Comparison of Six Commercial DNA Extraction Kits for Recovery of Cytomegalovirus DNA from Spiked Human Specimens

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We evaluated six commercially available DNA extraction kits for their ability to recover DNA from various dilutions of cytomegalovirus (CMV) added to four different specimens: bronchoalveolar lavage, cerebral spinal fluid, plasma, and whole blood. The kits evaluated included the Puregene DNA isolation kit (PG), Generation Capture Column kit, MasterPure DNA purification kit, IsoQuick nucleic acid extraction kit, QIAamp blood kit, and NucliSens isolation kit (NS). All six kits evaluated effectively removed PCR inhibitors from each of the four specimen types and produced consistently positive results down to a spiked concentration of 200 PFU of whole CMV per ml. However, the NS and PG resulted in the most consistently positive results at the lowest concentrations of spiked CMV (4 and 0.4 PFU/ml) and, in this evaluation, offered the most sensitive methods for extracting CMV DNA from the four different spiked specimens. Processing time and cost were also evaluated.

The use of nucleic-acid-amplification techniques for the detection of infectious agents in clinical specimens continues to expand, and these techniques promise to play an ever increasing role in diagnostic laboratories in years to come. While a great deal has been published regarding PCR applications, protocols, and optimization, less information is available addressing specimen processing for optimal DNA recovery prior to amplification (2, 5, 7). This issue is of critical importance in the diagnostic microbiology laboratory owing to the extremely small amount of DNA from pathogenic agents present in the typical volume of patient samples received. Ideally, an optimal DNA extraction procedure would offer a high degree of efficiency, could be used on a broad range of specimen types with little or no modification, and would be practical and affordable for use in a diagnostic clinical laboratory.

Six commercially available DNA extraction kits were tested for their ability to recover DNA from various dilutions of whole cytomegalovirus (CMV) spiked into four different specimens: bronchoalveolar lavage (BAL), cerebral spinal fluid (CSF), plasma (PLM), and whole blood (BLD). CMV was chosen as the model target for comparison of these extraction methods because we have several years of experience using our CMV PCR assay system in the clinical setting and because the time-resolved fluorescent signal from the hybridization detection probe is proportional to the number of target DNA copies in the PCR mixture. PCR assays for CMV in patient materials are widely offered in molecular diagnostic laboratories in the United States (1). Although most of the molecular assays for CMV are performed on CSF, BLD, or PLM, we occasionally perform CMV PCR assays on other specimen types. For this reason we thought that extraction of CMV DNA from CSF, BLD, PLM, and BAL specimens might serve as a reasonable test for the comparison of commercially available nucleic acid extraction kits. The kits included in the comparison were the Puregene DNA isolation kit (PG) (Gentra Systems, Inc., Min-

neapolis, Minn.), Generation Capture Column kit (GCC) (Gentra Systems, Inc.), MasterPure DNA purification kit (MP) (Epicentre Technologies, Madison, Wis.), IsoQuick nucleic acid extraction kit (IQ) (MicroProbe Corp., Bothell, Wash.), QIAamp blood kit (QIA) (QIAGEN, Inc., Valencia, Calif.), and NucliSens isolation kit (NS) (Organon Teknika Corp., Durham, N.C.). Sensitivity, processing time, and cost were compared between the different kits.

(These data were presented previously [G. A. Fahle and S. H. Fischer, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. C-334, p. 173, 1999].)

