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Evaluation of the COBAS TaqMan 48 Real-Time PCR System for Quantitation of Hepatitis B Virus DNA

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The purpose of this study is to evaluate the usefulness of the new real-time PCR COBAS TaqMan 48 analyzer, comparing it to the existing COBAS AMPLICOR HBV MONITOR based on conventional PCR technology. The study used 104 samples from different patients. No differences were found in the sensitivity of the tests. There was an excellent correlation between the sample with a viral load within the dynamic range of the two tests ($r = 0.938$). The COBAS TaqMan test has a wider linear range, and this fact enables quantifying of the viral load without diluting the sample.

Hepatitis B virus (HBV) is a member of the family *Hepadnaviridae* that causes acute and chronic hepatitis (6). It is estimated that 2 billion people have been infected by HBV all over the world and more than 350 million people have persistent infection (11). The availability of molecular diagnostic test has improved the handling of the disease and monitoring of the response to treatment (12). The main purpose of measuring HBV DNA is to confirm the activity of infection and possible candidacy of patients for antiviral treatment.

One of the present tests for amplification of target is the AMPLICOR HBV MONITOR test (Roche Molecular Systems) which is based on the amplification of DNA targets by the use of HBV-specific primers. The quantitative PCR system used in the MONITOR test calculates the initial amount of DNA by measuring it at the end of the amplification reaction (2). The results thus obtained are subject to errors caused by a "plateau" effect that occurs because of accumulation of the substance, exhaustion of the enzyme, dilution of reagents, and other reasons. This effect produces a reduction in the rate of exponential accumulation of final product (5).

The quantitation of real-time PCR is based on the evaluation of the threshold cycle (C_t) when the amplification products are detected for the first time. The amplified product is detected sooner when the initial viral load is higher. real-time PCR has a greater precision in quantitation because the C_t value is observed when the PCR is still in the exponential phase and so provides a more reliable measurement than when the amplified product is measured "at the endpoint" as in conventional PCR (8).

The aim of this study was to evaluate the validity of the COBAS TaqMan 48 real-time PCR analyser (Roche) for detection and quantitation of HBV DNA in plasma, comparing the results to those obtained with the same samples using COBAS AMPLICOR MONITOR (Roche) PCR analyzer. The study was carried out using 104 blood samples collected from individual patients. The extracted blood was introduced in BD VACUTAINER tubes with 1 ml of ACD anticoagulant. The plasma obtained was divided in two parts. The quantitation of HBV DNA was carried out in parallel by AMPLICOR and TaqMan analyzers.

The HBV DNA for COBAS TaqMan was isolated by a manual and generic preparation sample kit (High Pure System viral nucleic acid kit), based on the bonding of nucleic acid to glass fibers, in accordance with the manufacturer's instructions. TaqMan technology uses the 5′-3′ nuclease activity of thermostable Z05 DNA polymerase. It uses double-marked fluorescent hybridization probes that bond specifically to the sample between primers. The probe contains a fluorescent marker (reporter) and another colorant that inhibits the reporter fluorescence (quencher). The degradation of the hybridization probe releases the reporter, causing an increase of fluorescence emission. The COBAS TaqMan test quantifies the amplicons during the exponential phase of amplification. The appearance of specific fluorescent signal is considered a critical threshold value (C_t). The C_t is defined as the number of fractional cycles in which the fluorescence emitted by the sample exceeds a preset threshold (assigned level of fluorescence) and marks the beginning of an exponential growth phase of this signal.

Performance of the AMPLICOR test also requires isolating the HBV DNA using a manual system that involves many lysis reagents to obtain the extract through successive centrifugations and incubations at 100°C. The amplification and detection are performed by the COBAS AMPLICOR HBV MONITOR test, which also uses the highly conserved precore/core region of HBV as the target. A colorimetric enzymatic reaction at the end is used for detection.

Besides viral load, both tests were also compared in regard to the DNA extraction procedure used by each one. The first variable compared was the time involved in a complete extraction. The work unit was 24 samples to be processed (controls are handled in the same way as clinical samples). The AMPLICOR extraction kit requires an average of 2 h of work, the same as for the High Pure System for COBAS TaqMan. The extraction phase for COBAS TaqMan requires practically exclusive dedication throughout the process. However, extraction for AMPLICOR enables the technician to have some free time during the test to perform other routine laboratory functions, a very important aspect in clinical microbiology laboratories. A

