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Could Hepatitis B Surface Antigen (HBsAg) Quantitation Alternate Hepatitis B Viral Load (HBV) Quantification in Clinical Hepatitis Diagnosis? A Retrospective Study and Horizontal Quality Checks on Two Independent Assays Carried Out in Microbiology and Chemical Pathology Departments at National Hospital Abuja, Nigeria

Ilegogie, O. A[1*], Ajobiewe, J. O[2,] Akhaumere, E. O[1], Ajobiewe H. F[3] , Erukubami, J[2], Ojo, A. E[1], Dabi, E.C[1], Ndike, U.P[2], Alau, K[5], Sonibare, O. O[1], Agharanya, C. C[2], Okechuku, J. I[2] , Ajobiewe, T. P[6], Salami, A. O[7] , Dangana, A[8]

[1]Chemical Pathology Department, National Hospital Abuja, 265 Independence Ave, Central Business District 900103, Abuja, Nigeria
2Microbiology Department, National Hospital Abuja, 265 Independence Ave, Central Business District 900103, Abuja, Nigeria
3Bingham University Nasarawa State of Nigeria, Nigeria
4Defence Reference Laboratory, Asokoro, Abuja, Nigeria
5Society for Family Health Gimbiya Street Garki Area 11 Abuja, Nigeria
6Biological Science Department, University of Abuja, Nigeria
7United State Department of Defense, Walter Reed Program Nigeria and US Embassy Abuja, Nigeria
8University of Abuja Teaching Hospital Abuja, Nigeria

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***Corresponding author:** Ilegogie, O. A
Chemical Pathology Department, National Hospital Abuja, 265 Independence Ave, Central Business District 900103, Abuja, Nigeria

Abstract **Original Research Article**

This study retrospectively investigated the extent of correlation between HbsAg quantitation and HBV quantification, whether knowing the value of one could adequately serve as an indicator for the value of another. We also investigated the extent of reliability of our results output by way of horizontal quality checks on two assays independently conducted on a cohort of patients at both Microbiology and Chemical pathology departments of the Hospital named above. Hepatitis B surface antigen (HBsAg) is produced and secreted through a complex mechanism that is still not fully understood. In clinical fields, HBsAg has long served as a qualitative diagnostic marker for hepatitis B virus infection. Notably, advances have been made in the development of quantitative HBsAg assays, which have allowed viral replication monitoring, and there is an opportunity to make maximal use of quantitative HBsAg to elucidate its role in clinical fields. Yet, it needs to be underscored that a further understanding of HBsAg, not only from clinical point of view but also from a virologic point of view, would enable us to deepen our insights, so that we could more widely expand and apply its utility. HBV belongs to *Hepadnaviridae* and is composed of the envelope, core, DNA genome, and viral polymerase. It has a circular form of partially double-stranded DNA and is approximately 3200 nucleotides in length. A 42-45 nm long HBV spherical form (Dane particle), which is the full virion with infectivity, can be visualized under electron microscopy. Using the COBAS TAGMAN 48 PCR for HBV viral load technique in Microbiology and, the Cobas e 411 HBsAg II (Roche Diagnostics, Indianapolis, IN, USA) we could not observe much correlation between the two parameters, though the Spearman ranked correlation coefficient, r, was positive with value of 0.02. Serum HBeAg for this cohort showed a high positive correlation of r= 0.9 with high HBV DNA viral load; also, when HBeAg was weakly positive it correlated perfectly with relatively low HBV DNA viral load; we observed similar trend with quantitative HBsAg unisex across all ages tested. We therefore concluded that serum HBeAg was a better alternative/predictor assay for HBV DNA and HBsAg quantifications. While the correlation between HBV DNA and qHBsAg, though positive was very weak.

Keywords: Hepatitis B virus, Hepatitis B surface antigen, Quantitative assay, Virology.

STUDY BACKGROUND

Hepatitis B virus (HBV) causes a wide range of clinical consequences, from acute and chronic infection to cirrhosis and hepatocellular carcinoma, and represents a global public health problem [1]. Historically, HBV dates to 1967 when an unknown

antigen in Australia was recognized to be associated with hepatitis type B, which was later referred to as the hepatitis B surface antigen (HBsAg) [2]. Since then, HBsAg has served as a qualitative diagnostic marker for HBV infection. Notably, advances have been made in the development of quantitative HBsAg assays, which have allowed viral replication monitoring. A number of clinical studies have evaluated the clinical utility of HBsAg and suggested its potential roles. Yet, it needs to be underscored that a further understanding of HBsAg, not only from a clinical point of view but also from a virologic point of view, would enable us to deepen our insights, so that we could more widely expand and apply its utility. Therefore, in this article, we review current concepts and issues on the quantification of HBsAg titers (qHBsAg) with respect to their biologic nature, method principles, and clinically relevant topics.

STRUCTURE AND MOLECULAR VIROLOGY OF HBsAg

Components of the viral structure

HBV belongs to *Hepadnaviridae* and is composed of the envelope, core, DNA genome, and viral polymerase. It has a circular form of partially double-stranded DNA and is approximately 3200 nucleotides in length [3]. A 42-45 nm long HBV spherical form (Dane particle), which is the full virion with infectivity, can be visualized (Figure (Figure 1)1) under electron microscopy. It has two-layered shells. The outer shell is the envelope protein referred to as hepatitis B surface (HBs) protein, which is further divided into small, middle, and large HBs proteins (SHBs, MHBs and LHBs proteins, respectively), and the inner shell is a core protein referred to as the hepatitis B core protein in which viral polymerase and the HBV genome is enclosed. In addition to the abovementioned full virion, smaller non-infectious sub viral particles are present in the serum; 17-25 nm spherical particles, mainly composed of SHBs protein, constitute the most abundant form, which is as much as 10 000-fold in excess of the full infectious virion Filamentous (or tubular) particles are another form, with a 20 nm diameter and variable length, and are composed of SHBs, MHBs, and the LHBs protein. The form of the HBV particles appears to be determined by the proportion of LHBs protein [4]. All three forms can be detected in serum with commercial assays and are collectively referred to as HBsAg.

