

# World Journal of *Gastroenterology*

*World J Gastroenterol* 2017 October 7; 23(37): 6747-6922



**EDITORIAL**

- 6747 Microbial dysbiosis in spouses of ulcerative colitis patients: Any clues to disease pathogenesis?  
*Sorrentino D*

**REVIEW**

- 6750 Road to stemness in hepatocellular carcinoma  
*Flores-Téllez TNJ, Villa-Treviño S, Piña-Vázquez C*
- 6777 Intrahepatic vascular changes in non-alcoholic fatty liver disease: Potential role of insulin-resistance and endothelial dysfunction  
*Pasarin M, Abalde JG, Liguori E, Kok B, La Mura V*
- 6788 Epidemiological and clinical perspectives on irritable bowel syndrome in India, Bangladesh and Malaysia: A review  
*Rahman MM, Mahadeva S, Ghoshal UC*

**ORIGINAL ARTICLE****Basic Study**

- 6802 Estrogen receptor expression in chronic hepatitis C and hepatocellular carcinoma pathogenesis  
*Iyer JK, Kalra M, Kaul A, Payton ME, Kaul R*
- 6817 Glycosylation-related gene expression in HT29-MTX-E12 cells upon infection by *Helicobacter pylori*  
*Cairns MT, Gupta A, Naughton JA, Kane M, Clyne M, Joshi L*
- 6833 STAT3 deficiency prevents hepatocarcinogenesis and promotes biliary proliferation in thioacetamide-induced liver injury  
*Abe M, Yoshida T, Akiba J, Ikezono Y, Wada F, Masuda A, Sakaue T, Tanaka T, Iwamoto H, Nakamura T, Sata M, Koga H, Yoshimura A, Torimura T*
- 6845 Performance verification and comparison of TianLong automatic hypersensitive hepatitis B virus DNA quantification system with Roche CAP/CTM system  
*Li M, Chen L, Liu LM, Li YL, Li BA, Li B, Mao YL, Xia LF, Wang T, Liu YN, Li Z, Guo TS*

**Case Control Study**

- 6854 Association of insertion-deletions polymorphisms with colorectal cancer risk and clinical features  
*Marques D, Ferreira-Costa LR, Ferreira-Costa LL, Correa RS, Borges AMP, Ito FR, Ramos CCO, Bortolin RH, Luchessi AD, Ribeiro-dos-Santos A, Santos S, Silbiger VN*

**Retrospective Cohort Study**

- 6868 Hospital readmissions in decompensated cirrhotics: Factors pointing toward a prevention strategy  
*Seraj SM, Campbell EJ, Argyropoulos SK, Wegermann K, Chung RT, Richter JM*
- 6877 Measurement of biological age may help to assess the risk of colorectal adenoma in screening colonoscopy  
*Kim SJ, Kim BJ, Kang H*

**Retrospective Study**

- 6884 Prognostic factors of response to endoscopic treatment in painful chronic pancreatitis  
*Tantau A, Mandrutiu A, Leucuta DC, Ciobanu L, Tantau M*
- 6894 *In vivo* histological diagnosis for gastric cancer using endocytoscopy  
*Tsurudome I, Miyahara R, Funasaka K, Furukawa K, Matsushita M, Yamamura T, Ishikawa T, Ohno E, Nakamura M, Kawashima H, Watanabe O, Nakaguro M, Satou A, Hirooka Y, Goto H*

**CASE REPORT**

- 6902 Achalasia after bariatric Roux-en-Y gastric bypass surgery reversal  
*Abu Ghanimeh M, Qasrawi A, Abughanimeh O, Albadarin S, Clarkston W*
- 6907 Persistent severe hypomagnesemia caused by proton pump inhibitor resolved after laparoscopic fundoplication  
*Semb S, Helgstrand F, Hjørne F, Bytzer P*
- 6911 Rupture of small cystic pancreatic neuroendocrine tumor with many microtumors  
*Sagami R, Nishikiori H, Ikuyama S, Murakami K*

**LETTERS TO THE EDITOR**

- 6920 Resistance of *Helicobacter pylori* to furazolidone and levofloxacin: A viewpoint  
*Zamani M, Rahbar A, Shokri-Shirvani J*

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NAME OF JOURNAL  
*World Journal of Gastroenterology*

ISSN  
ISSN 1007-9327 (print)  
ISSN 2219-2840 (online)

LAUNCH DATE  
October 1, 1995

FREQUENCY  
Weekly

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PUBLISHER  
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7901 Stoneridge Drive, Suite 501,  
Pleasanton, CA 94588, USA  
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<http://www.wjgnet.com>

PUBLICATION DATE  
October 7, 2017

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Basic Study

# Performance verification and comparison of TianLong automatic hypersensitive hepatitis B virus DNA quantification system with Roche CAP/CTM system

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**Institutional review board statement:** The study was reviewed and approved by the 302 Military Hospital of China Medical Ethic Committee.

