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Evaluation of the COBAS TaqMan HCV Test with Automated Sample Processing Using the MagNA Pure LC Instrument

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The COBAS TaqMan HCV Test (TaqMan HCV; Roche Molecular Systems Inc., Branchburg, N.J.) for hepatitis C virus (HCV) performed on the COBAS TaqMan 48 Analyzer (Roche Molecular Systems) currently relies on a manual sample processing method. Implementation of an automated sample processing method would facilitate the clinical use of this test. In this study, we evaluated the performance characteristics of TaqMan HCV following automated sample processing by the MagNA Pure LC instrument (MP; Roche Applied Science, Indianapolis, Ind.). The analytical sensitivity of TaqMan HCV following sample processing by MP was 8.1 IU/ml (95% confidence interval, 6.1 to 15.2). The assay showed good linearity ($R^2 = 0.99$) across a wide range of HCV RNA levels (25 to 5×10^6 IU/ml), with coefficients of variation ranging from 10% to 46%. Among 83 clinical specimens, the sensitivity and specificity of TaqMan HCV were 100% and 95%, respectively, when compared to the COBAS AMPLICOR hepatitis C virus test, version 2.0 (COBAS AMPLICOR; Roche Molecular Systems), with TaqMan HCV detecting two more HCV RNA-positive specimens than COBAS AMPLICOR. Both specimens were confirmed to be HCV RNA positive by the VERSANT HCV RNA qualitative test (Bayer HealthCare LLC, Tarrytown, N.Y.). There was also strong correlation ($R^2 = 0.95$) and good agreement between the results from TaqMan HCV and the VERSANT HCV RNA 3.0 assay (bDNA) (Bayer HealthCare LLC) among a group of 93 clinical specimens. The MP is a versatile, labor-saving sample processing platform suitable for reliable performance of TaqMan HCV.

Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease in the United States. The prevalence of HCV infection has been estimated to be 1.8% in the general population, resulting in a conservative estimate of 2.7 million cases of chronic HCV infection in the United States (2). Current anti-HCV treatment algorithms continue to rely on the direct detection and quantitation of HCV RNA in serum or plasma for confirmation of chronic HCV infection and determining duration of anti-HCV therapy. Significant decreases in HCV RNA viral load and clearance of HCV RNA during the course of therapy are also important predictors of sustained virologic response with current anti-HCV therapies. As a result, there is a growing clinical need for rapid automated molecular techniques capable of providing both sensitive detection of HCV RNA and accurate quantitation of HCV RNA over a broad dynamic range.

The COBAS TaqMan HCV Test (TaqMan HCV; Roche Molecular Systems, Inc., Branchburg, N.J.) is a real-time nucleic acid amplification assay for the qualitative and quantitative detection of HCV RNA in human serum or plasma. Like the TaqMan HCV analyte specific reagent (TaqMan HCV ASR; Roche Molecular Systems), this assay has been developed for use with the recently introduced COBAS TaqMan 48 Analyzer (CTM 48; Roche Molecular Systems). While use of the TaqMan HCV ASR requires the testing laboratory to establish and validate the actual test method and provide appropriate test controls, TaqMan HCV is a complete test kit currently designated for research use only in the United States.

Currently, TaqMan HCV is comprised of two products, the High Pure System viral nucleic acid kit (Roche Molecular Systems) and the COBAS TaqMan HCV Test kit, which can be purchased separately. The High Pure System viral nucleic acid kit is a generic, manual specimen preparation method configured for processing multiple samples in batches. Specimen preparation is followed by automated reverse transcription (RT)-PCR amplification and real-time detection of cleaved dual fluorescent dye-labeled oligonucleotide (TaqMan) probes that allow the specific and simultaneous detection and quantitation of the target sequence as well as an internal HCV quantitation standard with the CTM 48.

