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# Comparative Evaluation of Three Commercial Systems for Nucleic Acid Extraction from Urine Specimens

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**A nucleic acid extraction system that can handle small numbers of specimens with a short test turnaround time and short hands-on time is desirable for emergent testing. We performed a comparative validation on three systems: the MagNA Pure compact system (Compact), the NucliSens miniMAG extraction instrument (miniMAG), and the BioRobot EZ1 system (EZ1). A total of 75 urine specimens submitted for polyomavirus BK virus detection were used. The human β-actin gene was detected on 75 (100%), 75 (100%), and 72 (96%) nucleic acid extracts prepared by the miniMAG, EZ1, and Compact, respectively. The miniMAG produced the highest quantity of nucleic acids and the best precision among the three systems. The agreement rate was 100% for BKV detection on nucleic acid extracts prepared by the three extraction systems. When a full panel of specimens was run, the hands-on time and test turnaround time were 105.7 and 121.1 min for miniMAG, 6.1 and 22.6 min for EZ1, and 7.4 and 33.7 min for Compact, respectively. The EZ1 and Compact systems processed automatic nucleic acid extraction properly, providing a good solution to the need for sporadic but emergent specimen detection. The miniMAG yielded the highest quantity of nucleic acids, suggesting that this system would be the best for specimens containing a low number of microorganisms of interest.**

Nucleic acid amplification techniques are being incorporated more and more into clinical laboratories due to the high sensitivity and specificity of these assays. Advances in these techniques, including implementation of real-time PCR, have significantly shortened the test turnaround time (TAT), which has significantly affected patient care for some immediately needed tests, such as herpes simplex virus and enterovirus detection in cerebrospinal fluid. A nucleic acid extraction system that can handle a small number of specimens and has a short test turnaround time and hands-on time provides another opportunity to maximally apply amplification techniques to clinical services.

A good specimen preparation is comprised of an efficient target recovery, establishment of the integrity of nucleic acid targets, optimal removal of amplification inhibitors, elimination of components which affect other enzymatic substrates, and sterilization of potentially hazardous organisms. This is especially critical for urine specimens, since urine has been found to be a particularly difficult substrate for PCR (2, 5, 10). Recently, several new commercial systems that are designed for daily, low-throughput nucleic acid extraction without complicated software interfaces and specialized user training have become available. Among them, the MagNA Pure compact system (Compact; Roche Diagnostic Corp., Indianapolis, IN), the NucliSens miniMAG extraction instrument (miniMAG; bioMerieux, Inc., Durham, NC), and the BioRobot EZ1 system (EZ1; QIAGEN Inc., Valencia, CA) have become attractive due to their flexibility, convenience, and ease of use. While each system has been used in the diagnostic molecular microbiology service, it is important to have a parallel validation of their performance in the clinical setting.

The detection and monitoring of polyomavirus load in the urine and blood of infected patients using a quantitative PCR technique have been shown to be useful tools in the diagnosis and subsequent management of BK virus (BKV) nephropathy associated with the deterioration of renal function following kidney transplantation (8, 9, 12). We have chosen urine specimens submitted for BKV detection and quantitation as the samples to validate the three nucleic acid extraction systems. The quantities of the extracted nucleic acids were measured, and the sensitivity and precision for BKV detection and quantitation were contrasted. In addition, TAT, technologist hands-on time, and cost were determined for each system.

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**Clinical specimens.** A total of 75 urine specimens submitted to the Clinical Microbiology Section of the Cleveland Clinic Foundation for polyomavirus screening, qualification, and quantitation were included in this study. Total viral DNA was extracted by a NucliSens Extractor (bioMerieux, Inc., Durham, NC), and BKV detection was performed by real-time PCR on a LightCycler (Roche Applied Science, Indianapolis, Ind.) and confirmed by pyrosequencing (1). Specimen aliquots were prepared and stored at −70°C until DNA extraction was performed.

**DNA extraction by miniMAG.** DNA extraction was performed by using NucliSens magnetic extraction reagents according to the manufacturer's instructions. Briefly, 200 μl of urine sample was added to a lysis buffer and incubated for 10 min at room temperature. Then 50 μl of magnetic silica was mixed with the lysis buffer-sample mixture for 10 min. The lysis buffer-silica-sample mixture was pelleted, and the supernatant

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was aspirated. The pellet was resuspended in 400 μl of wash buffer 1 and then transferred to a 1.5-ml centrifuge tube. Several wash steps were performed using the miniMAG semiautomated instrument. After the last wash buffer was aspirated, 50 μl of elution buffer was added and incubated for 5 min at 60°C. Tubes were moved against a magnetic rack while 50 μl of eluted DNA was pipetted. A maximum of 12 specimens can be processed during each run.

