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Detection of Highly Prevalent Hepatitis B Virus Coinfection among HIV-Seropositive Persons in Ghana[▽]

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Simple hepatitis B surface antigen (HBsAg) tests may facilitate ascertainment of hepatitis B virus (HBV) infection in settings with high endemicity but limited infrastructure. We evaluated two rapid HBsAg tests and characterized HBV coinfection in a Ghanaian HIV-positive cohort. Samples from 838 patients were tested by the rapid assays Determine and Vikia and the reference assays Architect, Murex version 3, and Liaison Ultra. The assays were also evaluated using the 2nd International Standard, a seroconversion panel, and two mutant panels. HBsAg-positive samples underwent HBV DNA quantification by real-time PCR and surface and polymerase gene population sequencing. Overall, 140/838 patients (16.7%; 95% confidence interval, 14.2 to 19.2%) were HBsAg positive, and of these, 103/140 (73.6%) were e-antigen negative and 118/140 (84.3%) showed an HBV DNA level of >14 IU/ml (median, 8,279 IU/ml). Assay sensitivities and specificities were as follows: Architect, 97.9 and 99.6%; Liaison, 97.1 and 99.4%; Murex, 98.6 and 99.3%; Determine, 69.3 and 100%; and Vikia, 70.7 and 100%. With Determine, the limit of detection was >1.5 to 3.4 HBsAg IU/ml, and the median HBV DNA loads were 598 and 10,905 IU/ml in Determine-negative and -positive samples, respectively ($P = 0.0005$). Results were similar with the Vikia assay. HBV DNA sequencing indicated infection with genotype E in 82/86 (95.3%) patients. HBsAg mutations affected assay performance, including a T123A mutant that escaped detection by Architect. Major drug resistance mutations were observed in 4/86 patients (4.6%). The prevalence of HBV coinfection was high in this HIV-positive Ghanaian cohort. The two rapid assays identified HBsAg-positive patients at risk for liver disease with high specificity, albeit with only moderate sensitivity.

In recent years, coinfection with hepatitis B virus (HBV) has emerged as a significant cause of morbidity and mortality among HIV-positive patients, due to the promoting effect of HIV on HBV replication and progression of hepatic damage (12, 34, 47, 50). Across Europe, approximately 9% of HIV-positive patients are coinfected with HBV (23). Data from sub-Saharan Africa are limited, primarily due to a lack of routine screening. A number of seroprevalence studies conducted in various HIV-positive populations using various screening and confirmatory modalities have reported coinfection rates of 2 to 20% in east and south Africa (Kenya, Uganda, Rwanda, Malawi, and South Africa) (17, 18, 36, 40) and 12 to 17% in west Africa (Senegal, Burkina Faso, Nigeria, and Ivory Coast) (15, 38, 42, 45). No data are available for Ghana, although a previous national multicenter study described a significant association between HIV and HBV infection among prison inmates and officers (2).

Treatment of coinfected patients is facilitated by the availability of nucleoside or nucleotide analogues with dual antiviral activity and can achieve HBV suppression and be of clinical benefit (29, 30, 39). Current guidelines recommend against the use of lamivudine as a single anti-HBV agent due to the high risk of drug resistance (6, 46). Tenofovir, typically used in combination with emtricitabine or lamivudine in the context of highly active antiretroviral therapy (HAART), is currently the preferred option for treating HBV coinfection (46). Expanded access to HAART is dramatically reducing HIV-related mortality in many resource-limited countries, including Ghana (13, 16, 24). In these settings, first-line therapy usually uses lamivudine in combination with either zidovudine or stavudine and nevirapine or efavirenz. Tenofovir is often available but reserved for use after failure of the initial regimen. Hepatitis B testing is not part of routine care. As a result, a substantial proportion of HIV- and HBV-coinfected patients are currently receiving lamivudine as a single anti-HBV agent and are at significant risk of HBV drug resistance and progression of liver disease (46). The lack of routine HBV testing, in turn a reflection of limited laboratory infrastructure, is one obstacle to the improved management of HBV coinfection in these settings.

Two previous studies have evaluated the rapid Determine hepatitis B surface antigen (HBsAg) test in African cohorts (36, 41). One small study recently raised significant concerns about the specificity of the assay when used in HIV-infected patients in Malawi (36). The aim of the study was to determine HBsAg seroprevalence in a large cohort of HIV-1-infected patients receiving routine HIV care in Kumasi, Ghana; characterize the HBV virological profile of coinfected patients;

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and, in this population, evaluate the performance of rapid and simple tests for HBsAg detection, which may allow the introduction of reliable screening in the absence of extensive laboratory infrastructure. The Determine test in particular was evaluated for its performance both on site in Kumasi and by repeat testing of the samples in London, United Kingdom. In addition, the Vikia test, not previously evaluated, was studied as a possible alternative to the Determine test.

