This correction factor is in accordance with the value that has been found by Vukovich et al. (10) and corresponds with what one would theoretically expect when 1 part citrate solution and 9 parts blood with a hematocrit of 42% are taken together (11).

The D-dimer concentration in lithium-heparin plasma in our study is unaffected by transport by pneumatic mail or by rough handling, simulated by placing the sample on an oscillating roller. This opens the possibility of pneumatic dispatch of the sample tubes and thus a gain in time at the emergency department. It also indicates that samples obtained by general practitioners or local hospitals with limited capacity can be transported to a central laboratory for D-dimer measurements without decreasing their quality. That means that patients do not necessarily have to go to the emergency room for D-dimer determinations.

We found that the D-dimer concentrations in citrate and heparin plasma were stable regardless of the time of measurement or freezing. The results obtained with different types of analyzers were comparable. This indicates that it is valid to perform studies on frozen material and that test results of the Tina-quant assay, obtained under different (pre)analytic conditions, can be compared without loss of reliability.

In conclusion, measurement of D-dimer concentrations with the Tina-quant D-dimer test using heparin plasma is valid and provides a reduction in TAT. The D-dimer assay remains valid under different (pre)analytic conditions.

References

Clinical Evaluation of an Automated Nucleic Acid Isolation System, Kristin R. Fiebelkorn, Brenda G. Lee, Charles E. Hill, Angela M. Caliendo, and Frederick S. Nolte* (Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322; * address correspondence to this author at: Emory University Hospital, Clinical Laboratories, Room F145, 1364 Clifton Rd. NE, Atlanta, GA 30322; fax 404-712-4632, e-mail fnolte@emory.edu)

Nucleic acid isolation is among the most technically demanding and labor-intensive procedures performed in molecular diagnostic laboratories. Although considerable progress has been made in automating the amplification and detection steps of nucleic acid amplification assays, sample preparation is still performed manually in many laboratories. Various automated systems have been developed to streamline these procedures, ranging from versatile robotic stations to systems dedicated to specific amplification platforms or formats.

The MagNA Pure LC system (Roche Applied Science, Indianapolis, IN) uses robotics, precision pipettors, and magnetic glass particles to purify DNA, RNA, mRNA, or total nucleic acid from various sample types. The samples are dissolved and simultaneously stabilized by incubation with a buffer containing denaturing agents and proteinase K. Nucleic acids are bound to the surface of the magnetic glass particles, and several washing steps remove the unbound substances. The purified nucleic acids are then eluted in a low-salt buffer, with elution volumes ranging from 50 to 100 μL. The instrument can process up to 32 samples in 1.5 h; it can also automate PCR set-up and transfer the purified nucleic acids directly into a wide variety of reaction vessels, including LightCycler capillary tubes (Roche Applied Science), 96-well microliter, standard PCR tubes, and COBAS AMPLICOR amplification rings (Roche Diagnostics).

We developed and verified MagNA Pure LC (software Ver. 2.1) protocols for use with a quantitative test for cytomegalovirus (CMV) DNA (COBAS AMPLICOR MONITOR CMV Test) (1) and a qualitative test for hepatitis C virus (HCV) RNA (AMPLICOR HCV Test version 2.0) (2). The MagNA Pure LC total nucleic acid reagent set was used to recover CMV DNA and HCV RNA from EDTA plasma. The starting sample volume in each case was 200 μL. The total nucleic acid isolation reagent set and instrument were used as recommended by the manufacturer with the following exceptions. The protocol for sample preparation for the COBAS AMPLICOR MONITOR CMV Test included the addition of the CMV internal quantification standard (QS) to the MagNA Pure LC lysis buffer in the ratio of 84 μL to 7.8 mL and a final elution volume of 100 μL. The protocol for the AMPLICOR HCV Test version 2.0 included addition of the HCV internal control (IC) to the MagNA Pure LC lysis buffer in the ratio of 162 μL to 7.8 mL and a final elution volume of 65 μL.

The manual specimen preparation, amplification, and detection steps of the COBAS AMPLICOR CMV MONI-
TOR Test were performed according to the manufacturer’s instructions (1). The manual specimen preparation method isolates CMV DNA from 200 μL of plasma by lysis of the virus with a chaotropic agent, guanidine thiocyanate, followed by precipitation of the DNA with isopropyl alcohol. The CMV QS is introduced into each specimen with the lysis reagent, and the total absorbance at 660 nm (A660) of the QS and CMV amplicons is used to calculate the CMV DNA copies/mL according to the equation: Total CMV A660/Total QS A660 × input QS copies/PCR × 40. All CMV viral load values were transformed to log10 before statistical analysis. The agreement between CMV viral load values determined in samples extracted by manual and automated methods was assessed by the method of Bland and Altman (3).

The manual specimen preparation, amplification, and detection steps of the AMPLICOR HCV Test were performed according to the manufacturer’s instructions (2). The manual specimen preparation method isolates HCV RNA from 200 μL of plasma by lysis of the virus with a chaotropic agent, guanidine thiocyanate, followed by precipitation of the RNA with isopropyl alcohol. The HCV IC is introduced into each specimen with the lysis reagent and serves as an extraction and amplification control for each specimen.

