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Evaluation of the MagNA Pure LC Instrument for Extraction of Hepatitis C Virus RNA for the COBAS AMPLICOR Hepatitis C Virus Test, Version 2.0

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The COBAS AMPLICOR system has played a major role in the transition of molecular diagnostics from research to routine clinical laboratory use by automating the nucleic acid amplification and detection processes. However, sample preparation remains a labor-intensive portion of the procedure. In this study, we evaluated the performance of the COBAS AMPLICOR Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems, Branchburg, N.J.) following manual hepatitis C virus (HCV) RNA extraction versus automated extraction with the MagNA Pure LC instrument (Roche Applied Science, Indianapolis, Ind.). Parallel replicate testing was performed with standard dilutions of 100, 75, 60, and 0 HCV IU/ml and 153 clinical specimens. An analytical sensitivity of 75 IU/ml was achieved with either the manual or the standard-volume (200 μl) automated extraction methodologies (25 of 26 [96.2%]; 95% confidence interval [95% CI], 80.4 to 99.9), whereas the clinical sensitivity and specificity were both 100% with either extraction method. A large-volume (1 ml) automated extraction method was also evaluated with standard dilutions of 40, 25, 10, and 0 IU/ml and the same 153 clinical specimens. The analytical sensitivity of the COBAS AMPLICOR assay with the large-volume extraction method was 25 HCV IU/ml (26 of 26 [100%]; 95% CI, 86.8 to 100), whereas the clinical sensitivity and specificity were both 100%. The MagNA Pure LC instrument is a versatile, labor-saving platform capable of integration with minimal modification of the existing assay procedure. The increased sensitivity of the COBAS AMPLICOR Hepatitis C Virus Test, version 2.0 performed in conjunction with large-volume HCV RNA extraction may be important in HCV diagnostic testing as new therapeutic strategies evolve.

The U.S. Food and Drug Administration-approved COBAS AMPLICOR Hepatitis C Virus Test, version 2.0 (COBAS AMP HCV 2.0; Roche Molecular Systems, Branchburg, N.J.) is among the most widely used commercially available molecular assays in the United States. While the introduction of the COBAS AMPLICOR system has played a major role in the transition of molecular diagnostics from research to routine clinical laboratory use by automating the amplification and detection process, sample preparation remains a labor-intensive portion of the procedure (2, 8) and has been reported to account for the majority of the hands-on time required to perform the assay (6, 8). The sample preparation process is the most technically demanding portion of the assay and a potential source of run-to-run variability, as well as sample contamination (11, 14). Implementation of a fully automated extraction system in clinical diagnostic laboratories is a potential labor-saving technique that may help to reduce the number of failed extractions and the potential for specimen-to-specimen contamination during processing. There may also be important advantages of automated specimen processing in the current laboratory environment with decreased laboratory space and a lack of qualified laboratory technologists skilled in molecular techniques (5).

Recent evaluations of the MagNA Pure LC instrument (MP; Roche Applied Science, Indianapolis, Ind.) for nucleic acid extraction have shown that it is a flexible and reliable platform suitable for a variety of applications (3, 4, 7, 12). The goal of the present study was to compare the performance of the COBAS AMP HCV 2.0 following manual (i.e., COBAS AMP HCV 2.0) hepatitis C virus (HCV) RNA extraction versus automated HCV RNA extraction by using the MP instrument. The analytical sensitivity of the COBAS AMP HCV 2.0 was assessed with each extraction method by using an HCV dilution panel prepared from the First World Health Organization (WHO) HCV International Standard for Nucleic Acid Amplification Technology Assays for HCV RNA (standard 96/790) (15). The clinical sensitivity, specificity, specimen-to-specimen contamination, amplification inhibition rate, and time requirements were evaluated by using a well-characterized panel of clinical specimens. Using these same methods, the feasibility of assaying, with the MP, a large-volume sample (i.e., five times that currently used with the manual COBAS AMP HCV 2.0 extraction) was also evaluated.

