Annex 5.10

PICO 8 - Diagnostic accuracy of HBsAg/HBeAg test versus NAT to confirm successful treatment response: a meta-analysis and review of the literature

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1. Executive summary

**Background:** Advances in hepatitis B virus detection technology create new opportunities for enhancing screening, referral and treatment. The purpose of this review was to determine the diagnostic accuracy of HBsAg or HBeAg test versus nucleic acid testing (NAT) to confirm successful treatment response among patients receiving treatment for HBV.

**Method:** A literature search was conducted focused on hepatitis B, diagnostic tests and diagnostic accuracy. Studies were included if they evaluated an assay to determine the sensitivity and specificity of a HBsAg or HBeAg test compared to a quantitative HBV RNA reference among humans. Two reviewers performed a quality assessment of the studies and extracted data for estimating test accuracy.

**Results:** It was found that, despite HBV NAT being considered the gold standard in confirming response to treatment, both HBsAg and HBeAg were useful in monitoring patients receiving treatment as in many resource-limiting settings NAT is not readily available. Studies showed that the kinetics of HBsAg and HBV DNA followed similar profiles during treatment with pegylated interferon (PEG-IFN) and follow up in patients who developed sustained virological response (SVR). Further studies determined that this correlation was present for all four genotypes. It was also reported that HBsAg quantification can allow for detection of active cases of chronic HBV from true inactive carriers, therefore reducing the need to rigorously monitor HBV DNA levels. Studies showed that HBeAg was capable of differentiating late responders from non-responders to HBV DNA after 24 weeks of treatment.

**Conclusions:** There is limited evidence for the sole use of HBsAg or HBeAg compared to HBV DNA for monitoring treatment response. More studies are needed to determine which tests for HBV antigen detection may be useful as a marker of treatment response for which therapeutic agent.

2. Background

An estimated 240 million individuals worldwide\(^1\) are chronically infected with hepatitis B virus (HBV) and there are an estimated 4 million acute HBV infections each year. Of those with chronic hepatitis B infection, 20–30% will develop cirrhosis\(^2\) or hepatocellular carcinoma,\(^3\) leading to approximately 650 000 deaths each year.\(^4\) However, most individuals with chronic HBV infection are not aware of their serostatus, contributing to delayed diagnosis and complications from advanced disease.\(^5\) HBV testing is critically important in order to refer infected individuals to HBV treatment and care, to refer uninfected individuals to vaccination and to mobilize prevention and control efforts.

The introduction of NAT is an integral step in the control of the disease as it allows for rapid diagnosis and early treatment of HBV. The virus can be transmitted by blood from asymptomatic donors with acute HBV infection before the development of HBsAg or an anti-HBc response. Therefore, NATs can used to detect HBV DNA in a donor’s blood before antigen or
antibody response are detected. Though NAT testing has been proven to be more sensitive in detecting viral infections, serological testing is better suited for the detection of active infections.

Treatment with tenofovir or entecavir is effective for HBV. Their efficacy can be measured by a sustained reduction in viral load, but the quantitative HBsAg response may remain high. The data for measuring HBsAg quantitatively largely works for interferon-based agents. Locarini reviewed the literature on quantitative HBsAg in hepatology and highlighted some of the challenges of quantitative HBsAg testing in using other therapeutic agents.

In March 2015, the World Health Organization (WHO) published the first guidelines for the prevention, care and treatment of individuals with chronic HBV infection. These guidelines focused on assessment for treatment eligibility, initiation of first-line therapies, switching and monitoring. These initial guidelines did not include screening recommendations. Given the large burden of HBV in low- and middle-income settings where there are limited or no existing HBV testing guidelines, there is a substantial need for HBV testing guidelines.

Advances in HBV detection technology create new opportunities for enhancing screening, referral and treatment. Previous systematic reviews on hepatitis B infection have focused on immunological responses, surveillance of cirrhosis and treatment. Existing systematic reviews on hepatitis B testing focused on point-of-care (POC) tests and included tests with unclear reference standards. No systematic reviews have examined the diagnostic accuracy of using HBsAg/HBeAg compared to HBV DNA detection to monitor treatment response.