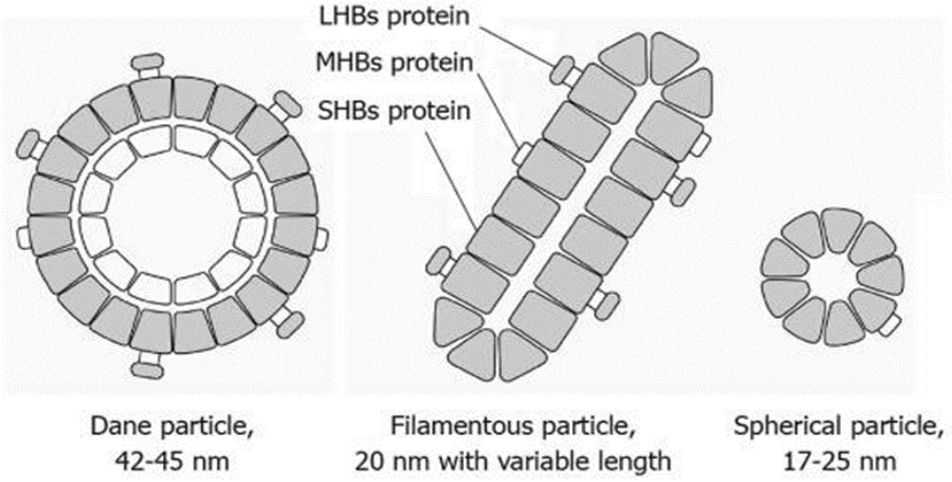

Figure 1

Schematic model of hepatitis B surface antigen structure. Three forms of hepatitis B surface (HBs) antigen (Dane particle, filamentous particle, and spherical particle) are visualized in serum by electron microscopy. These are composed of small, middle, and large hepatitis B surface proteins. LHBs: Large HBs proteins; MHBs: Middle HBs proteins; SHBs: Small HBs proteins.

KM NV

Synthesis and secretion

HBV has four distinct open reading frames (ORFs) that encode the envelope, core, polymerase, and X proteins. ORF S has three internal AUG codons encoding the SHBs, MHBs, and LHBs proteins, which correspond to the S, preS2 + S, and preS1 + preS2 + S domains, respectively (Figure 2). These proteins have a common carboxyl end but different amino ends [5].

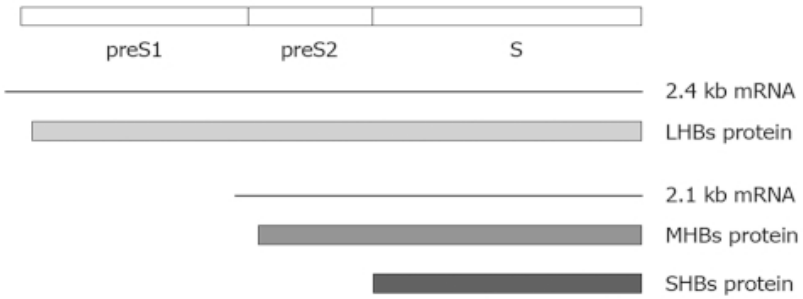

Figure 2

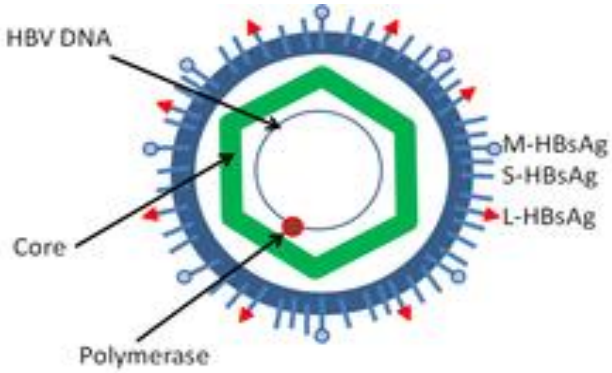

Figure 3: Structure of Hepatitis B Virus

Hepatitis B virus is a member of the Hepadnavirus family [6]. The virus particle, called Dane particle [7] (virion), consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. The nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses [8]. The outer envelope contains embedded proteins which are involved in viral binding of, and entry into, susceptible cells. The virus is one of the smallest enveloped animal viruses with a virion diameter of 42 nm, but pleomorphic forms exist, including filamentous and spherical bodies lacking a core. These particles are not infectious and are composed of the lipid and protein that forms part of the surface of the virion, which is called the surface antigen (HBsAg) and is produced in excess during the life cycle of the virus [9].

Schematic presentation of the S/preS1/preS2 gene, RNA transcripts, and translational products. Opening reading frame S has three internal AUG codons. Transcription to produce the 2.1 kb and 2.4 kb mRNAs first occurs after translation into small hepatitis B surface proteins (SHBs), middle hepatitis B surface proteins (MHBs), and large hepatitis B surface proteins (LHBs) ensues with different promoters.

