compatible with EQA programmes Verified with College of American Pathologists, NIBSC, Quality Control for Molecular Diagnostics, United States Nuclear Regulatory Commission, Accrometrix
Infrastructure requirements	Suitable for a centralized and some decentralized settings
User interface	Touchscreen user interface in English

Alere Inc.	
Alere™ q HCV VL	
Type of assay	RT RNA PCR system for the quantitation of HCV viral load from plasma
Equipment	POC: Alere™ q System
Alere™ q	
Type of technology	Alere™ q is a portable automated bench-top real-time RT RNA PCR system Alere™ q cartridges provide for sample collection, cell lysis, target capture, reverse transcription, real-time PCR amplification and real-time fluorescence detection based on competitive reporter probe hybridization on an integrated microchip array
Output	Alere™ q HCV VL test provides quantitation of HCV Viral Load
Turnaround time	<60 minutes
Capacity	Maximum of ~8 samples per day

Throughput per technician/per day	~8 samples per technician per day; no batching capabilities; walk-away operation
Sample needed and stability	Plasma assay: 0.5–1.0 mL plasma
Sample preparation and protocol complexity	Plasma: centrifugation of whole blood sample required Steps: (i) apply plasma sample to cartridge; (ii) close cartridge; (iii) insert cartridge into analyser; (iv) analysis starts automatically; (v) enter operator and sample ID; (vi) after assay is finished remove cartridge from analyser; and (vii) read result from screen Hands-on time <3 minutes
Reagent stability	Freeze-dried reagents require no refrigeration Stable for 12 months at 4–30 °C
Cost/test	TBD
Cost/instrument	TBD
Regulatory status	Alere Inc. will seek regulatory approval for CE-IVD marking and FDA approval
Physical dimensions (analyser only) (L x H x D)	Length: 20 cm (7.87 in) Height: 22 cm (8.66 in) Depth: 31 cm (12.2 in)
Weight	<7.8 kg
3rd party supplies	For capillary samples: sterile lancets, alcohol swabs, dry swabs (also available from Alere Inc.)
Electric power requirements	100–240 V (A/C) at 50–60 Hz mains power Analyser contains onboard rechargeable battery; additional external battery available
Environmental requirements	Operating temperature: 15–40 °C (59–104 °F) Humidity: <90% relative humidity Maximum altitude: N/A (permissible atmospheric pressure: 800–1060 hPa)
Data station	1000 test results can be stored on the instrument archive; results can be downloaded via USB Results can be printed immediately, but results also are stored in an onboard archive and can be viewed and printed at a later date, exported to a USB memory stick or exported to a remote server via the use of an optional USB connectivity package that makes use of GSM mobile telephone network infrastructure Data point connectivity solution for instrument management, QC and cartridge consumption provided
Monitor	Colour touchscreen integrated into instrument
Printer	Separate printer (prints on thermal paper); USB/battery powered Length 95 mm x width 93 mm x height 66 mm; weight: ~350 g, including paper roll
Barcode scanner	Integrated into instrument for test cartridges and compatible to external barcode readers
Training	Minimal training required Lay person can be trained in less than half a day Primary skill required is for correct lancet blood draw
Maintenance	Maintenance free instrument Care package for instrument is available If damaged portability of device allows for direct swap-out replacement rather than onsite repair
Internal QC	Yes
EQA	Will be fully compatible with existing EQA programmes
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities
User interface	Touchscreen colour display to enter patient information, view results, adjust settings, download results and navigate system software

Wave 80 Biosciences	
EOSCAPE-HCV Rapid RNA Assay System	
Type of assay	Nucleic acid amplification assay for the quantitation of HCV RNA
Equipment	POC: Wave 80 EOSCAPE System
WAVE 80 EOSCAPE System	
Type of technology	Nucleic acid test
Output	HCV RNA level (quantitative)
Turnaround time	60 minutes
Capacity	1 per analyser (EOSCAPE-1); 16 per analyser (EOSCAPE-16)
Throughput per technician/per day	8 samples per technician/per day for EOSCAPE 1; 128 samples per technician for EOSCAPE 16
Sample needed and stability	Fresh whole blood
Sample preparation and protocol complexity	Collect whole blood and transfer to cartridge
Reagent stability	Cartridges are shelf stable for one year at 40° C
Cost/test	TBD
Cost/instrument	<US$ 10 000 for EOSCAPE-1
Regulatory status	Company will seek WHO prequalification, CE-IVD marking and FDA approval; others are also planned
Physical dimensions (EOSCAPE 1) (W x D x H)	Width: 15.4 cm (6 in) Depth: 16 cm (6.3 in) Height: 15.4 cm (6 in) Auxilliary touchscreen: 26.5 cm (10.4 in) x 18.0 cm (7 in) x 1.1 cm (0.43 in)
Weight (EOSCAPE 1)	1.25 kg (~2.8 lbs)
3rd party supplies	Lancet, alcohol swab (supplied in kit)
Electric power requirements	Mains power with 8-hour rechargeable battery backup Solar charging capable
Environmental requirements	Operating temperature: <40 °C Humidity: N/A Maximum altitude: TBD
Data station	Standard laboratory information management systems; USB; integrated wireless connectivity
Monitor	
Printer	External
Barcode scanner	Integrated
Training	Eight hours training for United States high school education level; one hour training for medical professionals
Maintenance	Wipe down with diluted bleach solution; replace rechargeable batteries after extended cycling
Internal QC	Internal amplification/process control
EQA	
Infrastructure requirements	Biohazard disposal; cartridge storage
User interface	Touchscreen

