A comparative Study of HBV-DNA Quantification from Chronic HBV Infected Patients using COBAS TaqMan and GenoQuant HBV Real-Time PCR

ALAM, Md. Sohrab[a1*]; POON, LS[b1]; YANG, Mei[b]; LEE, Rebecca[b]; NABI, A.H.M. Nurun[c]; SAHA, Sajoy Kanti[c]; HASSAN, M. Sawkat[d]

[a] *Molecular Genetics Laboratory, Department of Immunology, BIRDEM General Hospital, Dhaka, Bangladesh*
[b] *Department of Research & Development, DiagCor Life Science Limited, Hong Kong SAR, China*
[c] *Laboratory of Population Genetics, Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh*
[d] *Department of Laboratory Medicine, Impulse Hospital, Dhaka, Bangladesh*
[1] *Both of the authors contributed equally to the work, and are considered as Co-first author.*
(Corresponding author: E-mail: sohrab.alam@gmail.com)*

ABSTRACT

Quantification of hepatitis B virus (HBV) DNA in human serum or plasma is of high value for detecting HBV infection and monitoring antiviral treatment efficacy. Due to the high cost of HBV diagnosis and treatment, a reliable, precise and sensitive diagnostic test is crucial to patients. In this study, the new Diagcor GenoQuant™ HBV Real-Time PCR assay was compared to the Roche COBAS® TaqMan® HBV assay with 369 serum samples from patients with chronic hepatitis B infection in Bangladesh. Results indicated a high concordant rate of 96.91% within the dynamic range of the two real-time PCR assays. The GenoQuant™ HBV Real-Time PCR test showed comparable sensitivity and a broader dynamic range than the COBAS® TaqMan® HBV assay. By coupling high sensitivity with an extended dynamic range, the GenoQuant™ HBV Real-Time PCR assay provides an accurate, reliable and low-cost nucleic acid amplification testing for HBV infected patients.

Keywords: *Hepatitis B Virus, DNA Quantification, Chronic Hepatitis, Molecular Diagnostics, Real-Time PCR, Antiviral Treatment Monitor*

INTRODUCTION

Hepatitis B virus (HBV) is a small partially double-stranded virus belongs to the family of the *Hepadnaviridae* that can induces acute or chronic hepatitis.[1] It has been reported that over 2 billion people throughout the world are HBV infected, and over 350 million of them are chronically infected carriers.[2] Chronic carriers are at increased risk of progression to chronic hepatitis, cirrhosis and hepatocellular carcinoma.[3-5] The determination of HBV-DNA levels in human serum or plasma has become the most straightforward and reliable method used for accurate diagnosis and prognosis of acute and chronic HBV infection.[6] Measurement of HBV levels in serum also plays a vital role in the management of patients receiving antiviral drugs, such as monitoring antiviral therapy response and identifying the occurrence of drug resistance in patients.[7, 8]

Unlike the antibody test, nucleic acid amplification testing (NAAT) is sensitive, specific and rapid, which allows early detection of HBV virus DNA before the production of HBV antibody by the human body. That is, NAAT reduces the duration of the pre-seroconversion window period.[9, 10] Real-time PCR is a new molecular tool that offers highly sensitive quantitative analysis. It is progressively replacing endpoint PCR systems for monitoring patients with chronic hepatitis B since it allows absolute quantification of the HBV viral load with a broad dynamic range, high sensitive, definite and rapid result.[11, 12] Quantification by real-time PCR is based on the determination of the threshold cycle (C_t) at which amplification is first detected at the early exponential phase.[13] In this case, the quantification of the viral load is much more decisive than that measured with endpoint PCR systems.[14, 15]

In therapeutic practice, patients suffering from HBV infection need to perform HBV DNA quantitative test to monitor the viral load repeatedly. Sometimes alternative

tests are required until the complete clearance of the hepatitis B virus represented by a negative result of HBs antigen.[16] As far as we know, numerous researchers and laboratories/companies have reported several in-house and commercial HBV real-time PCR tests/ kits.[11-13, 17-22] However, the cost of current HBV-DNA tests is prohibitive, which places a heavy medical burden on the patients and the public. Thus, a valid test with lower cost for HBV infected patients would be of great benefit. Our study aimed to evaluate the performance of DiagCor GenoQuant™ HBV Real-Time PCR assay for the detection and quantification of HBV-DNA in serum samples and to compare these results with COBAS® TaqMan® HBV test from Roche Molecular Diagnostics.