The BAL sample and CSF sample were each prepared by pooling several clinical specimens collected from patients admitted to the Warren G. Magnuson Clinical Center at the National Institutes of Health. The PLM sample and BLD sample (both EDTA anticoagulated) were obtained from a single healthy donor. Each sample was initially screened for the presence of CMV by processing six aliquots for each of the six extraction methods evaluated and performing the CMV PCR and detection method. In addition, an aliquot from each sample was cultured for CMV by inoculating mink lung shell vials (Intracel, Issaquah, Wash.) and performing an immunofluorescence antibody (IFA) stain (Intracel) for detection of the CMV immediate early antigen (IEA) following the manufacturer's guidelines, with the single modification of centrifuging shell vials for 15 instead of 60 min (3). All four samples tested were negative for CMV by cell culture and by CMV PCR using DNA prepared by each of the extraction methods. Each specimen was then spiked with a quantified stock culture of CMV (reference strain AD169) (6) to obtain concentrations of 10,000, 200, and 4 PFU/ml. Six aliquots of each concentration from the four specimens were processed by each extraction method. To further evaluate the two most sensitive extraction methods (NS and PG), three additional concentrations of 2, 0.8, and 0.4 PFU/ml were prepared in the CSF sample only and processed as stated above.

For each kit evaluated, the manufacturer's protocol for the specific sample type was followed. Sample volumes and recovered extraction volumes for each kit are listed in Table 3 below. In addition, any manufacturer-recommended modifications to

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TABLE 1. Comparison of DNA extraction kit sensitivity from spiked specimens as determined by mean time-resolved fluorescence values[a]

Specimen (concn [PFU/ml])	NS		PG		QIA		MP		IQ		GCC	
	Mean value	No. pos[b]	Mean value	No. pos[b]	Mean value	No. pos[b]	Mean value	No. pos[b]	Mean value	No. pos[b]	Mean value	No. pos[b]
BAL (4)	293,768	6/6	242,640	6/6	123,226	6/6	375,405	5/6	97,325	4/6	14,300	2/6
BLD (4)	292,375	6/6	79,808	5/6	587,679	6/6	42,176	4/6	63,402	4/6	220,722	6/6
Plasma (4)	359,998	6/6	296,304	6/6	123,173	6/6	303,136	6/6	47,882	2/6	8,894	1/6
CSF												
4	662,433	6/6	527,460	5/6	65,469	3/6	97,573	2/6	31,787	2/6	1,486	0/6
2	72,042	5/6	64,069	4/6								
0.8	35,542	5/6	25,847	3/6								
0.4	28,943	3/6	8,193	1/6								

[a] Six replicates from each sample. pos, positive.
[b] Number of replicates out of six with a DELFIA fluorescence value of >15,000.

increase DNA recovery were employed and are noted below. With the six kits evaluated, four basic methodologies were used to recover DNA and eliminate protein and other inhibiting substances from the sample. The PG and MP kits used a cell lysis reagent and proteinase K digestion followed by the addition of a protein precipitation reagent to free the DNA from cells and remove proteins from the sample. An alcohol precipitation was then used to recover the DNA. Additionally, PG instructions include recommended use of glycogen to aid in DNA precipitation. A DNA capture column was used in both the GCC and the QIA kits. For each test, the sample was added to the column (lysed prior to addition for QIA only) to allow DNA to bind, several wash steps were used to remove inhibiting substances, and then the DNA was eluted from the column. Instructions for QIA recommended eluting the column twice with the same elution buffer (200 μl) to increase DNA yield. In addition, the volume of elution buffer could be reduced to 50 μl, resulting in a more concentrated sample, but this modification was not employed due to concerns that there would be an increased possibility of PCR inhibition (as indicated by the manufacturer). However, since the completion of this evaluation, the QIA wash buffer has been modified to provide for more efficient removal of PCR inhibitors from the column prior to DNA elution, making this processing modification a more viable option. The IQ kit used a cell lysis reagent followed by the addition of an extraction reagent containing a nuclease-binding matrix. After centrifugation, the aqueous phase was transferred to a new tube, and an alcohol precipitation was used to recover DNA. For the NS kit, cells in the sample were lysed, and silica particles were added to bind with the DNA. After several washes, the DNA was eluted from the silica.