**Institutional animal care and use committee statement:** This study didn't involve animal subjects.

**Conflict-of-interest statement:** To the best of our knowledge, no conflict of interest exists.

**Data sharing statement:** No additional data are available.

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**Manuscript source:** Unsolicited manuscript

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**Received:** May 18, 2017

**Peer-review started:** May 19, 2017

**First decision:** June 8, 2017

**Revised:** August 1, 2017

**Accepted:** August 15, 2017

**Article in press:** August 15, 2017

**Published online:** October 7, 2017

## Abstract

### AIM

To investigate and compare the analytical and clinical performance of TianLong automatic hypersensitive hepatitis B virus (HBV) DNA quantification system and Roche CAP/CTM system.

### METHODS

Two hundred blood samples for HBV DNA testing, HBV-DNA negative samples and high-titer HBV-DNA mixture samples were collected and prepared. National standard materials for serum HBV and a worldwide HBV DNA panel were employed for performance verification. The analytical performance, such as limit of detection, limit of quantification, accuracy, precision, reproducibility, linearity, genotype coverage and cross-

contamination, was determined using the TianLong automatic hypersensitive HBV DNA quantification system (TL system). Correlation and Bland-Altman plot analyses were carried out to compare the clinical performance of the TL system assay and the CAP/CTM system.

## RESULTS

The detection limit of the TL system was 10 IU/mL, and its limit of quantification was 30 IU/mL. The differences between the expected and tested concentrations of the national standards were less than  $\pm 0.4 \text{ Log}_{10} \text{ IU/mL}$ , which showed high accuracy of the system. Results of the precision, reproducibility and linearity tests showed that the multiple test coefficient of variation (CV) of the same sample was less than 5% for  $10^2$ - $10^6 \text{ IU/mL}$ ; and for  $30$ - $10^8 \text{ IU/mL}$ , the linear correlation coefficient  $r^2 = 0.99$ . The TL system detected HBV DNA (A-H) genotypes and there was no cross-contamination during the "checkerboard" test. When compared with the CAP/CTM assay, the two assays showed 100% consistency in both negative and positive sample results (15 negative samples and 185 positive samples). No statistical differences between the two assays in the HBV DNA quantification values were observed ( $P > 0.05$ ). Correlation analysis indicated a significant correlation between the two assays,  $r^2 = 0.9774$ . The Bland-Altman plot analysis showed that 98.9% of the positive data were within the 95% acceptable range, and the maximum difference was -0.49.

## CONCLUSION

The TL system has good analytical performance, and exhibits good agreement with the CAP/CTM system in clinical performance.

**Key words:** Analytical performance; Hepatitis B virus DNA quantification; Clinical performance; Hepatitis B virus; Real-time quantification PCR

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**Core tip:** The TianLong automatic hypersensitive hepatitis B virus DNA quantification system achieved a limit of detection of 10 IU/mL, limit of quantification of 30 IU/mL and good analytical performance in terms of accuracy, precision, reproducibility, linearity, genotype coverage and cross-contamination. In clinical performance, the TL system showed good correlation and agreement with the Roche CAP/CTM system.

Li M, Chen L, Liu LM, Li YL, Li BA, Li B, Mao YL, Xia LF, Wang T, Liu YN, Li Z, Guo TS. Performance verification and comparison of TianLong automatic hypersensitive hepatitis B virus DNA quantification system with Roche CAP/CTM system. *World J Gastroenterol* 2017; 23(37): 6845-6853 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v23/i37/6845.htm> DOI: <http://dx.doi.org/10.3748/wjg.v23.i37.6845>

## INTRODUCTION

Hepatitis B virus (HBV) infection is a worldwide disease, and over 2 billion people once have been infected with HBV and of these, 240 million have chronic hepatitis B<sup>[1,2]</sup>. Although there are effective vaccines and anti-viral medicine, HBV infection is still a major condition threatening the human health<sup>[3-5]</sup>. Globally, 30% of patients with liver cirrhosis and 45% with liver cancer are caused by HBV infection, respectively. Nationally, 500-600 million people in China have been infected with HBV, accounting for nearly 40% of the total national population<sup>[6-8]</sup>. Currently, China has approximately 93 million chronic HBV infected individuals, and 20 million of these patients have chronic hepatitis B<sup>[9,10]</sup>. An even higher percentage of patients with liver cirrhosis (60%) and hepatocellular cancer (HCC) (80%) were caused by HBV infection<sup>[11,12]</sup>.

