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Current global applications of HBcrAg assays in the management of chronic hepatitis B

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Abstract: Hepatitis B core-related antigen (HBcrAg) is a vital marker for monitoring chronic hepatitis B (CHB) as it correlates with hepatitis B (HBV) DNA and covalently closed circular DNA (cccDNA). The iTACT-HBcrAg assay, approved in Japan, provides highly sensitive and automated testing, reducing patient burden by requiring smaller specimen volumes and offering shorter processing times. Crucial for managing HBV reactivation and predicting hepatocellular carcinoma, it delivers consistent and reliable results. In resource-limited regions, the HBcrAg-rapid diagnostic test (HBcrAg-RDT) facilitates early HBV detection and management. This point-of-care testing (POCT) tool requires no specialized equipment and provides results within 30 minutes, making it invaluable in areas lacking HBV DNA quantification. Trials in West Africa, Asia, and other developing regions demonstrate its sensitivity and specificity. Together, these advancements in iTACT-HBcrAg and HBcrAg-RDT assays enhance CHB patient care and contribute significantly to the global effort to eliminate HBV as a public health threat.

***Keywords*:** hepatitis B virus (HBV), covalently closed circular DNA (cccDNA), point-of-care testing (POCT)

Introduction

Hepatitis B virus (HBV) integrates into host liver cell nuclei as covalently closed circular DNA (cccDNA), playing a crucial role in chronic hepatitis B (CHB) progression (*1*). Current anti-HBV treatments using nucleos(t)ide analogues (NAs) or pegylated interferons inhibit HBV replication but cannot eliminate HBV from hepatocytes. The progression and prognosis of CHB are closely associated with the amount and activity of cccDNA (*2*).

HBV reactivation is commonly seen in patients receiving systemic chemotherapy for hematological malignancies or in hematopoietic stem cell transplantation recipients (*3*). Measuring serum HBV DNA is essential for preventing and diagnosing HBV reactivation. Novel biomarkers, such as high-sensitive HBsAg and hepatitis B core-related antigen (HBcrAg), help in early diagnosis of HBV reactivation. HBcrAg, correlating well with traditional HBV biomarkers, is valuable for screening and diagnosing HBV reactivation, especially in hepatitis B core antigen (HBeAg)-negative patients, even when HBV DNA is suppressed by NAs (*4*). A highly sensitive and automated HBcrAg assay iTACT-HBcrAg was approved in Japan in June 2022 (*5*). iTACT stands for Immunoassay for Total Antigen including Complex *via* pretreatment, and is an assay that analyzes the different molecular modalities present in the specimen (*6*).

Regions such as sub-Saharan Africa, Asia, and the Western Pacific are high-risk areas for HBV infections (*7*). The World Health Organization (WHO) recommends peripartum antiviral prophylaxis for HBV-infected pregnant women with high HBV DNA levels (> 200,000 IU/mL) to prevent mother-to-child transmission (*8-10*). Despite the importance of measuring HBV DNA, over 95% of HBV-infected individuals live in areas where HBV DNA quantification is not readily available (*11*). In these settings, a rapid and simple HBcrAg assay is effective as point-of-care testing (POCT) (*12*).

Currently, there are two main needs for HBV biomarkers: highly sensitive automated assays and systems suitable for POCT in resource-limited settings. This communication highlights the clinical application of the new HBcrAg marker in CHB and HBV reactivation treatment, focusing on the iTACT-HBcrAg assay and a novel HBV prevention strategy based on POCT.

Development of a fully automated highly sensitive HBcrAg assay

Characteristics of iTACT-HBcrAg

We have developed a new sensitive HBcrAg assay called iTACT-HBcrAg. This assay measures HBcrAg, which consists of hepatitis B core antigen, HBeAg, and empty particles (*13,14*). The iTACT-HBcrAg assay contains a reducing agent in the pretreatment solution that alters the molecular structure of HBcrAg and facilitates its measurement. iTACT-HBcrAg achieves a sensitivity about 10 times greater than the conventional assay and improves analytical performance at low concentrations compared to a conventional assay. Additionally, the sensitivity of iTACT-HBcrAg was improved by increasing the number of solid-phase antibodies and optimizing the reagent assay (Figure 1).

