# MagaZorb: A Simple Tool for Rapid Isolation of Viral Nucleic Acids

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**Effective isolation of nucleic acids from samples containing viral materials is an essential step for accurate diagnosis of viral infections. The necessity of this critical step before analytical identification and diagnosis of viral infections is paramount to screening programs and to identifying and monitoring epidemics and pandemics. With molecular assays rapidly evolving into routine practice, clinical laboratories face several challenges, including presence of small amounts of viral nucleic acids in abundant levels of genomic DNA and total RNA, processing of various sample types, and carry-over of polymerase chain reaction inhibitors, which could significantly affect polymerase chain reaction and microarray results. MagaZorb nucleic acid isolation technology overcomes these challenges and offers a simple and reliable method for isolation of high-quality and high-yield nucleic acids. Although the MagaZorb technology is readily adaptable to automated platforms, it is also well suited to laboratories in remote areas of resource-poor countries, because a simple magnet is the only device required to perform the procedure manually. Performance characteristics and clinical application of the MagaZorb technology are briefly described here.**

Sample preparation for molecular diagnostic assays is rapidly becoming a major bottleneck, because these tests are evolving into routine practice in research and clinical laboratories. The need for a fast, reliable, and cost-effective way to purify nucleic acids from a variety of sample types is a reality. Most existing nucleic acid purification techniques are based on the use of silica beads or various membranes [1–3]. These procedures are tedious, time consuming, and laborious and involve hazardous materials and organic solvents. They often require on-site preparation of ancillary reagents or need additional equipment, such as a centrifuge or vacuum aspiration device. The MagaZorb (Cortex Biochem, recently acquired by Promega) nucleic acid isolation system overcomes these limitations and provides high-quality and high-yield nucleic acids with speed, ease of use, and reliability. It is also well suited to the existing automated platforms for high-throughput applications. Moreover, because a simple magnet is the only device required to perform the procedure manually, the technique is suitable for laboratories in remote areas of resource-poor countries. Examples of studies conducted to demonstrate the performance characteristics and clinical application of the MagaZorb technology will be presented in this article.

## MAGAZORB TECHNOLOGY

### Principle

MagaZorb technology is based on specific interaction between nucleic acids and the proprietary magnetizable particles in the presence of specially formulated buffers. This simple technology is designed so that the binding of nucleic acids to MagaZorb magnetizable particles is not dependent on chaotropic agents. A schematic representation of MagaZorb technology is shown in Figure 1. In brief, the cells are lysed to release the nucleic acids, which will then bind to MagaZorb magnetizable particles in the presence of a binding buffer. The unwanted

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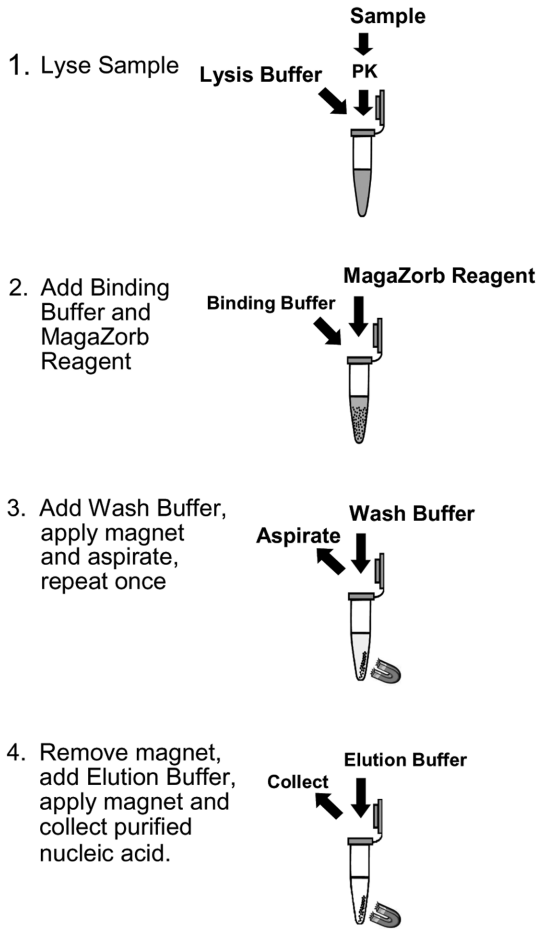


**Figure 1.** Principle of MagaZorb nucleic acid isolation technology.

materials are washed with a water-based wash buffer, and the purified nucleic acids are eluted with an elution buffer.

