

## COMMENTARY

## Where we are with point-of-care testing

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**SUMMARY.** Viral hepatitis claims one million lives each year. Scaling up treatment for hepatitis B and C in resource-limited settings is not possible without access to reliable diagnostic tools. This article gives an overview of current technologies and the pipeline for easy-to-use assays for serological and virological analyses, which can be performed at the site of patient care ('point-of-care assays'). Furthermore, the utility of dried blood spots for

hepatitis B and C viral load testing is discussed. In addition to simple and reliable diagnostics, there is a need for a sustainable funding scheme and generic production of antiviral drugs to reduce the burden of viral hepatitis worldwide.

**Keywords:** hepatitis B, hepatitis C, low-tech, resource-limited settings, viral load.

## THE DIAGNOSTIC CHALLENGE

Despite the huge burden of hepatitis B worldwide, with an estimated 350 million people chronically infected, very few patients in low- and middle-income countries are currently receiving antiviral treatment. This is largely to blame on lack of access to viral load quantification, because this is considered a mandatory component of the diagnostic work-up in all international liver society guidelines. Without a viral load measurement, it is virtually impossible to establish whether a hepatitis B surface antigen (HBsAg) positive individual has chronic hepatitis B, characterized by a level of hepatitis B virus (HBV) DNA >2000–20 000 IU/mL and continued necro-inflammation in the liver. These patients are at high risk of progression to cirrhosis and hepatocellular carcinoma in the absence of antiviral treatment, whereas inactive carriers, characterized by HBV DNA levels <2000 IU/mL, have an excellent prognosis in the absence of treatment [1,2].

The situation for hepatitis C is somewhat different because the main obstacle to treatment has been expensive and toxic treatment regimens. With the release of new direct acting antivirals for hepatitis C, opportunities are opening up in less developed countries where the disease burden is often high. An estimated 130–150 million people are chronically infected with hepatitis C virus (HCV) globally, and the prevalence is higher in certain areas such as

North Africa and Central and East Asia [3]. However, prior to starting treatment of hepatitis C, HCV RNA measurements are required to establish the diagnosis of chronic HCV infection [4], which is a major obstacle in many places in the world.

Lack of access to viral load testing and antiviral treatment of HBV and HCV in resource-limited settings is a silent epidemic with major consequences. The World Health Organization (WHO) estimates that about 1 million people die from chronic HBV and HCV infection each year, which places viral hepatitis on the top 10 leading causes of mortality globally [5].

## DO WE NEED POINT-OF-CARE ASSAYS?

Modern management of viral hepatitis relies heavily on laboratory support. First, serological tests are used to screen for hepatitis markers and exclude co-infections. Thereafter, a new battery of tests is needed to distinguish past or inactive infection from chronic hepatitis. This diagnostic work-up involves molecular biology laboratories and advanced target amplification methods such as polymerase chain reaction (PCR) assays. These tests are typically performed at large referral laboratories, because they require sophisticated equipment, highly specialized laboratory personnel and strict quality control measures. In resource-limited settings, however, PCR assays are rarely available due to their complexity and high costs. Furthermore, stringent requirements for storage and shipment of plasma to the referral laboratory are barriers in settings with limited infrastructure.

The solution to this challenge would be to develop reliable, cheap, and easy-to-use assays that can be performed at the site of patient care ('point-of-care' assays). Indeed,

Abbreviation: DBS, dried blood spots; HBeAg, hepatitis B e-antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction; RDTs, rapid diagnostic tests.

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for serological testing, such assays already exist. Numerous rapid diagnostic tests (RDTs) for hepatitis B and C are commercially available, most of which provide a test result within 5–30 minutes; however, the diagnostic accuracy varies from excellent to very poor. A recent meta-analysis by Shivkumar and colleagues found that the sensitivity of HBsAg RDTs used to screen for hepatitis B infection varied from 42 to 100% and the specificity from 0 to 100% [6]. The same authors also reviewed anti-HCV RDTs used for hepatitis C screening and found sensitivities ranging from 0 to 100% and specificities from 81.6 to 100% [7]. Hence, although high-quality RDTs can be used with confidence in screening for HBV and HCV, health authorities should warn against the use of unreliable (and often very cheap) RDTs with poor sensitivity and specificity.