MATERIALS AND METHODS

HCV standard dilutions. WHO standard 96/790, obtained from the National Institute for Biological Standards and Control (South Mimms, Potters Bar, Hertfordshire, United Kingdom) was used in the preparation of HCV standard

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TABLE 1. COBAS AMP HCV 2.0 analytical sensitivity after AMPLICOR manual standard-volume sample processing

Amt of HCV RNA[a] (IU/ml)	Replicates (*n*)	Negative result ($A_{660} < 0.15$)		Equivocal result ($0.15 \leq A_{660} < 1.0$)		Positive result ($A_{660} \geq 1.0$)	
		n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
100	26	1	3.9 (0.1–19.6)	0	0 (0–13.2)	25	96.2 (80.4–99.9)
75	26	1	3.9 (0.1–19.6)	0	0 (0–13.2)	25	96.2 (80.4–99.9)
60	26	0	0 (0–13.2)	4	15.4 (4.4–34.9)	22	84.6 (65.1–95.6)
0	26	26	100 (86.8–100)	0	0 (0–13.2)	0	0 (0–13.2)

[a] Determined with dilutions of the First WHO HCV International Standard for Nucleic Acid Amplification Technology Assays for HCV RNA (standard 96/790).

dilutions. The vial was reconstituted with 0.5 ml of distilled water, resulting in a solution that contained 10^5 HCV IU/ml. The following dilutions of WHO standard 96/790 were prepared by using pooled normal human plasma (NHP) determined to be anti-HCV- and HCV RNA-negative as diluent: 1,000, 500, 250, 100, 75, 60, 50, 40, 25, 10, 5, and 0 IU/ml. All dilutions were divided into aliquots (220, 220, and 1,050 μl) in duplicate for use in a preliminary evaluation of the extraction methods. An additional 48 aliquots (220 μl each) of selected dilutions (100, 75, 60, and 0 IU/ml), along with 24 aliquots (1,050 μl each) of selected dilutions (40, 25, 10, and 0 IU/ml), were prepared for use in the determination of the analytical sensitivity of the HCV RNA extraction methods studied. All dilution and aliquoting procedures were performed on ice, and all aliquots were frozen and held at −70°C prior to testing to minimize HCV RNA loss due to handling and multiple freeze-thaw cycles.

Clinical specimens. A total of 110 well-characterized HCV RNA-positive serum specimens, representing HCV genotypes 1, 2, 3, and 4, with viral loads ranging from <615 to >7,692,310 IU/ml (as determined by the VERSANT HCV RNA 3.0 Assay; Bayer HealthCare LLC, Berkeley, Calif.) and stored at −70°C, were studied, along with 43 anti-HCV- and HCV RNA-negative serum specimens. All specimens were thawed, divided into aliquots (220, 220, and 1,050 μl), and refrozen at −70°C prior to analysis to avoid HCV RNA loss during testing due to multiple freeze-thaw cycles.

MagNA Pure LC sample preparation. (i) Standard-volume extraction. HCV RNA was extracted from 200-μl aliquots of plasma or serum by utilizing the instrument's Total Nucleic Acid External_Lysis protocol and the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche Applied Science). The Total Nucleic Acid External_Lysis protocol was utilized in order to accommodate the COBAS AMP HCV 2.0 kit controls, with minimal modification of the manufacturer's recommended procedure. The COBAS AMP HCV 2.0 internal control (IC) was also utilized in the MP extraction process by adding it directly to the MP Lysis/Binding Buffer just prior to dispensing the reagent into the MP sample cartridge. For the extraction of 24 samples (including positive and negative controls), 190 μl of IC was added directly to 7.8 ml of MP Lysis/Binding Buffer and gently mixed prior to dispensing the reagent. External lysis of specimens was performed prior to the start of automated processing by adding 300 μl of MP Lysis/Binding Buffer, followed by the addition of 200 μl of each serum or plasma specimen to each appropriate well of the MP sample cartridge. For the assay controls, 30 μl of the COBAS AMP HCV 2.0 positive and negative controls were added to the appropriate MP sample cartridge wells containing a mixture of MP Lysis/Binding Buffer (300 μl) and HCV RNA-negative NHP (200 μl) just prior to beginning the automated extraction processing. Due to an apparent reagent incompatibility between the preserved NHP provided with the COBAS AMP HCV 2.0 kit controls and the MP reagents, fresh-frozen NHP (HCV RNA negative) was substituted in place of the NHP provided with the COBAS AMP HCV 2.0 kit. The final elution volume was 65 μl.