### Performance Characteristics

***Simplicity and speed.*** Figure 2 shows a comparison of the workflow involved in the MagaZorb technology with that involved in QIAamp methodology (Qiagen). As shown, the QIAamp procedure involves more hands-on work, including a reagent preparation step, which is a possible source of error by the operator. In addition, the QIAamp method involves the use of ethanol, a known polymerase chain reaction (PCR) inhibitor that also requires storage and waste disposal management. Ease of use and the fewer steps involved in the MagaZorb protocol result in a faster turn-around time; processing of 5 samples can be completed within 50 min, compared with 70 min with the QIAamp protocol.

***High yield/binding capacity.*** One of the major characteristics of MagaZorb particles is their high binding capacity for capturing nucleic acids. To demonstrate this feature, various amounts (25–500 $\mu$g) of calf thymus DNA (Sigma) in STE buffer (0.5 M NaCl, 100 mM Tris-HCl, and 1 mM EDTA; pH, 8.0) were processed by the MagaZorb DNA Isolation Kit (Cortex Biochem), according to the protocol outlined in Figure 1, except that the lysis step was eliminated. The purified DNA concentrations were determined by quantitation at 260 nm, and percentage of DNA recovery was calculated using the expected values as 100% recovery. As shown in Table 1, with 1 mg of MagaZorb particles, ~500 $\mu$g of calf thymus DNA was captured.

***Quality and integrity.*** Total RNA from buffy coats (prepared using Accu-Prep Kit; Accurate Chemical and Scientific Corp) was purified using the MagaZorb RNA Isolation Kit (Cortex Biochem), according to the manufacturer's instructions. Purified RNA samples were analyzed by agarose gel electrophoresis and Agilent 2100 Bioanalyzer. Integrity of the purified RNA (evident by presence of intact ribosomal peaks and bands [18S and 28S]) is shown in Figures 3 and 4.

***Reliability and applications.*** MagaZorb technology has been applied to purification of various nucleic acid targets from a wide variety of sample sources, producing reliable results [4–10]. The protocol can be performed in a manual [4–7], semi-automated [8, 9], or fully automated format [10]; thus, it is suitable for laboratories with low to medium or high throughput needs. Moreover, because the only device required is a simple magnetic stand, the technique is well suited to laboratories in remote areas of resource-poor countries. Two ap-

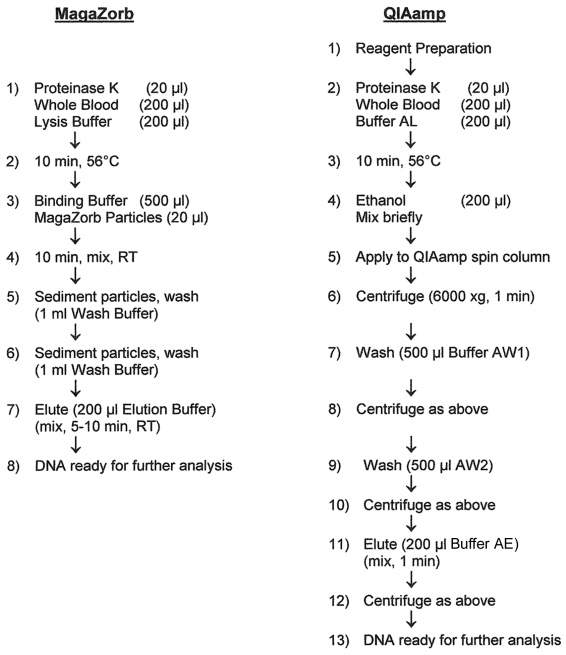


**Figure 2.** Comparison of MagaZorb DNA protocol with the QIAmp DNA protocol, showing the simplicity, speed, and adaptability to true automation of the MagaZorb protocol. The MagaZorb protocol involves less hands-on work, no reagent preparation, and no organic solvents or centrifugation step, eliminating potential sources of operator errors and offering a fast turn-around time.