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MATERIALS AND METHODS

Study population. Paired serum and plasma samples were collected from 838 consecutive, unselected HIV-infected patients with unknown HBsAg status attending for routine care at the Komfo Anokye Teaching Hospital (KATH) in Kumasi, Ghana. The samples were stored at −20°C prior to shipping on dry ice to the Royal Free Hospital in London, where they were kept at −70°C before testing, avoiding multiple freeze-thaw cycles. Stored serum samples from a further 1,300 HIV-infected patients were tested for HBsAg at the KATH serology laboratory using the rapid Determine assay. Of these, 178 samples tested HBsAg positive and were retrieved for repeat testing in London. The study was approved by the Committee on Human Research Publications and Ethics at the Kwame Nkrumah University of Science and Technology of Kumasi, Ghana.

Control panels. The World Health Organization (WHO) 2nd International Standard (IS) for HBsAg (genotype A, subtype adw2) was used to evaluate the sensitivity of the assays (National Institute for Biological Standards and Control, Potters Bar, United Kingdom; code no. 03/262). The panel included the positive controls A to D (encompassing 4-fold dilutions) and the negative control E, each reconstituted in 1 ml of distilled water. In addition, a seroconversion panel was tested comprising samples collected prospectively from a donor with acute hepatitis B infection, at days 0 (first sample), 36, and 43 (Boston Biomedica, Inc. [BBI], West Bridgewater, MA).

Detection of HBsAg mutants was investigated using the Coleman panel of recombinant HBsAg mutant samples, which was provided by Abbott (Abbott Laboratories, Abbott Park, IL) (10). The panel comprised five single mutants (E129H, M133L, D144A, G145R, and T123A), two double mutants (P142L G145R and P142S G145R), two insertion mutants (122NTins and 122RAins), and a wild-type positive control. A second panel of recombinant HBsAg mutants (genotype D) comprised 22 site-directed single mutants spanning codons 120 to 147 and a wild-type positive control. The expression vector, constructed using pCH under the cytomegalovirus immediate-early promoter-enhancer 078 pCHsAg An b-glob, was kindly donated by Ulrike Protzer (Technische Universität München, Munich, Germany). The wild-type and mutated HBsAg were tested by transfection of human hepatoma HuH7 cells with pCHsAg plasmids using FuGene6 (Roche Diagnostics GmbH, Mannheim, Germany). After 72 h, supernatants were collected for analysis. The same supernatant was used for all tests.

Serology assays. Serum samples and control panels were tested for the presence of HBsAg, using five assays. These comprised three reference assays, the automated chemiluminescent immunoassays Architect HBsAg (Abbott Diagnostics, Maidenhead, United Kingdom) and Liaison HBsAg Ultra (Diasorin, Bracknell, United Kingdom), and the manual Murex version 3 plate enzyme immunoassay (Abbott Diagnostics). In addition, the samples and control panels were tested by two rapid immunochromatography (lateral flow) assays, the Determine HBsAg assay (Inverness Medical, Stockport, United Kingdom) and the Vikia HBsAg assay (bioMérieux, Basingstoke, United Kingdom). The manufacturers' reported respective sensitivity and specificity are >99% and >99% for the three reference assays and >95% and >99% for the Determine test and >98% and >99% for the Vikia test. Serum samples were centrifuged at 10,000 relative centrifugal force (RCF) for 15 min prior to testing to remove debris. All assays were performed according to the manufacturers' instructions. In the Architect assay, results were reported as IU/ml and specimens with concentration values of ≥0.05 IU/ml were considered reactive. In the Liaison assay, results were reported as index values, and specimens with values of ≥0.9 were considered reactive. In the Murex assay, the mean absorbance of the replicates of the negative control (NC) was calculated and the reactivity was identified by the formula NC + 0.05; results were reported as sample absorbance/cutoff (s/co) values and regarded as reactive if the s/co value was ≥1. Reactivity was confirmed in neutralization assays by pretreatment of samples with anti-HBs antibodies, using the appropriate Architect, Liaison, or Murex HBsAg confirmatory assay according to the manufacturer's instructions; specimens showing neutralization values of >60% were considered positive. The Determine and Vikia rapid tests were read 15 min after addition of 50 μl and 75 μl of sample, respectively. A positive result was indicated by the presence of a clear antigen band and a clear control band on the strip; invalid results, defined by the lack of a clear control band, underwent repeat testing. The HBV e antigen (HBeAg) and anti-HBe antibody were measured by the Architect assay (Abbott Diagnostics).