**DNA extraction by EZ1.** DNA extraction was performed by using the EZ1 DNA tissue kit and EZ1 DNA bacterial card according to the manufacturer's instructions. Briefly, 200 μl of urine sample was pipetted into a sample tube, which was loaded on the instrument along with pipette tips, pipette tip holders, elution tubes, and a reagent cartridge. A predigestion step was not included in the study, and the third pipette tip position was left unloaded as recommended by the manufacturer. The automatic extraction was started by pressing the "start" button. After extraction was finished, the elution tubes were capped and removed for subsequent PCR amplification. An elution volume of 50 μl was chosen in the study. A maximum of six specimens can be processed during each run.

**DNA extraction by Compact.** DNA extraction was performed with Compact's nucleic acid isolation kit 1 according to the manufacturer's instructions. Briefly, urine specimens, reagent cartridges, pipette tips on holders, and elution tubes were loaded on the instrument. A user program was selected to define the processing protocol, which included sample volume (200 μl), elution volume (50 μl), and lot numbers. The automatic extraction was started by pressing the "start" button. After extraction was finished, the elution tubes were capped and removed for PCR usage. A maximum of eight specimens can be processed during each run.

**Housekeeping gene detection.** A colorimetric microtiter plate PCR assay was used to detect the human β-actin gene to ensure the quality of extracted DNA, as previously described (7). Output signals were measured at optical densities at 450 nm ($OD_{450}$) and 490 nm ($OD_{490}$). A positive result was defined as an $OD_{450}$ to $OD_{490}$ value greater than or equal to 0.1 (16).

**BKV amplification and quantitation.** A quantitation standard curve was achieved by performing serial dilutions of a plasmid standard containing the entire BKV genome (Advanced Biotechnologies Inc., Columbia, MD), covering a linear range from 1 to 1,000,000 copies/reaction (12). BKV DNA amplification was performed in a "real-time" format on the 7700 ABI Prism sequence detector (Applied Biosystems, Foster City, CA). An aliquot of 20 μl of the extracted nucleic acid was added to 5 μl of reaction mixture containing 0.8 μM of each primer and 0.4 μM fluorophore probe (final concentration) and was mixed with 25 μl of TaqMan universal PCR master mix (Applied Biosystems). A polyomavirus universal primer set (Pep-1 M, 5′-CAG GAA AGT CTT TAG GGT CTT CTA CC-3′, and Pep-2 M, 5′-GGT GCC AAC CTA TGG AAC AGA-3′) which amplified a 180-base-pair portion of the polyomavirus T-antigen gene (15) was briefly modified from the one published previously (8). The BKV-specific TaqMan minor groove binder probe (TAg63MGB, 5′-TGC TGT TGC TTC TTC-3′), whose 5′ end was labeled with 6-carboxyfluorescein, was designed by using the Primer Express program (Applied Biosystems, Foster City, Calif.). The TaqMan cycling conditions were a 2-min degradation of the preamplified templates at 50°C and then 40 cycles of PCR that consisted of denaturation at 95°C for 15 s and annealing and extension at 60°C for 60 s. BKV-specific nucleic acid recovery rates among the three systems were estimated by quantitating BKV loads on one DNA sample extracted by each system from a pooled negative urine specimen spiked with the known copy numbers of plasmids containing the entire BKV genome. The DNA samples were run in triplicate along with an unextracted control sample, and the recovery rate was calculated by comparing the BKV loads in the control.

TABLE 1. Specific-target detection and quantitation of DNA in 75 urine specimens subjected to the three systems

| Analysis | Value determined by: | | |
|---|---|---|---|
| | Compact | EZ1 | miniMAG |
| β-Actin gene detection [no. (% positivity)] | 72 (96.0) | 75 (100.0) | 75 (100.0) |
| BKV detection[a] [no. (% positivity)] | 24 (32.0) | 24 (32.0) | 24 (32.0) |
| BKV quantitation (no. of copies/reaction)[b] | 1,332.8 ± 90.1 | 1,635.9 ± 104.7 | 3,789.2 ± 86.5 |

[a] Agreement among the three systems was 100%.
[b] Means ± standard deviations are presented. There were no statistical differences in BKV loads between Compact and EZ1 ($P > 0.05$). The miniMAG detected significantly higher BKV loads than the Compact or EZ1 ($P < 0.002$).

**Cost, TAT, and hands-on-time analysis.** The cost per test was calculated for each system, which included test kit, materials, and reagents. TAT was calculated from a uniform start time until the time of result entry. Each step listed by the manufacturer was timed by the processing technologist and tabulated for the total hands-on time needed to perform each system. Hands-on time and TAT were estimated as minutes per specimen under a full run from two technologists on runs performed on different days. Time was separated into hands-on time and TAT when the incubation or centrifugation step was equal to or less than 5 min, whereas only TAT was calculated when the incubation or centrifugation step was greater than 5 min.