**Table 1. MagaZorb Particles Binding Capacity**

| Sample number | DNA yield, μg | | Recovery, % |
|---|---|---|---|
| | Expected value | Observed value | |
| 1 | 25.0 | 20.9 | 83.6 |
| 2 | 50.0 | 47.7 | 95.4 |
| 3 | 100.0 | 88.0 | 88.0 |
| 4 | 250.0 | 258.0 | 103.0 |
| 5 | 500.0 | 497.0 | 99.4 |

**NOTE.** DNA recovery of >80% is obtained with 1 mg MagaZorb particles and input DNA of 25–500 μg, showing the high capacity of these unique particles.

plication examples have been selected for further discussion: recovery of MS2 viral RNA spiked into serum samples and isolation and early detection of HIV-1 in dried blood spots (DBSs) from infants.

***MS2 viral RNA detection in serum samples.*** MS2 viral RNA, a bacteriophage RNA of 3569 nucleotides (Boehringer Mannheim), was spiked at $1 \times 10^7$–$1 \times 10^8$ copies into 200 μL of 3 different serum samples, and the spiked samples were processed using the MagaZorb RNA Kit (Cortex Biochem) and the RNeasy Kit (Qiagen). The purified RNA samples were quantitated by real-time reverse-transcription PCR (RT-PCR; GeneAmp EZ rTth RNA PCR Kit; Perkin-Elmer) with use of the primers and probes (Oswel) shown in Table 2, master mix, and the Cepheid Smart Cycler. The SUPERase-IN was from Ambion. Reaction mixtures were cycled in the Smart Cycler with use of the following conditions: 60°C for 30 min, followed by 95°C for 120 s and 45 cycles at 95°C for 15 s and at 60°C for 30 s with Optics ON. Figures 5*A* and 5*B* show that the RNA purified by the MagaZorb RNA Isolation Kit performs superior to the RNA purified by RNeasy in terms of both PCR amplification profile (lower cycle thresholds, 20 vs 28) and the recovery of viral RNA (45%–50% vs 10%–15%).

***Early detection of HIV in DBS samples from infants.*** It has been well established that, without the intervention of antiretroviral therapy, 20%–45% of HIV-1–infected women transmit HIV to their infants [11]. Maternal antibodies against HIV can cross the placenta and persist in infants for ⩾18 months; thus, antibody tests cannot be reliably used to identify HIV infection in infants. The most reliable detection method currently available is the nucleic acid–based assay to detect the presence of HIV nucleic acid in blood samples from infants [12]. Because most of the infected infants reside in remote areas of resource-poor countries, to make the detection service available to these infants, blood is collected on a filter paper (eg, Whatman 903 paper) by finger or toe pricking, dried at room temperature, and packaged in a humidity-proof bag with desiccants to prevent the build-up of humidity, which causes degradation of RNA and DNA in DBS specimens after prolonged storage at −20°C. These DBS specimens are transported to a central laboratory for processing and detection using PCR-based assays. The most frequently used PCR product in resource-limited countries in Africa and Asia is the qualitative Amplicor DNA Assay (version 1.5; Roche) [12].

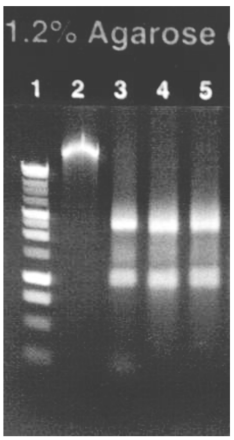


**Figure 3.** Image of 1.2% agarose gel electrophoresis of RNA samples isolated from buffy coats with use of the MagaZorb RNA Isolation Kit. *Lane 1,* 1 Kb DNA ladder; *lane 2,* calf thymus DNA control; *lane 3,* human liver RNA control; *lane 4,* buffy coat (BC180C2); *lane 5,* buffy coat (BC180E). Intact, distinct ribosomal RNA bands were present in all buffy coat samples.