During the last decade, with the advent of new type interferon and antiviral drugs with a high genetic barrier to resistance, the antiviral treatment of chronic HBV infection has achieved significant progress<sup>[13-16]</sup>. HBV DNA quantification is one of the most important indicators used in HBV antiviral therapy, and can be used to determine whether the patient is suitable for antiviral therapy, monitor antiviral treatment response and virus resistance<sup>[17-19]</sup>. Use of hypersensitive HBV DNA quantification system to accurately detect HBV DNA in serum is the key factor in determining the curative effect and endpoints of hepatitis B antiviral therapy.

International associations for the study of liver diseases have required HBV DNA quantification detection, with hypersensitivity (a HBV detection kit should reach the sensitivity criterion of 10-15 IU/mL), wide linearity range (1-9  $\log_{10} \text{ IU/mL}$ ), and high specificity and good repeatability<sup>[20]</sup>.

Many automatic HBV DNA detection systems are currently available in the Chinese market, including COBAS TaqMan HBV assay used in combination with COBAS AmpliPrep, abbreviated to CAP/CTM (Roche Molecular Diagnostics, Pleasanton, CA, United States), and Abbott Real Time HBV assay used in combination with Abbott m2000sp (Abbott Molecular, Des Plaines, IL, United States). However, due to their high cost and slow detection speed, these assays are not widely applied.

Most of the real-time HBV DNA qPCR detection kits in China have common shortcomings, such as poor specificity, low detection sensitivity, poor quantitative accuracy, narrow quantitative linear range and difficulty in detecting low or high viral load. To address these problems, the Chinese Food and Drug Administration (CFDA) released "The guideline principles for the technical review of hepatitis B virus DNA quantitative detection reagents registration" in

**Table 1** Detail information of national standard materials

Art. No.	Standard Material No.	Batch No.	Standard material name	Reference Conc.
HS-0001	GBW (E) 090137	201511003	HBV DNA serum standard material	$1.41 \times 10^3$ IU/mL
HS-0002	GBW (E) 090138	201512004	HBV DNA serum standard material	$5.9 \times 10^4$ IU/mL
HS-0003	GBW (E) 090139	201511003	HBV DNA serum standard material	$4.6 \times 10^5$ IU/mL

2013, which clearly put forward the requirements for HBV DNA detection. These principles will also guide the development direction of hypersensitive HBV DNA detection kits and prompt the rapid development of automatic hypersensitive HBV DNA quantitative detection systems.

The TianLong automatic hypersensitive HBV DNA quantification system (TL system) owns proprietary intellectual property rights, and has been approved by the CFDA. This study evaluates the analytical performance of the TL system and its clinical performance compared with the CAP/CTM system.

## MATERIALS AND METHODS

### Clinical samples

HBV-DNA positive samples: 200 residual blood samples were obtained from HBV infected patients in the clinical laboratory at 302 Military Hospital of China. These samples included HBV A genotype (4 cases), HBV B genotype (70 cases), HBV C genotype (66 cases), HBV D genotype (28 cases) and unknown genotype (32 cases).

HBV-DNA negative samples: These serum samples were obtained after immunology testing in the clinical laboratory at 302 Military Hospital of China, and were retested using the CAP/CTM system and confirmed to be negative.

High-titer HBV-DNA mixture samples: High-titer HBV-DNA samples were collected from the clinical laboratory at 302 Military Hospital of China. The high-titer HBV-DNA mixture samples were diluted 100 times and measured three times with the CAP/CTM system. The original concentration of the high-titer HBV-DNA mixture samples was calculated to be  $2.5 \times 10^8$  IU/mL.

The clinical serum samples were tested for co-infections with HIV, HCV and HDV. All results were negative.

### Standards samples (reference serum)

The worldwide HBV DNA performance panel (WW-HD301, SeraCare, Milford, Massachusetts, United States) which contains HBV DNA (A-H) genotypes was used in this study. National standard materials for HBV serum were used to verify the assay performance including accuracy, limit of detection and limit of quantification. Details of the national standard materials are shown in Table 1.

### TianLong automatic hypersensitive HBV DNA quantification system assay

The TL system consists of a PANA 9600E automatic nucleic acid workstation, Gentier 96E real-time quantitative PCR system (Xi'an TianLong Science and Technology, Xi'an, Shaanxi, China), nucleic acid extraction kit (magnetic bead method) and a HBV DNA quantitative detection kit (fluorescence qPCR method).

The experimental method for the TL system assay followed the manufacturer's instructions. Serum of 200  $\mu$ L was used for HBV DNA extraction, and for PCR reaction assessment, 40  $\mu$ L for each sample was prepared on the PANA 9600E automatic nucleic acid workstation. The Gentier 96E real-time quantitative PCR system was used for quantitative detection as follows: 50  $^{\circ}$ C for 2 min, 95  $^{\circ}$ C for 3 min, [94  $^{\circ}$ C for 15 s, 60  $^{\circ}$ C for 30 s (read fluorescence)]  $\times$  45 cycles. The data were automatically analyzed by software and the results were expressed in international units per milliliter (IU/mL). The TL system detected 96 samples (89 samples, 4 quantitative standards, a negative control, a weak positive control and a strong positive control) within 140 min (80 min for nucleic acid extraction and 60 min for real-time PCR detection).