Like all other proteins, mRNA transcription is the first event to occur. Two 2.1 kb mRNAs for the M/SHBs proteins and a 2.4 kb mRNA for the LHBs protein are formed, and take a separate pathway from viral replication. Diverse transcription factors are involved and act on promoters, enhancers, and other regulatory elements, such as the glucocorticoid responsive element [10]. LHBs and M/SHBs expression are thought to be independently regulated with different promoters; a typical TATA box is present in the LHBs promoter (S promoter I, SPI), whereas the TATA-less promoter, which usually has multiple initiation sites, is associated with the M/SHBs promoter, thus accounting for synthesis of distinct proteins from one mRNA. In patients with active viral replication, the protein expression pattern shows a predominance of the M/SHBs protein in contrast to a predominance of the LHBs protein in inactive carriers [11]. After transcription, protein synthesis and glycosylation follows at the endoplasmic reticulum (ER) membrane resulting in a 226 amino acid SHBs protein, the MHBs protein with an additional 55 amino acids, and the LHBs protein with an additional 108-119 amino acids. Although the LHBs mRNA includes the M/SHBs sequence, it does not translate into the M/SHBs protein, and the ratio between the MHBs and SHBs protein is controlled by a complex mechanism, which is not fully understood [12]. To form a full virion, a mixture of HBs proteins in a well-balanced ratio is utilized to envelop core particles in which SHBs and LHBs protein are indispensable [13]. The virion is transported to the cell membrane through vesicles, and several conditions must be satisfied for successful secretion, because excess SHBs protein is required, whereas excess LHBs protein prevents secretion and causes dilatation of the ER with a ground-glass appearance [14].

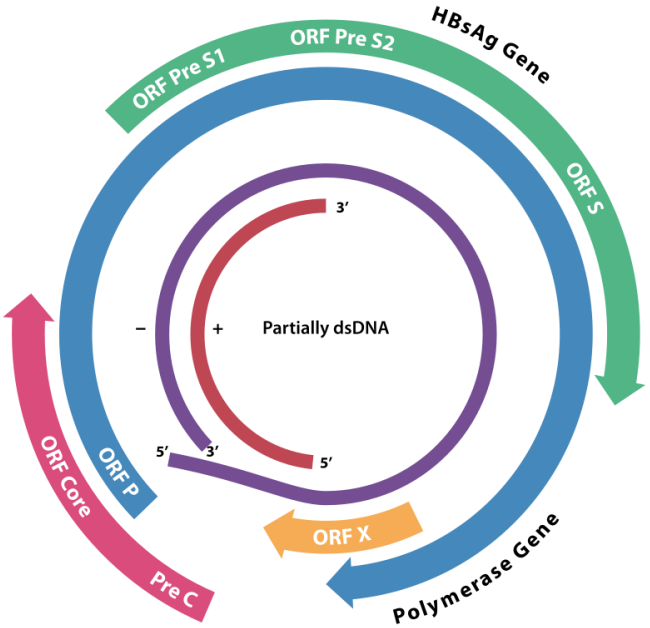

Figure 4: The Genome Organization of HBV. The Genes Overlap

Size

The genome of HBV is made of circular DNA, but it is unusual because the DNA is not fully double-stranded. One end of the full length strand is linked to the viral DNA polymerase. The genome is 3020–3320 nucleotides long (for the full length strand) and 1700–2800 nucleotides long (for the short length strand) [15].

Encoding

The negative-sense, (non-coding) strand is complementary to the viral mRNA. The viral DNA is found in the nucleus soon after infection of the cell. The partially double-stranded DNA is rendered fully double-stranded by completion of the (+) sense strand by cellular DNA polymerases (viral DNA polymerase is used for a later stage) and removal of the viral polymerase protein (P) from the (−) sense strand and a short sequence of RNA from the (+) sense strand. Non-coding bases are removed from the ends of the (−) sense strand and the ends are rejoined.

The viral genes are transcribed by the cellular RNA polymerase II in the cell nucleus from a covalently closed circular DNA (cccDNA) template. Two enhancers designated enhancer I (EnhI) and enhancer II (EnhI) have been identified in the HBV genome. Both enhancers exhibit greater activity in cells of hepatic origin, and together they drive and regulate the expression of the complete viral transcripts [16].

There are four known genes encoded by the genome called C, P, S, and X. The core protein is coded for by gene C (HBcAg), and its start codon is preceded by an upstream in-frame AUG start codon from which the pre-core protein is produced. HBeAg is produced by proteolytic processing of the pre-core protein. The DNA polymerase is encoded by gene P. Gene S is the gene that codes for the surface antigen (HBsAg). The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three sections, pre-S1, pre-S2, and S. Because of the multiple start codons, polypeptides of three different sizes called large, middle, and small (pre-S1 + pre-S2 + S, pre-S2 + S, or S) are produced [17].

The function of the protein coded for by gene X is not fully understood [18], but some evidence suggests that it may function as a transcriptional Trans activator. Interestingly, a 40 kDa X-Core fusion protein is encoded by a long viral 3.9-kb transcript, whose function remains unclear [18]. Synthesis of the 3.9 kb RNA initiates at the X gene promoter region and the transcript is polyadenylated only after the second round of transcription. Similar behavior is shared by other long pregenomic/pre-core (pg/pc) RNA species. Thus, the viral transcription machinery must ignore the poly (A) signal at the first transcription round.

Several non-coding RNA elements have been identified in the HBV genome. These include: HBV PR alpha, HBV PREbeta and HBV RNA encapsulation signal epsilon [19].

Genotypes

Genotypes differ by at least 8% of the sequence and have distinct geographical distributions and this has been associated with anthropological history. Within genotypes subtypes have been described: these differ by 4–8% of the genome.

There are eight known genotypes labeled A through H [20]. A possible new "I" genotype has been described [6], but acceptance of this notation is not universal [7].

Two further genotypes have since been recognized [8]. The current (2014) listing now runs A though to J. Several subtypes are also recognized.

There are at least 24 subtypes. Different genotypes may respond to treatment in different ways [21].

Individual Genotypes

Type F which diverges from the other genomes by 14% is the most divergent type known. Type A is prevalent in Europe, Africa and South-east Asia, including the Philippines. Type B and C are predominant in Asia; type D is common in the Mediterranean area, the Middle East and India; type E is localized in sub-Saharan Africa; type F (or H) is restricted to Central and South America. Type G has been found in France and Germany. Genotypes A, D and F are predominant in Brazil and all genotypes occur in the United States with frequencies dependent on ethnicity.