Molbio Diagnostics Pvt Ltd	
Truenat™ HCV quantitative assay	
Type of assay	RT-PCR assay for the quantitation of HCV RNA
Equipment	POC: Truelab™ Real Time micro PCR system
Truelab™ Real Time micro PCR system	
Type of technology	NAAT (real-time PCR)
Output	HCV RNA level (quantitative)
Turnaround time	60 minutes (sample to result)
Capacity	One chip per processing unit (company plans a four chip version)
Throughput per technician/per day	About 12 per 8-hour day (about 50 with four chip version)
Sample needed and stability	100 µL plasma for HCV viral load Sample must be processed immediately upon collection or stored at -20 °C Alternatively, for transport, 100 µL of specimen can be transferred to a tube to which 500 µL of lysis reagent has been pre-added
Sample preparation and protocol complexity	Extraction process currently involves multiple pipetting steps that require operator interventions, including adding reagents, aspirating liquid, adding buffer, etc. (an automatic sample preparation is expected to be introduced soon) Once extracted, the nucleic acid is dispensed into a chip that is inserted into the PCR analyser and the thermal cycling and analysis takes place automatically within the analyser
Reagent stability	Reagents are ready to use, shelf stable for one year when stored at 2–30 °C and for three months at temperatures up to 40 °C
Cost/test	US$ 14 per chip; US$ 2 per extraction
Cost/instrument	US$ 8000 (includes sample preparation, PCR analyser, printer, pipettes)
Regulatory status	Manufacturing facility is ISO 13485 and ISO 9001 certified Indian test manufacturing license obtained and registration process under way
Physical dimensions (analyser) (W x H x D)	Length: 21 cm (8.27 in) Width: 14 cm (5.5 in) Height: 10.9 cm (4.29 in)
Weight (analyser)	0.9 kg (~2 lbs)
3rd party supplies	Powder-free disposal gloves, waste disposal container with lid, sterile lancets, alcohol swabs, dry swabs
Electric power requirements	Continuous power supply not required Rechargeable lithium ion battery pack: 7.5 V; 2200 mAh provides for over 8 hours of backup on a full charge
Environmental requirements	Operating temperature: 15–~35 °C Humidity: 10–80% Maximum altitude: N/A
Data station	Dedicated CPU integrated into instrument; approximately 5000 test results can be stored on the instrument archive Support wireless connectivity (Wi-Fi, Bluetooth, GPRS)
Monitor	Integrated touchscreen colour monitor (3.2 in); touchscreen interface; power on/off switches on analyser unit
Printer	External 2 in Bluetooth Thermal Printer
Barcode scanner	No
Training	Two–three hours; high school diploma or equivalent

Maintenance	Yearly contract, warranty one year
Internal QC	Full process internal control that validates the sample preparation and PCR
EQA	Universal control kit containing positive and negative controls must be ordered separately Positive and negative controls should be run from time to time; it is advised to run controls under the following circumstances: (i) whenever a new shipment of test kits is received; (ii) when opening a new test kit lot; and (iii) by each new user prior to performing testing on a clinical specimen
Infrastructure requirements	None