Another significant advantage of the high-sensitivity iTACT-HBcrAg assay is the fully automated sample preparation, which saves assay time. Previously, about 30 minutes of manual sample preparation was required, but iTACT-HBcrAg automates this process, reducing the assay time to only 6.5 minutes. The new assay provides results in just 33 minutes. Furthermore, automation eliminates the variability inherent in manual processes, ensuring consistent and reliable results, which is especially important for longitudinal studies and CHB monitoring. Furthermore, iTACT-HBcrAg can reduce the required specimen amount for measurement by one-third, thereby reducing the burden on patients (*5,6*).

Basic performance evaluation of iTACT-HBcrAg

Our initial report highlighted the analytical performance of iTACT-HBcrAg. Compared to a conventional assay, iTACT-HBcrAg accurately measures low-concentration areas below 2.7 log U/mL. The cutoff values are 2.1 log U/mL (limit of quantification: 1.8 log U/mL) for iTACT-HBcrAg and 2.8 log U/mL for a conventional assay (*5*). Serial sera from 161 HBeAg-negative patients with CHB and persistently undetectable HBV DNA were measured using iTACT-HBcrAg and a conventional HBcrAg assay. HBcrAg was detectable in the sera of 97.5% (157/161) of these patients by iTACT-HBcrAg, with 75.2% (121/161) having > 2.8 log U/mL HBcrAg and 22.4% (36/161) having 2.1–2.8 log U/mL HBcrAg, which was undetectable by a conventional assay (*5*). Furthermore, correlation studies with samples from 389 HBeAg-positive or negative patients show that iTACT-HBcrAg correlates well with conventional HBcrAg values ranging from 2.8 log U/mL to 7 log U/mL (*5*).

In summary, iTACT-HBcrAg detects HBcrAg levels between 2.1 log U/mL and 2.8 log U/mL that are undetectable using a conventional HBcrAg assay. Performance evaluation studies have demonstrated that iTACT-HBcrAg has excellent specificity and reproducibility, making it a reliable tool for clinical use. This enhanced detection capability improves the ability to monitor and manage CHB more effectively (*5*).