The E and F strains appear to have originated in aboriginal populations of Africa and the New World, respectively.

Type A has two subtypes: Aa (A1) in Africa/Asia and the Philippines and Ae (A2) in Europe/United States.

Type B has two distinct geographical distributions: Bj/B1 ('j'—Japan) and Ba/B2 ('a'—Asia). Type Ba has been further subdivided into four clades (B2–B4).

Type C has two geographically subtypes: Cs (C1) in South-east Asia and Ce (C2) in East Asia. The C subtypes have been divided into five clades (C1–C5). A sixth clade (C6) has been described in the Philippines but only in one isolate to date [21]. Type C1 is associated with Vietnam, Myanmar and Thailand; type C2 with Japan, Korea and China; type C3 with New Caledonia and Polynesia; C4 with Australia; and C5 with the Philippines. A further subtype has been described in Papua, Indonesia [22].

Type D has been divided into 7 subtypes (D1–D7)

Type F has been subdivided into 4 subtypes (F1–F4). F1 has been further divided into 1a and 1b. In Venezuela subtypes F1, F2, and F3 are found in East and West Amerindians. Among South Amerindians only F3 was found. Subtypes Ia, III, and IV exhibit a restricted geographic distribution (Central America, the North and the South of South America respectively) while clades Ib and II are found in all the Americas except in the Northern South America and North America respectively.

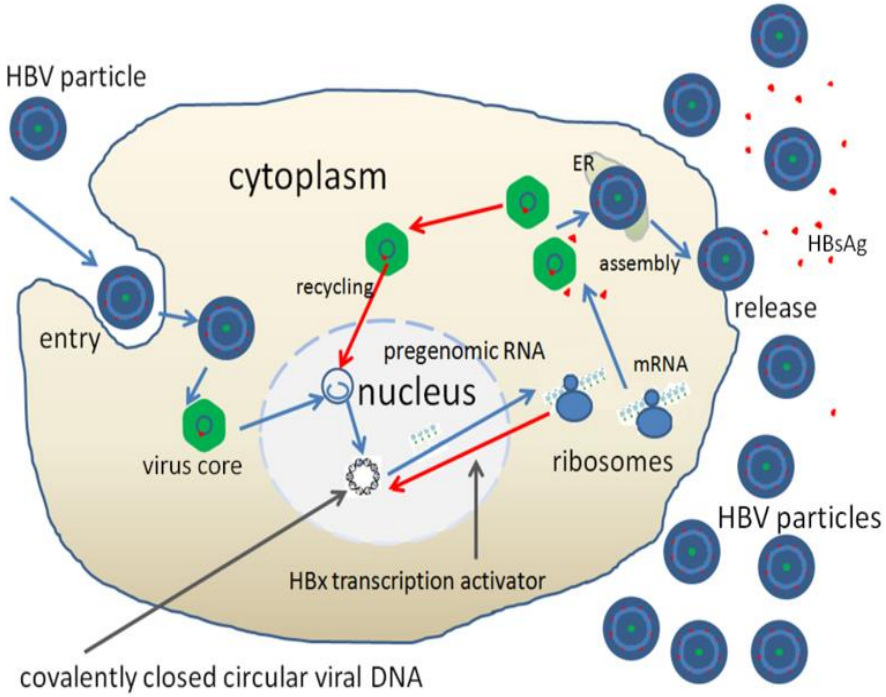

Figure 5: Life Cycle

***Hepatitis B virus* replication**

The life cycle of *Hepatitis B virus* is complex. Hepatitis B is one of a few known non-retroviral viruses which use reverse transcription as a part of its replication process.

Attachment

The virus gains entry into the cell by binding to receptors on the surface of the cell and entering it by endocytosis mediated by either clathrin or caveolin-1 [23]. HBV initially binds to heparin sulfate proteoglycan. The pre-S1 segment of the HBV L protein then binds tightly to the cell surface receptor sodium taurocolate transporting polypeptide (NTCP), encoded by the SLC10A1gene [24]. NTCP is mostly found in the sinusoidal membrane of liver cells. The presence of NTCP in liver cells correlates with the tissue specificity of HBV infection [3].

Penetration

Following endocytosis, the virus membrane fuses with the host cell's membrane, releasing the nucleocapsid into the cytoplasm [25].

Uncoating

Because the virus multiplies via RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus. It is thought that the capsid is transported in the microtubules to the nuclear pore. The core proteins dissociate from the partially double stranded viral DNA, which is then made fully double stranded (by host DNA polymerases) and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs.

Replication

The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral RNA-dependent-DNA-polymerase.

Assembly

These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and re-cycled to produce even more copies [26].

Release

The long mRNA is then transported back to the cytoplasm where the virion P protein synthesizes DNA via its reverse transcriptase activity.

Trans activated genes

HBV has the ability to transactivate [27].

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Function

The primary function of the HBs protein as a virologic structure is to enclose the viral components. It also plays a major role in cell membrane attachment to initiate the infection process. Several studies have confirmed the idea that the peptide in the preS1 domain

is essential in this process, showing that it specifically binds to the human liver plasma membrane and can be inhibited by a monoclonal antibody [28, 29]. However, participation of the SHBs protein in attachment has also been suggested following identification of hepatocyte-bound endonexin II, which specifically binds the SHBs protein [28]. Additionally, from the host perspective, the HBs protein has the major antigenic components, including the determinant, which is important for host-activated immunity. However, from a virologic perspective, it is postulated that excess HBs protein may divert such neutralizing antibody immune function away from the infectious virion [29].