### Roche COBAS HBV v2.0 assay (CAP/CTM)

HBV DNA was extracted from 650  $\mu$ L of serum by the Cobas AmpliPrep instrument according to the manufacturer's instructions. The Cobas TaqMan 48 analyzer was used for automated real-time PCR amplification and detection of PCR products according to the manufacturer's instructions. The data thus generated were analyzed with AmpliLink software. HBV DNA levels were expressed in international units per milliliter (IU/mL).

### Limit of detection

National standard materials for HBV serum [GBW (E) 090137, Batch No. 201511003] at a concentration of  $1.41 \times 10^3$  IU/mL were diluted with HBV DNA negative serum to nominal concentrations of 30 IU/mL, 15 IU/mL, 10 IU/mL, 5 IU/mL, and 2.5 IU/mL. 25 replicates per run were tested with three different batches of reagents in three runs, and the test results were log transformed to verify whether the limit of detection of the TL system met the standard (10-15 IU/mL) recommended by the European Association for the

**Table 2** Limit of detection test results

Sample Conc. (IU/ml)	1 <sup>st</sup> Batch of reagent			2 <sup>nd</sup> Batch of reagent			3 <sup>rd</sup> Batch of reagent			Average detection rate
	NO. detected	NO. not detected	Detection rate	NO. detected	NO. not detected	Detection Rate	NO. detected	NO. not detected	Detection rate	
2.5	10	15	40%	12	13	48%	11	14	44%	44.0%
5	21	4	84%	20	5	80%	22	3	88%	84.0%
10	25	0	100%	24	1	96%	25	0	100%	98.7%
15	25	0	100%	25	0	100%	25	0	100%	100.0%
30	25	0	100%	25	0	100%	25	0	100%	100.0%

Study of Liver (EASL) [21].

### Limit of quantification

National standard materials for HBV serum [GBW (E) 090137, Batch No. 201511003], at a concentration of  $1.41 \times 10^3$  IU/mL were diluted with HBV DNA negative serum to nominal concentrations of 30 IU/mL, 20 IU/mL, and 10 IU/mL. 25 replicates per run were tested with three different batches of reagents in three runs, and the test results were log transformed to verify whether the limit of detection of the TL system met the standard (30 IU/mL) required in "The guideline principles for the technical review of hepatitis B virus DNA quantitative detection reagents registration".

### Accuracy

National standard materials for HBV serum were used as samples and three replicates were tested at each concentration in three runs with three different batches of reagents. The sample results were log transformed to evaluate the accuracy of the TL system assay.

### Comparison of clinical samples with CAP/CTM assay

A total of 200 clinical HBV positive samples with a uniform distribution of concentrations were tested with the TL system and the CAP/CTM system. The quantitative results from the two assays were counted and the Statistical Program for Social Sciences (SPSS 13.0 for Windows; SPSS, Chicago, IL, United States) was used to assess the agreement between the positive results obtained with the two assays.

### Precision and reproducibility

High-titer HBV-DNA mixture samples were diluted with HBV DNA negative serum to nominal concentrations of  $1 \times 10^7$  IU/mL,  $1 \times 10^4$  IU/mL and  $1 \times 10^2$  IU/mL. Twenty replicates were tested at each concentration with the same batch of reagent to assess the intra-assay precision. The inter-assay precision was evaluated by three tests with different batches of reagents, and five replicates at each concentration were tested per run. The inter-assay precision test was repeated over 4 d, in order to validate the reproducibility of the TL system assay. The results were log transformed for analysis.

### Linearity

High-titer HBV-DNA mixture samples were diluted to nominal concentrations of  $1 \times 10^8$  IU/mL,  $1 \times 10^7$  IU/mL,  $1 \times 10^6$  IU/mL,  $1 \times 10^5$  IU/mL,  $1 \times 10^4$  IU/mL,  $1 \times 10^3$  IU/mL,  $1 \times 10^2$  IU/mL, 60 IU/mL and 30 IU/mL. Triplicate measurements were made at each concentration level, the sample results were log transformed and then analyzed by Pearson's correlation analysis and linear regression analysis to assess the quantitative linear range of the TL system.

### HBV genotypes coverage

The HBV DNA performance panel (WWHD301, Sera-Care, Milford, Massachusetts, United States) which contains all the genotypes of the HBV DNA (A-H) was used, triplicate measurements were continuously carried out for each HBV genotype for 3 days with the TL system to validate its capability of detecting all HBV DNA (A-H) genotypes.