(ii) Large-volume extraction. This protocol was performed with 1-ml aliquots of plasma or serum utilizing the instrument's Total Nucleic Acid Large Volume Serum_Plasma protocol in conjunction with the MagNA Pure LC Total Nucleic Acid Isolation Kit–Large Volume (Roche Applied Science). Since no external lysis protocol exists in the large-volume format due to the physical limitations of the instrument, the COBAS AMP HCV 2.0 positive and negative controls (30 μl each) were added to wells containing HCV RNA-negative NHP (800 μl) and 20% MP Lysis/Binding Buffer (200 μl, without IC). The actual lysis of specimens was ultimately performed in the MP sample cartridge after the start of automated processing. The COBAS AMP HCV 2.0 IC was also utilized in the MP large-volume extraction process by adding it directly to the MP Lysis/Binding Buffer used by the MP instrument just prior to the start of automated processing. For the extraction of 24 samples (including positive and negative controls), 190 μl of IC was added to 35.8 ml of MP Lysis/Binding Buffer and gently mixed prior to dispensing it into the appropriate MP reagent reservoir. Again, due to the apparent incompatibility between the preserved NHP provided with the COBAS AMP HCV 2.0 kit controls and the MP extraction reagents, fresh-frozen NHP (HCV RNA negative) was substituted in place of the NHP provided with the COBAS AMP HCV 2.0 kit. The final elution volume was 65 μl.

(iii) Postelution handling. After completion of the extraction process, two tubes of COBAS AMP HCV 2.0 working amplification reagent and two AMPLICOR A-rings were added to the integrated AMPLICOR cooling block designed for use with the MP instrument. The addition of working amplification reagent (50 μl) and sample extracts (50 μl) to each AMPLICOR A-ring tube was automatically performed by using the MP's postelution handling capability. After the addition of all reagents and specimen extracts was complete, the A-rings were removed and manually sealed prior to loading onto the COBAS instrument.

Manual sample preparation. HCV RNA was isolated from plasma preparations and serum specimens according to the manufacturer's instructions for the COBAS AMP HCV 2.0. Specimen extracts (50 μl) were manually loaded into the A-ring tubes containing the working amplification reagent (50 μl) and sealed prior to loading onto the COBAS instrument.

Amplification and detection. Amplification and detection of the manual and automated HCV RNA preparations was performed according to the manufacturer's instructions for the COBAS AMP HCV 2.0. Detection of the IC was attempted in all reactions regardless of HCV RNA reactivity. Optical density (OD) readings for all samples were performed at A_{660} and interpreted as follows: <0.15, negative; 0.15 to <1.0, equivocal; and ≥1.0, positive. The OD readings for the IC were also performed at A_{660} and interpreted as follows: <0.15, negative; and ≥0.15, positive. The analytical sensitivity (95% threshold) of the COBAS AMP HCV 2.0 is 60 and 100 HCV IU/ml with EDTA plasma and serum, respectively (according to the COBAS AMP HCV 2.0 product insert [Roche Molecular Systems]). Duplicate testing of equivocal specimens (as specified in the manufacturer's instructions) was not performed as a part of the present study.