To assess the ability of the DNA extraction kits to remove impurities that may contribute to PCR inhibition, an internal control (IC) was included in each amplification reaction tube. The IC was constructed by first linearizing the *Escherichia coli* plasmid cloning vector pBR322 (New England BioLabs, Beverly, Mass.) with *Eco*RI restriction endonuclease (New England BioLabs). A unique probe binding sequence on the vector (5′-G CG-ATG-CTG-TCG-GAA-TGG-ACG-3′) was selected to minimize homology with primers and target sequences but to have similar hybridization parameters to those of the CMV target probe. Primer sites along the vector were then selected to amplify the IC probe site region to produce a 249-bp product, which is 40 bp longer than the 209-bp CMV amplicon, thus avoiding preferential amplification of the IC over the CMV target region. During construction of the mimic, these primers were extended on their 5′ ends to include the sequence of either the upstream or the downstream CMV primer (des-

ignated in bold below) used in the CMV amplification reaction. This technique makes it possible to coamplify both the IC and CMV target using the single CMV primer pair. PCR was performed on the linearized vector by using these modified primers (IC-UP, 5′-**CGC-TCG-CTG-CTC-TGC-GTC-CAG-AC G-GG**T-AGT-TTA-TCA-CAG-TTA-AAT-TGC-TAA-CG-3′; IC-DWN, 5′-**CCG-CCG-ACG-GGA-CCA-CCG-TGA-CG**C-AT A-TAG-CGC-TAG-CAG-CAC-GCC-3′ (Research Genetics, Inc., Huntsville, Ala.), the product was electrophoresed on a 1% agarose gel, and the appropriately sized band was removed and purified with the QIAquick gel extraction kit (QIAGEN, Inc., Valencia, Calif.). After confirming amplification of the proper product when this DNA was used as a template with the CMV primers, the IC was ligated into the pCR 2.1 vector and transformed into INVαF′ One Shot competent *E. coli* cells by using the Original TA Cloning kit (Invitrogen Corp., San Diego, Calif.) following the manufacturer's guidelines. A transformed colony was selected, verified for proper plasmid insertion by a PCR assay, and then used to maintain a stock culture of the vector containing the IC. By using the Plasmid Miniprep kit (QIAGEN, Inc.), IC plasmids were isolated and then quantified based on the 260:280 ratio as determined on the DU-64 spectrophotometer (Beckman Coulter, Inc., Fullerton, Calif.). A 1-μg aliquot was then linearized using *Xba*I restriction endonuclease (New England BioLabs) to permit more efficient amplification of the IC site. Based on the concentration of linearized DNA and the length of the vector, the number of copies of IC per microliter was calculated. The IC was then diluted in TBE buffer to obtain a working stock concentration.

For each amplification reaction, a 5-μl aliquot of the extracted sample was amplified in a total reaction volume of 50 μl, as described previously (4), with the addition of 50 copies of IC per reaction mixture. Detection of the amplification products was then performed using the DELFIA plate hybridization assay (Perkin-Elmer Wallac, Inc., Gaithersburg, Md.) (4). Briefly, the biotinylated amplicons were bound to streptavidin-coated microdilution wells. After denaturation, the CMV-specific and IC-specific europium-labeled probes were allowed to hybridize to their complementary regions on the bound single-stranded amplicons. Enhancement solution was then added to the wells, and the resulting time-resolved fluorescence signals were measured on a time-resolved fluorometer.

To evaluate and compare sensitivities, six replicates from each sample were processed by each of the six DNA extraction kits. The CMV PCR was performed on the extracted DNA, and the mean time-resolved fluorescence values obtained with the CMV target probe were then calculated. A fluorescence level that was at least threefold higher than the highest negative control result was used to determine the positive cutoff

TABLE 2. Comparison of the NucliSens isolation kit sensitivity to other extraction methods from spiked specimens
as determined by mean time-resolved fluorescence values[a]