### Cross-contamination test

To assess the anti-cross contamination performance of the PANA 9600E automatic nucleic acid workstation, the "checkerboard" test was performed twice, using high-titer mixture samples interspersed with aliquots of HBV negative serum samples during DNA extraction. Each high-titer sample was included with HBV DNA negative plasma samples in the batch of 96 tests (48 positive samples and 48 negative samples) and the positions of positive samples and negative samples in the second round of testing were interchanged.

## RESULTS

### Limit of detection

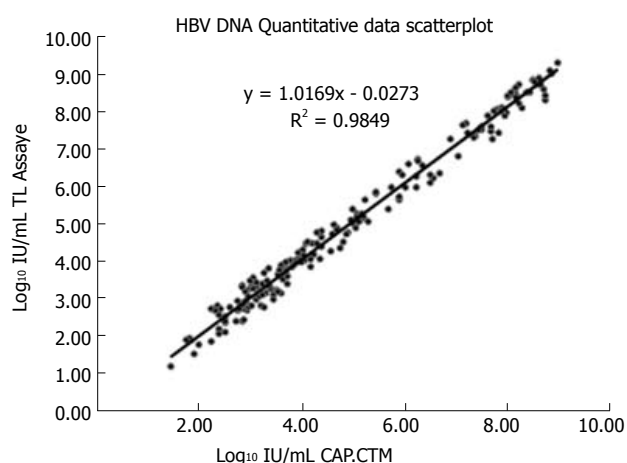
The test results of the limit of detection are shown in Table 2, and a detection rate  $\geq 95\%$  was taken as an acceptable detection limit criterion. According to the test results, the average detection rates of the samples at five concentration levels were 44.0%, 84.0%, 98.7%, 100%, and 100%, respectively. Of these results, the detection rates for the 10 IU/mL samples were above 95% in all three tests. Therefore, the concentration of 10 IU/mL was determined as the detection limit of the TL system, which meets the

**Table 3** Limit of quantification test results

Sample Conc. (IU/mL)	Test batch	Test result (Log <sub>10</sub> IU/mL)			Accurate rate	
		Avg. value	CV	No. ≤ ± 0.4	per batch	Average
10	1	0.79	40.57%	19	76.00%	65.33%
	2	0.59	47.58%	13	52.00%	
	3	0.71	36.53%	17	68.00%	
20	1	1.45	14.11%	21	84.00%	82.67%
	2	1.45	16.65%	20	80.00%	
	3	1.36	20.55%	21	84.00%	
30	1	1.49	6.09%	25	100.00%	100.00%
	2	1.40	8.51%	25	100.00%	
	3	1.46	5.55%	25	100.00%	

**Table 4** Accuracy test results

National standard materials for Hepatitis B virus serum		Expected	mean ± SD,	Absolute value of Log <sub>10</sub> IU/mL difference
Standard material No.	Reference Conc.	Log <sub>10</sub> IU/mL	Log <sub>10</sub> IU/mL	
GBW (E) 090137	1.41 × 10 <sup>3</sup> IU/mL	3.15	3.11 ± 0.02	0.04
GBW (E) 090138	5.9 × 10 <sup>4</sup> IU/mL	4.77	4.80 ± 0.07	0.07
GBW (E) 090139	4.6 × 10 <sup>5</sup> IU/mL	5.66	5.70 ± 0.05	0.05



**Figure 1** The correlation of measured hepatitis B virus DNA concentrations between the TL system assay and the CAP/CTM assay. HBV: Hepatitis B virus.

standard (10-15 IU/mL) recommended by the EASL.

### Limit of quantification

The test results of the limit of quantification are shown in Table 3. Log<sub>10</sub> IU/mL difference represents the log value difference between the detected and the expected concentrations. Taking ± 0.4 Log<sub>10</sub> IU/mL as an acceptable value range in the detection results, this was converted using detected values of traceable national standard materials according to the National Center for Clinical Laboratories (NCCL) in China. When the detection accuracy rate of certain concentration was ≥ 95%, this was considered the system quantification limit. According to the test results, all three test results for the 30 IU/mL samples showed 100% accuracy rate and the Log<sub>10</sub> IU/mL difference values were between -0.22 Log<sub>10</sub> IU/mL and 0.19 Log<sub>10</sub> IU/mL. Therefore, the concentration of 30 IU/mL was determined as the quantification limit of

the TL system, which meets the standard (30 IU/mL) required in "The guideline principles for the technical review of hepatitis B virus DNA quantitative detection reagents registration".

### Accuracy

The accuracy test results are shown in Table 4. The Log<sub>10</sub> IU/mL difference represents the log value difference between the detected and the expected concentrations, and when the Log<sub>10</sub> IU/mL difference was less than ± 0.4, the test result was considered accurate. According to the test results, for three concentration levels of HBV national standard serum, the absolute values of Log<sub>10</sub> IU/mL differences were 0.04, 0.07, and 0.05, respectively; therefore, the test results meet the accuracy requirement.