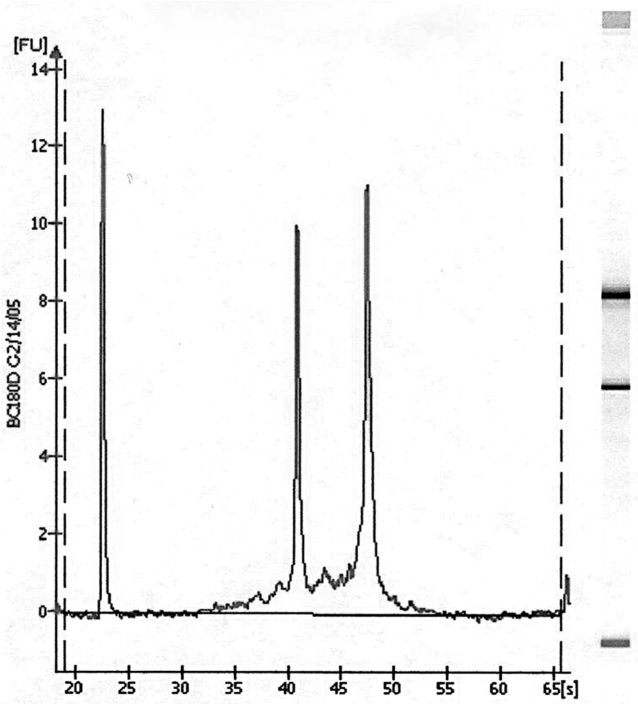


**Figure 4.** Integrity of RNA samples isolated from buffy coat with use of the MagaZorb RNA Isolation Kit; evaluation was performed using the Agilent 2100 Bioanalyzer. Distinct ribosomal RNA peaks and bands [18S and 28S] are present.

**Table 2. Details of Real-Time Reverse-Transcription Polymerase Chain Reaction (GeneAmp EZ rTth RNA PCR Kit; Perkin-Elmer) of Purified RNA Samples**

| Reagent | Volume, μL/25 μL reaction | Sequence |
|---|---|---|
| DEPC-treated water | 10.125 | … |
| 5X EZ buffer | 5.0 | … |
| MS2 forward primer (1029F, 10 μM) | 0.75 | 5′-GGAGAGACAGGGCACTGCTA-3′ |
| MS2 reverse primer (1096R, 10 μM) | 0.75 | 5′-TTGGCCATACGGATTGTACC-3′ |
| MS2 probe (1052T, 10 μM) | 0.375 | 5′-CCCAAATCTCAGCCATGCATCGAG-3′ |
| SUPERase-IN (20 U/μL) | 0.5 | … |
| dNTPs (2.5 mM) | 3.0 | … |
| rTth DNA polymerase | 1.0 | … |
| $Mn(OAc)_2$ (25 mM) | 2.5 | … |
| MS2RNA | 1.0 | … |

Ou et al [4] recently developed a real-time fluorescent RT-PCR assay for HIV-1 detection in infants and young children. This method uses a small disk of 6 mm in diameter from DBS. Total nucleic acids (DNA and RNA) are extracted using MagaZorb DNA Kit (Cortex Biochem), excluding the RNase treatment step. HIV-1 DNA and RNA are detected using a fluorescent real-time RT-PCR assay with a primer pair to generate an amplicon of ~110 base pairs [4]. In this report, a total of 443 DBS samples collected from infants and young children in Uganda ($n = 128$) and Cameroon ($n = 315$) were examined and compared with the existing standard protocol using the HIV load assay and the Roche Amplicor DNA assay, with a concordance of 99.2% (127 of 128 children) and 99.4% (313 of 315 children), respectively. This report revealed the high