A total of 75 urine specimens were used for the comparative validation. Using a colorimetric microtiter plate PCR assay, we first detected a "housekeeping" gene, human β-actin, to determine whether the extracted DNA specimens were free of amplification inhibitors. The β-actin gene was detected on 75 (100%), 75 (100%), and 72 (96%) nucleic acid extracts prepared by the miniMAG, EZ1, and Compact, respectively (Table 1). We next assessed test sensitivity by detecting and quantitating BKV using a real-time TaqMan PCR. BKV was detected in 24 DNA specimens extracted by all three systems with the same sensitivity and an agreement rate of 100% (Table 1). The three human β-actin-negative DNA specimens extracted by the Compact were also negative for BKV. Test precisions of the three systems were determined on a spiked urine specimen ($3 \times 10^4$ plasmids/reaction) which was extracted and run at different times for BKV nucleic acid recovery. The coefficients of variation were 4.8%, 46.6%, and 49.6% for the miniMAG, EZ1, and Compact, respectively (Table 2). These data suggest that the three extraction systems possess similar sensitivities and that the miniMAG presents the best precision for nucleic acid detection and quantitation.

TABLE 2. Reproducibility and BKV-specific nucleic acid recovery of the three systems

| Sample or parameter | Value determined by: | | |
|---|---|---|---|
| | Compact | EZ1 | miniMAG |
| Expt 1, sample 1[a] | $8.75 \times 10^2$ (2.9) | $1.63 \times 10^3$ (5.4) | $4.60 \times 10^3$ (15.3) |
| Expt 1, sample 2[a] | $3.71 \times 10^2$ (1.2) | $4.77 \times 10^2$ (1.6) | $4.51 \times 10^3$ (15.0) |
| Expt 1, sample 3[a] | $3.16 \times 10^2$ (1.1) | $1.05 \times 10^3$ (3.5) | $4.90 \times 10^3$ (16.3) |
| Expt 2, sample 1[a] | $4.85 \times 10^2$ (1.6) | $9.39 \times 10^2$ (3.1) | $4.82 \times 10^3$ (16.1) |
| Expt 2, sample 2[a] | $1.16 \times 10^3$ (3.9) | $1.71 \times 10^3$ (5.7) | $5.15 \times 10^3$ (17.2) |
| Expt 2, sample 3[a] | $7.88 \times 10^2$ (2.6) | $6.59 \times 10^2$ (2.2) | $4.87 \times 10^3$ (16.2) |
| Mean ± SD[b] | $(6.7 \pm 3.3) \times 10^2$ (2.2 ± 1.1) | $(1.1 \pm 0.5) \times 10^3$ (3.6 ± 1.7) | $(4.8 \pm 0.2) \times 10^3$ (16.0 ± 0.8) |
| Coefficient of variation (%) | 49.6 | 46.6 | 4.8 |

[a] Values are numbers of copies per reaction (recovery percentages). The original number of plasmid copies per reaction was $3 \times 10^4$.
[b] There were no statistical differences in recovery rate between Compact and EZ1 ($P > 0.05$). The miniMAG had a significantly higher recovery rate than Compact or EZ1 ($P < 0.001$).

The BKV-specific nucleic acid recovery rates for the three systems were determined on a pooled urine specimen spiked with the known BKV genome-containing plasmids. The specimen DNA extraction was run in triplicate in two separate experiments. The recovery rates for the Compact, EZ1, and miniMAG were 2.2%, 3.6%, and 16.0%, respectively (Table 2). There were no statistical differences in recovery rates between Compact and EZ1 ($P > 0.05$); however, the miniMAG had the highest recovery rate among the three systems ($P < 0.001$). Based on the quantitation of 24 BKV-positive urine specimens, the mean BKV loads were determined to be 1,332.8, 1,635.9, and 3,789.2 copies/reaction on DNA specimens extracted by the Compact, EZ1, and miniMAG, respectively (Table 1). There were no statistical differences in BKV loads between Compact and EZ1 ($P > 0.05$), and the DNA extracted by the miniMAG yielded much higher BKV loads than that extracted by the Compact/EZ1 ($P < 0.002$).