MATERIALS AND METHODS

Three hundred and sixty-nine blood samples were obtained from patients with chronic HBV infection who visited the Molecular Diagnostic Laboratory of Popular Diagnostic Centre, Dhaka, Bangladesh. Blood samples were collected in a BD Vacutainer tube. After centrifugation, the serum was divided into two aliquots of 1 ml each and then extracted for DNA. A known DNA copy number of HBV quantitation standard (QS) were introduced in each specimen during the extraction.[11] Roche High Pure Viral Nucleic Acid (manual) extraction kit was used to extract HBV-DNA from 500 µL of blood serum. The obtained DNA samples were further examined by DiagCor and COBAS® TaqMan® HBV Real-time PCR assays, respectively. **Figure 1** shows the flowchart of clinical samples detection method. Both systems utilize a hybridization probe with a fluorescent moiety covalently linked to the 5′ end of the probe (reporter) and a quenching moiety bound to the 3′ end of the probe (quencher). Probe hybridization and primer extension coincide in the presence of target. The fluorescent signal is generated by removing the reporter through the 5′→3′ exonuclease activity of a thermostable *Taq* DNA polymerase.[5, 11] Quantitation of HBV-DNA is performed using the HBV QS. The system quantifies the amplicons during the exponential phase of amplification by recording the fluorescence signal with a fluorescence detector in real time condition. During the PCR cycle, an increase in normalized fluorescence of a sample exceeds the background noise is considered as the critical C_t, which indicates the beginning of the exponential growth phase of the detection signal.

COBAS® TaqMan® HBV Test

Extracted DNA samples were subjected to the COBAS® TaqMan® HBV real-time PCR assay according to the user manual (Roche, USA). In this assay, HBV DNA and the HBV QS were amplified simultaneously. Results were displayed as an international unit per milliliter (IU/mL). The limit of detection of the assay was set as 6 IU/mL with the dynamic range from 29 to 1.10E+08 IU/mL. The test fulfills the current requirements of a highly sensitive HBV DNA detection method according to the International WHO Standard to provide reliable quantification of HBV genotypes A–G with extensive measuring range. Laborious repeat testing is minimized as well.

GenoQuant™ HBV Real-Time PCR Kit

Extracted DNA samples were tested using GenoQuant™ HBV Real-Time PCR (DiagCor, Hong Kong) following the manufacturer's instructions. A total of 369 specimens were blind tested by DiagCor using ABI ViiA7 real-time

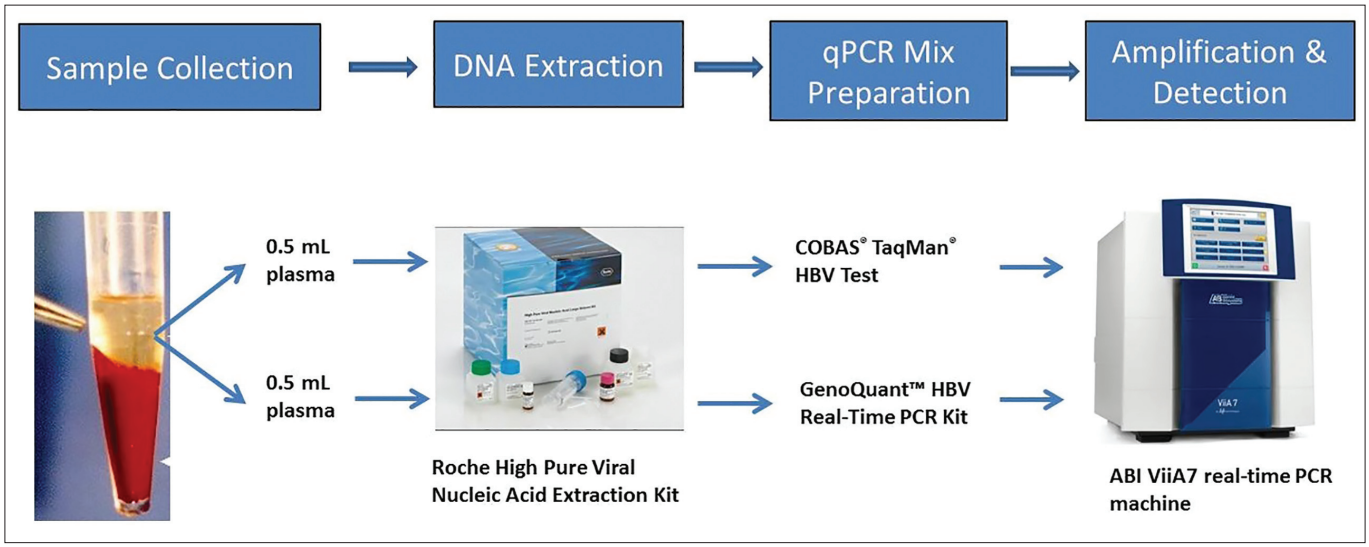

Figure 1. Flowchart of clinical samples detection method.

PCR machine (Applied Biosystems, Foster City, CA, USA). Two targets of HBV DNA and internal amplification control were amplified simultaneously in each sample. A four-point external standard set was used to calculate the initial copy number of the samples. The result was reported in IU/mL. The limit of detection was set at 10 IU/mL with a dynamic range from 10 to 1.0E+08 IU/mL.

RESULTS AND DISCUSSION

A total of 369 samples from a Bangladesh laboratory were determined by using our DiagCor GenoQuant™ HBV Real-Time PCR kit for the presence and quantity of HBV DNA. The results were further compared with COBAS® TaqMan® HBV Test. Of the 369 samples, 94 samples were negative or with viral load values below the limit of detection (LOD, <6 IU/mL). A total of 338 samples were detected by GenoQuant™ HBV Real Time assay, with a relative sensitivity of 95.21%.