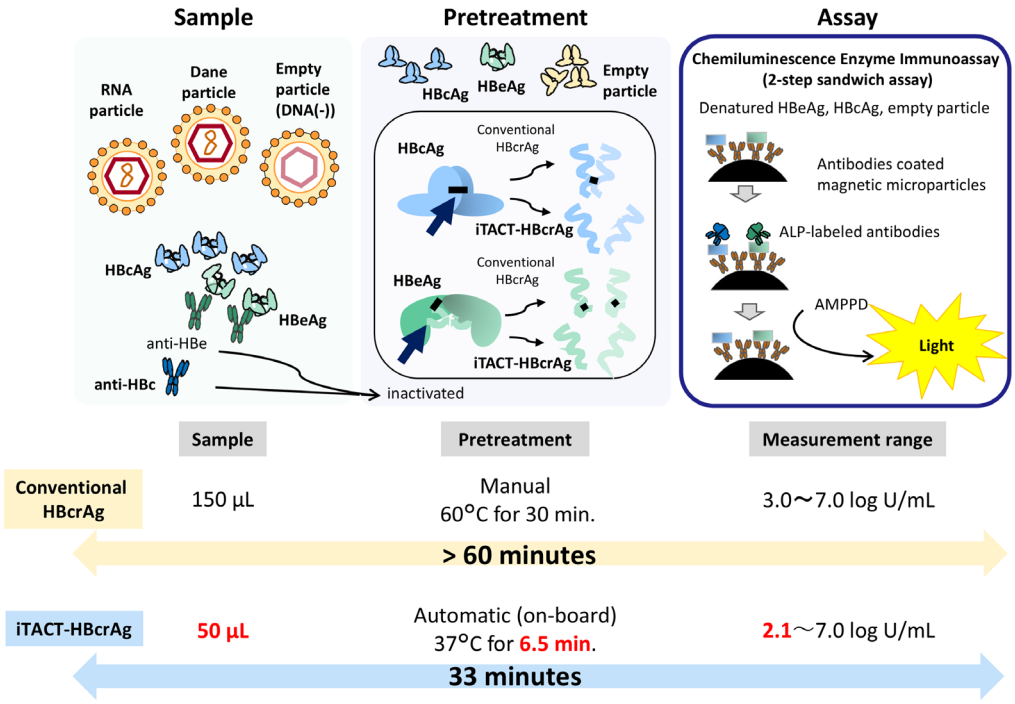

Figure 1. Comparison of the conventional and highly sensitive HBcrAg assays. The figure compares the iTACT-HBcrAg assay with a conventional assay, divided into sample, pretreatment, and assay sections. The illustration at the top of the figure shows a schematic of the iTACT-HBcrAg measurement system. The bottom of the figure contrasts these two assays in terms of sample amount required for measurement, measurement time, and measurement range (sensitivity). *Abbreviations:* HBcrAg, hepatitis B core-related antigen; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; anti-HBc, hepatitis B core antibody; anti-HBe, hepatitis B e antibody; ALP, alkaline phosphatase; AMPPO, 3-(2'-spiroadamantane)4-methoxy-4-(3"-phosphoryloxy)phenyl-1,2-dioxetane.

Clinical utility of iTACT-HBcrAg in the diagnosis of HBV reactivation

In our initial studies comparing serum HBcrAg and HBV DNA from samples of 13 patients diagnosed with HBV reactivation, iTACT-HBcrAg detected HBV reactivation earlier than HBV DNA in 9 of the 13 patients. Furthermore, a comparison between HBcrAg and high-sensitivity hepatitis B surface antigen (HBsAg) showed that iTACT-HBcrAg detected serum HBcrAg earlier than HBsAg in 7 cases, highlighting its superior sensitivity in early HBV reactivation detection (*5*).

Analysis of serum from one patient using the OptiPrep density gradient ultracentrifugation method revealed that during the early stages of HBV reactivation, HBcrAg primarily detects empty particles derived from cccDNA. In serum samples taken before HBV DNA detection, HBcrAg levels increased, indicating HBeAg presence due to HBV reactivation. The serum analysis after HBV DNA detection showed further increases in HBcrAg, demonstrating the clinical utility of iTACT-HBcrAg in early HBV reactivation diagnosis (*5*).

iTACT-HBcrAg and HBV DNA quantification results show that iTACT-HBcrAg detection could serve as markers for initiating NA treatment in HBV reactivation (Figure 2). We categorized 44 HBV reactivation cases based on serum HBV DNA levels, with 27 cases quantifiable and 17 cases non-quantifiable. HBcrAg was detectable by iTACT-HBcrAg before HBV DNA was quantifiable in 15 of the 27 patients. Of the 11 patients with HBV reactivation and undetectable HBcrAg by iTACT-HBcrAg at HBV reactivation and/or thereafter, 10 had unquantifiable HBV DNA and none developed hepatitis (*15*).

In another of our papers, among 25 HBV reactivation patients, iTACT-HBcrAg became negative in 68% (17/25) after NA treatment. Eight patients who achieved iTACT-HBcrAg loss or anti-HBs seropositivity had no recurrence of HBV reactivation after NA discontinuation, except for one patient who did not have anti-HBs after allogeneic transplantation (*16*).

Detecting HBV reactivation in its early phase and safely discontinuing treatment in patients with post-reactivation is crucial to prevent disease progression and improve patient outcomes. The ability of iTACT-HBcrAg to detect HBV reactivation enables clinicians to start treatment sooner, potentially preventing complications and improve the overall prognosis for CHB patients.

Clinical utility of the HBcrAg assay in resource-limited regions

In 2016, WHO called for the elimination of viral hepatitis by 2030 (*17*). The WHO published guidelines in March 2024 outlining NA therapy indications for pregnant women with CHB. In areas where measuring HBV DNA or HBeAg is challenging, all HBsAg-positive pregnant women are eligible for NA therapy, even if they are HBeAg-negative, which may lead to overtreatment.