QUANTITATIVE HBsAg ASSAYS

Methods to detect HBsAg were first described in the 1970s using radioimmunoassay and enzyme immunoassays [23]. Since then, various diagnostic techniques have been developed, which are mostly confined to qualitatively diagnose HBV in clinical practice. Recently, quantitative assay of HBsAg has been developed, and two commercially available assays will be briefly introduced here. The Architect HBsAg QT (Abbott Diagnostic, Wiesbaden, Germany) is a chemiluminescent micro particles immunoassay, which is currently the method most widely used in clinical studies [30]. The Architect HBsAg QT assay is a two-step immunoassay with flexible assay protocols, referred to as Chemiflex, for quantitatively determining human serum and plasma HBsAg concentrations. In the first step, the sample and hepatitis B surface antigen antibody (anti-HBs) coated with paramagnetic micro particles are combined. HBsAg present in the sample binds to the anti-HBs coated micro particles. After washing, acridinium-labeled anti-HBs conjugate is added. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the Architect Immunoassay System optics. The Architect HBsAg is a fully automated system and can detect as low as 0.2 ng/mL of HBsAg with a dynamic range of 0.05-250.0 IU/mL [31].

Elecsys HBsAg II (Roche Diagnostics, Indianapolis, IN, USA) is another method for quantitatively determining HBsAg [25]. In the first incubation step, the antigen in the sample reacts with two biotinylated monoclonal HBsAg-specific antibodies and a monoclonal/polyclonal (sheep) HBsAg-specific antibody, labeled with a ruthenium complex, to form a sandwich complex. In the second step, streptavidin-coated micro particles are added, and the complex binds to the solid phase *via* interaction with biotin and streptavidin. The results are reported as a cutoff index (signal sample/cutoff), and the sample is considered reactive if the index is greater than 1.0.

CLINICAL APPLICATION OF QUANTITATIVE HBsAg

Correlation with serum HBV DNA

Although measuring serum HBV DNA is the gold standard for monitoring viral load, it is relatively expensive and not yet readily available in some areas. By contrast, the technique for detecting qHBsAg is fairly easy and inexpensive, and the primary aim of initial clinical studies was to determine the relationship between qHBsAg and serum HBV DNA. In 2004, Deguchi *et al.,* [27] first reported the clinical significance of a high qHBsAg in patients who were hepatitis B e antigen (HBeAg) positive as opposed to those with an antibody positive to the hepatitis B e antigen (anti-HBe), and that qHBsAg correlated well with the serum HBV DNA level (r = 0.862). Although there are some contradicting results on whether qHBsAg is correlated with serum HBV DNA [26, 27], it seems that they are correlated based on a number of studies [28]. Further studies are required to investigate the possibility of using qHBsAg as an aid, if not an alternative, for HBV DNA.

METHODS

In HBsAg Quantitation, the Elecsys HBsAg II (Roche Diagnostics, Indianapolis, IN, USA); technique was used While COBAS TAGMAN 48 PCR was used for the HBV quantification according to the manufacturer's instruction**.** Total duration per essay was18 minutes. In the 1st round of incubation cycle ,50µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti HBsAg antibodies labeled with a ruthenium complex were compounded together to form a sandwich complex. In the 2nd round of incubation, after the addition of streptavidin-coated micro-particles, the complex was bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture was aspirated into the measuring cell where the micro-particles were magnetically captured onto the surface of the electrode. Unbound substances were then removed with ProCell/ProCell M. Voltage was applied to the electrode which induced chemiluminescent emission which was measured by a photomultiplier. The results were determined via a calibration curve which was instrumental and specifically generated by 2- point calibration and a master curve provided via the reagent barcode. The reagents-working solutions were; the reagent rack pack (M, R1, and R2) that was labeled as HBSAG-QN. Where M was Streptavidin-coated micro-particles (in transparent cap), 1 bottle, 6.5mL: Streptavidin- coated micro-particles 0.72mg/mL; preservative. R1 Anti-HBs Ag- Ab- biotin (in gray cap), 1 bottle, 8 mL; Two biotinylated monoclonal anti-HBsAg antibodies (obtained from mouse)> 0.5mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative. R2 was Anti-HBsAg – Ab-Ru $(bpy)^2{}_3+$ (in black cap), 1 bottle, 7ml. Monoclonal anti-HBsAg antibody (obtained from

mouse), polyclonal anti-HBsAg antibodies (from sheep) labeled with ruthenium complex >1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative. HBSAG-QN Cal 1 was Negative calibrator 1 (in white cap), 2 bottles of 1.3 mL each. Also used was Human serum and a preservative. HBSAG-QN Cal 2 was used as the Positive calibrator 2 (in black cap), purchased in 2 bottles of 1.3 mL each: There was also HBsAg approx. 0.5 IU/mL in human serum; and a preservative. HBSAG-QN DilHepB supplied in 2 bottles of 36 mL each (in white cap): We also had negative Human serum for HBsAg and anti-HBs, buffered, pH 6.5; preservative.

HYPOTHESES

HYPOTHES1S ONE

Ho: There is no correlation between quantitative hepatitis B surface antigen (qHBsAg) estimation and Hepatitis B Viral load DNA quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria.

Ha : There is correlation between quantitative hepatitis B surface antigen (qHBsAg) estimation and Hepatitis B Viral load quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria

HYPOTHES1S TWO

Ho: There is no correlation between quantitative hepatitis B surface antigen (qHBsAg) estimation and Hepatitis Be-antigen (HBeAg) quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria.

Ha: There is correlation between quantitative hepatitis B surface antigen (qHBsAg) estimation and hepatitis Be-antigen (HBeAg) quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria.

HYPOTHES1S THREE

Ho: There is no correlation between quantitative hepatitis B Viral load estimation and Hepatitis Be - antigen (HBeAg) quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria.