Molecular assays. To quantify the HBV DNA load, HBsAg-positive samples underwent nucleic acid extraction by the *m*2000sp automated system (Abbott Laboratories), followed by quantitative real-time PCR using an assay that was calibrated against the 2nd World Health Organization (WHO) International Standard for HBV DNA and showing a lower limit of quantification of 14 IU/ml. Samples with detectable HBV DNA underwent population sequencing of the polymerase gene reverse transcriptase (RT) domain (amino acids 1 to 344) and the surface (S) gene (amino acids 1 to 226) as previously described (5). Briefly, plasma DNA was extracted using the NucliSens EasyMag platform (bioMerieux). A 1.4-kb amplicon comprising RT and surface gene was amplified by nested PCR and sequenced using a 3730 DNA analyzer (Applied Biosystems, Warrington, United Kingdom). Sequence analysis was performed using SeqScape software V 2.6 (Applied Biosystem).

Analysis. Confirmed positive results were defined by reactivity in all three reference assays or reactivity in one or two reference assays followed by confirmation in the respective neutralization assay. Invalid results in the two rapid tests were analyzed with the negative results. Sensitivity for each assay was defined by the number of correctly identified positive (true-positive) results divided by the total number of confirmed positive results (true positive + false negative). Specificity was defined by the number of correctly identified negative (true-negative) results, divided by the total number of confirmed negative results (true negative + false positive). The 95% confidence intervals (CI) for specificity and sensitivity were calculated. The positive predictive value (PPV) of the assays was calculated by the formula true positives/(true positives + false positives), whereas the negative predictive value (NPV) was defined by the formula true negatives/(true negatives + false negatives). To facilitate the comparative analysis, the strength of the signal expressed as fold reactivity above the assay interpretative cutoff was categorized as follows: Architect and Liaison signals 1- to 10-fold above cutoff, +; >10- to 40-fold above cutoff, ++; >40- to 100 fold above cutoff, +++; and >100-fold above cutoff, ++++; Murex s/co value of 1 to 3, +; s/co value of >3 to 10, ++; s/co value of >10 to 20, +++; and s/co value of >20, ++++. Discrepant results were defined as a marked reduced reactivity in one or two of the three reference assays relative to the third assay. The latter was defined as a signal strength of + relative to a strength of +++ or ++++ in one other test or a signal strength of ++ relative to a strength of ++++ in one other test. Among coinfected patients who tested either negative or positive with the rapid tests, proportions with HBV DNA of >14 and >2,000 IU/ml were compared by the *z*-test, whereas the median HBV DNA load values were compared using the Kruskall-Wallis test.

RESULTS

HBsAg seroprevalence in HIV-1-infected patients with unknown HBsAg status. Among 838 samples from HIV-1-infected patients with unknown HBsAg status, 140 showed confirmed HBsAg positivity, including 134 specimens that were HBsAg positive in all three reference assays and 6 specimens that were HBsAg positive in one ($n = 2$) or two ($n = 4$) reference assays. This yielded an HBsAg seroprevalence of 16.7% (95% CI, 14.2 to 19.2%).

Characteristics of HBsAg-positive patients. Among HBsAg-positive patients, 37/140 (26.4%) were HBeAg positive and 67/140 (47.9%) were anti-HBe antibody positive; the remainder lacked HBV e antigen markers. The majority of HBsAg-positive patients (118/140; 84.3%) showed a detectable (>14 IU/ml) HBV DNA load, and the median HBV DNA loads were 5,346 IU/ml overall and 8,279 IU/ml (range, 23 to 506,000,000 IU/ml) among viremic patients. HBV DNA sequencing was attempted with all samples showing a detectable

TABLE 1. Sensitivity and specificity of five HBsAg assays for 838 samples from HIV-infected patients from Kumasi, Ghana[a]