The potential benefits of nucleic acid amplification and detection with real-time PCR have been well documented (6, 14, 16). Among the benefits are substantial reductions in labor as well as decreased test turnaround time. The disadvantages of manual specimen preparation methods have also been well documented. Labor-intensive manual sample preparation methods have been reported to account for the majority of the hands-on time required to perform current commercially available PCR-based assays for HCV (1, 12, 13). In many cases, sample preparation is the most technically demanding portion of the assay and a potential source of run-to-run variability as well as sample contamination. Implementation of an automated sample processing system in clinical diagnostic laboratories provides a labor-saving approach to reducing the number of failed preparations and limiting the occurrence of specimen-to-specimen contamination during processing. Other important advantages of automated specimen processing include reduced laboratory space

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TABLE 1. Analytical sensitivity of TaqMan HCV combined with sample processing by MP

HCV RNA concn (IU/ml)	No. of replicates tested	No. of positive replicates	% positive (95% confidence interval)
25	10	10	100 (69–100)
10	10	10	100 (69–100)
5	10	6	60 (26–88)
2.5	10	3	30 (7–65)
0	10	0	0 (0–31)

requirements and decreased dependence on laboratory technologists skilled in molecular techniques.

Recent evaluations of the MagNA Pure LC instrument (MP; Roche Applied Science, Indianapolis, Ind.) have shown that it is a flexible and reliable platform suitable for the extraction and purification of HCV RNA from clinical specimens (7, 9). In this study, we evaluated the performance characteristics of TaqMan HCV with HCV standards and clinical specimens processed by the MP. Analytical sensitivity and precision of the assay were determined with HCV standards, while clinical sensitivity, specificity, correlation, and agreement were assessed with previously characterized clinical specimens.

MATERIALS AND METHODS

HCV analytical standards. Commercially available panels of HCV standards at 5,000,000, 500,000, 50,000, 5,000, 500, 50, and 0 IU/ml (OptiQuant HCV RNA; AcroMetrix Corp., Benicia, Calif.) were used in this evaluation. Four additional dilutions at 25, 10, 5, and 2.5 IU/ml were also prepared from these standards with NAT Dilution Matrix (AcroMetrix Corp.). The 25 and 10 IU/ml dilutions were prepared and stored at −70°C prior to analysis by TaqMan HCV, while the 5 and 2.5 IU/ml dilutions were prepared and analyzed in a single test run on the day of preparation. With the exception of the 5 and 2.5 IU/ml dilutions, all analytical standards were tested daily (in duplicate) for a total of 5 days.

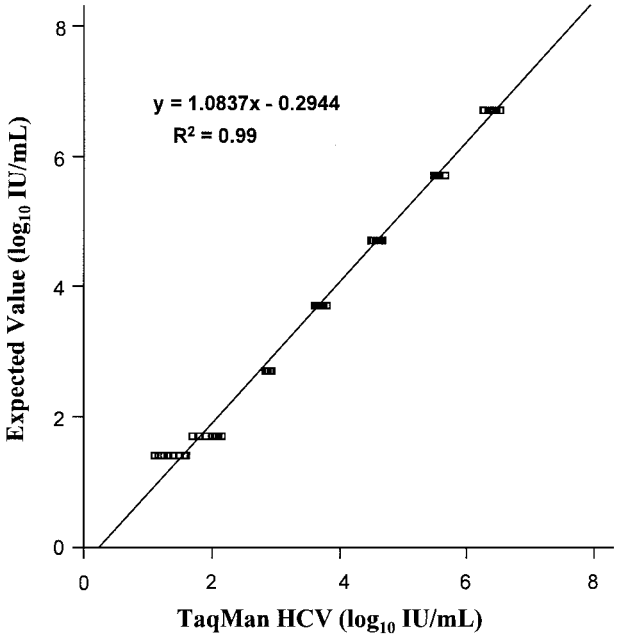

FIG. 1. Correlation between observed and expected results of HCV standards tested by TaqMan HCV combined with sample processing by MP.