<table>
<thead>
<tr>
<th>PICO 8</th>
<th>Among patients receiving treatment for HBV, what is the diagnostic accuracy of HBsAg/HBeAg test versus NAT to confirm successful treatment response?</th>
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<tbody>
<tr>
<td>P</td>
<td>Patients receiving treatment for HBV</td>
</tr>
<tr>
<td>I</td>
<td>HBsAg/HBeAg testing</td>
</tr>
<tr>
<td>C</td>
<td>NAT for HBV DNA detection</td>
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</table>
| O      | Diagnostic accuracy  
True negatives (TNs) – who are screen negative and have cleared the HBV infection.  
False negatives (FN) – who are screen negative but have HBV infection. These will be misclassified and treatment will be stopped resulting in disease progression leading to liver-related morbidity (fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma), progression of liver disease and mortality.  
True positives (TP) – who are screen positive and truly have HBV infection. This will increase the number of treated cases and cure rate.  
False positives (FP) – who are screen positive, but do not have HBV infection. (These will continue treatment inappropriately, and will have unnecessary referral).  
Costs – cost of testing strategy, including lab reagents and running costs, cost of further evaluation of a false positive.  
Cost–effectiveness |
3. Objectives

The purpose of this review was to identify evidence on the sensitivity and specificity of HBsAg/HBeAg compared to HBV DNA detection for HBV treatment monitoring and to summarize the key test characteristics associated with detection of HBsAg/HBeAg.

4. Methodology

We followed standard guidelines and methods for systematic review and meta-analyses of diagnostic tests.\textsuperscript{14,15} We prepared a protocol for the literature search, article selection, data extraction and assessment of methodological quality.

Selection criteria

\textbf{i. Types of studies}

We included observational studies and randomized controlled trials (RCTs) that provide original data from patient specimens, including cross-sectional and case–control studies, and studied HBsAg/HBeAg testing compared to a reference standard of HBV DNA detection.

\textbf{ii. Participants}

Little information on participants was provided in the selection of papers included in the systematic review; therefore, we set a wide inclusion criterion. We included patients of all age groups from all settings and countries as well as all types of specimens.

\textbf{iii. Index tests}

Studies that utilized commercially available HBsAg/HBeAg and HBV DNA assays were eligible for inclusion. The following four are the index tests included:

- Architect HBsAg assays, Abbott
- COBAS AMPLICOR TM HBV Test v2.0 assay, Roche Diagnostics Systems
- IMx HBeAg assay, Abbott
- Iprobe, Abbott
iv. Reference standard

The reference standards acceptable for a definitive diagnosis included tests for detection of HBV by the following HBV DNA detection techniques—polymerase chain reaction (PCR), branched-chain DNA (bDNA), or transcription-mediated amplification (TMA) and DNA hybridization assays.

Outcome measures

Sensitivity refers to the proportion of samples with true HBV infection diagnosed with positive HBsAg/HBeAg test confirmed with a positive HBV DNA detection method.

Specificity refers to the proportion of samples with negative HBsAg/HBeAg test confirmed with a negative HBV DNA detection method.

Search methods

A database search of LILACS, MEDLINE, EMBASE, PubMed, Scopus, Web of Science, Cochrane and WHO Global Index Medicus was performed through April 2015. No language restriction was applied. The references of published articles found in the above databases were searched for additional pertinent materials.

Study selection proceeded in three stages. First, titles/abstracts were screened by a single reviewer according to standard inclusion and exclusion criteria. Second, full manuscripts were obtained and assessed against inclusion criteria. Papers were accepted or rejected and reasons for rejection were specified. Third, two independent reviewers assessed each manuscript and differences were resolved by a third independent reviewer.

Data extraction

Information on the following variables were extracted by a reviewer if the study met the exclusion and inclusion criteria—first author, total sample size, country (and city) of sampling, sample type (oral fluid, finger-prick, venous blood, etc.), point-of-care (Y/N), eligibility criteria, reference standard, manufacturer, raw cell numbers (true positives, false negatives, false positives, true negatives), sources of funding and reported conflicts of interest. We define point of care as being able to give a result within 60 min and having the results guide clinical management at the same encounter.