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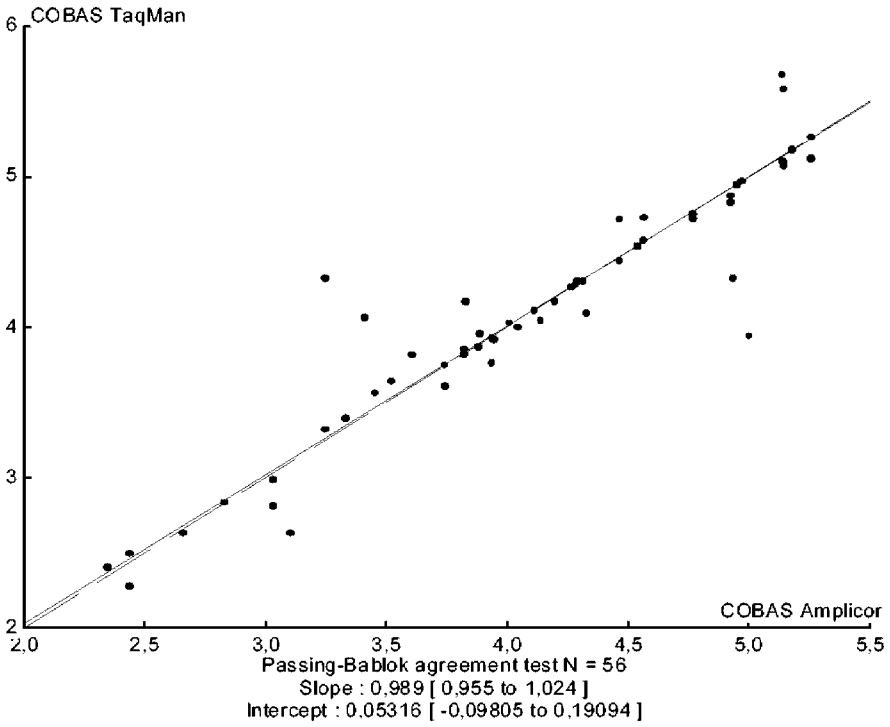

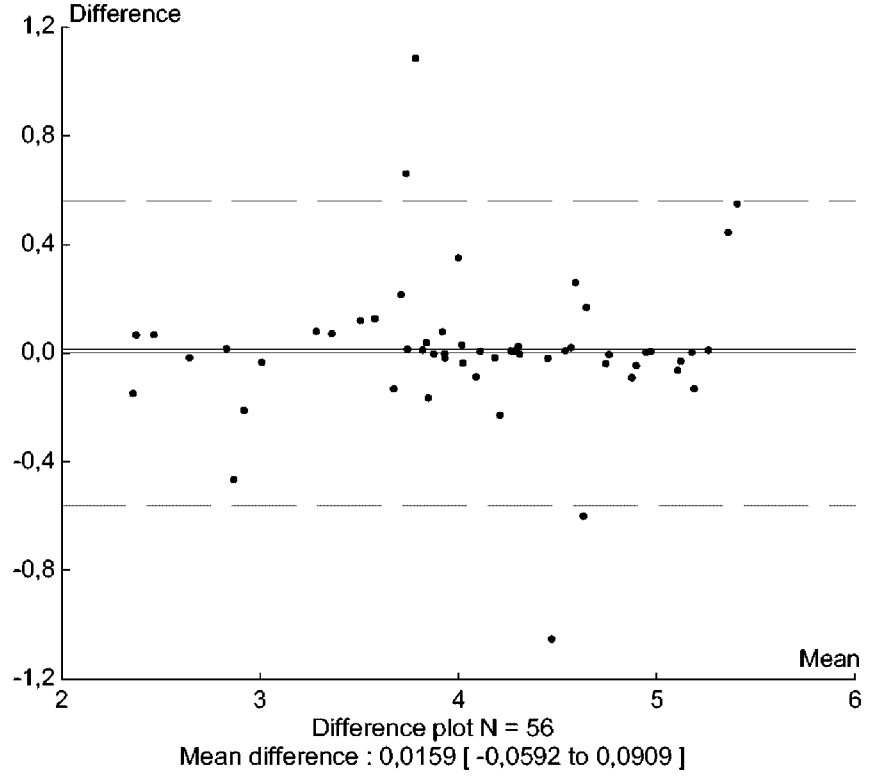

FIG. 1. Correlation between viral loads detected by the two tests in group A samples (Passing-Bablok and Bland-Altman).