Specimen (concn [PFU/ml])	Mean time-resolved fluorescence values (P value) for paired tests:				
	NS/PG	NS/QIA	NS/MP	NS/IQ	NS/GCC
BAL (4)	293,768/242,640 (0.3807[b])	293,768/123,226 (0.0255)	293,768/375,405 (0.3637[b])	293,768/97,325 (0.0654[b])	293,768/14,300 (0.0009)
BLD (4)	292,375/79,808 (0.0265)	292,375/587,679 (0.5139[b])	292,375/42,176 (0.0235)	292,375/63,402 (0.0087)	292,375/220,722 (0.4887[b])
Plasma (4)	359,998/296,304 (0.6317[b])	359,998/123,173 (0.0105)	359,998/303,136 (0.3558[b])	359,998/47,882 (0.0022)	359,998/8,894 (0.0027)
CSF					
4	662,433/527,460 (0.4165[b])	662,433/65,469 (0.0121)	662,433/97,573 (0.0416)	662,433/31,787 (0.0085)	662,433/1,486 (0.0076)
2	72,042/64,069 (0.7741[b])				
0.8	35,542/25,847 (0.4816[b])				
0.4	28,943/8,193 (0.1353[b])				

[a] Six replicates were performed from each sample. Results are presented as pairwise comparisons and P values (by paired t test; calculated with StatView).
[b] Not significant (P > 0.05).

value of 15,000 for the hybridization assay. Results of the sensitivity comparison are shown in Table 1. At the two highest concentrations of spiked CMV (10,000 and 200 PFU/ml), the extracted DNA from all six kits produced very high positive results in all samples for each of the six replicates. However, at the spiked CMV concentration of 4 PFU/ml, the NS was the only kit to produce positive results in the six replicates from all four specimen types evaluated. The PG kit performed almost as well as the NS, missing only one replicate each from the BLD and CSF. The QIA performed well when used to process the BAL, BLD, and PLM (all six replicates positive) but produced only positive results in half of the 4-PFU/ml CSF replicates. The GCC produced positive results for all six replicates from the 4-PFU/ml spiked BLD specimen but performed poorly at this CMV concentration when used on the other specimen types, particularly the CSF (none of the six replicates positive). To compare the lower limits of detection between the NS and PG, three additional dilutions of CMV were prepared in the CSF. For each dilution, the NS resulted in a higher number of positive replicates and a higher mean time-resolved fluorescence value. Each amplification reaction performed in this study resulted in a clearly positive time-resolved fluorescence signal from the IC probe (data not shown). These

data indicated that the six evaluated kits were able to effectively remove proteins and other PCR inhibitors from each of the four different specimen types.

These results suggest that the NS kit offers the highest degree of CMV DNA recovery with the broad range of specimen types evaluated. To determine whether these data are statistically significant (P > 0.05), the NS mean time-resolved fluorescence values were compared to those of the other extraction kits using the paired t test (calculated with StatView) and are shown in Table 2. While the mean fluorescence values were all higher with the NS compared to the PG, only one sample—BLD spiked with 4 PFU/ml—produced a statistically significant higher value. The NS resulted in significantly higher mean-fluorescence values in half of the spiked samples at the 4-PFU/ml concentration when compared to those from MP and, in three of four samples, when compared to QIA, IQ, and GCC. As shown in Table 3, each of these kits has different recommended sample sizes and final extraction volumes which would, obviously, affect the overall sensitivity of the individual system. However, this evaluation was designed to compare sensitivities of the kits when used as specified by the manufacturer for optimal performance, and no effort was made to standardize input/output volumes.

TABLE 3. Comparison of cost, processing time, and other miscellaneous aspects between the evaluated DNA extraction kits

Extraction method	Cost/test[a]	Processing time[b] (h:min)		Maximum sample vol (μl)	Recovered extraction vol (μl)	Required reagents not included in kit	Special equipment[e]
		Hands-on[c]	Total[d]				
NS[f]	$4.00	02:28	03:08	200[g]	50	70% ethanol Acetone	Vacuum aspirator
PG	0.23	01:49	04:39	100	20	Proteinase K 100% isopropanol 70% ethanol Glycogen	
QIA	1.10	01:35	01:55	200	200	100% ethanol	
MP	0.69	01:04	01:59	150 (BAL, CSF) 50 (PLM) 12.5 (BLD)	35	100% isopropanol 75% ethanol	Refrigerated centrifuge
IQ	0.84	01:53	02:38	100	100	100% isopropanol 70% ethanol	
GCC	1.08	00:45	00:55	200	200		Fitted heat block[h]