Assessment of methodological quality
Study quality was evaluated using the QUADAS-2 tool,\textsuperscript{16} the STARD checklist\textsuperscript{17} and the GRADE method.\textsuperscript{18} QUADAS includes domains to evaluate bias in the following categories—risk of bias (patient selection, index test, reference standard, flow and timing); applicability concerns (patient selection, index test, reference standard). The GRADE method evaluates the strength of evidence by assessing the risk and probability of bias, imprecision and inconsistency as well as dose–respondent gradient and residual confounding.\textsuperscript{18}

5. Results

PRISMA flowchart

\textbf{Fig. 1.} PRISMA flow diagram outlining study selection examining diagnostic accuracy of HBV antibody tests compared to HBV DNA in confirming successful treatment response
Characteristics of included studies

Only two of the studies analysed met the PICO criteria and data were extracted from both these studies. These studies took place in Sweden and the United States of America. The patient population for the Larsson 2013 study was derived from a clinical setting; there was no information on the patient population for the Perrillo 1993 study. The assays evaluated for this
systematic review were Architect HBsAg assays, COBAS AMPLICOR TM HBV Test v2.0 assay, IMx HBeAg assay and Iprobe. Of these two studies, only one reported sensitivity/specificity of HBsAg and one reported sensitivity/specificity of HBeAg.

The lack of information on these diagnostic accuracy measures is a large limitation in the quality of the studies. Other issues with the quality of these studies were insufficient information on the populations studied, randomization and sample collection.

**Table 1.** Description of study design, study population and setting of all studies (n=2)

<table>
<thead>
<tr>
<th>First author</th>
<th>Sample type &amp; size</th>
<th>Country</th>
<th>Treatment</th>
<th>Study population</th>
<th>Eligibility criteria</th>
<th>Index diagnostic test</th>
<th>Reference test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larsson, 2013</td>
<td>Liver tissue and blood sample N= 160</td>
<td>Sweden</td>
<td>INF</td>
<td>Infectious Disease clinic N =160</td>
<td>Patients with chronic HBV</td>
<td>Architect HBsAg assays, Abbott</td>
<td>COBAS AMPLICORT M HBV Test v2.0 assay. Roche</td>
<td>34%</td>
<td>89%</td>
</tr>
<tr>
<td>Perrillo, 1993</td>
<td>Plasma N= 34</td>
<td>United States of America</td>
<td>INF</td>
<td>?</td>
<td>29 patients on treatment and 5 neg. controls</td>
<td>IMx HBeAg assay, Abbott</td>
<td>Iprobe, Abbott</td>
<td>95%</td>
<td>44%</td>
</tr>
</tbody>
</table>

Larsson et al. (2013) monitored HBsAg levels (Architect assays, Abbott) and HBV DNA quantitation (COBAS AMPLICOR™ HBV monitor, Roche) in 160 patients treated for chronic HBV infection at the Infectious Disease Clinic at Sahlgrenska University Hospital between 1993 and 1995. Sensitivity of HBsAg compared to HBV DNA was 34% and the specificity was 89%. A correlation between HBsAg and HBV DNA in serum samples ($R^2 = 0.39; P< 0.0001$) was also noted, in that a 90% reduction of HBV DNA corresponded to a 48% decline in HBsAg. The authors also measured HBeAg levels and found that HBeAg-positive patients had a 300 times higher HBV DNA/HBsAg ratio compared to those who were HBeAg-negative. These results indicate that HBsAg quantification could be complementary to HBV DNA quantification for treatment monitoring and confirming successful treatment response.

Perrillo et al. (1993) evaluated whether the HBeAg assay (Abbott IMX) was capable of providing comparable information to HBV DNA assays (Iprobe, Abbott) during and after IFN therapy in 29 consecutive, IFN-treated patients and five untreated controls. The authors found that decremental and incremental changes in HBeAg concentration during and after therapy mirrored those observed with HBV DNA with a significant correlation ($R = 0.768 P>0.0001$). Only 56% of HBV DNA-negative patients tested positive for HBeAg but 95% of HBV DNA-positive samples were also positive for HBeAg. Though this information allows us to understand that HBeAg concentrations can provide similar clinically relevant information compared to HBV DNA
assays, it is difficult to state the accuracy of these tests against each other as they are traditionally used to measure different indicators.

**Narrative summary of each systematic review’s findings**

Monitoring response to treatment is an essential mechanism in the control of HBV and requires both the sustained disappearance of HBV DNA and the clearance of HBsAg/ HBeAg from the blood. The systematic review showed that monitoring of HBeAg concentration can provide clinically relevant information, though only two of the 6464 studies identified for screening (Larrson et al. 2013 and Perrillo et al. 1993) were included in the systematic review as they were the sole articles that met both the inclusion and exclusion criteria for PICO 8 (showed sensitivity and specificity of assays).