TABLE 2. Precision of TaqMan HCV combined with sample processing by MP

HCV RNA concn (IU/ml)	Observed mean HCV RNA titer (IU/ml)	No. of replicates tested	Coefficient of variation (%)
25	22	10	46
50	102	10	29
500	770	10	10
5,000	4,958	10	14
50,000	40,100	10	13
500,000	345,600	10	13
5,000,000	2,452,000	10	19

Clinical specimens. Eighty-three clinical serum specimens submitted to the Mayo Clinic Hepatitis/HIV Laboratory for routine qualitative HCV RNA testing by the COBAS AMPLICOR hepatitis C virus test, version 2.0 (COBAS AMPLICOR; Roche Molecular Systems), during January 2004 were retrospectively selected for this study. This group of specimens included 41 and 42 specimens with and without detectable HCV RNA, respectively, as determined by COBAS AMPLICOR performed according to the manufacturer's instructions. The specimens were stored at −70°C for up to 2 weeks between COBAS AMPLICOR testing and analysis by TaqMan HCV. Specimens yielding discordant TaqMan HCV and COBAS AMPLICOR results were tested by transcription-mediated amplification with the VERSANT HCV RNA qualitative test (Bayer HealthCare LLC, Tarrytown, N.Y.).

Ninety-three well-characterized HCV RNA-positive serum specimens submitted to our laboratory between September 2002 and January 2004 for routine quantitative HCV RNA testing were also retrospectively selected for this study. These specimens included HCV genotypes 1 to 6, with HCV viral loads ranging from 730 to 4,987,210 IU/ml as determined by the VERSANT HCV RNA 3.0 assay (bDNA; Bayer HealthCare LLC) performed according to the manufacturer's instructions. Specimens were stored at −70°C from 2 to 68 weeks following bDNA testing and prior to analysis by TaqMan HCV.

Twelve of the 93 well-characterized HCV RNA-positive serum specimens previously described (two each of genotypes 1 to 6) were also diluted 1:100 and 1:1,000 with NAT dilution matrix. These diluted specimens were tested by TaqMan HCV for use in the comparison of amplification efficiencies among HCV genotypes.

This study was reviewed and approved by the Mayo Foundation Institutional Review Board.

HCV genotype determination. HCV genotyping was performed on all 93 HCV RNA-positive clinical specimens submitted for quantitative HCV RNA testing. Direct DNA sequence analysis of the HCV 5′ noncoding region was performed with the TRUGENE HCV 5′NC genotyping kit (Bayer HealthCare LLC) following amplification by COBAS AMPLICOR.

MP sample processing and TaqMan HCV. HCV RNA was extracted from 500-μl aliquots of HCV analytical standards, clinical specimens, and assay controls with the MP total nucleic acid isolation kit–large volume (Roche Applied Science) and MP software version 3.03. The TaqMan HCV quantitation standard was also used in MP sample processing by adding it directly to the MP lysis/binding buffer just prior to the start of automated processing. For the processing of the 24 samples, 114 μl of TaqMan HCV quantitation standard was added to 37.686 ml of MP lysis/binding buffer and gently mixed prior to dispensing it into the appropriate MP reagent reservoirs. The final MP elution volume for each sample was 75 μl.

Following the completion of MP sample processing, a tube containing Taq-

TABLE 3. Clinical sensitivity and specificity of TaqMan HCV with sample processing by MP compared to COBAS AMPLICOR

TaqMan HCV result	No. of specimens with COBAS AMPLICOR result:	
	Positive	Negative
Positive	41	2
Negative	0	40