[a] Based on pricing quote (early 1999) from each manufacturer for large-volume orders.
[b] Determined for an 18-sample run. Timing began with aliquoting of first reagent or sample and concluded with recovery of extracted DNA.
[c] Time required to perform manual manipulation of tubes, reagents, and samples.
[d] Time required to obtain extracted DNA, including hands-on time and incubation periods.
[e] Excluding standard laboratory equipment.
[f] An automated extractor is available for laboratories with high sample volumes.
[g] Kit available for larger-sample-volume processing.
[h] Bore size must be large enough to completely contain capture column. Manufacturer recommends specific models.

We performed a preliminary evaluation of the modified QIA kit by comparing the results to the best performing kit, the NS. A dilution series of CMV was spiked into whole blood (EDTA anticoagulated), and six aliquots were extracted from each dilution, using both systems. DNA was eluted from the QIA column with a 50-μl aliquot of elution buffer. CMV PCR and the DELFIA assay were performed on the extracts as described previously. These results indicated a comparable level of sensitivity for both kits. However, a more comprehensive evaluation using multiple specimen types and a broader dilution range of CMV would be required to fully evaluate the new QIA kit.

While the sensitivity of a DNA extraction kit is important, many factors must be considered when selecting the most practical and appropriate methodology for the clinical laboratory. Several of these aspects are summarized in Table 3. Regarding the cost per test, four of the six kits (QIA, MP, IQ, and GCC) varied by only about $0.40 per test, ranging from $0.69 to $1.10 per test. However, the PG offered the most economical method at just $0.23 per test, while the NS, priced at $4.00 per test, was considerably more costly than any of the other kits evaluated. The time required to process an 18-sample run was measured for each kit. Timing began with aliquoting of the first reagent or sample and concluded with recovery of the extracted DNA. The processing (hands-on) times required for manual manipulation of tubes, reagents, and samples were tabulated separately from nonmanipulation time (e.g., incubation, drying, etc.). Processing time varied considerably between the kits, ranging from as little as 55 min for completion of the GCC to as long as 4 h and 39 min for the PG. None of the kits required the use of expensive or unusual chemicals or reagents not supplied by the manufacturer. With the exception of the GCC, which comes complete, the other kits did require the user to supply various alcohols or other common reagents. All of the protocols were easy to perform and used standard equipment commonly available in most clinical laboratories. The only exception was the requirement in the GCC procedure for a heat block with a bore size large enough to accommodate the capture column. Although not that uncommon, a vacuum aspirator is highly recommended for use with the NS, and a refrigerated centrifuge is required for the MP.

In summary, all six kits evaluated effectively removed PCR inhibitors from each of the four specimen types and produced consistently positive results down to a spiked concentration of 200 PFU/ml of whole CMV. The NS and PG kits resulted in the most consistently positive results at the lowest concentrations of spiked CMV and, in this evaluation, offered the most sensitive methods for extracting CMV DNA from the four different spiked-specimen types. Although generally not statistically significant, the NS resulted in higher and more consistent fluorescent values in all samples evaluated when compared to the next best method, extraction with the PG kit. The cost per test for each of these kits varied considerably, with the biggest difference being between the PG ($0.23 per test) and the NS ($4.00 per test). Likewise, the processing time varied between kits from as little as 55 min for the GCC to as long as 4 h 39 min for the PG. The results from three previously published articles (2, 5, 7) indicate that the QIAamp columns performed as well as or better than all of the other commercial and noncommercial methods evaluated for the extraction of DNA for PCR. However, none of these evaluations included the NS or PG in their comparisons. Over the past 12 months, we have used the NS extraction method with assays designed to detect several parasites, viruses, and bacterial pathogens. The NS method has worked well with all of these assays.

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