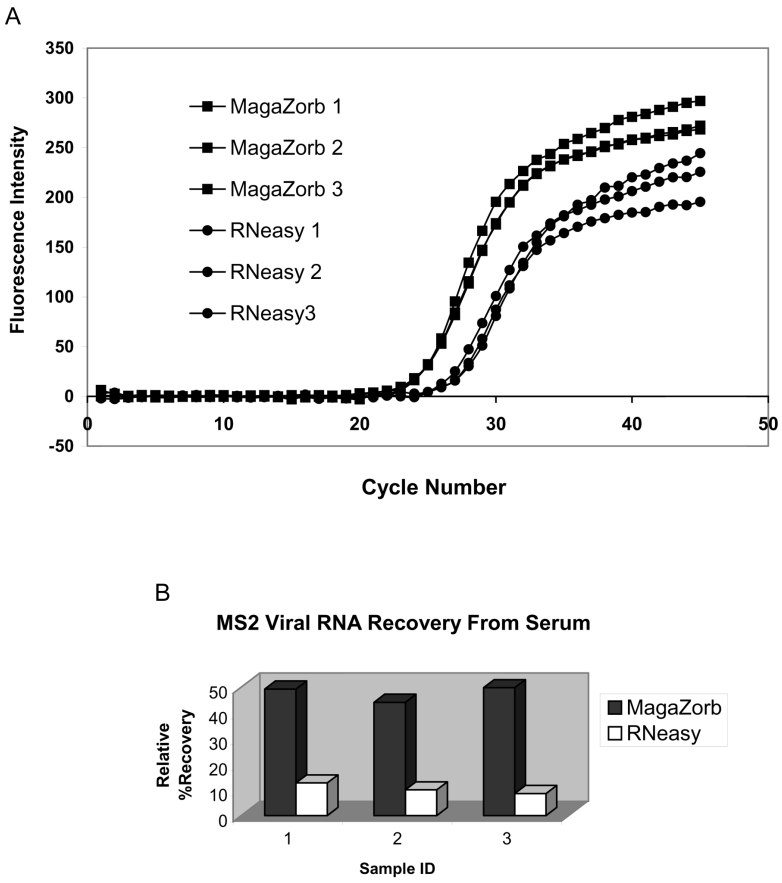


**Figure 5.** *A,* MS2 viral RNA reverse-transcription polymerase chain reaction growth curves (MagaZorb RNA vs RNeasy). *B,* MS2 viral RNA recovery.

performance of the MagaZorb technology to isolate total nucleic acids from DBS samples for subsequent real-time PCR assay.

## CONCLUSIONS

Because molecular assays are rapidly evolving into routine practice in research and clinical laboratories, the need for a fast, reliable, and cost-effective way to purify nucleic acids from a wide variety of sample types has become a reality. Most of the existing sample preparation techniques are tedious, lengthy, and subject to carry-over of PCR inhibitors. MagaZorb nucleic acid isolation technology was designed to overcome these limitations. As exemplified in this report, MS2 viral RNA isolated by MagaZorb technique exhibited superior performance, compared with the well-established procedure of RNeasy. Moreover, the clinical usefulness of MagaZorb nucleic acid isolation technique has been well demonstrated by the representative study presented here on isolation and detection of HIV-1 total nucleic acids (RNA and DNA simultaneously, using a single protocol) in DBS samples from infants. Other examples of clinical applications of MagaZorb technology include isolation of coronavirus RNA [5], norovirus [8], and *Mycobacterium avium Paratuberculosis* [6] from fecal specimens and parainfluenza virus type 4 [7] from respiratory samples.

In conclusion, MagaZorb technology offers a fast, reliable, and cost-effective method to isolate high-quality and high-yield nucleic acids. It can be used in manual, semi-automated, and fully automated formats and, thus, is suitable for small and large laboratories with varied throughput requirements. In addition, because a simple magnetic stand is the only device needed for sample processing by MagaZorb technique, it is well suited to laboratories in remote locations of resource-poor countries.

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