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Bringing quantitative HBsAg to the US provider, drug development and patient network

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Disclosures:

- Robert G Gish MD acts as a consultant to Quest Diagnostics

- For all disclosures: see robertgish.com

Presentation Overview

- Quantitative HBsAg assays have not been available in the US

- qHBsAg from Abbott: Architect and Roche Elecsys have been available world-wide for more that 5-10 years

HBsAg levels can help determine the appropriate treatment

- Treatment for HBV consists of PEG-IFN or Nucleoside Analogues (NA). The duration and treatment effect for these medications are different with different phases of disease
- Patients who had an on-treatment decline in HBsAg were more likely to achieve sustained virologic response regardless of treatment choice.[1]
- HBsAg levels can be used to differentiate different phases of Hepatitis B disease.
- HBsAg levels can predict what the response to treatment will be, which can provide information on continuing therapy or stopping therapy. Patients who had the greatest decrease in HBsAg concentration along with a decrease in HBV DNA and HBeAg were more likely to achieve off treatment response.[1,2]
- Patients who did not have a decrease in HBsAg at 12 weeks, had a low likelihood of achieving treatment response, and other treatments should be considered.[2]

1. Seth AK. HBsAg Quantification in Clinical Practice. Journal of Clinical and Experimental Hepatology. 2012;2(1):75-80. doi:10.1016/S0973-6883(12)60084-X. 2. Sonneveld, M. J et al. Prediction of sustained response to peginterferon alfa-2b for hepatitis B e antigen–positive chronic hepatitis B using on-treatment hepatitis B surface antigen decline. Hepatology, 52: 1251–1257.

HBsAg Quant, Therapeutic Monitoring (TC 94333(X))

Samples used for validation

- 20 commercially-available plasma samples with known HBsAg levels
- 3rd WHO International Standard (NIBSC 12/226)
- WHO HBsAg Reference Panel (NIBSC 03/262)
- NIBSC 13/B625 QC sample
- 15 low-level HBsAg positive samples (commercially available) with known values
- Serum samples with positive and negative results as previously determined by the current qualitative HBsAg test (Note: where serum volume was limited, positive samples were spiked into pooled negative sera and used for linearity, precision and sample stability studies)
- 40 blood donor sera

HBsAg Quant, Therapeutic Monitoring (TC 94333(X)) cont.

Standards used in the validation study

- In house standards were prepared using purified HBsAg containing known levels of ad & ay subtypes, respectively
- On the date of testing, a cocktail of the ad and ay proteins was prepared and serially diluted; these standards were tested in conjunction with samples and used to construct a polynomial standard curve whereby unknown SCR values could be transformed into qualitative IU/mL results.
- The standards consisted of known values ranging from 250 IU/mL – 0.037 IU/mL, making the final analytic range 0.05 – 25,000 IU/mL where the testing algorithm included samples tested both undiluted and diluted 1:100.

Monitoring Response to Therapy

- Monitoring of ALT levels during treatment helps to assess treatment response[1]
  - Falling ALT levels are consistent with response to treatment
- Quantitative HBV DNA assays can help monitor response to therapy and predict the emergence of resistance to antiviral agents[1]
  - Results are used to guide changes in drug selection after therapy initiation: a change in regimen may be appropriate for patients receiving a nucleoside analogue who do not achieve a primary response (<2-log decrease in HBV DNA within 6 months)[2]
- In HBeAg-positive patients, the loss of HBeAg is an indicator of treatment response[1]

1. Andersson KL, et al. *Hepatology*. 2009;49(5 suppl):S166-S173. 2. Lok A, McMahon BJ. *Hepatology*. 2009;50(3):661-662.

Verification of standard values

- To assess the accuracy of the initially-determined in-house standard assignments (based on ad & ay values determined by the manufacturer), the WHO 3rd International Standard 12/226 was run as an unknown for quantitative HBsAg.
- Acceptance criteria for the 3rd International standard is within 75 – 125% of its assigned value.
- **Our *Observed IU/mL (40.30) was within 85% of the original provided (47.3) to us.***

Method validation

- 20 commercially-available samples with known HBsAg values previously determined by the Abbott Architect.
- The test values were log-transformed to normalize the distortion effect at the high measureable values;
- The linear regression observed was (R^2: 0.9874, slope: 1.0256)
- These findings meet the acceptance criterion of a coefficient of determination (R^2) >0.90, with the slope of the line 0.80-1.20.

Linearity

5 positive samples were serially diluted twofold in Vitros Sample Diluent B

Initial sample composition:	Serial dilutions:	Range evaluated:	R^2:	Slope:
a. High positive sample	2-fold	230 - 0.1	0.9995	1.0071
b. ay-spiked into negative serum	2-fold	71.6 - 0.07	0.9986	1.0083
c. Positive spiked in pooled negative sera	2-fold	168 - 0.08	0.9930	1.0279
d. Positive spiked in pooled negative sera	2-fold	192 - 0.09	0.9808	1.0482
e. Positive serum in pooled negative sera	4-fold	201 - 0.05	0.9999	1.0605

- The combined linear regression was (R^2: 0.9924, slope: 1.0196)
- These findings meet the acceptance criterion of a coefficient of determination (R^2) >0.90, with the slope of the line 0.80-1.20.
- Linearity studies for undiluted and low level samples also met the acceptance criteria mentioned above.

Blood donor seroprevalence

- Seroprevalence for HBsAg in blood donor samples was evaluated by testing 43 sera obtained from healthy blood donors (samples tested undiluted).
- None of the samples showed measureable HBsAg, meeting the acceptance criteria that 100% of samples show levels of HBsAg below the lowest limit of quantitation

Guideline recommendations

Currently in the United States, there are no guidelines that recommend utilizing quantitative HBsAG measurements in clinical practice.

- Clinical practice guidelines are in consensus that the qualitative HBsAg test is the primary way to definitively diagnose chronic HBV infection.

- Hepatitis B treatment guidelines, from areas of the world where HBV is endemic, do have HBsAg monitoring to predict treatment type and outcomes included as a guide for management of HBV infection.[1-3]

    - Asian Pacific Association for Study of the Liver (APASL)
    - World Health Organization (WHO)
    - National Institute for Health & Care Excellence (NICE)

1. Asian Pacific Association for Study of Liver Hepatol Int (2016) 10:1–98 DOI 10.1007/s12072-015-9675-4 /; 2. National Institute for Health & Care Excellence https://www.nice.org.uk/sharedlearning/using-the-hepatitis-b-surface-antigen-hbsag-quantitative-assay-test-to-evaluate-patient-response-to-treatment-for-chronic-hepatitis-b-virus-infection; 3. World Health Organization: http://www.who.int/hiv/pub/hepatitis/hepatitis-b-guidelines/en/; .

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Thank You!
To the Quest Development Team
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