HBsAg assay	Sensitivity		Specificity		% PPV (95% CI)	% NPV (95% CI)
	No. of samples false negative	% (95% CI)	No. of samples false positive	% (95% CI)		
Architect	3	97.9 (95.5–100)	3	99.6 (99.1–100)	97.9 (95.5–100)	99.6 (99.1–100)
Liaison	4	97.1 (94.4–99.9)	4	99.4 (98.9–100)	97.1 (94.4–99.9)	99.4 (98.9–100)
Murex	2	98.6 (96.6–100)	5	99.3 (98.7–99.9)	96.5 (93.5–99.5)	99.7 (99.3–100)
Determine	43[b]	69.3 (61.6–76.9)	0	100 (100–100)	100 (100–100)	94.2 (92.5–95.9)
Vikia	41[c]	70.7 (63.2–78.3)	0	100 (100–100)	100 (100–100)	94.5 (92.8–96.1)

[a] A total of 140 of these 838 samples were confirmed as HBsAg positive.
[b] Includes one invalid result.
[c] Includes three invalid results.

HBV DNA load. However, the success rate was low for samples with HBV DNA levels of <200 IU/ml despite a nested PCR, due to the small volume of sample available for testing. Among 86/118 (72.9%) viremic patients with an HBV DNA sequence, the distribution of HBV genotypes was E in 82/86 (95.3%) and A in 4/86 (4.7%). Antiviral treatment status was not known for individual coinfected patients, but in the whole cohort, one-third of patients were receiving lamivudine-based antiretroviral therapy. The overall prevalence of major drug resistance mutations in RT was 4/86 (4.6%), comprising the lamivudine resistance mutations V173L, L180M, and M204V in three patients and the unusual combination of V173L plus the tenofovir resistance mutation A194T in the fourth patient.

Assay performance with serum samples. The sensitivity and specificity of the five HBSAg assays are summarized in Table 1. The two rapid assays showed a sensitivity of 69.3% for Determine and 70.7% for Vikia, whereas specificity and PPV were 100% for both assays. The NPVs were 94.2% and 94.5% for Determine and Vikia, respectively. Among 140 HBsAg-positive samples, invalid results (no control band) requiring repeat testing were obtained in 5 (3.6%) samples by Determine and 20 (14.3%) samples by Vikia. After repeat testing, one sample showed a persistently invalid result by Determine, and three samples showed a persistently invalid result by Vikia. The lowest HBsAg concentration (as measured by the Architect assay) that showed reactivity in the Determine and Vikia assays was 1.5 IU/ml. The median HBsAg concentrations of samples that tested falsely negative in the rapid assays (excluding invalid results) were 0.16 IU/ml (range, 0.05 to 3.4 IU/ml) for Determine and 0.15 IU/ml (range, 0.05 to 1.0 IU/ml) for Vikia. The proportion of patients with a detectable HBV DNA load was 34/43 (79.1%) among those who tested falsely negative by Determine (including one invalid result) and 84/97 (86.6%) among those who tested positive ($P = 0.38$); the median HBV DNA loads were 598 IU/ml (range, <14 to 950,405 IU/ml; interquartile range [IQR], 30 to 4,908 IU/ml) and 10,905 IU/ml (range, <14 to 506,000,000 IU/ml; IQR, 252 to 1,750,000 IU/ml) in the two groups, respectively ($P = 0.0005$); 15/43 (34.9%) and 60/97 (61.9%) patients, respectively, showed an HBV DNA level of >2,000 IU/ml ($P = 0.006$). Similarly, the proportions of patients with a detectable HBV DNA load were 31/41 (75.6%) among those who tested falsely negative by Vikia (including three invalid results) and 86/99 (86.9%) among those who tested positive ($P = 0.17$); the median HBV DNA loads were 587 IU/ml (range, <14 to 950,405 IU/ml; IQR, 30 to 3,848 IU/ml) and 10,905 IU/ml (range, <14 to 506,000,000 IU/ml; IQR, 252 to 1,750,000 IU/ml) in the two groups, respectively ($P = 0.0003$); 13/41 (31.7%) and 61/99 (61.6%) patients, respectively, showed an HBV DNA level of >2,000 IU/ml ($P = 0.002$).

The 178 samples that had tested HBsAg positive by the Determine assay in Ghana were tested by the Architect and Liaison assays, and all were confirmed as HBsAg positive, yielding 100% specificity for the test when performed on site.