Table 1 shows the detailed comparisons between Diagcor GenoQuant™ test and Roche Cobas® TaqMan® assay. For the latter assay, 233 of the 369 samples (63.14%) were detected with HBV DNA within the dynamic range of 29 to 1.10E+08 IU/mL. About 118 samples were below the lower limit of the linear range of the test, in which 94 samples were below the LOD of the assay (<6 IU/mL) and 24 samples were detected within the low HBV viral load range of 6-29 IU/mL. Eighteen samples were above the upper limit of quantification (>1.10E+08 IU/mL). HBV DNA was detected and quantifiable in 286 samples (77.51%) using Diagcor GenoQuant™ Real-Time assay, showing a slightly broader linear quantification range than that of Cobas® TaqMan® assay. Results indicated that 46 (30+16) out of 286 samples had viral loads below the lower limit of the dynamic range (<29 IU/mL) while 7 out of 286 samples showed viral loads above the upper limit of the dynamic range (>1.10E+08 IU/mL) of the Cobas® TaqMan® assay. For those 30 samples with viral loads below LOD and 7 samples above the upper dynamic range by Cobas® TaqMan® assay, their HBV viral loads were found to be ranged from 10.3 to 420 IU/mL, and from 2.43E+06 to 9.72E+07 IU/mL using Diagcor GenoQuant™ Real-Time assay, respectively. Using Diagcor GenoQuant™ Real-Time assay, 72 samples were below the LOD of the test (<10 IU/mL), and 11 samples were above the upper limit of quantification (>1.10E+08 IU/mL).

An excellent correlation (R^2 = 0.9691) was obtained when the linear regression analysis was carried out according to the 233 paired quantitative results available for both tests **(Figure 2)**. The regression line showed the following equation: GenoQuant™ HBV Real-Time (log IU/mL) = 0.972 x COBAS® TaqMan® HBV (log IU/mL) + 0.3289. The mean difference of assay result (GenoQuant™ – COBAS®) was -0.24 log IU/mL ranging from -1.46 to +1.00, with a standard deviation of 0.27 log IU/mL. Comparing the results from both assays, it showed that 91 and 99% of the samples were different by less than 0.5 and 1 log, respectively. Detailed results of each sample are listed in the supplementary data **Table 1S**.

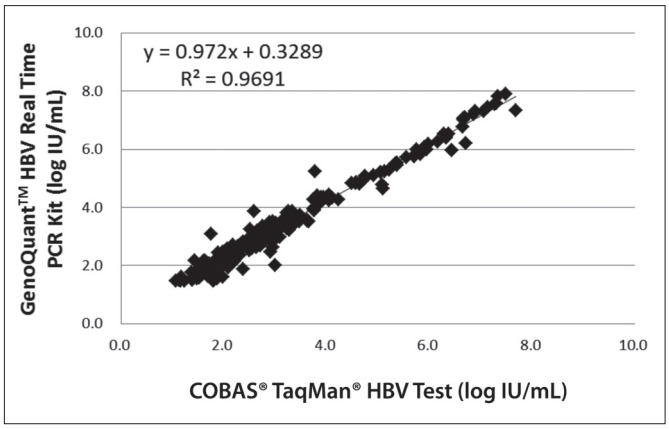

Figure 2. Linear regression analysis performed on 233 samples with quantitative results obtained by the two assays.

Table 1. Comparison between GenoQuant™ HBV Real-Time PCR Kit and COBAS® TaqMan® HBV Test

		COBAS® TaqMan® HBV Test				
		< 6 IU/mL	6-29 IU/mL	Within dynamic range (29- 1.10E+08 IU/mL)	>1.10E+08 IU/mL	Total
GenoQuant™ HBV Real-Time PCR	<10 IU/mL	64	8[a]			72
	Within dynamic range (10-1.0E+08)	30[b]	16[c]	233	7[d]	286
	>1.0E+08 IU/mL				11	11
	Total	94	24	233	18	369

[a] COBAS® TaqMan® results ranged from 6 to 15.9 IU/mL.
[b] GenoQuant™ HBV results ranged from 10.3 to 420 IU/mL.
[c] COBAS® TaqMan® results ranged from 6.13 to 25.1 IU/mL; GenoQuant™ HBV results ranged from 10.1 to 87.6 IU/mL.
[d] GenoQuant™ HBV results ranged from 2.43E+06 to 9.72E+07 IU/mL.
Remark: Detailed results are listed in supplementary data (Table 1S).