Cepheid	
GeneXpert® HCV quantitative assay	
Type of assay	RT-PCR assay for the quantitation of HCV RNA
Equipment	POC: GeneXpert® System
HCV Viral Load Assay for GeneXpert® System	
Type of technology	RT-PCR-based NAAT test
Output	Quantitative HCV RNA
Turnaround time	<95 minutes
Capacity	Dependent on GeneXpert® system and number of modules ranging 1–80 per system, comparable to GeneXpert® MTB
Throughput per technician/per day	Dependent on GeneXpert® system and number of modules; e.g. 397 results per 8-hour shift with an Infinity-80* (80 modules)
Sample needed and stability	Quantitative HCV: 1 mL plasma sample input volume
Sample preparation and protocol complexity	Automated within cartridge
Reagent stability	No refrigeration required; in development
Cost/test	TBD
Cost/instrument	Comparable to GeneXpert® MTB
Regulatory status	CE-IVD marking expected 1H 2015
Physical dimensions (W x H x D)	Specifications for GX-IV Processing Unit: Length: 11.00 in Height: 12.00 in Depth: 13.25 in
Weight	GX-IV Processing Unit: 25 lbs
Electric power requirements	Mains power required: 100–240 V; UPS compatible
Environmental requirements	Operating temperature: 15–30 °C Relative humidity: 10–95%, non-condensing
Barcode scanner	Included with system
Training	Minimal training required Lay person can be trained in less than half a day Primary skill required is for correct blood draw
Maintenance	Remote calibration kit for onsite user calibration If damaged, modules are exchangeable

Internal QC	Internal to the cartridge
EQA	Will be fully compatible with existing EQA programmes
Infrastructure requirements	Can be used at all levels of health facilities that have electricity, including health centres or in mobile facilities

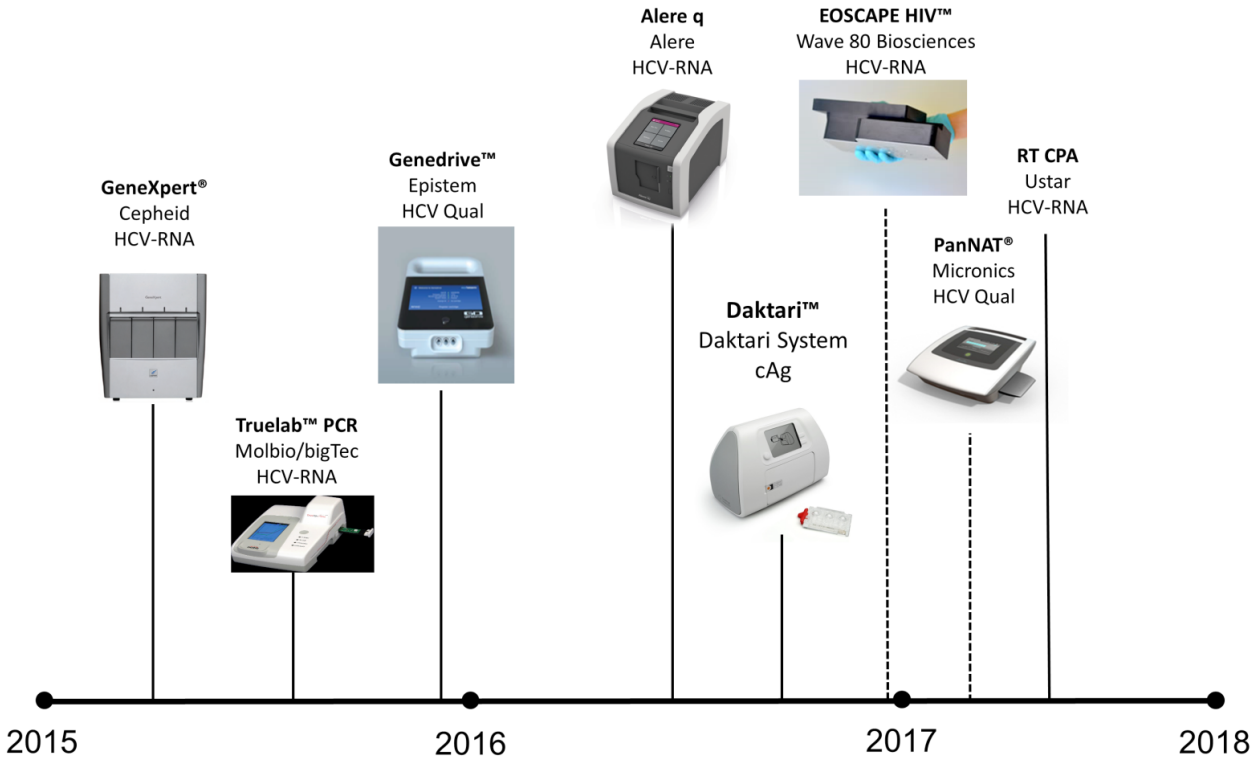
Ustar Biotechnologies	
RT-CPA HCV Viral Load Test	
Type of assay	Isothermal Cross Priming Amplification (CPA) NAAT for the quantitation of HCV RNA
Equipment	POC: Ustar NAAT Platform
Ustar Platform for RT-CPA HCV Viral Load Test	
Type of technology	Nucleic acid amplification – isothermal CPA
Output	HCV RNA level (quantitative)
Turnaround time	<1 hour
Capacity (per run)	Four tests plus internal controls
Throughput per technician/per day	>24 tests
Sample needed and stability	100 µL plasma
Sample preparation and protocol complexity	No more than three–five steps from sample to result (fully automated, just add sample to cartridge)
Reagent stability	Stable for 24 months at 0–40 ˚C, 90% humidity, including transport stress (48 hours with fluctuations up to 50 ˚C and down to 0 ˚C
Cost/test	<US$ 5
Cost/instrument	<US$ 5000
Regulatory status	Under development
Physical dimensions	Width: TBD Depth: TBD Height: TBD
Weight	<5 kg (<11 lbs)
3rd party supplies	None
Electric power requirements	110–220 V AC mains current or DC power with rechargeable battery lasting 8 hours
Environmental requirements	Temperature: 0–40 ˚C Humidity: no requirement Maximum altitude: >3000 metres (9800 feet)
Peripherals/supporting instrumentation	None
Barcode scanner	Yes
Training	Approximately half a day
Maintenance	System is swapped for a new one upon malfunction
Internal QC	Internal amplification control; fluorescent control to ensure probes are working
EQA	Three quantitative standards and one negative control
Infrastructure requirements	Intermittent power; bench to store instrument; incineration for medical waste