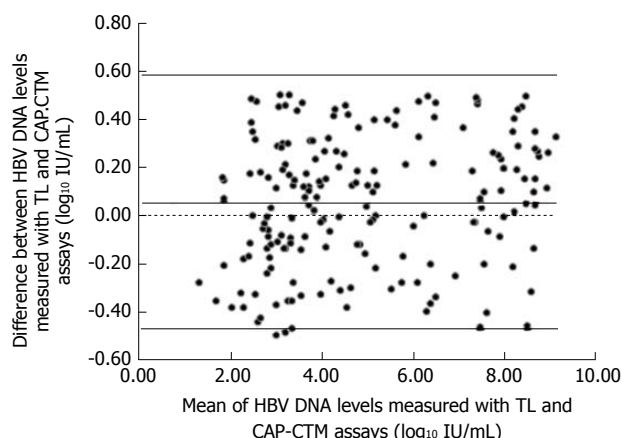
### Comparison of clinical samples with CAP/CTM assay

In December 2015, 200 HBV samples were collected and used to compare the results of the TL system assay with the CAP/CTM assay. The results of the two assays showed 100% consistency for both negative and positive results (15 negative samples, 185 positive samples), and the concentrations of positive samples were uniformly distributed between 60 IU/mL and 8.7 × 10<sup>9</sup> IU/mL. The Statistical Program for Social Sciences (SPSS 13.0 for Windows; SPSS, Chicago, IL, United States) was used to assess the agreement between the positive results obtained with the two assays. The normality test results indicated that the concentration distribution did not conform to the normal distribution ( $P = 0.00$ ,  $P < 0.05$ ). The hypothesis test summary indicated that there were no statistical differences between the TL system and CAP/CTM assays ( $P = 0.817$ ,  $P > 0.05$ ). The scatter plot for the two assays is shown in Figure 1, with the linear equation  $y = 1.0286x - 0.0829$  and correlation

**Table 5** Precision and reproducibility test results

Sample Conc. (IU/mL)	Tested Results Log <sub>10</sub> (IU/mL)	1.0 × 10 <sup>7</sup>		1.0 × 10 <sup>4</sup>		1.0 × 10 <sup>2</sup>	
		Mean Value	CV	Mean Value	CV	Mean Value	CV
Intra assay precision <sup>1</sup>		6.97	0.23%	4.06	1.10%	2.29	3.10%
Inter assay precision	1 <sup>st</sup> Day <sup>2</sup>	6.94	0.91%	4.19	1.82%	2.26	3.79%
	2 <sup>nd</sup> Day <sup>2</sup>	6.95	1.04%	4.31	1.49%	2.41	3.25%
	3 <sup>rd</sup> Day <sup>2</sup>	7.08	0.64%	4.31	1.67%	2.39	3.72%
	4 <sup>th</sup> Day <sup>2</sup>	7.10	0.81%	4.32	1.63%	2.42	3.47%
Reproducibility <sup>3</sup>		7.02	1.36%	4.28	2.08%	2.37	4.41%

<sup>1</sup>n = 20; <sup>2</sup>n = 15; <sup>3</sup>n = 60 (15 tests/d × 4 d).



**Figure 2** The difference of measured hepatitis B virus DNA concentrations between the TL system assay and the CAP/CTM assay. HBV: Hepatitis B virus.

coefficient  $r^2 = 0.9774$ , which indicated a significant correlation between the two assays. The Bland-Altman plot analysis was used to assess the difference between the positive results obtained with the two assays (Figure 2). The results showed that 98.9% (183/185) of the positive data were within the 95% acceptable range, the average difference value was 0.06, the maximum difference was -0.49, and 82.3% (153/185) of the difference values were between  $\pm 0.4$  Log<sub>10</sub> IU/mL.

### Precision and reproducibility

The precision and reproducibility test results are shown in Table 5. As shown in this Table, in the sample concentration range of  $1.0 \times 10^2$  IU/mL to  $1.0 \times 10^7$  IU/mL, the CV of intra-assay precision, inter-assay precision and reproducibility was all less than 5%.

### Linearity

The linearity test results are shown in Figure 3. The linear regression analysis of eight concentration levels of HBV-DNA samples showed good linearity (the linear equation  $y = 0.9848x - 0.0775$  and correlation coefficient  $r^2 = 0.99$ ).

### HBV genotypes coverage

The HBV genotype coverage test results indicated

that the TL system was capable of detecting all eight HBV DNA (A, B, C, D, E, F, G, H) genotypes as shown in Table 6. This almost covers all common genotypes worldwide, and meets the clinical requirements.