Ha: Ho: There is correlation between quantitative hepatitis B Viral load estimation and Hepatitis Be antigen (HBeAg) quantification among infected patients in both Microbiology and Chemical pathology departments at the National Hospital Abuja Nigeria.

RESULTS

Table 1

S/N	HbsAg Result (X)	Rank of HbsAg (R_x)	HBViral Load Result (Y)	Rank of HBViral Load (R_y)	D= R_x- R_y	D^2
1	382	12	102	24	-12	144
2	1188	22	116	34	-12	144
3	22640	180	125	37	143	20449
4	21360	178	106	26	152	23104
5	6804	96	9428	185	-89	7921
6	1377	28	274	70	-42	1764
7	19358	167	144915	201	-34	1156
8	2368	50	820	109	-59	3481
9	13351	135	22	1	134	17956
10	13602	136	15407649	209	-73	5329
11	27400	190	351	79	111	12321
12	5184	81	266	67	14	196
13	18306	160	8159	181	-21	441
14	34820	206	155	39	167	27889
15	1053	18	106	27	-9	81
16	4886	76	999	122	-46	2116
17	3128	57	222	59	-2	4
18	27680	192	109	29	163	26569
19	4112	70	59	15	55	3025
20	18532	162	1509	137	25	625
21	5390	83	159	40	43	1849
22	2318	48	53816769	210	-162	26244
23	33100	203	2503	167	51	2601
24	7916	107	1051	126	-19	361
25	7924	108	5571260	208	-100	10000
26	7398	102	991	121	-19	361
27	16034	147	122	35	112	12544
28	6356	92	44812	197	-105	11025
29	24460	184	2210	184	38	1444
30	9150	114	345	114	37	1369
31	9144	113	269	113	44	1936

S/N	HbsAg Result (X)	Rank of HbsAg (R_x)	HBViral Load Result (Y)	Rank of HBViral Load (R_y)	D= R_x- R_y	D^2
32	5178	80	7100	80	-99	9801
33	14002	139	303100	139	-65	4225
34	20760	173	3011	156	17	289
35	32340	202	8990	183	19	361
36	9074	112	2756	155	-43	1849
37	38640	209	2310	147	62	3844
38	26420	188	1100	128	52	2704
39	10200	125	900	115	10	100
40	9694	122	266	67	55	3025
41	11940	129	163	71	88	7744
42	17216	154	169	43	111	12321
43	12298	131	576	92	39	1521
44	7560	103	1107	130	-27	729
45	3552	63	2730	154	-91	8281
46	4084	69	676	99	-30	900
47	4211	71	777	105	-34	1156
48	1565	32	341	76	-44	1936
49	9544	118	75	19	99	9801
50	15768	144	11779	187	-43	1849
51	20180	170	61	16	154	23716
52	9550	119	361	7	112	12544
53	16232	149	281	71	78	6084
54	3890	66	1091	127	-61	3721
55	17502	155	661	97	58	3364
56	5071	79	59498	199	-120	14400
57	12384	132	856	113	19	361
58	34820	207	177	44	163	26569
59	9026	111	417	81	30	900
60	3480	62	11199	186	-124	15376
61	2286	46	1644	139	-93	8649
62	19392	168	305	73	95	9025
63	27780	193	144	38	155	24025
64	11036	127	4963205	207	-80	6400
65	15946	146	3210	161	-151	225
66	17720	156	610	93	63	3969
67	18352	161	7001	178	-17	289
68	211.8	9	77307	200	-191	36481
69	1362	26	402	80	-54	2916
70	1450	29	35	6	23	529
71	16508	150	55	14	136	18496
72	40720	210	4020	165	45	2025
73	3932	68	649	96	-28	784
74	1802	39	250	64	-25	625
75	28260	196	93	21	175	30625
76	22500	179	1665	141	38	1444
77	7226	99	221	58	41	1681
78	2.438	1	42	10	-9	81
79	15496	143	2437	148	-5	25
80	18548	163	83	20	143	20449
81	5600	84	19110	189	-105	11025
82	33640	204	284	72	132	17424
83	17790	158	2492	151	7	49
84	3216	59	22904	190	-131	17161
85	1800	38	1001	124	-86	7396
87	1674	35	122	36	-1	1
88	957.4	16	30	4	12	144
89	29880	200	9415	184	16	256
90	6088	89	345	77	12	144
91	21140	177	5839	174	3	9
92	2748	52	69	17	35	1225
93	1011	17	317322	205	-188	35344

S/N	HbsAg Result (X)	Rank of HbsAg (R_x)	HBViral Load Result (Y)	Rank of HBViral Load (R_y)	D= R_x- R_y	D^2
94	2310	47	167	42	5	25
95	4734	75	1322	136	-61	3721
96	21020	175	3205	160	15	225
97	37180	208	70	18	190	36100
98	1454	30	1275	135	-105	11025
99	17152	152	24481	19	-39	1521
100	14334	141	6380	176	-35	1225
101	31920	201	896	114	87	7569
102	9460	117	736	103	14	196
103	3800	65	558	90	-25	625
104	34220	205	846	111	94	8836
105	7.61	2	23	2	0	0
106	3168	58	1003	125	-67	4489
107	9.884	3	261	66	-63	3969
108	17004	152	930	119	33	1089
109	1896	41	310	74	-33	1089
110	13972	138	540	87	51	2601
111	149.6	7	8721	182	-175	30625
112	19064	164	710	101	63	3969
113	16142	148	110	31	117	13689
114	1834	40	421	82	-42	1764
115	26620	189	1952	143	46	2116
116	29320	198	110	32	166	27556
117	6994	98	1170	131	-33	1089
118	9640	121	1731	142	-21	441
119	11666	128	247924	203	-75	5625
120	2004	42	2485	150	-108	11664
121	3344	61	844	110	-49	2461
122	20180	170	189	45	125	15625
123	2130	43	205	49	-6	36
124	4390	73	33138	195	-122	14884
125	1343	24	109	29	-5	25
126	56.4	5	107	28	-23	529
127	29560	199	234	61	138	19044
128	19138	165	662	98	67	4489
129	9458	116	195	47	69	4761
130	7644	104	34	5	99	9801
131	1143	21	2100	145	-124	15376
132	2358	49	1100	129	-80	6400
133	10966	126	210168	202	76	5776
134	17758	157	189	46	111	12321
135	5726	85	510	86	-1	1
136	7386	101	239	62	39	1521
137	691.2	15	221	57	-42	1764
138	6780	95	3179	159	-64	4096
139	25020	186	327	75	111	12321
140	27540	191	96	22	169	28561
141	1751.2	36	29	3	33	1089
142	6522	93	2100	146	-53	2809
143	5758	86	5670	173	-87	7569
144	10100	124	29059	192	-68	4624
145	9928	123	241	63	60	3600
146	15860	145	221	56	89	7921
147	1633	34	974	120	-86	7396
148	28760	197	49	13	184	33856
149	19502	169	47	12	157	24649
150	1594	33	103	25	8	64
151	578.4	14	210	51	-37	1369
152	7242	100	219	55	45	2025
153	2272	45	31930	194	-149	22201
154	2250	44	37	8	36	1296