Assay performance with reference reagents. With the 2nd International HBsAg standard, the Architect and Liaison assays showed identical levels of performance, with reactivity that declined from 187- and 181-fold above the cutoff at a 1:4 dilution to 46- and 44-fold, 12- and 12-fold, and 3- and 3-fold above the cutoff at 1:16, 1:64, and 1:256 dilutions, respectively (Table 2). The Murex assay also detected all dilutions, whereas the two rapid assays were positive with the 1:4 dilution but negative with the 1:16 dilution, corresponding to an HBsAg concentration of 2.3 IU/ml. With the seroconversion panel, the levels of performance of the three reference assays were sim-

TABLE 2. Assay performance with the 2nd International HBsAg standard

HBsAg standard (subtype adw1, genotype A)	Dilution	Result for assay[a]:				
		Architect (IU/ml)	Liaison (index value)	Murex (s/co value)	Determine	Vikia
A	1:4	9.4 (++++)	162.8 (++++)	27.5 (++++)	Positive	Positive
B	1:16	2.3 (+++)	41.7 (+++)	15.0 (+++)	−	−
C	1:64	0.6 (++)	11.2 (++)	4.2 (++)	−	−
D	1:256	0.2 (+)	2.7 (+)	1.6 (+)	−	−
E (NC)[b]		−	−	−	−	−

[a] Interpretative cutoffs: Architect, ≥0.05 IU/ml; Liaison index value, ≥0.9; Murex sample absorbance/cutoff (s/co) value, ≥1. The signal strength (given in parentheses) indicates fold reactivity above the assay cutoff. −, negative.
[b] NC, negative control.

TABLE 3. Assay performance with an HBsAg seroconversion panel

Code	Day	Result for assay[a]:				
		Architect (IU/ml)	Liaison (index value)	Murex (s/co value)	Determine	Vikia
1-1	0[b]	−	−	−	−	−
1-2	36	−	−	−	−	−
1-3	43	0.3 (+)	3.0 (+)	3.8 (++)	−	−

[a] Interpretative cutoffs: Architect, ≥0.05 IU/ml; Liaison index value, ≥0.9; and Murex sample absorbance/cutoff (s/co) value, ≥1. The signal strength (given in parentheses) indicates fold reactivity above the assay cutoff. −, negative.
[b] Day 0 represents the first sample.

ilar overall, whereas the rapid tests remained negative up to day 43 after the first sampling (Table 3).

Assay performance with mutant recombinant HBsAg. With the Coleman HBsAg mutant panel, all mutants were detected by the three reference assays, with the highest relative strength of reaction seen with the Murex assay (Table 4). Low signal strength with Architect relative to Murex was seen with T123A and the 122NT and 122RA insertion mutants. The rapid assays detected some but not all of the mutants, although it should be noted that overall HBsAg concentrations were generally low relative to the expected assay sensitivities of >1.5 to 3.4 IU/ml for Determine and >1.5 to 2.3 for Vikia. With the Cologne HBsAg mutant panel, all mutants were detected by the Architect assay and HBsAg concentrations were generally higher than those seen with the Coleman panel. Of the 22 mutants, 16 (72.7%) were detected by Determine and 18 (81.8%) by Vikia. The samples that escaped detection by Determine despite an HBsAg concentration of >3.4 IU/ml comprised the P120G, C137W, and C147S mutants. The Vikia assay detected both the P120G and C137W mutants but missed the C147S mutant.

Analysis of false-negative and discrepant samples. We analyzed the HBV surface gene sequence of samples that gave false-negative or discrepant results and had detectable HBV DNA (Table 5). Among six samples that showed a false-negative result in one ($n = 4$) or two ($n = 2$) reference assays, the HBV DNA loads were <14 IU/ml in two samples and 23, 24, 199, and 6,479 IU/ml in the other four samples, respectively. A sequence was obtained from the latter sample, which gave a false-negative result in the Architect assay; this showed the