Supplementary data

Table 1S. Results of GenoQuant™ HBV Real-Time PCR Kit and COBAS® TaqMan® HBV Test

Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)	Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)
1	8850	1.44E+03	3.96E+03	39	9448	1.08E+02	3.23E+02
2	9409	4.33E+01	1.44E+02	40	9449	2.70E+01	1.47E+02
3	9410	7.23E+02	2.15E+03	41	9450	8.42E+00	<6.00E+00
4	9411	1.26E+03	1.94E+03	42	9451	3.58E+02	1.07E+03
5	9412	4.98E+06	1.26E+07	43	9452	1.50E+02	5.01E+02
6	9413	2.24E+05	3.23E+05	44	9453	4.52E+03	3.37E+03
7	9414	4.47E+00	<6.00E+00	45	9454	2.80E+08	>1.10E+08
8	9415	7.40E+01	3.58E+01	46	9455	1.22E+03	9.52E+02
9	9416	1.84E+02	4.39E+02	47	9456	4.14E+01	6.50E+01
10	9417	8.86E+02	4.13E+02	48	9457	8.63E+00	<6.00E+00
11	9418	2.19E+03	7.37E+03	49	9458	6.70E+03	2.54E+04
12	9419	5.60E+05	9.74E+05	50	9459	4.71E+00	<6.00E+00
13	9420	4.18E+02	8.07E+02	51	9460	2.45E+00	<6.00E+00
14	9421	2.36E+02	7.54E+01	52	9461	1.35E+01	3.15E+01
15	9422	2.33E+02	6.54E+02	53	9462	1.68E+03	1.85E+03
16	9423	0.00E+00	N.D.	54	9463	1.27E+02	3.63E+02
17	9424	2.06E+02	4.68E+02	55	9464	0.00E+00	N.D.
18	9425	8.01E+00	7.84E+00	56	9465	9.09E+02	3.22E+03
19	9426	2.16E+00	<6.00E+00	57	9466	0.00E+00	<6.00E+00
20	9427	9.31E+01	2.64E+02	58	9467	2.39E+01	3.14E+01
21	9428	4.22E+00	<6.00E+00	59	9468	7.88E+02	3.00E+03
22	9429	1.71E+01	2.90E+01	60	9469	1.79E+03	7.24E+03
23	9430	8.10E+05	1.19E+06	61	9470	0.00E+00	N.D.
24	9431	7.66E+00	N.D.	62	9471	2.43E+06	>1.10E+08
25	9432	0.00E+00	<6.00E+00	63	9472	2.36E+06	3.43E+06
26	9433	0.00E+00	N.D.	64	9473	2.89E+02	<6.00E+00
27	9434	1.29E+04	2.07E+04	65	9474	1.83E+08	>1.10E+08
28	9435	5.72E+00	<6.00E+00	66	9475	1.49E+01	<6.00E+00
29	9436	3.67E+04	6.82E+04	67	9476	4.30E+02	7.79E+02
30	9437	4.12E+00	N.D.	68	9477	8.53E+02	6.10E+02
31	9438	1.92E+03	6.40E+03	69	9478	4.58E+01	1.29E+02
32	9439	0.00E+00	N.D.	70	9479	9.00E+02	1.26E+03
33	9440	4.40E+02	1.56E+03	71	9480	5.51E+04	9.10E+04
34	9442	2.98E+01	5.37E+01	72	9481	6.14E+01	1.15E+02
35	9443	1.05E+01	<6.00E+00	73	9482	4.60E+08	>1.10E+08
36	9444	6.43E+03	1.61E+04	74	9483	6.33E+03	2.08E+04
37	9445	9.71E+01	2.64E+02	75	9484	4.09E+02	6.33E+02
38	9447	6.09E+02	7.60E+02	76	9485	1.36E+07	2.75E+07

Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)	Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)
77	9486	2.64E+02	4.78E+02	118	10269	2.79E+02	5.07E+02
78	9487	5.60E+04	1.16E+05	119	10270	2.04E+02	4.82E+02
79	9488	1.46E+01	3.97E+01	120	10271	8.52E+01	1.20E+02
80	9489	1.55E+01	2.38E+01	121	10272	8.70E+00	<6.00E+00
81	9490	1.01E+04	2.26E+04	122	10273	6.24E+00	1.25E+01
82	9491	1.50E+02	2.94E+02	123	10274	0.00E+00	N.D.
83	9492	1.33E+01	<6.00E+00	124	10275	2.21E+00	<6.00E+00
84	9493	1.99E+08	>1.10E+08	125	10276	2.09E+03	3.13E+03
85	9494	7.48E+00	6.00E+00	126	10277	4.22E+03	3.42E+03
86	9495	2.58E+01	4.15E+01	127	10278	1.30E+01	1.01E+01
87	9496	7.22E+06	1.61E+07	128	10279	0.00E+00	N.D.
88	9497	0.00E+00	N.D.	129	10280	1.93E+03	3.36E+03
89	9498	1.75E+03	4.65E+03	130	10281	2.93E+03	3.26E+03
90	9499	4.23E+02	4.34E+02	131	10282	1.89E+07	3.41E+07
91	9500	2.39E+05	3.29E+05	132	10283	4.60E+00	<6.00E+00
92	9501	0.00E+00	N.D.	133	10284	1.51E+01	<6.00E+00
93	9502	9.20E+00	<6.00E+00	134	10285	3.36E+01	<6.00E+00
94	9503	5.52E+00	<6.00E+00	135	10286	2.04E+08	>1.10E+08
95	9504	5.11E+01	8.41E+01	136	10287	3.87E+00	1.06E+01
96	9505	1.85E+00	<6.00E+00	137	10288	3.38E+01	<6.00E+00
97	9506	5.79E+02	1.40E+03	138	10289	2.37E+05	2.77E+05
98	9507	0.00E+00	<6.00E+00	139	10290	5.61E+03	8.96E+03
99	9508	5.46E+00	<6.00E+00	140	10291	1.33E+05	1.77E+05
100	9509	8.03E+01	1.25E+02	141	10292	1.52E+01	6.13E+00
101	9510	3.62E+01	7.23E+01	142	10293	5.61E+05	6.99E+05
102	9511	5.19E+02	8.29E+02	143	10294	1.98E+01	2.51E+01
103	9512	8.17E+02	2.99E+02	144	10295	1.91E+02	2.27E+02
104	9513	1.29E+01	<6.00E+00	145	10296	3.18E+02	6.51E+02
105	9514	5.93E+03	9.77E+03	146	10297	3.96E+01	5.31E+01
106	9515	1.68E+03	2.66E+03	147	10298	1.79E+02	2.73E+02
107	10258	1.01E+01	1.36E+01	148	10299	7.38E+02	1.16E+03
108	10259	9.90E+01	1.33E+02	149	10300	4.97E+01	<6.00E+00
109	10260	5.76E+02	2.23E+03	150	10301	6.84E+05	6.67E+05
110	10261	4.81E+01	1.21E+01	151	10302	1.51E+03	2.64E+03
111	10262	1.16E+02	<6.00E+00	152	10303	2.07E+02	3.99E+02
112	10263	4.68E+06	1.18E+07	153	10304	1.29E+02	2.93E+02
113	10264	2.39E+08	>1.10E+08	154	10305	0.00E+00	N.D.
114	10265	1.23E+05	6.04E+04	155	10306	7.32E+02	1.26E+03
115	10266	2.51E+03	5.21E+03	156	10307	5.24E+01	1.16E+02
116	10267	0.00E+00	<6.00E+00	157	10308	1.33E+01	<6.00E+00
117	10268	1.20E+03	2.20E+03	158	10309	1.43E+02	3.77E+02

Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)	Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)
159	10310	4.41E+01	<6.00E+00	200	10351	1.03E+03	2.91E+03
160	10311	0.00E+00	<6.00E+00	201	10352	2.11E+00	<6.00E+00
161	10312	2.98E+01	3.55E+01	202	10353	0.00E+00	<6.00E+00
162	10313	2.15E+00	N.D.	203	10354	4.10E+00	<6.00E+00
163	10314	1.24E+02	8.34E+01	204	10355	0.00E+00	<6.00E+00
164	10315	6.00E+03	1.73E+05	205	10356	1.54E+02	2.42E+02
165	10316	2.51E+00	<6.00E+00	206	10357	7.68E+01	2.80E+02
166	10317	3.81E+02	7.40E+03	207	10358	3.44E+01	<6.00E+00
167	10318	8.22E+01	1.11E+02	208	10359	8.70E+03	2.21E+04
168	10319	4.14E+09	>1.10E+08	209	10360	2.11E+07	>1.10E+08
169	10320	5.21E+06	1.63E+06	210	10361	8.70E+02	1.95E+03
170	10321	8.76E+01	6.44E+00	211	10362	4.55E+06	5.85E+06
171	10322	0.00E+00	<6.00E+00	212	10363	3.29E+02	1.75E+03
172	10323	2.06E+02	2.26E+02	213	10364	2.00E+07	3.62E+07
173	10324	1.15E+07	2.02E+07	214	10365	9.42E+00	7.58E+00
174	10325	8.94E+00	1.59E+01	215	10366	8.46E+02	9.59E+02
175	10326	6.18E+01	1.21E+02	216	10367	9.00E+05	9.76E+05
176	10327	8.16E+04	1.23E+05	217	10368	1.63E+02	1.37E+02
177	10328	9.42E+01	2.59E+02	218	10369	5.20E+01	1.08E+02
178	10329	1.75E+02	3.60E+02	219	10370	7.98E+03	2.14E+04
179	10330	1.33E+01	<6.00E+00	220	10371	2.00E+00	<6.00E+00
180	10331	1.13E+02	2.42E+02	221	10372	5.60E+03	1.88E+04
181	10332	4.94E+07	2.15E+07	222	10373	1.97E+01	1.40E+01
182	10333	1.24E+05	4.41E+04	223	10374	7.86E+03	2.25E+04
183	10334	2.96E+01	1.11E+01	224	10375	0.00E+00	<6.00E+00
184	10335	0.00E+00	<6.00E+00	225	10376	9.54E+05	1.54E+06
185	10336	1.13E+02	2.69E+02	226	10377	1.67E+03	6.58E+03
186	10337	0.00E+00	<6.00E+00	227	10378	2.52E+02	5.49E+02
187	10338	1.39E+00	<6.00E+00	228	10379	3.33E+01	3.87E+01
188	10339	3.66E+01	5.25E+01	229	10380	1.87E+00	<6.00E+00
189	10340	8.22E+01	7.44E+01	230	10381	2.49E+01	<6.00E+00
190	10341	9.72E+07	>1.10E+08	231	10382	3.57E+01	<6.00E+00
191	10342	6.00E+03	1.84E+04	232	10383	0.00E+00	<6.00E+00
192	10343	1.24E+08	>1.10E+08	233	10384	2.90E+02	5.23E+02
193	10344	3.86E+02	1.07E+03	234	10385	0.00E+00	<6.00E+00
194	10345	1.36E+03	2.36E+03	235	10386	1.73E+01	1.50E+01
195	10346	5.69E+01	4.08E+01	236	10387	1.95E+06	3.38E+06
196	10347	7.32E+07	>1.10E+08	237	10388	3.63E+01	8.19E+01
197	10348	0.00E+00	N.D.	238	10389	8.22E+00	1.25E+01
198	10349	0.00E+00	<6.00E+00	239	10390	9.84E+00	N.D.
199	10350	1.67E+03	2.92E+03	240	10391	4.65E+01	<6.00E+00

Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)	Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)
241	10392	2.16E+03	5.39E+03	282	10433	3.56E+07	>1.10E+08
242	10393	3.54E+02	3.75E+02	283	10434	0.00E+00	<6.00E+00
243	10394	3.06E+02	7.92E+02	284	10435	7.62E+08	>1.10E+08
244	10395	3.14E+02	3.36E+02	285	10436	4.72E+06	9.97E+06
245	10396	3.12E+02	8.90E+02	286	10437	1.72E+01	1.31E+01
246	10397	4.41E+02	6.46E+02	287	10438	4.95E+00	<6.00E+00
247	10398	5.90E+02	7.73E+02	288	10439	1.04E+02	2.02E+02
248	10399	1.16E+02	3.67E+02	289	10440	7.74E+03	1.47E+04
249	10400	3.58E+05	5.37E+05	290	10441	8.40E+02	1.71E+03
250	10401	3.50E+01	8.31E+01	291	10442	4.64E+02	7.45E+02
251	10402	0.00E+00	N.D.	292	10443	0.00E+00	N.D.
252	10403	1.62E+01	N.D.	293	10465	1.21E+02	2.22E+02
253	10404	7.92E+06	1.98E+07	294	10466	4.42E+01	8.53E+01
254	10405	6.24E+01	6.32E+01	295	10467	1.89E+03	1.70E+03
255	10406	1.14E+05	1.61E+05	296	10468	3.07E+07	8.06E+07
256	10407	2.00E+02	4.88E+02	297	10469	4.21E+03	3.78E+03
257	10408	2.28E+03	5.57E+03	298	10470	9.06E+02	1.20E+03
258	10409	4.13E+02	1.19E+03	299	10471	1.71E+01	<6.00E+00
259	10410	2.94E+03	3.41E+03	300	10472	4.35E+01	6.57E+00
260	10411	3.95E+00	N.D.	301	10473	1.00E+03	2.07E+03
261	10412	9.48E+01	2.96E+02	302	10474	6.06E+07	>1.10E+08
262	10413	4.46E+04	6.54E+04	303	10475	3.11E+04	6.74E+04
263	10414	1.32E+03	2.31E+03	304	10476	1.65E+02	3.62E+02
264	10415	1.12E+04	1.76E+04	305	10477	1.03E+01	<6.00E+00
265	10416	5.07E+04	8.20E+04	306	10478	4.13E+01	9.25E+01
266	10417	4.99E+02	1.66E+03	307	10479	8.16E+00	<6.00E+00
267	10418	1.49E+00	N.D.	308	10480	1.18E+03	1.92E+03
268	10419	1.96E+01	N.D.	309	10481	4.05E+00	<6.00E+00
269	10420	3.78E+02	9.08E+02	310	10482	1.00E+00	<6.00E+00
270	10421	9.66E+01	4.04E+01	311	10483	1.14E+04	2.68E+04
271	10422	1.93E+01	N.D.	312	10484	2.70E+06	9.38E+05
272	10423	2.94E+03	4.93E+03	313	10485	3.83E+04	7.38E+04
273	10424	4.15E+00	<6.00E+00	314	10486	2.48E+00	1.36E+01
274	10425	0.00E+00	<6.00E+00	315	10487	1.54E+01	<6.00E+00
275	10426	2.29E+00	<6.00E+00	316	10488	1.67E+01	<6.00E+00
276	10427	1.36E+03	2.18E+03	317	10489	6.24E+01	2.92E+01
277	10428	1.47E+06	1.81E+06	318	10490	7.02E+05	7.90E+05
278	10429	2.34E+01	5.70E+01	319	10491	1.15E+01	3.04E+01
279	10430	1.00E+02	1.87E+02	320	10492	3.35E+02	5.76E+02
280	10431	0.00E+00	N.D.	321	10493	1.99E+02	3.30E+02
281	10432	3.03E+03	5.53E+03	322	10494	4.87E+01	4.21E+01

Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)	Number	Sample ID	GenoQuant™ Result (IU/mL)	COBAS® TaqMan® Result (IU/mL)
323	10495	2.32E+03	3.40E+03	347	10519	6.18E+07	>1.10E+08
324	10496	8.10E+03	1.68E+04	348	10520	5.12E+05	5.70E+05
325	10497	1.01E+03	2.45E+03	349	10521	1.12E+01	<6.00E+00
326	10498	3.87E+01	4.87E+01	350	10522	6.78E+08	>1.10E+08
327	10499	2.44E+03	2.96E+03	351	10523	3.60E+02	6.84E+02
328	10500	9.06E+01	1.86E+02	352	10524	1.64E+01	2.06E+01
329	10501	1.70E+04	1.83E+04	353	10525	1.68E+03	3.53E+03
330	10502	1.36E+01	<6.00E+00	354	10526	4.20E+02	<6.00E+00
331	10503	3.93E+01	1.41E+02	355	10527	6.60E+01	1.44E+02
332	10504	6.24E+01	7.71E+01	356	10260 qsi	8.28E+02	2.23E+03
333	10505	9.66E+02	2.44E+03	357	10260 qsir	1.01E+03	2.23E+03
334	10506	4.74E+01	<6.00E+00	358	10484 R	2.78E+06	9.38E+05
335	10507	0.00E+00	<6.00E+00	359	8952 R	5.68E+01	1.23E+03
336	10508	1.42E+01	3.03E+01	360	9327 b	5.25E+02	1.01E+03
337	10509	2.19E+01	1.36E+01	361	9328 rb	2.18E+06	2.46E+06
338	10510	3.55E+01	6.19E+01	362	9373 rc	1.07E+01	1.38E+01
339	10511	1.37E+01	<6.00E+00	363	9374 rc	3.56E+08	>1.10E+08
340	10512	3.63E+01	8.95E+00	364	9375 rc	6.84E+03	1.67E+04
341	10513	1.88E+01	<6.00E+00	365	9376 rc	5.18E+02	4.95E+02
342	10514	5.76E+03	7.69E+03	366	9377 r	6.60E+01	1.36E+02
343	10515	1.73E+03	2.62E+03	367	9378 r	4.47E+02	7.86E+02
344	10516	1.67E+05	1.96E+05	368	9379 r	2.18E+07	6.42E+07
345	10517	1.41E+02	1.19E+02	369	9380 r	6.36E+01	2.90E+01
346	10518	1.00E+03	9.99E+01				

Remark: N.D. = Not detected.
COBAS® TaqMan® HBV-DNA conversion factor is: 1IU/mL = 5.82 copies/mL.
GenoQuant™ HBV-DNA conversion factor is: 1IU/mL = 3.99 copies/mL.

CONCLUSION

A total of 369 DNA samples were extracted from serum samples of chronic hepatitis B infection patients in Bangladesh and then amplified by two commercial HBV real-time PCR tests Roche COBAS® TaqMan® HBV Test and Diagcor GenoQuant™ HBV Real-Time assay, respectively. Results indicated that the newly developed GenoQuant™ HBV Real-Time PCR Kit showed similar performance but slightly broader dynamic range than that of COBAS® TaqMan® HBV Test. Moreover, results of Diagcor GenoQuant™ HBV Real-Time assay correlated exceptionally well (R^2 = 0.9691) with those of COBAS® TaqMan® HBV Test. By coupling an exquisite sensitivity with an extended linear quantification range of HBV DNA, we demonstrated the potential clinical usefulness of both assays in monitoring the antiviral therapy of patient with chronic hepatitis B.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Joseph Wing-On TAM and Dr. Rebecca LEE from DiagCor Life Science Limited for their arrangement of the GenoQuant™ HBV real-time PCR test. Their comments and suggestions on the manuscript are highly appreciated.

DECLARATION

The authors declare that the research methods and the results obtained in this study are pertinent and do not deliberately favor any brand mentioned in the manuscript.

Author's background

Dr. ALAM Md. Sohrab *is currently working as Lab In-Charge and Senior Research Officer in the Molecular Genetics Laboratory, Department of Immunology, BIRDEM General Hospital, Dhaka, Bangladesh. He got his PhD in Cancer research from the University of Dhaka, Bangladesh. He has over 16 years working experience in molecular diagnostic laboratory and clinical research in the diagnosis of different infectious diseases, such as, Hepatitis B, C virus and viral load (DNA/RNA) quantification, HCV Genotyping, HPV-DNA High Risk Screening for cervical cancer, HLA Typing for organ transplant, HLA B27 Genotyping for ankylosing spondylitis & spondyloarthropathies. His email address is sohrab.alam@gmail.com.* ***Ms. POON LS*** *obtained her MPhil degree in Biochemistry from the Hong Kong University of Science and Technology.* ***Dr. YNAG Mei*** *obtained her PhD in Biomedical Sciences from the City University of Hong Kong. Both Ms. POON LS and Dr. YANG Mei are working as a scientist in the department of R&D at the DiagCor Life Science Limited.* ***Dr. LEE Rebecca*** *obtained her PhD from the National Institute for Basic Biology, Japan. She worked as an R&D manager in DiagCor.* ***Dr. NABI A.H.M. Nurun*** *is currently working as a Professor in the Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh. He obtained his PhD degree from the United Graduate School of Agricultural Science of Gifu University, Japan.* ***Mr. SAHA, Sajoy Kanti*** *is currently working as a PhD candidate at the Population Genetics Laboratory of the University of Dhaka. Now he is working to find out the association of mitochondrial DNA polymorphism with type 2 diabetes in the Bangladeshi population.* ***Dr. HASSAN, M. Sawkat*** *obtained PhD in Clinical Immunology from Karoliska Institute, Staockholm, Sweden. He has published over 33 peer review papers, and focus on the researches related to infectious immunity and cancer genomics.*

References

1. Ganem D and Prince AM (2004). Hepatitis B virus infection—natural history and clinical consequences. *New England Journal of Medicine*, 350(11): 1118-1129.