S/N	HbsAg Result (X)	Rank of HbsAg (R_x)	HBViral Load Result (Y)	Rank of HBViral Load (R_y)	D= R_x- R_y	D^2
155	27980	194	501	85	109	11881
156	182.0	8	5151	171	-163	26569
157	23400	183	3291	162	21	441
158	1371.8	27	4879	170	-143	20449
159	4890	77	210	53	24	576
160	14084	140	3710	163	-23	529
161	1540	31	210	52	-21	441
162	24680	185	778	107	78	6084
163	27980	195	799	108	87	7569
164	6250	91	614	94	-3	9
165	6986	97	4501	168	-71	5041
166	13752	137	3100	157	-20	400
167	8744	110	7980	180	-70	4900
168	2446	51	100	23	28	784
169	3044	56	547	89	-33	1089
170	9568	120	110	33	87	7569
171	5374	82	540	88	-6	36
172	1355	25	1001	123	-98	9604
173	1062	20	500	84	-64	4096
174	23340	182	1594	138	44	1936
175	21040	176	2570	153	23	529
176	7660	105	618	95	10	100
177	6214	90	704	100	-10	100
178	13108	134	735	102	32	1024
179	6068	88	3100	158	-70	4900
180	19200	166	250	65	101	10201
181	570.6	13	14094	188	-175	30625
182	25940	187	1219	133	54	2916
183	18052	159	6100	175	-16	256
184	14378	142	4098238	206	-64	4096
185	9196	115	909	117	-2	4
186	6042	87	1654	140	-53	2809
187	20700	172	209	50	122	14884
188	3010	55	575	91	-36	1296
189	16954	151	40	9	142	20164
190	20.15	4	43037	196	192	36864
191	1290	23	5600	172	-149	22201
192	12420	133	2450	149	-16	256
193	11966	130	4201	166	-36	1296
194	6592	94	4666	169	-75	5625
195	8342	109	3830	164	-55	3025
196	2932	54	4310	167	-113	12769
197	7706	106	909	118	-12	144
198	22660	181	195	48	133	17689
199	20880	174	778	106	68	4624
200	4296	72	906	116	-44	1936
201	33.8	11	852	112	-101	10201
202	4598	74	31791	193	-119	14161
203	1050	18	750	104	-86	7396
204	2792	53	101	36	17	289
205	3238	60	225	60	0	0
206	1791	37	1200	132	-95	9025
207	23.5	10	213	54	-44	1936
208	4914	78	444	83	-5	25
209	142	6	51261	198	-192	36864
210	3740	64	1243	134	-70	4900

Spearman ranked correlation, $r = 1 - 6 \sum D^2$
$n (n^2 - 1)$ r = 0.02
Where D= Difference in ranks, n = size of samples

Although there was a positive correlation, the association was quite weak, - being only 0.02 Due to the high numbers of samples tested in this current study we randomly matched HBeAg positivity colour intensity scored as ++++, +++, ++, + (visually according to the test, and control bands on the cassettes used for the assays) respectively as extremely high, very high, high and faintly indicative of HBeAg presence. Negative assays served as controls. While the manufacturer's inner cassette band served as intrinsic positive control. The following few randomly selected results were observed;

Table 2: HBeAg Versus qHBsAg

HBeAg	Rank Rx	Pooled qHBsAg	Mean qHBsAg	Rank Ry	D = Rx-Ry	D^2
++++	4	10,966; 17,758; 7,386, ……e.t.c.	13,700	3	1	1
+++	3	43,037; 27,980; 24, 680,…e.t.c.	40,100	4	-1	1
++	2	575; 219; 182; ……e.t.c.	570	2	0	0
+	1	40; 47; 96;…….e.t.c.	45	1	0	0