TABLE 4. HBsAg assay performance with two HBsAg mutant panels

HBsAg mutant	Mutation	Result for assay[a]:				
		Architect (IU/ml)	Liaison (index value)	Murex (s/co value)	Determine	Vikia
Panel 1 (Coleman)						
3-1	E129H	1.2 (++)	40.1 (++)	17.2 (+++)	Pos	Pos
3-2	M133L	1.1 (++)	34.1 (++)	14.1 (+++)	−	−
3-3	D144A	0.6 (++)	16.0 (++)	11.6 (+++)	−	−
3-4	G145R	0.8 (++)	10.1 (+)	11.8 (+++)	Pos	Pos
3-6	P142L + G145R	0.7 (++)	11.3 (++)	13.1 (+++)	−	Pos
3-7	P142S + G145R	0.7 (++)	10.7 (+)	9.7 (++)	−	−
3-9	T123A	0.4 (+)	60.1 (+++)	21.0 (++++)	−	Pos
3-10	122NT insertion	0.2 (+)	29.1 (++)	28.9 (++++)	−	−
3-11	122RA insertion	0.3 (+)	27.2 (++)	20.1 (++++)	−	Pos
3-12	Positive control	1.6 (++)	53.5 (+++)	19.2 (+++)	Pos	Pos
Panel 2 (Cologne)						
2-1	P120G	4.9 (+++)	ND	ND	−	Pos
2-2	P120S	14.0 (++++)	ND	ND	Pos	Pos
2-3	P120T	8.0 (++++)	ND	ND	Pos	Pos
2-4	C121Y	0.3 (+)	ND	ND	−	−
2-5	R122I	5.3 (++++)	ND	ND	Pos	Pos
2-6	T123N	0.2 (+)	ND	ND	−	−
2-7	C124R	0.4 (+)	ND	ND	−	−
2-8	T125 M	14.6 (++++)	ND	ND	Pos	Pos
2-9	T126I	13.3 (++++)	ND	ND	Pos	Pos
2-10	T126V	12.8 (++++)	ND	ND	Pos	Pos
2-11	P127T	12.5 (++++)	ND	ND	Pos	Pos
2-12	G130D	9.2 (++++)	ND	ND	Pos	Pos
2-13	M133T	12.2 (++++)	ND	ND	Pos	Pos
2-14	F134N	26.3 (++++)	ND	ND	Pos	Pos
2-15	C137W	9.2 (++++)	ND	ND	−	Pos
2-16	K141W	14.5 (++++)	ND	ND	Pos	Pos
2-17	D144E	9.4 (++++)	ND	ND	Pos	Pos
2-18	D144G	5.1 (++++)	ND	ND	Pos	Pos
2-19	D144H	12.0 (++++)	ND	ND	Pos	Pos
2-20	G145K	6.0 (++++)	ND	ND	Pos	Pos
2-21	G145R	8.6 (++++)	ND	ND	Pos	Pos
2-22	C147S	7.6 (++++)	ND	ND	−	−
2-23	Positive control	8.2 (++++)	ND	ND	Pos	Pos

[a] Interpretative cutoffs: Architect, ≥0.05 IU/ml; Liaison index value, ≥0.9; Murex sample absorbance/cutoff (s/co) value, ≥1. The signal strength (given in parentheses) refers to fold reactivity above the assay cutoff. Pos, positive; ND, not done; −, negative.

TABLE 5. HBsAg mutations in samples with detectable HBV DNA load[a] that gave false-negative or discrepant results in one or more reference HBsAg assays and yielded a sequence for analysis

Sample	Result for assay[b]:						S gene mutations[c]
	Architect (IU/ml)	Liaison (index value)	Murex (s/co value)	Determine	Vikia	HBV DNA (IU/ml)	
1	−	4.5 (+)	5.1 (++)	−	−	6,479	T27KT L49P F80S **T123A**
2	0.05 (+/−)	23.0 (++)	10.0 (+++)	−	−	135,772	M103KM L104LM **T123A** T189IT
3	2.0 (++)	37.2 (+++)	26.3 (++++)	−	Pos	31,295	S31N F220L
4	1.0 (++)	44.5 (+++)	23.7 (++++)	−	−	5,494	V96G S113ST T126I/T L127LV T189IT P203Q
5[d]	0.6 (++)	12.8 (++)	26.3 (++++)	−	−	4,543	S3N F20S N40S G44E L49R L127I **E164D** S210T
6	1.0 (++)	8.7 (+)	22.0 (++++)	−	−	360	S3N G44E L49R **T131N M133T**
7	7.0 (++++)	20.0 (++)	28.8 (++++)	Pos	Pos	860	Y72S F93FY Q101KQ L104LM L127P N146D L175S Q181R F220FI

[a] A detectable HBV DNA load was defined as >14 IU/ml.
[b] Interpretative cutoffs: Architect, ≥0.05 IU/ml; Liaison index value, ≥0.9; Murex sample absorbance/cutoff (s/co) value, ≥1. The signal strength (given in parentheses) indicates fold reactivity above the assay cutoff. Pos, positive; −, negative.
[c] All sequences were genotype E and were compared to a consensus genotype E reference sequence constructed using 82 sequences from the cohort, with recognized major HBsAg immune escape mutations in boldface.
[d] Sample 5 showed the major reverse transcriptase drug-resistance mutations V173L plus A194T.