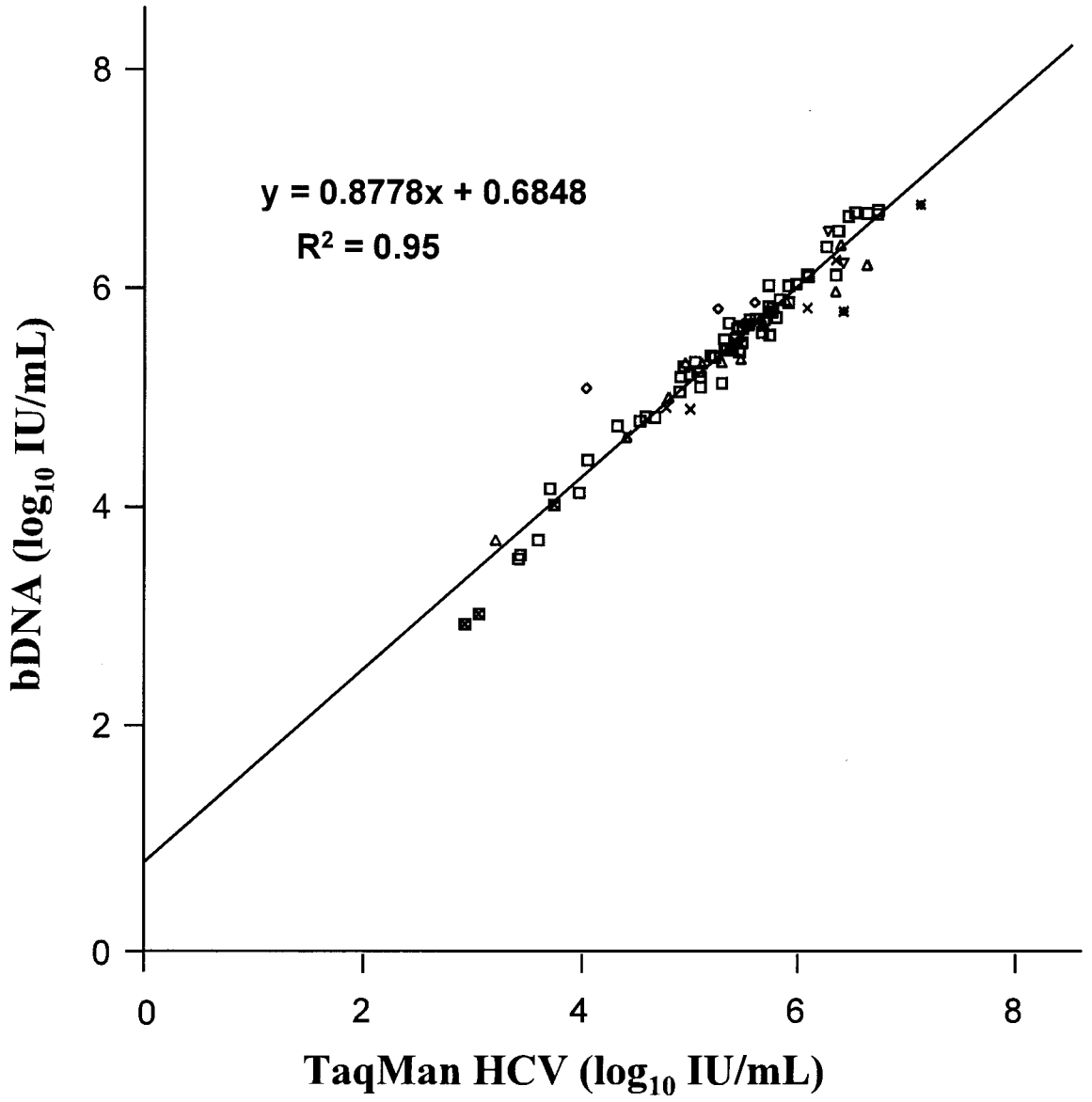

FIG. 2. Correlation between the results of 93 clinical specimens tested by TaqMan HCV combined with sample processing by MP and bDNA. HCV genotypes: (□) genotype 1; (△) genotype 2; (×) genotype 3; (◇) genotype 4; (▽) genotype 5; (star) genotype 6; and (boxed X) unable to genotype.

Man HCV working master mix and 24 open COBAS TaqMan kinetic reaction tubes (K-tubes) contained in a K-tube holder (K-carrier) were placed onto a prototype (noncooling) postelution handling block specifically designed to hold a K-carrier (containing K-tubes) in the MP. The addition of working master mix (50 μl) and sample eluate (50 μl) to each K-tube (followed by a mixing step) was automatically performed by the MP. K-carriers were removed from the MP and individual K-tubes were manually sealed prior to loading onto the CTM 48. Amplification and detection were performed according to the manufacturer's instructions for TaqMan HCV with the CTM 48 with AMLILINK software v 3.0.1 (Roche Molecular Systems).

Statistical analysis. Observed results of HCV analytical standards tested by TaqMan HCV were compared with the expected results by linear regression, while the lower limit of detection with 95% confidence interval was determined by probit analysis (95% hit rate) (8). The precision of TaqMan HCV was estimated by determining the coefficients of variation at seven different HCV RNA concentrations (25 to 5×10^6 IU/ml) tested over a total of 5 days. Correlation and strength of agreement between TaqMan HCV and bDNA results were determined by linear regression and Bland-Altman plot (4), respectively. Potential differences in target amplification efficiency (quantitation) by TaqMan HCV were examined further by comparing the slopes of the linear regression lines from 12 clinical specimens (HCV genotypes 1 to 6) tested undiluted and at 1:100 and 1:1,000 dilutions.