Spearman ranked correlation coefficient, rs =

$$\frac{\sum 6\,D^2}{N\,(N^2-1)}$$

$$\frac{6 \times 1\ 6/60}{4\ (16-1)} = 0.9$$

Test Quantification

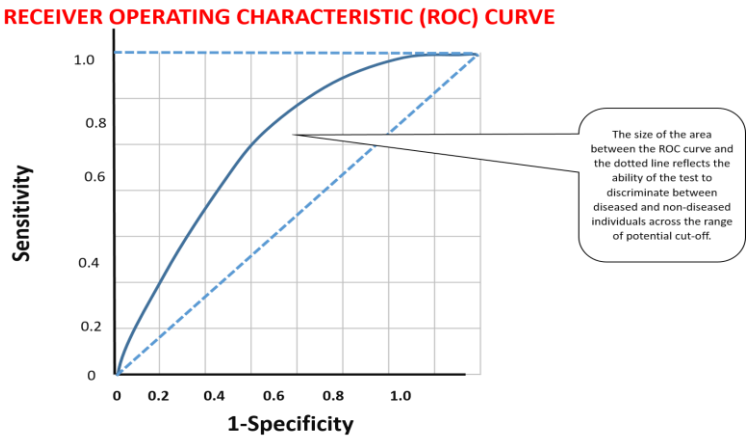
		NUMBER OF TOTAL VIRAL LOAD RESULTS		
TEST RESULTS		CONFIRMED	REFUTED	
HBeAg screening	POSITIVE	88	65	153
	NEGATIVE	42	15	57
		130	80	210

SENSITIVITY = 88/130 = 68%
SPECIFICITY = 15/80 =19%
POSITIVE PREDICTIVE VALUE, PPP; = 88/153 = 57.5%
NEGATIVE PREDICTIVE VALUE, NPP = 15/57 = 26.3%

Sensitivity: Proportion of those people who have the disease who are correctly detected by the test.
Specificity: Proportion of those people who do not have the disease who correctly left undetected by the test.
Positive Predictive Value ('*yield'*): Proportion of those testing positive who truly have the disease.
Negative Predictive Value (NPV): Proportion of those testing negative who are truly disease free.

Difference in Sensitivity and Specificity significant at 95% confidence limits; ($P < 0.05$)

Difference in Positive Predictive Value(PPV) and Negative Predictive Value(NPV) significant at 99% confidence limits; ($P < 0.01$)



P < 0.01

Likelihood ratio of the positive test results

$$LR+ = \frac{\text{True +ve rate}}{\text{True –ve rate}} = \frac{\text{sensitivity}}{\text{(1- Specificity)}}$$

Likelihood ratio of the negative test results

$$LR+ = \frac{\text{True - ve rate}}{\text{True + ve rate}} = \frac{\text{1-Sensitivity}}{\text{Specificity}}$$

Difference between likelihood ratio of the positive test results and those of the likelihood ratio of the negative test results are significant at 99% confidence limits: ($P<0.01$)

Table 3: HBeAg versus HBV DNA VIRAL LOAD

HBeAg	Rank Rx	Pooled HBV DNA Viral Load m/ml	mean	Rank Ry	D = Rx-Ry	D^2
++++	4	29059; 24481 …….. e.t.c.	29059	3	1	1
+++	3	31930……..e.t.c.	31930	4	-1	1
++	2	3179…….e.t.c	3179	2	0	0
+	1	906…….e.t.c,	906	1	0	0
RS	$1-\frac{\sum 6 D^2}{N (N^2 -1)}$ $1- \frac{6 X 1 6/60}{4 (16-1)} = 0.9$					

Perfect correlation thus existed between HBeAg and HBV DNA likewise we observed similar trend between HBeAg and qHBsAg.

DISCUSSION

Two hundred and ten hepatitis samples for HBeAg, HBV load, and HBsAg quantification were extensively analyzed and tested for their extent of correlations. In resource poor settings where serum HBeAg could easily be diagnosed using simple test cassettes by reagent manufactures, we tried to see whether the extent of positivity or negativity on these cassettes could accurately predict HBV DNA viral load and quantitative HBsAg values. We also investigated the extent of reliability of our results output by way of horizontal quality checks on two assays independently conducted on a cohort of patients at both Microbiology and Chemical pathology departments of the Hospital named above from our independently generated results output. Hepatitis B surface antigen (HBsAg) is produced and secreted through a complex mechanism that is still not fully understood. In clinical fields, HBsAg has long served as a qualitative diagnostic marker for hepatitis B virus infection. Notably, advances have been made in the development of quantitative HBsAg assays, which have allowed viral replication monitoring, and there is an opportunity to make maximal use of quantitative HBsAg to elucidate its role in clinical fields. Yet, it needs to be underscored that a further understanding of HBsAg, not only from clinical point of view but also from a virologic point of view, would enable us to deepen our insights, so that we could more widely expand and apply its utility. Our results agreed in parts and also contradicted the submissions by Deguchi *et al.,* [23] In 2004 who first reported the clinical significance of a high qHBsAg in patients who were hepatitis B e antigen (HBeAg) positive as opposed to those with an antibody positive to the hepatitis B e antigen (anti-HBe): and also reported that qHBsAg correlated well with the serum HBV DNA level (r = 0.862). Our work only supported some earlier researchers results who investigated whether qHBsAg was correlated with serum HBV DNA [26, 27], These researchers observed poor and highly weak correlation between qHBsAg and qHBV DNA in their studies [28-31]. We want to emphasize that in further studies attention should be paid to the sample size used; in this current study, considering the local period prevalence of hepatitis B virus in our hospital environment we hold our findings at high level of confidence. Thus, far more multicenter studies are still required to investigate the possibility of using qHBsAg as an aid, if not an alternative, for HBV DNA before a hard line of decision shall be drawn on this. We were not surprised at the consistent high level of correlation between HBeAg, qHBsAg and HBV DNA viral load (r=0.9) being a product of proteolytic cleavage of Hepatitis B pre core protein. Procedures and processes for obtaining the value of HBeAg are quite cheap and easily obtainable in resource limited settings, hence we suggest that it should be a better alternative assay in determining the values of qHBsAg and HBV DNA viral load.

CONCLUSION

We therefore concluded that serum HBeAg was a better alternative/predictor assay for HBV DNA and HBsAg quantifications. HBeAg values could be used as alternative assay in resource limited /poor settings to monitor the clinical progress of a hepatitis patient to therapy. While the correlation between HBV DNA and qHBsAg, though positive was very weak.

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