### THE PIPELINE FOR POINT-OF-CARE VIRAL LOAD ASSAYS

Even though serological point-of-care assays for hepatitis B and C are widely available, the same is not the case for virological analyses. However, if we look over the fence into the HIV landscape, there has been an active development of point-of-care kits for viral load quantification over the past few years. Because of major international investments in the fight against HIV/AIDS, there is an attractive commercial market for such kits. The first products were recently launched, including the *Alere q Analyzer*<sup>®</sup> (Alere Inc., Waltham, MA, USA) and the *SAMBA*<sup>®</sup> (Diagnostics for the Real World, Sunnyvale, CA, USA), both of which have undergone field testing in sub-Saharan Africa and shown excellent performance [8,9]. As there are no similar funding mechanisms for viral hepatitis in resource-limited settings, commercial actors have not shown the same interest in low-tech diagnostics for HBV and HCV; however, many of the technological advances for HIV can be modified to detect other viruses.

Currently, there is one product, *Truenat*<sup>®</sup> (Molbio Diagnostics, Goa, India), for HBV DNA quantification which is advertised as a point-of-care assay. A similar HCV RNA kit from the same manufacturer is in the pipeline. The *Truenat* kit provides a PCR result within an hour; however, it seems rather complex, involving several manual steps and multiple reagents, making it less ideal for outside laboratory settings. Furthermore, there are no peer-reviewed publications on its performance. An independent evaluation of the *Truenat* kit under field conditions would be of major interest.

A few other promising tools are in the pipeline. The US-based company Wave 80 Biosciences is developing a point-of-care kit for HBV DNA quantification as well as a qualitative assay for HCV RNA detection, building on their existing HIV viral load assay *EOSCAPE-HIV*<sup>®</sup> (Wave 80 Biosciences, San Francisco, CA, USA). *EOSCAPE-HIV* is a fully automated, cartridge format system which is said to

be robust and easy to operate. However, *EOSCAPE-HIV* is still not commercially available, so the timing for the release of the HBV and HCV kits is still uncertain.

The UK biotechnology company Epistem received a prestigious grant award in 2013 to develop an HCV point-of-care device for viral load testing and genotyping. This novel assay will build on their existing *Genedrive*<sup>®</sup> (Epistem, Manchester, UK) device, which is a rapid, easy to use, sensitive, handheld PCR platform already CE-IVD marked for other genotype tests, such as *Mycobacterium tuberculosis* identification and antibiotic resistance testing. However, it is still uncertain when such a product will be commercially available.

### WHAT DOES AN IDEAL POINT-OF-CARE ASSAY LOOK LIKE?

Far too often advanced technology has been shipped to low-income countries with good intentions, but ended up in a storeroom because of insufficient training, lack of maintenance, and shortage of reagents. Partly, this has to be blamed on the manufacturers, who rarely offer training or service agreements to low- and middle-income countries. It is important, therefore, that point-of-care assays should be designed specifically to operate under basic conditions with minimal maintenance requirements and not just be high-tech solutions forced to fit low-tech settings. The international nongovernmental organization Médecins Sans Frontières published their desired specifications for a point-of-care HIV viral load assay a few years ago, and the same specification would also apply to an ideal HBV or HCV assay [10]:

- No need for specialized laboratory facilities
- Closed system to avoid contamination
- Long shelf life in tropical climate
- No need for cold chain transportation or refrigerated storage
- Ease of use
- Adequate sensitivity
- Cheap

Affordability is an important issue if treatment for viral hepatitis is to be scaled-up globally. Both the initial cost for the machine, but also the consumables thereafter must be priced reasonably. With regard to HBV, repeated tests over time are usually required, and the accumulated cost of viral load testing can be high. For hepatitis C, on the other hand, one viral load measurement prior to treatment and another 24 weeks after treatment would be sufficient if patients are being treated with the new direct acting antivirals.