RESULTS

The lower limit of detection or analytical sensitivity of TaqMan HCV combined with sample processing by MP was determined based on the results of replicate testing of HCV analytical standards at concentrations of 25, 10, 5, 2.5, and 0 IU/ml (Table 1). With probit analysis with a 95% hit rate, the lower limit of detection was 8.1 IU/ml (95% confidence interval, 6.1 to 15.2 IU/ml). Excellent linearity ($R^2 = 0.99$) was also present across a wide range of HCV RNA levels extending

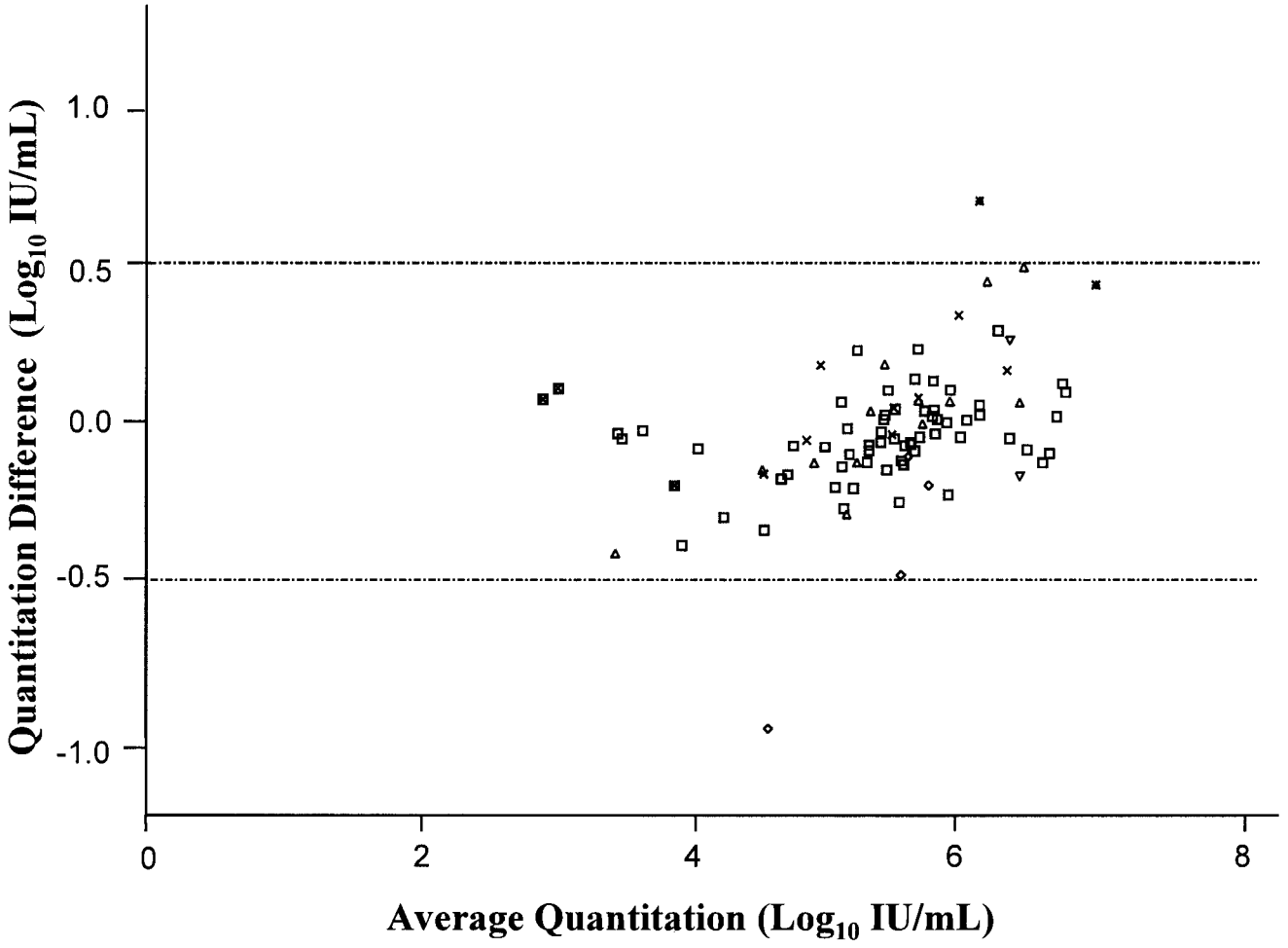

FIG. 3. Log_{10} differences in quantitation between TaqMan HCV combined with sample processing by MP and bDNA among 93 clinical specimens (Bland-Altman plot). HCV genotypes: (□) genotype 1; (△) genotype 2; (×) genotype 3; (◇) genotype 4; (▽) genotype 5; (star) genotype 6; and (boxed X) unable to genotype.

from 25 to 5×10^6 IU/ml (Fig. 1), with interassay coefficients of variation ranging from 10% to 46% among the seven concentrations tested over 5 days (Table 2).

When the results of TaqMan HCV combined with sample processing by MP were compared to those of COBAS AMPLICOR among the 83 clinical serum specimens, the clinical sensitivity and specificity of TaqMan HCV combined with sample processing by MP were 100% (95% confidence interval, 91% to 100%) and 95% (95% confidence interval, 84% to 99%), respectively. TaqMan HCV detected two more HCV RNA-positive specimens than COBAS AMPLICOR (Table 3). These two discordant specimens were also tested by the VERSANT HCV RNA qualitative test, with both specimens yielding positive HCV RNA results.