mutation T123A (Table 5). There were 12 samples that showed discrepant results in one or two of the reference assays relative to the third reference assay, as indicated by marked differences in the strength of the signal. These included 10 samples with reduced reactivity by Architect, 6 samples with reduced reactivity by Liaison, and 1 sample with reduced reactivity by Murex. The HBV DNA load was <14 in four samples and ranged between 82 and 135,772 IU/ml in the remaining eight samples, of which six yielded a sequence (Table 5). The T123A mutation again appeared to have a significant impact on detection by the Architect assay. Major HBsAg immune escape mutants were detected in two samples, including E164D in the sample with the RT mutations V173L and 194T. In addition, three samples with an HBsAg concentration of >1.5 IU/ml escaped detection by one or both rapid assays, as confirmed by repeat testing. The HBV DNA loads were 23, 59, and 4,596 IU/ml in the three samples, respectively; a sequence was obtained from the latter sample, but no mutations were identified in the surface gene.

DISCUSSION

Like the rest of sub-Saharan Africa, Ghana is a region of high HBV endemicity. Over 15% of the population is chronically infected, with most infections occurring before 16 years of age (3). The predominant route of HBV transmission is horizontal, while vertical transmission is estimated to account for only 8% of chronic infections (8). This study is the first to document the prevalence of HBV infection in a cohort of HIV-positive patients in Ghana. We conducted the study in Kumasi, the second-largest city in Ghana, which has over 6,000 HIV-positive patients under follow-up at the KATH HIV clinic. At 16.7%, coinfection was common, frequently characterized by lack of HBeAg, and associated predominantly with genotype E. While the HBV serological profile and prevalent genotype were similar, the median HBV DNA load was higher than that measured in HBsAg-positive blood donors attending the same hospital in Kumasi (8,279 versus 848 IU/ml) (7), most likely a reflection of the promoting effect that HIV has on HBV replication (12). The median HBV DNA load was also above the threshold of 2,000 IU/ml that indicates possible disease activity (46). The liver disease burden in the Kumasi HIV cohort is unknown. Hepatic transaminases are routinely available, but they do not provide a good indication of liver disease in HIV-infected patients (46) or a measure of liver fibrosis. Regrettably, we did not have access to information about the antiretroviral treatment status of coinfected patients. However, across the whole cohort one-third of the patients were receiving first-line therapy with the standard combination of lamivudine plus stavudine or zidovudine and nevirapine or efavirenz. The high HBV DNA detection rate suggests poorly controlled HBV replication and risk of emergence of HBV resistance to lamivudine (6) and raises concerns about progressive liver damage in this population. With an estimated HIV prevalence of 2% (14) and an HBV coinfection prevalence of 16.7%, there may over 6,000 coinfected people potentially at risk of HBV-related liver disease in Kumasi alone.

Despite high seroprevalence, HBsAg screening is not part of routine HIV care in Ghana. There is, however, interest in adopting a simple test that may allow the rapid identification of HBsAg-positive patients in the context of limited laboratory infrastructure. For those patients who test HBsAg positive, tenofovir, although more expensive, is available to replace zidovudine or stavudine in the first-line antiretroviral regimen. A pilot study conducted in Kumasi with the Determine HBsAg test identified 178 HBsAg-positive patients among 1,300 persons, yielding a prevalence of coinfection of 13.7%. The finding was encouraging, suggesting that screening with the rapid test was likely to identify a high number of coinfections. A recent study, however, raised concerns about the specificity of the Determine HBsAg test when applied to a small HIV-infected population in Malawi (36). Using a laboratory-based plate assay as reference, the sensitivity of Determine was 56%, while the specificity was only 69%. The authors proposed as possible explanations operator errors, effect of tropical climate on kit stability, or sample-related interference with test performance in areas of high HIV

and/or malaria prevalence. We investigated the performance of two rapid HBsAg tests, the Determine assay and the recently developed Vikia assay, as a potential alternative, using as a reference three assays widely used in high-income countries. The two rapid tests detected HBV infection with high specificity, albeit with only moderate sensitivity. For the Determine test, our findings are in line with those of previous studies in Madagascar (41) and Vietnam (25). Importantly, the Determine test showed equally high specificity regardless of whether it was used in Kumasi or in London. Thus, our results appear to point to operator errors as a significant determinant of the poor specificity shown by Determine in the Malawi study, highlighting the importance of training and quality assurance for rapid tests.