Study design. In the first part of the study, parallel COBAS AMPLICOR HCV 2.0 manual and MP standard-volume (200 μl) extractions were performed and analyzed in duplicate utilizing four sets of HCV standard dilutions ranging in concentration from 1,000 to 0 IU/ml in a preliminary evaluation of the two extraction methods (data not shown). After successful completion of the preliminary evaluation, parallel, replicate testing was performed with standard dilutions of 100, 75, 60, and 0 IU/ml (24 replicates each) in order to establish the analytical sensitivity of the COBAS AMP HCV 2.0 by using both extraction methods and assess the overall assay performance. All extraction, amplification, and detection steps were performed in batches of 24 samples (22 specimens and 2 controls) or less, which were performed in parallel utilizing two COBAS instruments each processing the two corresponding A-rings (manual and MP) from each run. Extraction, amplification, and detection of HCV RNA and the IC was performed in six parallel, consecutive runs in order to minimize the number of variables associated with the comparison of the manual and MP standard-volume extraction methods.

Performance of the manual and MP standard-volume extraction methods was further evaluated by processing and analyzing 110 HCV RNA-positive clinical serum specimens and 43 HCV RNA-negative serum specimens in parallel. The HCV-negative specimen aliquots were interspersed among the HCV RNA-positive specimen aliquots at regular intervals prior to specimen processing. Specimens were kept in the same relative positions throughout the entire procedure, including the extraction (manual and automated), amplification, and detection processes, in order to evaluate and compare the potential for specimen-to-specimen contamination by both extraction methods.

In the second part of the study, an MP large-volume (1 ml) extraction method was evaluated and compared to both the manual and the MP standard-volume (200 μl) extraction methods. The MP large-volume extraction was performed with two sets of HCV standard dilutions ranging in concentration from 1,000 to

TABLE 2. COBAS AMP HCV 2.0 analytical sensitivity after MP standard-volume sample processing

Amt of HCV RNA[a] (IU/ml)	Replicates (*n*)	Negative result ($A_{660} < 0.15$)		Equivocal result ($0.15 \leq A_{660} < 1.0$)		Positive result ($A_{660} \geq 1.0$)	
		n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
100	26	0	0 (0–13.2)	1	3.9 (0.1–19.6)	25	96.2 (80.4–99.9)
75	26	0	0 (0–13.2)	1	3.9 (0.1–19.6)	25	96.2 (80.4–99.9)
60	26	2	7.7 (1.0–25.1)	5	19.2 (6.6–39.4)	19	73.1 (52.2–88.4)
0	26	26	100 (86.8–100)	0	0 (0–13.2)	0	0 (0–13.2)

[a] See Table 1, footnote *a*.

0 IU/ml in a preliminary evaluation of this extraction method (data not shown). Replicate testing was then performed with standard dilutions of 40, 25, 10, and 0 IU/ml (24 replicates each) in order to establish the analytical sensitivity of the COBAS AMP HCV 2.0 by using the large-volume extraction method and to assess the overall performance of the assay. Performance of the IC was also evaluated in these samples. As previously stated, all extraction, amplification, and detection steps were performed in six consecutive runs containing batches of 24 samples (22 specimens and 2 controls) or less.

Performance of the MP large-volume extraction method was further evaluated by using the panel of 153 clinical specimens and methods described previously.

Data analysis. Estimates of sensitivity and specificity are reported, along with the exact binomial 95% confidence interval (95% CI). The sensitivity comparison for IC detection by the manual extraction method versus the MP large-volume extraction method was made by using a Fisher exact test.

RESULTS

Analytical evaluation. Data detailing the results of the replicate testing by using the manual and MP standard-volume extractions are presented in Tables 1 and 2, respectively. These data include the results obtained from 24 replicate aliquots (100, 75, 60, and 0 IU/ml), plus two additional results for each dilution obtained from the preliminary test panels (26 replicates total). The data indicate an analytical sensitivity of 75 IU/ml for the COBAS AMP HCV 2.0 by using either of these standard-volume HCV RNA extraction methodologies (25 of 26 [96.2%]; 95% CI, 80.4 to 99.9). In addition, none of the HCV RNA-negative (0 IU/ml) aliquots was found to be positive by either method, and the IC was successfully detected in all amplification reactions.