The volume capacities, reagent costs, hands-on times, and TATs of the three systems were contrasted (Table 3). The reagent costs, including the listed kit price and additional materials, ranged from $5.65 to $6.35 per specimen for processing costs, which were similar among the three systems. A maximum of 12, 6, and 8 specimens could be processed during each run on the miniMAG, EZ1, and Compact, respectively. When a full panel of specimens was run, the hands-on time and TAT for each specimen processed were 105.7 and 121.1 min for miniMAG, 6.1 and 22.6 min for EZ1, and 7.4 and 33.7 min for Compact, respectively (Table 3). The EZ1 and Compact processed automatic nucleic acid extraction properly, offering a good solution to the need for emergent but sporadic specimen detection.

TABLE 3. Summary of capacity, cost per test, hands-on time, and TAT

| Validation parameter | Result for: | | |
|---|---|---|---|
| | Compact | EZ1 | miniMAG |
| Maximum no. of specimens/run | 8 | 6 | 12 |
| Cost per test[a] | $5.65 | $6.35 | $5.70 |
| Hands-on time (min)[b] | 7.4 ± 1.8 | 6.1 ± 2.4 | 105.7 ± 3.8 |
| TAT (min)[b] | 33.7 ± 2.6 | 22.6 ± 1.9 | 12.1 ± 7.6 |

[a] Based on manufacturers' listed kit price plus materials.
[b] Hands-on times and TATs are listed as means ± standard deviations. There was a significant difference in TAT between the miniMAG and either the Compact or the EZ1 (for both pairwise comparisons, $P$ was <0.001).

The development and availability of real-time PCR on a variety of platforms and in conjunction with user-friendly kits have facilitated the common use of this technology in the modern microbiology laboratory. Although these assays continue to be used for the detection and quantitation of fastidious microorganisms, e.g., Epstein-Barr virus and BKV (8, 14), they are also being used as more-rapid and -sensitive assays for the detection of microorganisms that may be readily cultivated, e.g., group B streptococcus and enteroviruses (4, 6). The development of rapid-cycle PCR technology affords the implementation of rapid molecular diagnostics, which have implications for patient care and cost savings (11).

Coincident with the development of these rapid and user-friendly methods of PCR has been the development of more-efficient methods and often automated methods of nucleic acid extraction. Unfortunately, most of the original automated systems were designed for the extraction of batches of clinical specimens rather than a single specimen (3, 13). Although a single specimen could be extracted on these systems, it was often costly and was associated with a significant waste of reagents. More recently, efficient automated systems that can accommodate fewer specimens have been devised.

We have evaluated three of these systems with residual urine samples that were shown to contain or be free of the polyomavirus BKV using Nuclisens extractor and LightCycler real-time PCR. The three systems were challenged in an equitable manner according to the respective manufacturer's instructions. All extractors examined produced DNA in sufficient quality and quantity to afford the qualitative detection of BKV in urine samples where it was previously determined to be present. However, a statistically significant higher quantity of BKV was detected following extraction using the miniMAG than either the Compact or the EZ1. The miniMAG also appeared superior to the Compact and the EZ1 in test precision when the coefficient of variation was assessed.

The miniMAG can process between 1 and 12 specimens; the Compact can process between 1 and 8 specimens; and the EZ1 can process between 1 and 6 specimens. While the costs per test were similar, the miniMAG required significantly more hands-on time than either the Compact or the EZ1. Similarly, TAT was significantly longer for the miniMAG than for either the Compact or the EZ1 systems, whereas there were no sig-

nificant differences detected between the TATs of the Compact and the EZ1.

In summary, new automated nucleic acid extraction platforms that can process a low number of clinical specimens are available. When linked with real-time-PCR-based methods of pathogen detection, these should allow for the more-timely and -efficient detection of the agents of many infectious diseases. Although different platforms are attractive to users for a variety of reasons, it is important to generate comparative data between different methods so that users may be aware of the strengths and limitations of the system they have chosen. We have compared similar instruments with respect to the extraction of the polyomavirus BKV from urine specimens. Similar studies should be performed with other human tissues and fluids; the data presented here should not be extrapolated to other types of clinical specimens. The data generated in this study suggest that the miniMAG is superior to the Compact and the EZ1 for the extraction of BKV from urine specimens using real-time PCR, even though the miniMAG required more hands-on time and a longer TAT than the other systems studied.

## ADDENDUM IN PROOF

Since the submission of the manucript, bioMérieux has launched an automated extraction system (easyMAG), which can process 1 to 24 specimens each run. We validated its performance by using the same 75 urine specimens included in this study. Its sensitivity for the detection of BKV was the same as that of the three systems evaluated. Reproducibility was similar to the miniMag, and the human β-actin gene was detected in 71 (94.7%) of the urine specimens. When a full panel of specimens (24 specimens) was processed, the TAT and technologist's hands-on times improved from 121.1 and 105.7 min, respectively, by the miniMag to 67.1 and 24.9 min, respectively, by the easyMAG.

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