### Cross-contamination

Following two rounds of “checkerboard” tests with the TL system assay, the detection rate of positive samples was 100%, and the detection rate of negative samples was 0. Therefore, throughout the entire test process, the negative samples were not contaminated.

## DISCUSSION

HBV includes ten genotypes (A-I)<sup>[22-24]</sup>. According to its distribution in China, genotype B is the most frequent genotype in northern areas, genotype C is commonly seen in southern areas, mixed C/D genotypes are frequent in the Tibetan population in west China and genotype D is prevalent among Uighurs in Xinjiang, where genotype C is rare<sup>[25]</sup>. Infection with other HBV genotypes has not been reported in China. The 200 clinical HBV positive samples tested in this study were mainly composed of genotype B (70 cases), genotype C (66 cases) and genotype D (28 cases), genotype A (4 cases) and unknown genotypes. In order to verify the HBV genotype coverage using the TL system assay, the worldwide HBV DNA performance panel was used. The test results showed a 100% detection rate, which indicated that the TL system assay was capable of detecting not only the four genotypes in clinical samples, but also the eight HBV DNA (A, B, C, D, E, F, G, H) genotypes included in the worldwide HBV DNA performance panel.

In this study, the quantification limit test criterion adopted the analytical standard, which refers to the acceptable range  $\leq \pm 0.4$  Log<sub>10</sub> IU/mL of external quality assessment used by the NCCL of China. The results of three rounds of 30 IU/mL quantification limit tests in this study complied with this criterion. However, there is another international frequently-used criterion that considers  $CV \leq 5\%$  as the acceptable quantification limit test criterion. In this study, the CV in three rounds of 30 IU/mL quantification limit tests was 6.09%, 8.51%, and 5.55%, which failed to

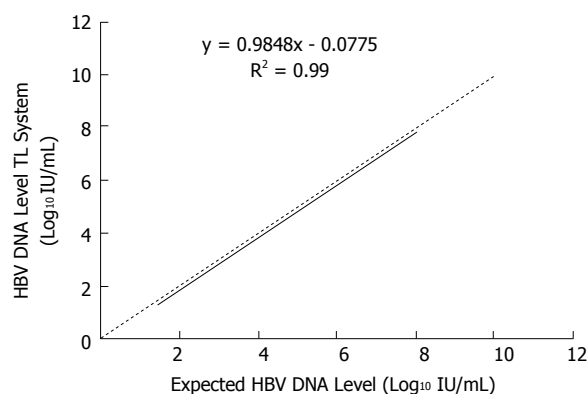
**Table 6** Hepatitis B virus genotypes coverage test Ct value

Hepatitis B virus DNA genotypes	Average tested Ct value			
	1 <sup>st</sup> day	2 <sup>nd</sup> day	3 <sup>rd</sup> day	Negative
A	25.62	25.89	25.71	Undetected
B	25.58	25.78	25.78	Undetected
C	25.94	25.86	25.99	Undetected
D	22.61	22.78	22.78	Undetected
E	22.67	22.70	22.68	Undetected
F	22.68	22.72	22.71	Undetected
G	24.50	24.78	24.76	Undetected
H	24.81	24.80	24.85	Undetected

meet this criterion. However, during the precision and reproducibility tests, the CV values for intra-assay and inter-assay tests of  $1.0 \times 10^2$  IU/mL samples were both less than 5%. These findings indicate that the TL system assay still requires further optimization for the 30 IU/mL concentration test.

In this study, during the comparison test of 200 clinical samples, the upper limit of linearity range of both the TL assay and CAP/CTM assay was  $1 \times 10^8$  IU/mL. Of 185 positive clinical samples, the test results of 23 samples obtained using both the TL and CAP/CTM assays exceeded the upper limit of linearity range. Four samples had the test results by the TL assay exceeded the upper limit of linearity range (8.08, 8.03, 8.08, 8.40), while the corresponding test results for the CAP/CTM assay were close to the upper limit of the linearity range (7.88, 7.80, 7.88, 8.00), but the  $\text{Log}_{10}$  IU/mL differences between the two assays were all less than  $\pm 0.4$ . Following elimination of these 27 samples, the correlation analysis results ( $P = 0.575$ ) showed no significant statistical differences between the two methods and good consistency. However, there were differences between the two assays in the detection of samples at a concentration over or close to the linearity range upper limit. In order to obtain more effective quantitative results, it is recommended that clinical samples are diluted and tested again.