The data from the replicate testing performed by using the MP large-volume extraction method is presented in Table 3. Again, these data include results obtained from 24 replicate aliquots (40, 25, 10, and 0 IU/ml) plus two additional results for each dilution obtained from the preliminary test panels (26 replicates total). The analytical sensitivity of the MP large-volume extraction method was determined to be 25 IU/ml (26 of 26 [100%]; 95% CI, 86.8 to 100). Again, all HCV RNA-negative (0 IU/ml) aliquots yielded negative results, and the IC was successfully detected in all amplification reactions.

All positive and negative control reactions included with each of the six analytical runs and performed by each of the three extraction methods reacted appropriately.

Clinical evaluation. The data obtained from the analysis of clinical specimens by the manual and MP extraction methods yielded comparable results. The sensitivity of the COBAS AMP HCV 2.0 to detect HCV RNA in clinical specimens by either the manual or MP standard-volume extraction was 100% (110 of 110; 95% CI, 96.7 to 100). The sensitivity of the COBAS AMP HCV 2.0 to detect the IC was calculated to be 99.4% (152 of 153; 95% CI, 96.4 to 100) for the manual and 100% (153 of 153; 95% CI, 97.6 to 100) for the MP standard-volume extraction methods. The specificity was found to be 100% (43 of 43; 95% CI, 91.8 to 100) with either extraction method.

The sensitivity of the COBAS AMP HCV 2.0 after MP large-volume extraction of clinical specimens was also 100% (110 of 110; 95% CI, 96.7 to 100), with a specificity of 100% (43 of 43; 95% CI, 91.8 to 100). The sensitivity of the IC detection was calculated to be 98.0% (150 of 153; 95% CI, 94.4 to 99.6) by using MP large-volume extraction. Of the three specimens yielding a failed IC amplification, two were HCV RNA positive, whereas the third was HCV RNA negative and would have required repeat testing under normal circumstances. It is important to note that, although we increased the specimen volume by a factor of five with the MP large-volume extraction method, there was no statistically significant decrease in IC positivity among 153 clinical specimens (150 of 153 [98.0%; 95% CI, 94.4 to 99.6] versus 152 of 153 [99.4%; 95% CI, 96.4 to 100] for the manual extraction [$P = 0.63$]).

All positive and negative control reactions included with each of the seven clinical runs and performed by using each of the three extraction methods reacted appropriately.

TABLE 3. COBAS AMP HCV 2.0 analytical sensitivity after MP large-volume sample processing

Amt of HCV RNA[a] (IU/ml)	Replicates (*n*)	Negative result ($A_{660} < 0.15$)		Equivocal result ($0.15 \leq A_{660} < 1.0$)		Positive result ($A_{660} \geq 1.0$)	
		n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
40	26	0	0 (0–13.2)	0	0 (0–13.2)	26	100 (86.8–100)
25	26	0	0 (0–13.2)	0	0 (0–13.2)	26	100 (86.8–100)
10	26	1	3.9 (0.1–19.6)	3	11.5 (2.5–30.2)	22	84.6 (65.1–95.6)
0	26	26	100 (86.8–100)	0	0 (0–13.2)	0	0 (0–13.2)

[a] See Table 1, footnote *a*.