The sensitivity of the two rapid tests was approximately 70%, and the estimated detection limit was an HBsAg concentration above 1.5 to 3.4 IU/ml for Determine and 1.5 to 2.3 for Vikia. This is consistent with a previous study indicating detection limits of 2.0 to 3.5 for the Determine assay and 1.6 to 3.2 IU/ml for genotype E specifically (26). While the Vikia assay appeared to have a slight sensitivity advantage over the Determine and also greater resilience in the presence of HBsAg mutations, it suffered from more frequent invalid results requiring repeat testing. The combined use of the two rapid tests would not substantially improve sensitivity in this cohort, and the choice of one test over the other should be based on considerations of cost and availability. Rapid and simple tests with improved sensitivity are clearly required to allow HBsAg screening where laboratory infrastructure is limited and in populations that may have low HBsAg concentrations (26). It is noteworthy that the HBV DNA load was significantly higher in coinfected patients who tested positive by the rapid tests compared with those who tested negative. This indicates that patients identified as HBsAg positive by the rapid tests are likely to have high levels of virus replication and a significant risk of liver disease, thus highlighting the potential clinical utility of testing.

The major hydrophilic region of HBsAg (amino acids ~103 to 169) contains multiple antigenic sites, including the immunodominant "a" determinant (amino acids 124 to 147) and other clusters at positions upstream of 120, 120 to 123, and 149 to 169 (19, 51). Mutations in this region can affect anti-HBsAb binding and cause discrepant reactivity between diagnostic assays that employ different reagent configurations, reducing or abrogating the sensitivity of detection (1, 9, 11, 20, 22, 28, 53). While the glycine-to-arginine G145R substitution is the predominant HBsAg immune escape variant described in the literature (9, 53), a wide range of other mutants have also been described (1, 21, 27, 33, 44, 49, 52). Genotype E shows approximately 8% amino acid divergence from genotype A, which is commonly used as the reference virus type for the development of HBsAg diagnostic assays (35). Previous studies indicated overall good performance of commercial assays with genotype E samples (31, 32, 43) but also suggested that amino acid changes within and outside the "a" determinant may affect HBsAg recognition (37). The performance of the three reference assays in this study was overall consistent with that reported by previous studies using European or reference samples (27, 32, 43). However, we identified a significant impact of the T123A mutation on the performance of the Architect assay. This mutant showed low signal strength when testing recombinant HBsAg and was either missed or showed very low reactivity when testing clinical samples. These findings are consistent with the observation that amino acid positions 120 to 123 are crucial for HBsAg recognition (48) and indicate that despite overall good performance the Architect fails to detect some HBsAg variants. Two other samples with the major mutations E164D and T131N plus M133T showed reduced but not abrogated reactivity in both the Architect and Liaison assays. Several samples and recombinants were missed by the rapid tests, which mostly reflected low HBsAg concentration rather than reduced HBsAg recognition. Discrepant Determine-negative and Vikia-positive results were seen with recombinant HBsAg carrying the P120G, T123A, and C137W single mutations, the P142L G145R double mutation, and the 122RA insertion. Taken together, these results indicate that both HBsAg concentration and the presence of certain HBsAg mutations affect the performance of the Determine test. HBsAg mutations may emergence as the result of immune pressure. In addition, due to the overlapping of surface and polymerase genes in the HBV genome, antiviral therapy with RT inhibitors such as lamivudine can lead to the emergence of variants carrying mutations in both the polymerase and surface genes. In our cohort, three patients had evidence of lamivudine resistance. One other patient carried the RT mutation V173L (with the corresponding HBsAg mutation E164D) plus A194T. V173L is usually seen together with M204V in patients receiving lamivudine. A194T is a rare mutation that has been associated with reduced susceptibility to tenofovir (4); we were able to retrieve the medical records for this patient, and the patient was reportedly naive to antiretroviral therapy.

This study has limitations. Due to the difficulty of collating data on site, antiviral treatment status was not generally available for individual HBV-coinfected patients; exposure to lamivudine as a component of HAART in approximately one-third of patients most certainly had an impact on HBV DNA levels and possibly HBsAg levels and HBeAg status of coinfected patients. Further studies are planned in the Kumasi cohort to investigate HBV coinfection. Meanwhile, this large study provides evidence of a high prevalence of coinfection commonly associated with HBV DNA levels above 2,000 IU/ml and indicates that screening of HIV-infected patients with either the Determine or Vikia rapid HBsAg test can reliably identify HBsAg-positive patients at risk of liver disease.

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