Initial experiments indicated that the automated method was only 50% as efficient as the manual method for recovery of both CMV genomic DNA and the QS, as judged by the total absorbance of the amplified products (data not shown). The initial volume of plasma and the concentration of the QS in the lysis buffer were the same in both of the sample preparation methods. The methods differed in the volume of lysis buffer added to the sample (300 μL in the automated method; 600 μL in the manual method) and in the final volume of the processed sample (100 μL in the automated method; 400 μL in the manual method). For each processed sample, 50 μL of the sample was added to 50 μL of the working master mixture for amplification. The decreased extraction efficiency of the automated processing method was offset by the reagent volume differences and the increased concentration of final processed sample. Therefore, the number of QS molecules in samples processed by both methods was essentially the same, and the amount of CMV target DNA in samples processed by MagNA Pure LC should be twice that of samples processed manually. As a result, the CMV DNA copies/mL calculated by the COBAS analyzer for samples processed by MagNA Pure LC were divided by 2 to compensate for the increased effective sample volume.

Ten replicates of plasma samples containing ~10^3, 10^4, and 10^5 copies/mL CMV DNA were processed by each method, and viral load was determined. We found no significant difference between the means of values determined on samples processed by different methods at the three concentrations tested (Supplemental File 1; available through the Clinical Chemistry Online web site at http://www.clinchem.org/content/vol48/issue9/). These concentrations span the dynamic range of the assay. The CVs for samples at the different concentrations were essentially the same regardless of which method was used to process the samples, indicating that the automated sample processing method had no effect on the precision of the COBAS (Supplemental File 1).

We also assessed the agreement between the viral load results for 45 CMV-positive clinical specimens that were processed by both methods. The results were in excellent agreement. The mean difference between the viral load values for specimens processed by both methods was 0.009 log10, and the SD was 0.153 log10. The limits of agreement (mean ± 2 SD) were 0.315 to (±) 0.297 log10. In other words, the results were within twofold agreement for 95% of the specimens. The relationship between the difference and the mean values is shown in Fig. 1. The differences did not vary in any systematic way over the range of measurements.

In the MagNA Pure LC protocol for the AMPLICOR HCV Test, ~35% more HCV IC (by volume) was added to the MagNA Pure LC lysis buffer than to the lysis buffer used for the manual method to compensate for the less efficient recovery of this small RNA transcript with the automated method (data not shown). Initial experiments with a 100-μL elution volume for the automated method showed a small decrease in the recovery of the HCV target RNA and a small increase in the dropout rate for the IC (data not shown). The elution volume was decreased to 65 μL to further compensate for the diminished recovery of the HCV and IC RNA.

Ten replicates of plasma samples containing 1000, 100, 50, and 25 nominal HCV RNA International Units (IU)/mL were processed by both methods, and the numbers positive for the HCV target RNA and IC were recorded (Table 1). All of the replicates were positive for HCV RNA for samples containing 1000 and 500 IU/mL regardless of the method used for sample processing. All samples processed by the automated method and 80% of samples processed manually were positive at 100 IU/mL.

Fig. 1. Difference in log10 viral load (automated minus manual extraction) plotted against the average log10 viral load for 45 clinical specimens extracted with both automated and manual methods. The mean difference in viral load (0.009) and mean ± 2 SD (0.306) are shown with dashed lines.
Ninety percent of the samples processed with MagNA Pure LC and 20% of samples processed manually were positive at 50 IU/mL. According to the manufacturer, 50 IU/mL is the limit of detection of the AMPLICOR HCV Test. There were no IC dropouts in samples processed by either method.

We also processed 77 clinical specimens by both methods and compared the results in the AMPLICOR HCV Test. We found 30 specimens to be positive and 47 to be negative for HCV RNA when the automated processing was used. Similarly, 30 specimens were positive, 2 specimens were equivocal, and 45 specimens were negative when manual processing was used. The two equivocal specimens were restested in duplicate, and both sets of duplicates were negative for HCV RNA. We saw no IC dropouts in any of the specimens tested.

We used the MagNA Pure LC protocol to process an additional 303 clinical specimens in 10 separate analytical runs to collect more data on the IC recovery. Among the 265 HCV RNA-negative specimens, there were 4 (1.5%) IC failures. The failure rate in a large multicenter clinical trial of the AMPLICOR HCV Test with the manual extraction method was similar at 1.1% (4).

A full analytical run of 32 specimens on the MagNA Pure LC instrument requires only 15 min of hands-on time compared with up to 2 h with the manual methods described here. The throughput of 32 samples in 1.5 h may be a limitation for those laboratories with larger batch sizes. The unit list cost for the reagents and disposables associated with MagNA Pure LC extraction is US$3.77/sample. On balance, it is a cost-effective alternative to manual methods when large numbers of specimens are processed. Batches of fewer than eight specimens in the MagNA Pure LC system may be neither time- nor cost-effective compared with manual methods. We found the instrument to be easy to operate and reliable in more than 12 months of continuous operation with only one malfunction during that period. However, we did experience a performance problem with one lot of the total nucleic acid isolation reagent set, which was promptly addressed by the manufacturer.

Although there is considerable need in molecular diagnostic laboratories for automated nucleic acid extraction systems, few critical evaluations of these systems have been published. Espy et al. (5) evaluated MagNA Pure LC and the BioRobot 9604 (Qiagen) as potential replacements for the manual IsoQuick (Orca Research) method for extraction of herpes simplex virus DNA from genital and dermal specimens for use in a real-time PCR assay. They found that both automated systems provided standardized, reproducible, and cost-effective methods for processing large numbers of specimens. Kessler et al. (6) demonstrated that the MagNA Pure LC system could replace a manual protocol for recovery of herpes simplex virus DNA from serum, plasma, and whole blood with a slight improvement in the analytical sensitivity of their real-time PCR assay. We have extended and expanded these observations by demonstrating that MagNA Pure LC can be used with AMPLICOR and COBAS AMPLICOR PCR platforms and for both viral DNA and RNA targets. We also demonstrated that automated nucleic acid isolation does not compromise the performance characteristics of these tests and produced substantial labor savings.

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References