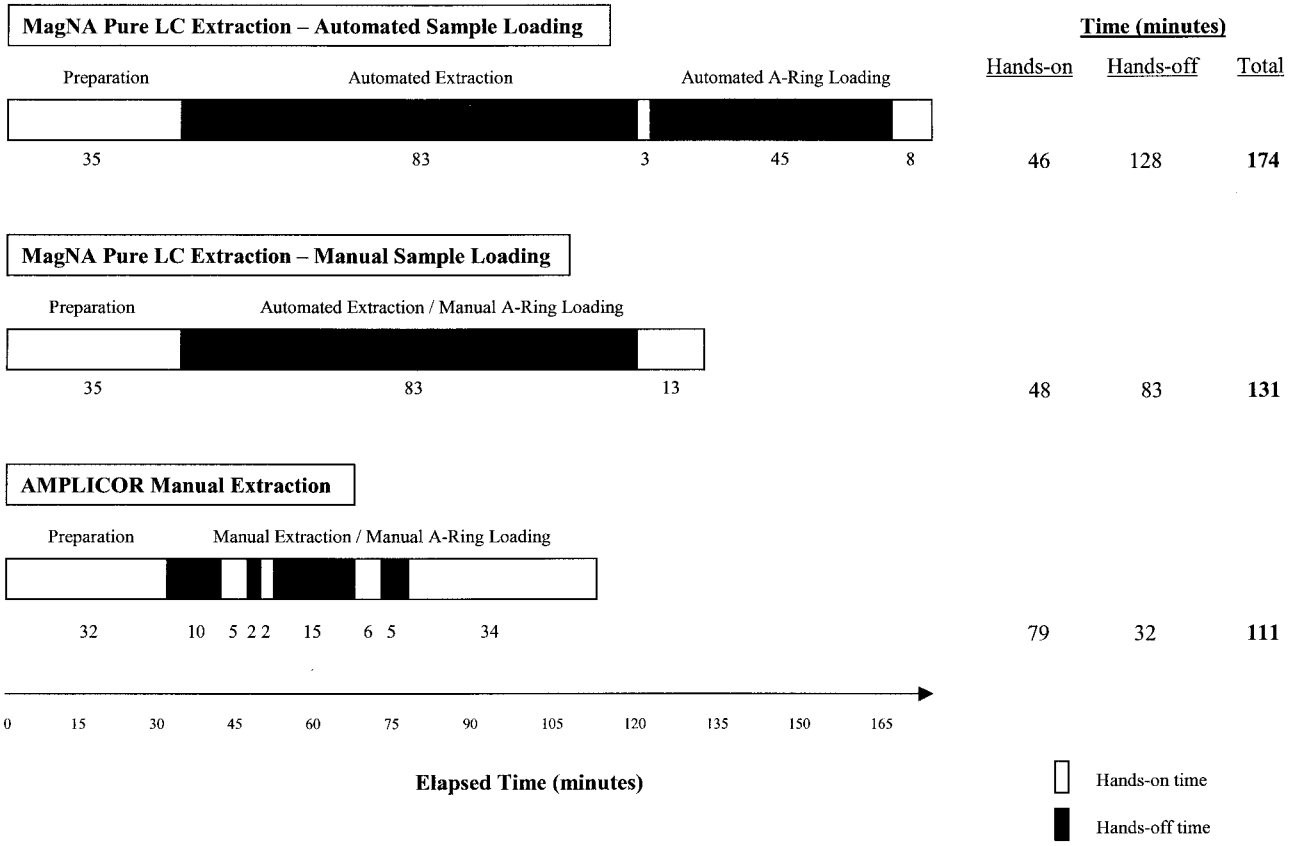

FIG. 1. MagNA Pure LC and manual AMPLICOR HCV RNA extraction workflow analysis. The bars represent the general workflow for each of the extraction methods, extending from sample preparation through completion of A-ring loading. A breakdown of the time required for the various processes is provided below each bar. All times are based on the processing of 24 samples (22 specimens and 2 controls) by each method.

Specimen cross-contamination. The extraction of 153 (110 HCV RNA-positive and 43 HCV RNA-negative) specimen aliquots by using the MP standard- and large-volume extraction methods revealed no evidence of specimen-to-specimen contamination during the MP standard-volume, large-volume, or postelution handling processes. No evidence of specimen-to-specimen contamination was observed among the 153 specimen aliquots extracted by the manual method.