In addition, this study also performed a cross-reaction test to analyze the effect of interfering substances on the TL system performance. However, we were unable to obtain the corresponding drug pharmacokinetic samples; thus, the HBV positive serum samples were adopted as the control group in the cross-reaction test, and these HBV positive serum samples were added to the interfering substances as the test group. The cross-reaction test results showed that the TL system was capable of detecting HBV positive serum samples containing four typical endogenous interfering substances (free hemoglobin, triglyceride, bilirubin and IgG) and four typical exogenous interfering substances (IFN $\alpha$ , lamivudine, adefovir dipivoxil, telbivudine) and the  $\text{Log}_{10}$  IU/mL differences between the corresponding samples from the test group and the control group were all less than  $\pm 0.4$ . Therefore, it can be concluded that the TL system has anti-interference capability for both

**Figure 3** Linear correlation test standard curve. HBV: Hepatitis B virus.

endogenous and exogenous interfering substances. For more precise evaluation of the anti-interference performance of the TL system, more tests should be carried out with drug pharmacokinetic samples and clinical samples containing interfering substances.

Qiu *et al.*<sup>[26]</sup> reported a comparison of the Abbott and Da-an real-time PCR for the quantitation of serum HBV DNA. The Abbott assay had a higher sensitivity, shorter assay time, and wider dynamic range compared with the Da-an assay. However, the costs of the Abbott assay limited its routine use in clinical molecular laboratories in China. The clinical performance of the TL system was comparable to the CAP/CTM system, with reasonable costs.

There are some deficiencies in the TL system found by performance verification. First, although no negative samples were detected in the cross-contamination test, two amplification curves for negative samples showed an increased tendency at 43-45 cycles, but did not reach the threshold line. Thus, cleaning and maintenance processes are particularly important in the case of a long run time, and the possibility of accumulated contamination should be validated over this period. Second, the TL system assay adopts pre-packaged nucleic acid extraction kits (24T $\times$ 4); thus, when the amount of test sample is not a multiple of 24, some nucleic acid extraction reagent is wasted and can increase costs, and this is one of the main deficiencies of the TL system which needs to be improved.

Only 200  $\mu$ L of sample were required by the TL system, its limit of detection (10 IU/mL) meets the standard (10-15 IU/mL) recommended by the EASL, and its limit of quantification (30 IU/mL) also meets the standard required in "The guideline principles for the technical review of hepatitis B virus DNA quantitative detection reagents registration" issued by the CFDA. The differences between the expected and tested concentration values of national standards were less than  $\pm 0.4 \text{ Log}_{10}$  IU/mL, which demonstrated the high accuracy of the system. In addition, there were no statistically significant differences between the TL system assay and the CAP/CTM assay. This

study also assessed the precision, reproducibility, and linearity of the TL system, and the results showed that between  $10^2$  IU/mL and  $10^6$  IU/mL, the multiple test CV of the same sample was less than 5%; and between 30 IU/mL and  $10^8$  IU/mL, the linear correlation coefficient  $r^2 = 0.99$ . Furthermore, the worldwide HBV DNA performance panel, which contains HBV DNA genotypes (A-H), was used to validate the capability of the TL system to detect all eight genotypes. Finally, two rounds of “checkerboard” tests with 96 samples (48 strongly positive and 48 negative) were tested to validate the anti-cross-contamination performance of the TL system, and the results showed a 100% coincidence rate. In summary, the TL system has good analytical performance, clinical performance and stability.

## COMMENTS

### Background

Use of the hypersensitive hepatitis B virus (HBV) DNA quantification system to accurately detect HBV DNA in serum is important in hepatitis B antiviral therapy. International associations for the study of liver diseases have required HBV DNA quantification detection with hypersensitivity (limit of detection: 10-15 IU/mL), a wide linearity range ( $1-9 \log_{10}$  IU/mL), high specificity and good repeatability. The CAP/CTM system is regarded as a worldwide standard for HBV DNA quantification. This study was conducted to validate and compare the automatic hypersensitive HBV DNA quantification system produced in China with the internationally accepted CAP/CTM system.

### Research frontiers

The sensitivity, reproducibility and system automaticity for HBV DNA quantification have improved in recent years, and are important factors in determining the curative effect and endpoints of hepatitis B antiviral therapy.

### Innovations and breakthroughs

This is the first study to evaluate the analytical performance of the TianLong automatic hypersensitive HBV DNA quantification system, which is the first CFDA approved system produced in China, and to compare its clinical performance with the CAP/CTM system. The TL system showed good analytical performance, and good correlation and quantitative agreement with the CAP/CTM system.

### Applications

This study will be helpful in improving the performance of this system and its clinical applications.

### Peer-review

The authors have evaluated the performance characteristics of the domestic TianLong HBV DNA Quantification System by testing 200 clinical HBV samples and compared the results with the international automatic HBV DNA detection systems including Roche CAP/CTM System. There was no statistically significant difference between TianLong and CAP/CTM systems. This study reports valuable results to validate the detection performance of TianLong HBV DNA Quantification System, which can pave the way to reduce cost of HBV DNA quantitative detection in the Chinese market.

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**P- Reviewer:** Bock CT, Farshadpour F, Jarcuska P, Osna NA, Vaughan G  
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ISSN 1007-9327



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