Daktari Diagnostics Inc.	
Daktari™ HCV cAg Assay	
Type of assay	Assay for quantitative determination of HCV cAg for diagnosis of active infection and treatment monitoring
Equipment	POC: Daktari™ system
Daktari™ System	
Type of technology	Small, portable device that uses cartridge microfluidic-based system to quantify HCV cAg
Output	HCV cAg
Turnaround time	Approximately 30 minutes
Capacity	16 samples per day per instrument
Throughput per technician/per day	One technician can operate six instruments without difficulty, or 96 samples per technician per day; no batching capabilities; walk-away operation
Sample needed and stability	50 µL of plasma
Sample preparation and protocol complexity	Approximately 30 seconds of hands-on time; whole blood is collected via fingerstick, introduced to the cartridge, and the cartridge is inserted into the instrument
Reagent stability	Dried reagents require no refrigeration
Cost/test	US$ 15–20
Cost/instrument	<US$ 8000 (estimated)
Regulatory status	ISO 13485 certification
Physical dimensions (cytometer only) (W x D xH)	Width: 22.9 cm (9.0 in) Depth: 12. 7 cm (5.0 in) Height: 17.8 cm (7.0 in)
Weight	2.5 kg (~5.5 lbs)
3rd party supplies	Alcohol swabs, gauze, adhesive bandage (lancets and capillary transfer tubes are provided)
Electric power requirements	Regular AC mains long-life rechargeable battery self-contained in device that can operate for up to three days on a single battery charge Solar recharging option
Environmental requirements	Operating temperature: 4–40 °C Humidity: up to 90% relative humidity Maximum altitude: up to 3280 metres (10 000 feet)
Data station	Daktari™ System includes a data management system, with a touchscreen user interface, wireless data transmission and a back-end data package that can stand alone or be integrated with customer databases
Monitor	LCD screen integrated into instrument Results stored on instrument and can be downloaded, if needed, and can be automatically uploaded to a remote server for analysis
Printer	Daktari™ System includes an optional USB printer accessory for printed results
Barcode scanner	Daktari™ System includes an optional USB barcode scanner
Training	Minimal training required Lay person can be trained in less than 90 minutes Primary skill required is for correct lancet blood draw
Maintenance	No daily calibration required; assays can be run as soon as the instrument is powered on The device does not use lasers, but rather employs an electronic measurement system similar to a glucose meter and might be less prone to damage If damaged, the company plans to swap out the device rather than repair it onsite

Internal QC	Internal QC of instrument performed with each assay run; internal QC of cartridge with each run includes checks on sensors, assay protocol and key reagents No calibration required Instrument also will perform QC of capillary blood draw and inform user if fingerstick is inadequate prior to running assay
EQA	TBD
Infrastructure requirements	Can be used at all levels of health facility, including health centres or in mobile facilities

Appendix 2: Pipeline for point-of-care diagnostics

Hepatitis C virus point-of-care diagnosis and treatment monitoring platforms: pipeline*



*Estimated as of September 2014 - timeline and sequence may change. ---- No market launch date set by company.