Time estimates. The estimated time required to perform the MP (standard- or large-volume) and manual extractions is presented in Fig. 1. The estimated hands-on setup time for the MP was ca. 15 min. The time required to manually load specimens into the MP sample cartridge prior to automated extraction was ca. 14 min. Use of the MP large-volume protocol required slightly less setup time because of the additional hands-on time (3 min) required for the manual addition of MP Lysis/Binding Buffer to each well of the MP sample cartridge when the MP standard-volume external lysis method was used. The total time required to perform the MP extraction protocols with the postelution handling option was 174 min, 63 min longer than the time required for manual extraction. However, the actual hands-on time was reduced from 79 min for the manual method to just 46 min with the automated extraction and postelution handling and included two large blocks of hands-off time (83 and 45 min), potentially allowing the operator time to perform additional tasks within the laboratory during the actual extraction process.

In addition to the time required for sample extraction and preparation for amplification, the actual amplification and detection process on the COBAS instrument required ca. 4 h of hands-off time per run (22 samples and 2 controls).

DISCUSSION

Comparison of the manual and MP standard-volume extraction methods demonstrates the ability of the MP instrument and the MP Total Nucleic Acid External_Lysis protocol, with minimal modification of the COBAS AMP HCV 2.0 procedure, to provide equivalent performance when directly compared to the manual extraction method. Using the MP standard-volume extraction with an external lysis step, we were able to utilize both the COBAS AMP HCV 2.0 IC and controls directly in the MP extraction process. No additional reagents, beyond those provided in the COBAS AMP HCV 2.0 and MP Total Nucleic Acid Isolation kits and the substitution of fresh-frozen NHP, were required for performance of the MP standard-volume extraction.

To further increase the sensitivity of the COBAS AMP HCV 2.0, a large-volume MP extraction method was evaluated in the second part of the present study. The large-volume extraction

utilized the same final concentrations of the COBAS AMP HCV 2.0 IC and controls as the MP standard-volume extraction. Again, no additional reagents, beyond those provided with the COBAS AMP HCV 2.0 and MP Total Nucleic Acid Isolation–Large Volume kits and the substitution of fresh-frozen NHP, were required for performance of the MP large-volume extraction.

Successful use of an MP standard-volume total nucleic acid protocol in combination with the manual AMPLICOR Hepatitis C Virus Test, version 2.0 (Roche Molecular Systems) has recently been described by Fiebelkorn et al. (4). In contrast to that study, we present here our evaluation of two alternate MP protocols used in conjunction with the automated (COBAS) version of the AMPLICOR Hepatitis C Virus Test. Importantly, the use of the standard-volume MP Total Nucleic Acid External_Lysis protocol and a modification of the MP Total Nucleic Acid Large Volume Serum_Plasma protocol allowed us to use the COBAS AMP HCV 2.0 kit controls in the extraction process, thus minimizing modification of the COBAS AMP HCV 2.0 procedure, as well as the need to provide alternative assay controls.

It is important to emphasize that the order in which reagents are added to the positive and negative control extractions is critical for proper performance of these controls regardless of the extraction method used. The RNA control transcripts are rapidly degraded in NHP or serum in the absence of a potent RNase inhibitor (chaotropic lysis reagent). To accommodate the COBAS AMP HCV 2.0 kit controls in the MP standard-volume extraction method, we utilized an MP external lysis procedure; this allowed the addition of MP Lysis/Binding Buffer, specimen (HCV RNA-negative NHP in the case of the controls), and finally the positive and negative control transcript to the respective wells of the MP sample cartridge (in that order) prior to the start of automated processing on the MP instrument. In the large-volume extraction method, the COBAS AMP HCV 2.0 control RNA transcripts were protected from degradation by adding them to NHP containing 20% MP Lysis/Binding Buffer just prior to automated extraction. This alternate procedure also successfully protected the control RNA transcripts in the presence of NHP until complete inactivation of RNase activity occurred once the MP extraction process began.