The HBcrAg-rapid diagnostic test (HBcrAg-RDT),

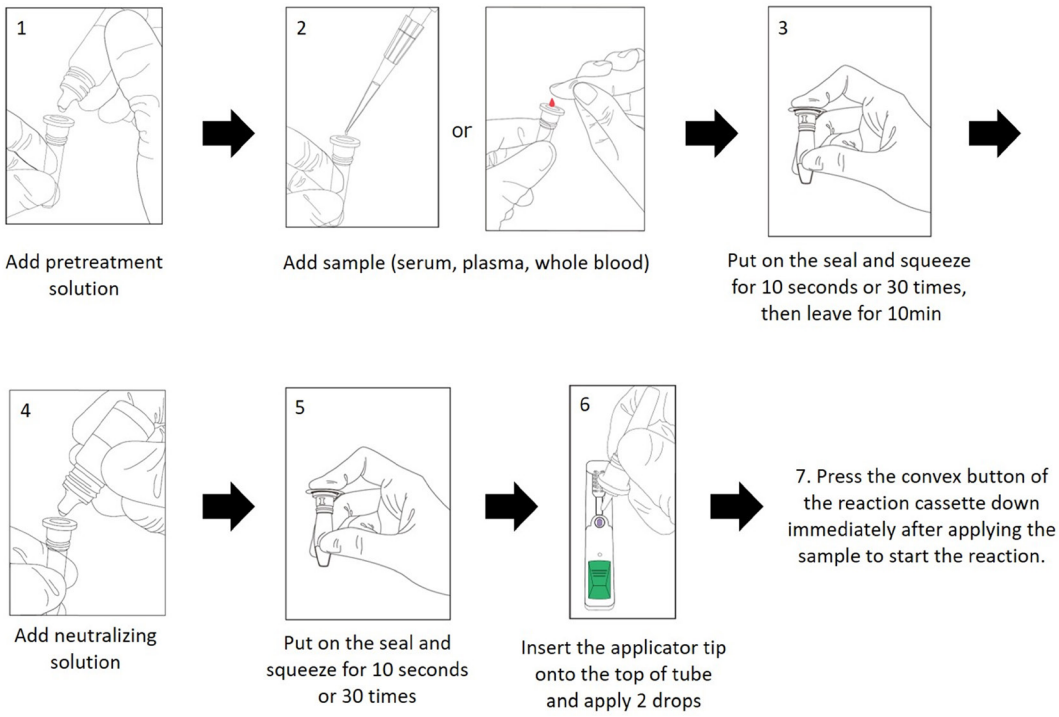

Figure 2. HBcrAg detection procedure by HBcrAg-RDT. The measurement process using HBcrAg-RDT is shown in a simplified diagram. HBcrAg-RDT is for the detection of HBcrAg in serum, plasma, whole blood or dried blood spots. This figure is based on Figure 4 in "Fundamental performance and clinical utilities of LumipulsePresto® iTACT® HBcrAg, a novel highly sensitive immunoassay for hepatitis B core-related antigen" by Yagi (*6*). *Abbreviations:* HBcrAg, hepatitis B core-related antigen; HBcrAg-RDT, HBcrAg rapid diagnostic test.

based on immunochromatography, is being developed to quickly identify HBeAg-positive cases. This easy-to-use kit requires no specialized equipment and delivers results within 30 minutes (*18*). Priced at under $15 per assay, HBcrAg provides a cost-effective alternative for diagnosing clinically significant HBV DNA thresholds (≥ 2,000, ≥ 20,000, and ≥ 200,000 IU/mL). Thus, HBcrAg represents an accurate, simple, and inexpensive substitute for HBV DNA quantification in resource-limited settings (*19*). Its low cost, minimal preparation requirements, lack of need for specialized equipment, and rapid turnaround time make it particularly suited for use in these regions (*20*).

Trials conducted in West Africa, Asia, and other developing regions have demonstrated that HBcrAg-RDT is sufficiently sensitive and specific, supporting effective CHB management (*19*). The implementation of HBcrAg-RDT in resource-limited regions facilitates early detection, monitoring, and management of HBV infections, improving patient outcomes. Its cost-effectiveness ensures broader accessibility, increasing public health impact. The success of the HBcrAg assay highlights its potential as a vital tool in the global effort to combat HBV.

Conclusion

The iTACT-HBcrAg assay is a highly sensitive, fully automated HBcrAg test suitable for outpatient pre-consultation screening. Offering approximately ten times the sensitivity of traditional methods, it has proven effective in managing HBV reactivation cases. The HBcrAg-RDT, in contrast, offers rapid diagnostic capabilities in resource-limited regions, facilitating the prompt identification of cases requiring treatment. The development and implementation of these assays mark significant advancements in the management and prevention of HBV, particularly in settings with limited healthcare resources.

Current HBcrAg assays not only enhance the accuracy and efficiency of HBV diagnosis but also have the potential to significantly impact public health by facilitating early detection and timely intervention. As these advanced diagnostic tools become more widely adopted across regions, the global burden of HBV can be substantially reduced.

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