The group of 93 well-characterized specimens consisted of HCV genotype 1 ($n = 61$), genotype 2 ($n = 13$), genotype 3 ($n = 8$), genotype 4 ($n = 4$), genotype 5 ($n = 2$), and genotype 6 ($n = 2$) as well as three HCV RNA-positive specimens that could not be genotyped, presumably due to low viral titer (862 to 5,720 IU/ml by bDNA). Comparison of TaqMan HCV and bDNA results among this group of specimens demonstrated strong correlation ($R^2 = 0.95$) of HCV RNA quantitative results obtained by TaqMan HCV and bDNA (Fig. 2). Bland-Altman plotting showed that differences between the $\log_{10}$ IU/ml results obtained from TaqMan HCV and bDNA were within ± 0.5 $\log_{10}$ IU/ml of the averaged $\log_{10}$ results of the two tests for 98% of the specimens tested (Fig. 3). The two specimens with differences of > 0.5 $\log_{10}$ IU/ml contained HCV genotypes 4 and 6. Repeat testing (TaqMan HCV and bDNA) of the specimen containing HCV genotype 4 yielded similar results, while the specimen containing HCV genotype 6 could not be retested due to insufficient volume. The mean slope of the linear regression lines derived from the 12 clinical specimens tested undiluted, and at 1:100 and 1:1000 dilutions was -1.4, with the mean genotype-specific slope ranging from -1.1 for genotype 4 to -1.6 for genotypes 1 and 6.

The hands-on setup time for the MP was ≈20 min. The time required to prepare and manually load specimens into the MP sample cartridge prior to automated processing was also ≈20 min. With the postelution handling option, the total time required for processing 24 reactions by MP was 192 min, an increase of 37 min relative to the estimated time required to