The increased initial concentrations of the COBAS AMP HCV 2.0 IC and positive and negative control transcripts (relative to the COBAS AMP HCV 2.0 manual extraction) utilized in this evaluation were based upon recently published data regarding the efficiency of nucleic acid extraction with the MP instrument by Fiebelkorn et al. (4) and on our own experience with the extraction efficiency of small RNA transcripts on the MP instrument (data not shown). Increased quantities of these small RNA transcripts used in the MP extraction methods were necessary to compensate for their decreased recovery relative to the larger HCV RNA molecules on the MP instrument.

We experienced no significant problems with the MP instrument during the present study, and it proved to be quite user-friendly. In addition, the actual hands-on time required to perform the MP specimen extraction protocols (both standard and large volume), compared to the manual extraction, was reduced by more than 30 min with or without the use of the MP instrument's postelution handling capability (run size of 24). Importantly, there are two large blocks of hands-off time with the complete MP procedure, which potentially allow technologists time to perform other laboratory duties, without interruption, during these time periods.

The MP instrument was designed to work with the LightCycler system (Roche Applied Science) and efficiently processes specimens in multiples of 8 (32-specimen maximum). The MP instrument can simultaneously extract two batches of 8 specimens in a staggered fashion, making the extraction of 16 or 32 specimens the most efficient batch sizes, whereas the COBAS AMP HCV 2.0 is most efficiently performed in batch sizes of 12 or 24. This difference in instrument configuration limits the efficiency of COBAS AMP HCV 2.0 specimen processing on the MP instrument. Although the MP instrument performed well in this evaluation utilizing a run size of 24, the efficiency of the complete process may be affected by other factors (i.e., laboratory test volume). It may be advantageous to use multiple MP instruments in a high-volume setting. In some situations it may also be advantageous to omit the postelution handling in favor of manual A-ring loading in order to maximize throughput on the MP instrument.

The MP large-volume extraction utilizing the MP Total Nucleic Acid Large Volume Serum_Plasma protocol, while requiring a larger specimen volume, resulted in an analytical sensitivity of 25 IU/ml, a threefold increase in assay sensitivity compared to both the manual and the MP standard-volume extraction methods. The analytical sensitivity achieved with this large-volume extraction method, combined with the COBAS AMP HCV 2.0, is comparable to the sensitivity of a recently described HCV TaqMan assay (9). It also approaches the sensitivity of the VERSANT HCV RNA Qualitative Assay (Bayer HealthCare), a transcription-mediated amplification assay with a detection limit of ca. 5 to 10 IU/ml (10; L. Sawyer, K. Leung, M. Friesenhahn, D. Duey, M. McMorrow, and B. Eguchi, Abstr. 35th Annu. Meet. Eur. Assoc. Study Liver, abstr. 116, 2000). The increased sensitivity associated with the large-volume extraction method may increase the clinical utility of the COBAS AMP HCV 2.0 (1, 13, 16, 17). Typically, more than 1 ml of serum or plasma is submitted for HCV RNA detection; therefore, the increased volume of patient specimen required should be easily accommodated.

In summary, our findings demonstrate that the MP instrument is a versatile platform capable of integration with the COBAS AMP HCV 2.0 with minimal modification of the existing assay procedure. The MP instrument's ability to calculate the quantity and volumes of disposables and reagents required for processing and to perform contamination-free extraction and/or postelution processing, together with the COBAS AMP HCV 2.0, provides diagnostic laboratories with a qualitative HCV RNA detection assay format that is now fully automated and well suited for use in routine diagnostic testing. The use of the MP large-volume extraction method resulted in a substantial increase in the analytical sensitivity of the COBAS AMP HCV 2.0 without an increased rate of amplification inhibition. The increased sensitivity of the COBAS AMP HCV 2.0 performed in conjunction with large-volume HCV RNA extraction may be increasingly important in HCV diagnostic testing as new therapeutic strategies evolve.

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