2. Lee WM (1997). Hepatitis B virus infection. *New England Journal of Medicine*, 337(24): 1733-1745.

3. Beasley RP (1988). Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer*, 61(10): 1942-1956.

4. Wong DK, Cheung AM, O'rourke K, Naylor CD, Detsky AS and Heathcote J (1993). Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B: A meta-analysis. *Annals of Internal Medicine*, 119(4): 312-323.

5. Zuckerman A and Lavanchy D (1999). Treatment options for chronic hepatitis: Antivirals look promising.

6. Mommeja-Marin H, Mondou E, Blum MR and Rousseau F (2003). Serum HBV DNA as a marker of efficacy during therapy for chronic hbv infection: Analysis and review of the literature. *Hepatology*, 37(6): 1309-1319.

7. Locarnini S, Hatzakis A, Heathcote J, Keeffe EB, Liang TJ, Mutimer D, Pawlotsky JM and Zoulim F (2004). Management of antiviral resistance in patients with chronic hepatitis B. *Antiviral Therapy*, 9(5): 679-693.

8. Ranki M, Schätzl HM, Zachoval R, Uusi□Oukari M and Lehtovaara P (1995). Quantification of hepatitis B virus DNA over a wide range from serum for studying viral replicative activity in response to treatment and in recurrent infection. *Hepatology*, 21(6): 1492-1499.

9. Jongerius J, Wester M, Cuypers H, Van Oostendorp W, Lelie P, Van Der Poel C and Van Leeuwen E (1998). New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion*, 38(1): 56-59.

10. Roth WK, Weber M, Petersen D, Drosten C, Buhr S, Sireis W, Weichert W, Hedges D and Seifried E (2002). Nat for HBV and anti-HBC testing increase blood safety. *Transfusion*, 42(7): 869-875.

11. Chen RW, Piiparinen H, Seppänen M, Koskela P, Sarna S and Lappalainen M (2001). Real-time PCR for detection and quantitation of hepatitis B virus DNA. *Journal of Medical Virology*, 65(2): 250-256.

12. Zanella I, Rossini A, Domenighini D, Albertini A and Cariani E (2002). Quantitative analysis of hepatitis B virus DNA by real-time amplification. *European Journal of Clinical Microbiology and Infectious Diseases*, 21(1): 22-26.

13. Gordillo RM, Gutiérrez J and Casal M (2005). Evaluation of the COBAS TaqMan 48 real-time PCR system for quantitation of hepatitis B virus DNA. *Journal of Clinical Microbiology*, 43(7): 3504-3507.

14. Gibosn U, Heid C and Williams P (1996). A novel method for real time quantitative real-time PCR. *Genomes Res*, 6: 995-1001.

15. Heid CA, Stevens J, Livak KJ and Williams PM (1996). Real time quantitative PCR. *Genome Research*, 6(10): 986-994.

16. Liver EAFTSOT (2017). Easl 2017 clinical practice guidelines on the management of hepatitis B virus infection. *Journal of Hepatology*, 67(2): 370-398.

17. He ML, Wu J, Chen Y, Lin MC, Lau GK and Kung HF (2002). A new and sensitive method for the quantification of HBV cccDNA by real-time PCR. *Biochemical and Biophysical Research Communications*, 295(5): 1102-1107.

18. Abe A, Inoue K, Tanaka T, Kato J, Kajiyama N, Kawaguchi R, Tanaka S, Yoshiba M and Kohara M (1999). Quantitation of hepatitis b virus genomic DNA by real-time detection PCR. *Journal of Clinical Microbiology*, 37(9): 2899-2903.

19. Brechtbuehl K, Whalley S, Dusheiko G and Saunders N (2001). A rapid real-time quantitative polymerase chain reaction for hepatitis B virus. *Journal of Vrological Methods,* 93(1-2): 105-113.

20. Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Valdes A, Galimany R, Esteban R and Guardia J (2001). Quantitative detection of hepatitis B virus DNA in serum by a new rapid real-time fluorescence PCR assay. *Journal of Viral Hepatitis*, 8(6): 465-471.

21. Pas SD, Fries E, Robert A, Osterhaus AD and Niesters HG (2000). Development of a quantitative real-time detection assay for hepatitis B virus DNA and comparison with two commercial assays. *Journal of Clinical Microbiology,* 38(8): 2897-2901.

22. Yeh SH, Tsai CY, Kao JH, Liu CJ, Kuo TJ, Lin MW, Huang WL, Lu SF, Jih J and Chen DS (2004). Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. *Journal of Hepatology*, 41(4): 659-666.