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TABLE 1. Samples whose viral load by COBAS AMPLICOR was outside the dynamic range of the technique but within that of the COBAS TaqMan analyzer[a]

Place in relation to dynamic range	No. of copies/ml (log_{10})	
	AMPLICOR	TaqMan
Above the range	47,700,000 (7.6785)	169,000,000 (8.2278)
	455,000 (5.6580)	670,450 (5.8263)
	2,040,000 (6.3096)	16,207,400 (7.2097)
	2,730,000 (6.4361)	43,068,000 (7.6341)
	5,690,000 (6.7551)	11,814,000 (7.0723)
	27,800,000 (7.4440)	295,074,000 (8.4699)
	4,080,000 (6.6106)	281,688,000 (8.4497)
Below the range	73 (1.8633)	52 (1.7160)
	114 (2.0569)	273 (2.4361)

[a] 5.82 copies/IU.

comparison was also made of the technical difficulty of both options. The High Pure System proved to be more laborious and requires equipment that is not normally found in a laboratory.

In amplification and detection, the first variable of interest was the time taken to process 24 samples. The results in this regard were favorable for the COBAS TaqMan test. Results can be obtained using AMPLICOR in 5 hours, whereas the average time taken with the TaqMan unit was 2.5 hours. Another point of interest is the difference between both assays regarding the sensitivity of the test and its dynamic range.

The correspondence between results was studied by dividing the 104 samples into the following groups: group A, patients with viral loads within the range of both tests (56 samples); group B, patients with viral loads below the detection limit of both tests (34 samples); group C, patients with viral loads outside the range of the AMPLICOR test (14 samples); Viral loads were converted into logarithms for easier handling. Passing-Bablock and Bland-Altman statistical tests were used to establish the agreement between both tests (1, 3) (Fig. 1). The resulting correlation coefficient was $r = 0.938$. In group B samples the viral load was undetectable with both assays, therefore there was complete correlation between AMPLICOR and TaqMan. In the group C samples an attempt was made to assess the importance of having a test with a wide dynamic range such as COBAS TaqMan. With the COBAS AMPLICOR MONITOR HBV analyzer there were 14 samples whose viral load was outside the range limits of technique (200 copies/ml to 200,000 copies/ml), 12 above the upper limit, and 2 below the lower limit. Using the COBAS TaqMan unit was possible to quantify the viral load within the limits of its dynamic range (110,000,000 IU/ml [660,000,000 copies/ml] to 30 IU/ml [180 copies/ml]) in nine samples (64%). (Table 1).

Many techniques have been described for quantifying HBV DNA by PCR technology (4, 7, 9) and some of them have come onto the market. This ios the case of the semiautomatic AMPLICOR HBV MONITOR and its predecessor (AMPLICOR HBV MONITOR in microtiter plates). Many studies mention their great sensitivity (13, 14). The commercial introduction of real-time PCR technology has meant an advance in the handling of HBV viral load. In the samples used in our study there was no difference in sensitivity of the tests, as all the samples in which COBAS AMPLICOR did not detect viral load, neither was it detected by COBAS TaqMan analyzer.

In the group of patients where the viral load was within the range of the AMPLICOR test there was excellent correlation with the results of the TaqMan unit. This coincides with results published in other studies which show that real-time PCR technology has a good degree of correlation with conventional PCR techniques (16, 17).

One inconvenience found with the AMPLICOR test in previous studies was its narrow dynamic range (14, 10, 15). This means that in a lot of samples previous dilution is necessary to extend the test range (10, 15, 16, 17). One of the technical characteristics of real-time PCR is its greater dynamic range (16). Our results show that 9 of 14 samples whose viral loads were outside the range of determination for the AMPLICOR test could be quantified using the TaqMan assay without diluting the sample. In conclusion, it can be said that the COBAS TaqMan 48 analyser provides faster results than AMPLICOR, both tests have excellent correlation in samples with viral load within the dynamic range of both assays ($r = 0.938$) and the greater dynamic range of COBAS TaqMan test provides more exact results without diluting the sample, especially in patients with very high viral loads.

The results obtained in this and other studies do not invalidate the use of COBAS AMPLICOR in laboratories that do not have real-time technology available. The quantitation of HBV DNA offered by COBAS AMPLICOR is extremely important in the management of large numbers of patients for confirming the condition of active replication as well as for evaluating response to treatment. Its use would therefore be justified and its validity demonstrated as a first step in follow-up of these patients. However, the characteristics of the test in real time justify its use in patients whose evaluation or follow-up cannot be considered certain within a linear range narrower than the conventional test. The fact that the procedure for the extraction of DNA for the AMPLICOR test is less laborious could be an element in its favor for use in smaller laboratories with fewer technical staff. An automatic system for the extraction of HBV DNA will become available in the near future, and this will further simplify this process.

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