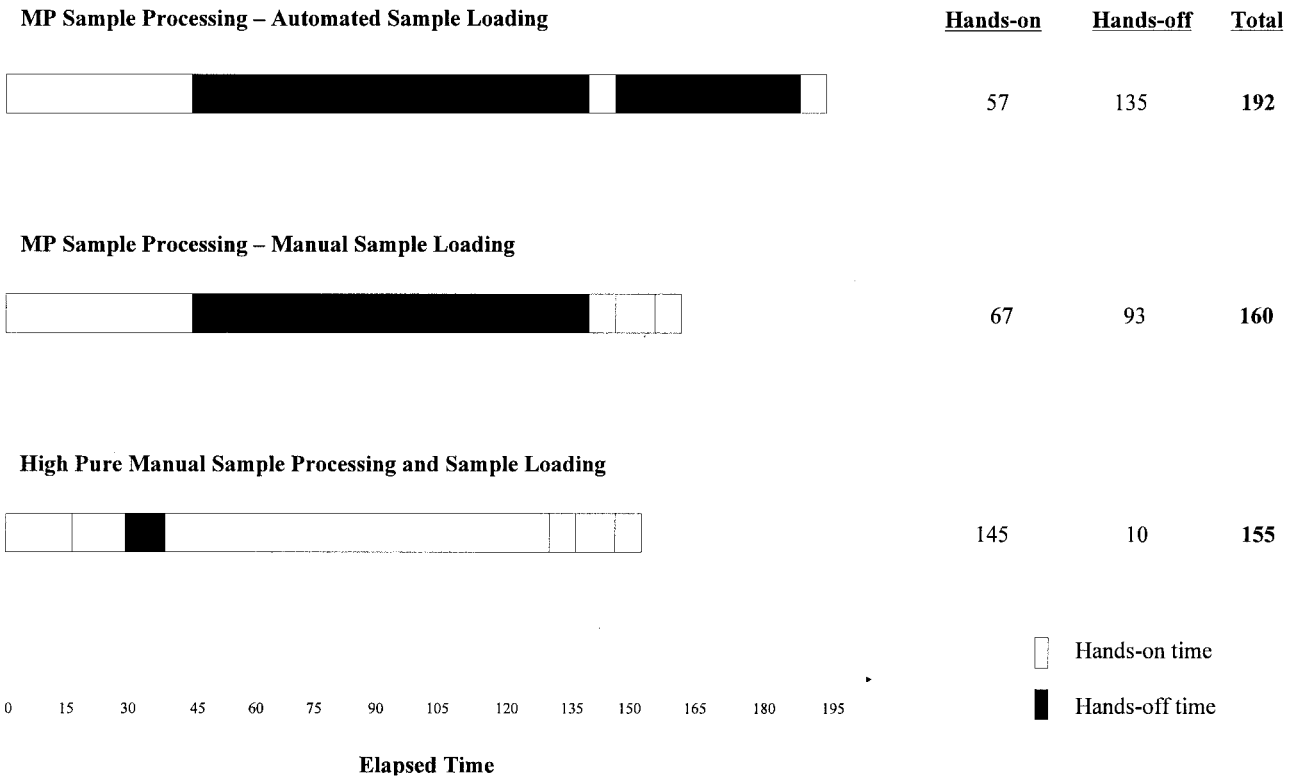

FIG. 4. Comparison of automated (MP) and manual (High Pure) sample processing workflow. The bars represent the general workflow for each sample processing method extending from sample preparation through completion of K-tube loading. The hands-on time, hands-off time, and total time required for the processing of 24 samples (21 specimens and 3 controls) by each method are also indicated (in minutes).

perform the manual method. However, the actual hands-on time was reduced from an estimated 145 min required by the manual method to just 57 min with MP sample processing and postelution handling, including two periods (93 and 42 min) of hands-off time (Fig. 4). The total duration for the entire procedure (21 samples plus three controls), including sample preparation, MP sample processing, and amplification/detection, was ≈6 h, of which ≈1 h was actual hands-on time. During the course of this study, there were no failures of sample processing associated with the MP, and the overall rate of TaqMan HCV failure (an invalid test result as defined by the APLILINK 3.0.1 software) was <1% (2 of 313) over the 17 runs completed for this study.

DISCUSSION

Sensitive, accurate detection and quantitation of HCV RNA is essential for the diagnosis and management of chronic HCV infection. With projected increases in the diagnosis of chronic HCV infection in the United States over the next several decades (3), clinical laboratories will face growing pressure to efficiently accommodate increasing test volumes, while continuing to provide both sensitive detection and accurate quantitation of HCV RNA in clinical specimens. The results of this study have demonstrated that TaqMan HCV used in conjunction with sample processing by MP is an efficient and reliable method capable of providing both qualitative and quantitative results with a single test.