Sustainability is another major concern with all technological devices, and it should be a prerequisite that the manufacturers provide adequate training and service agreements locally. Furthermore, sensitivity of the assay should be high enough to ensure treatment for those who

need it, and even more importantly, specificity must be close to 100% to avoid unnecessary and expensive treatment in uninfected individuals.

#### POINT-OF-CARE ASSAYS VERSUS DRIED BLOOD SPOTS

As described by Greenman and colleagues in the current issue of *JVH*, dried blood spots (DBS) can be a feasible and reliable alternative to point-of-care assays for viral hepatitis [11]. The main advantage of DBS is that it solves the problem of storage and shipment of samples in places with poor infrastructure. DBS can be stored for weeks at ambient temperature without clinically significant degradation of nucleic acids [12]. A drawback with DBS is the delayed reporting back of results. With viral hepatitis, however, this might be less of a concern, as viral load quantification is part of the pretreatment work-up of each patient and not the day-to-day monitoring of treatment effect (as in HIV). And in viral hepatitis, the decision to start treatment is rarely a matter of urgency.

Use of DBS is limited by the small amount of plasma per blood spot and less efficient nucleic acid extraction, which gives a reduced sensitivity in samples with low-level viraemia [13]. With regard to hepatitis C, this rarely has any practical consequences, as most untreated patients have viral loads (far) above 1000 IU/ml. For hepatitis B, however, the situation is rather different as hepatitis B e-antigen (HBeAg) negative hepatitis is now the main type of chronic hepatitis B worldwide [14]. These patients typically have fluctuating viral load levels in the lower to medium range, and it is often difficult to distinguish them from inactive carriers. A lack of precision in DBS could therefore jeopardize the management of these patients.

Previous studies of HBV DNA quantification in DBS have shown inconsistent results. A recent study by Mohamed and colleagues showed that DBS yielded viral loads 0.65  $\log_{10}$  lower than plasma [15], and an older study by Jardi and colleagues found that viral load levels were 1  $\log_{10}$  lower in DBS compared to plasma [16]. On the contrary, Lira and colleagues found no significant difference between DBS and plasma (0.21  $\log_{10}$  lower in DBS) [17]. Thus, more studies are needed to evaluate the precision of DBS for HBV DNA quantification, especially in the lower range around the decision threshold of 2000–20 000 IU/mL.

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In conclusion, DBS can be a valuable tool for the pre-treatment evaluation of patients with hepatitis C, but might be more troublesome in hepatitis B due to a reduced sensitivity in the lower range of viral loads.

#### STEPS TO ACTION

Management of viral hepatitis is going through a revolution with the launch of new direct acting antivirals for hepatitis C treatment. The cost of nearly 100 000 USD per patient, however, keeps these drugs out of reach for most patients worldwide. Recently, the pharmaceutical company Gilead announced that they work with generic drug manufacturers in India to produce high-quality, low-cost sofosbuvir for developing countries, raising expectations that this game-changing drug might become available in low- and middle-income countries. Other companies should follow Gilead's lead in this matter.

Drug availability, however, is not the only issue. In the absence of viral load measurements, doctors in resource-limited settings are left virtually blindfolded in the management of their patients with hepatitis. Point-of-care viral load assays for hepatitis B and C have the potential to bridge this gap and prove valuable tools for expansion of treatment globally. However, assay performance under standardized conditions in Europe or North America does not necessarily reflect real-life application in sub-Saharan Africa, and it is crucial to carry out independent field testing of these instruments before large scale use can be recommended.

Finally, sustainable funding mechanisms for diagnostics and treatment of viral hepatitis must be established. Scaling up of HIV treatment globally would have been impossible without major donor programs such as *PEPFAR*, *UNITAID* and *The Global Fund to fight AIDS, Tuberculosis, and Malaria*. It is time to consider a similar funding scheme for viral hepatitis. Then our colleagues in resource-limited settings might be able to treat viral hepatitis in the not so distant future.

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