i. **Diagnostic accuracy of nucleic acid testing**

HBV DNA is essential when determining the presence of the virus as it is quantitatively expressed and allows for prompt detection of HBV. With the advent of reverse transcriptase-polymerase chain reaction (RT-PCR), it quickly became regarded as the gold standard or confirming response to therapy due to its accuracy and cost-effectiveness. However, in many resource-limited settings, such assays are not widely available, therefore it is important to determine if HBsAg or HBeAg can be used for monitoring response to treatment.\(^{19}\)

ii. **Diagnostic accuracy of HBsAg test compared to HBV DNA**

Although they did not include specific accuracy values, three supplemental studies provided useful information on the quantitation of HBsAg for treatment monitoring in chronic HBV patients. Martinot-Peignoux et al. (2015) reported that the kinetics of HBsAg and HBV DNA followed similar profiles during treatment with PEG-IFN and follow up in patients who developed SVR (solid line) (see Fig. 2).\(^{18}\)

**Fig. 2.** Serum HBV DNA and HBsAg kinetics during treatment with PEG-IFN and follow up in patients who developed SVR (solid line) \(^{20}\)
This was confirmed by Ganji et al. (2011) who showed HBsAg had strong correlation with HBV DNA \( (r = 0.69; \ P < 0.01) \) for both genotypes investigated.\(^\text{20}\) Larsson et al. 2014 further proved that there was a correlation between HBsAg and HBV DNA for all four genotypes (Fig. 3).\(^\text{19}\) This highlights the potential for HBsAg to be a useful serological marker to predict response to treatment.

**Fig. 3 (A–D).** Correlation between HBsAg and HBV DNA in genotypes A–D

**Fig. 3 (E).** Box plot of HBsAg levels in HBeAg-positive and -negative patients by genotype (no significant differences)\(^\text{19}\)
Another important use for HBsAg assays is in monitoring treatment response for HBeAg-negative chronic patients with low HBV DNA levels. Sonneveld et al. (2011) reported that when monitoring PEG-IFN treatment in patients with chronic hepatitis B, HBsAg reduction is most pronounced in patients who achieve a response to therapy at 6 months post treatment. This suggests that HBsAg quantification can allow for detection of active cases of chronic HBV from true inactive carriers, thereby reducing the need to rigorously monitor HBV DNA levels.
iii. 4.5.3 Diagnostic accuracy of HBeAg test compared to HBV DNA

Monitoring HBeAg has been shown to be important due to its association with the disappearance of replicative viral intermediates and its persistence in the blood once HBV DNA has cleared. Using PEG-IFN alfa-2a, Fried et al. (2008) showed that HBeAg levels proved to be a stronger indicator of non-response compared to HBV DNA after 24 weeks of treatment. Lower levels of HBV DNA were seen to closely predict seroconversion (Table 2).

Table 2. Serum HBV DNA at weeks 12 and 24 of treatment: relationship to HBeAg seroconversion
It was also shown that those who reached HBeAg seroconversion had a consistent decline in their levels of HBeAg and remained at the lowest levels while under the follow-up period. This was in contrast to those who failed to achieve seroconversion after treatment was discontinued, as a rebound was observed allowing for better determining of seroconversion and a higher negative predictive value (Fig. 5). This highlights the importance of HBeAg in differentiating late responders from non-responders and is an important aspect of treatment.\textsuperscript{22}

**Fig. 5.** HBV DNA levels: responders versus non-responders at 24 weeks post treatment – HBeAg seroconversion\textsuperscript{23}

However, monitoring treatment response using HBeAg can be complicated as the response may vary with the therapy used. Non-interferon agents rarely cause HBeAg loss or might cause only a transient HBeAg loss while on therapy. Interferon agents are toxic and might convert ~35% of
HBeAg positives to negatives but only in a subset of people with high alanine aminotransferase (ALT).\textsuperscript{23}

Monitoring HBV treatment response remains a challenge. Guidelines for chronic HBV management and treatment state that the ideal end-point of treatment should be dictated by a lack of detectable HBsAg. The use of HBsAg/HBeAg as a marker to detect sustained virological response is essential, because on-treatment decrease in HBV DNA shows similar patterns for both sustained responders and relapers (Fig. 3).\textsuperscript{20} Due to the infrequency of obtaining this point with the current anti-HBV agents, the primary goal of antiviral therapy is defined as viral remission, PCR non-detectability (<300 copies/mL [57 IU/mL]).\textsuperscript{19,21}

For the time being, NAT can be used as the reference standard to confirm this response to therapy. More studies are needed to determine which tests for HBV antigen detection may be useful as effective markers of treatment response for therapeutic agents.

References

A. Reference list of studies that met criteria for inclusion in the analysis


B. Reference list from background