Our analytical data suggest that TaqMan HCV, when used in combination with sample processing by MP (with a lower limit of detection of 8.1 IU/ml), is more sensitive than COBAS AMPLICOR, which has a reported lower limit of detection ranging from 50 to 75 IU/ml (9, 15). Our clinical data also suggest that TaqMan HCV combined with sample processing by MP was more sensitive than COBAS AMPLICOR (Table 3), although the difference in clinical sensitivity between the two assays was not statistically significant ($P = 0.438$; one-tailed Fisher's exact test). When used in conjunction with MP, TaqMan HCV yielded a lower limit of detection comparable to that of the VERSANT HCV RNA qualitative test, which has been shown to reliably detect HCV RNA at concentrations of <10 IU/ml (11). The increased sensitivity of these ultrasensitive assays could be important in the management of HCV-infected patients, since the increased sensitivity of transcription-mediated amplification has been shown to improve the detection of extremely low levels of HCV RNA in end-of-treatment specimens and improve the prediction of treatment failure or virologic relapse in patients receiving anti-HCV therapy (5, 10, 17, 18).

In the current study, evaluation of HCV RNA quantitation by TaqMan HCV was limited to the analysis of analytical standards ranging from 25 to 5×10^6 IU/ml and a group of 93 well-characterized serum specimens previously tested by bDNA. While our study was limited in scope and did not evaluate the entire reportable range of TaqMan HCV (10 to 2×10^8 IU/ml) defined by the assay manufacturer, it did

demonstrate that the results of TaqMan HCV were in good agreement with those of bDNA and that there were no substantial differences in quantitation among the various HCV genotypes. In addition, TaqMan HCV provides a broader dynamic range than bDNA (615 to 7.69×10^6 IU/ml). The increased dynamic range of TaqMan HCV improves clinician's ability to monitor changes in HCV viral load in patients undergoing anti-HCV therapy, particularly when HCV RNA titers are <615 IU/ml or $>7.69 \times 10^6$ IU/ml.

During the course of this study, we experienced no significant problems related to MP performance. The lower limit of detection and precision of TaqMan HCV with sample processing performed by MP were comparable to those reported in a previous evaluation of TaqMan HCV used in conjunction with the High Pure System viral nucleic acid kit (J. Birkett, K. Demartin, C. Harkleroad, D. Romo, D. Liao, A. Maxwell, H. Sandhu, and S. Tsang, Abstr. 19th Annual Clinical Virology Symposium, abstr. M43, 2003). While our study was not specifically designed to evaluate specimen contamination with exogenous nucleic acids, we found no evidence of sample-to-sample contamination during evaluation of either the analytical or clinical samples, despite the processing of both HCV RNA-negative and high-titer samples within the same run on the MP. In addition to these findings, the hands-on time required for sample processing by MP, compared to manual sample processing, was reduced by $\approx$1 h with or without use of the postelution handling capability of MP (run size of 24). Importantly, with MP sample processing and postelution handling, there are two extended periods of hands-off time, which allows technologists time to perform other laboratory duties.

Although the MP performed well in this evaluation with a run size of 24, the efficiency of the complete process may be effected by other factors, such as laboratory test volume. Differences in instrument configuration limit the overall efficiency of MP specimen processing for TaqMan HCV. The MP instrument was originally designed to work with the LightCycler instrument (Roche Applied Science), and it efficiently processes eight specimens simultaneously with a maximum batch size of 32, such that processing batches of 8, 16, or 32 samples is most efficient. However, TaqMan HCV is performed most efficiently in batch sizes of 12, 24, and 48 samples on the CTM 48. It would be advantageous to use multiple MP instruments in clinical laboratories with high specimen throughput demands. Alternatively, omission of postelution handling by the MP in favor of manual K-tube loading could further decrease the total time required for MP sample processing and increase specimen throughput.

In summary, this study demonstrated that the MP is a versatile, automated, and labor-saving sample processing platform useful for the reliable performance of TaqMan HCV. Our analytical and clinical data suggest that TaqMan HCV combined with sample processing by MP may be more sensitive than COBAS AMPLICOR for the qualitative detection of HCV RNA. In this study, TaqMan HCV also provided quantitative HCV RNA results that were in good agreement with those of bDNA. Our findings, generated with a research-use-only test kit (TaqMan HCV), suggest that the MP would also be suitable for use with the TaqMan HCV ASR. However, the performance characteristics of assays utilizing TaqMan HCV ASR in conjunction with MP sample processing require further validation by individual clinical diagnostic laboratories.

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