

Automating Nucleic Acid Isolation for In Vitro Use Provides Improved Assay Performance in the Molecular Diagnostics Lab



Traditionally, nucleic acid isolation for use with in vitro assays has been labor intensive and technically involved. More recent approaches have employed magnetic particles with surface silica to offer advantages that are tempered by inconsistent results. A new methodology, incorporating a modified bead composition and nanotechnology, permits high pipetting reproducibility, quick magnetization, good particle suspension behavior, and high purification yields.

By Guido Hennig, PhD, Christoph Petry, PhD, and Ellen Sampson, MS, MBA

Isolation of an analyte prior to assaying for it is a principle not generally pursued with in vitro diagnostics. Most in vitro diagnostic assays have an analytical specificity that allows for assaying low-concentration analytes even in the presence of a background of 2,000 or more different and often more abundant proteins. Molecular diagnostics by no means depends on nucleic acid extraction prior to analysis. For example, branched DNA (bDNA) technology allows for detecting and quantifying minute amounts of nucleic acids without sample purification. Accordingly, bDNA molecular tests are exceedingly easy and convenient to use in the laboratory. Nevertheless, some methods such as polymerase chain reaction (PCR) or Sanger-sequencing that are routinely employed in many diagnostic laboratories often require initial purification of the nucleic acids from the specimens prior to analysis. For these enzyme-based molecular assays, nucleic acid isolation is a critical step and, thus, a core technology for the molecular laboratory.

The classic method of nucleic acid isolation, referred to as guanidinium thiocyanate-phenol-chloroform extraction, was developed by Piotr Chomczynski and Nicoletta Sacchi and published in 1987.^{1,2} Although still used in research environments, the method is difficult for clinical testing laboratories, primarily due to the use of hazardous reagents but also due to the extensive and highly manual procedural steps. A major improvement and technological breakthrough, described more than 20 years ago, was the discovery of the "Boom-Method."³ This approach is based on the use of chaotropic salts that, under certain conditions, mediate the binding of nucleic acids to silica. There are numerous technical implementations of this principle, including the use of silica-coated tubes, membranes, packed columns, or granulate. Although all of these methods have shown acceptable performance for the isolation of nucleic acids, they all share the common feature of being difficult to automate. This lack of automation limits their practical use in today's highly automated clinical laboratories.

Magnetic Particle-Based Isolation Technologies Leave Room for Improvement

More recently, the introduction of magnetic particle-based isolation technologies have provided for several key advantages, including (a) allowing specimens to be processed by state-of-the-art pipetting robots; (b) no requirement for manual interventions or process steps such as

pressure filtration or centrifugation, both of which are difficult to automate; and (c) allowing for a higher degree of freedom with regard to selecting sample type and sample volume, thereby resulting in greater flexibility for the automated molecular laboratory. Basically, there are four different approaches to specifically binding nucleic acids to magnetic bead surfaces: (a) the use of specific capture oligos to bind the complementary target

Bead Technology Improves CT/GC Testing as Global Prevalence of Sexually Transmitted Infection Rises

Sexually transmitted infections (STIs) are a major global cause of acute urogenital infection. Left untreated, these infections can lead to serious sequelae such as life-threatening pelvic inflammatory disease (PID) and chronic conditions such as infertility, with severe medical and psychological consequences for millions of men, women, and infants. STI rates continue to rise in most countries.⁷ During 2008 in the United Kingdom, over 200,000 cases of chlamydia alone were diagnosed in men and women 15-24 years of age.⁸ In the United States in 2008, more than 1.5 million total cases of chlamydia and gonorrhea were reported to the Centers for Disease Control.⁹

The VERSANT® CT/GC DNA 1.0 Assay (kPCR)* is a qualitative in vitro diagnostic assay for the detection of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC), both STIs. The assay is used with the VERSANT® kPCR Molecular System, which combines kinetic PCR (kPCR) DNA technology with Siemens proprietary magnetic bead particles for use in molecular clinical diagnostics applications. The VERSANT CT/GC assay is designed to detect the presence of CT and GC in both symptomatic and asymptomatic individuals from female endocervical swab specimens, male urethral swab specimens, and female and male urine specimens. The nucleic acid isolation and the kinetic PCR steps are fully automated, providing results for up to 96 specimens in under six hours, thereby improving the quality of the NA isolations and improving laboratory productivity.

*CE-marked. Not available for sale in the U.S.



sequence;⁴ (b) the use of positively charged polymers coated to the surface, which bind the negatively charged phosphate backbone of nucleic acids by ionic interaction;⁵ (c) solid phase reversible immobilization (SPRI) technology, which immobilizes the nucleic acid to a carboxylated bead surface under specific buffer conditions;⁶ and (d) the use of magnetic particles with silica on the surface using the “Boom-Method.” The last is the most common approach and often involves particles manufactured by co-precipitation of conglomerates of silica and basic iron oxides from silicate solutions. However, this procedure, which seems to be straightforward, is actually difficult to control during the manufacturing process. The crystallization of the gel-like, silica-rich precipitates results in agglomerates that show nonhomogeneous size distributions and variable silica-to-iron compositions (Figure 1). The use of such particles for nucleic acid analysis typically results in nonreproducible yields and inconsistent analyte recoveries.

Nanotechnology Production Step Provides for Improved Assay Performance

Siemens Healthcare Diagnostics has chosen an entirely different approach in synthesizing silica-containing magnetic particles for nucleic acid isolation.

Unlike most other magnetic beads, these particles are based on iron oxide rather than silica and use a photolithographic toner as the base. These particles are produced in industrial quantities employing highly optimized procedures to meet extremely narrow specifications. Under an electron microscope, these magnetic particles resemble “golf balls” that are nearly identical in size (<1 μm) and spherical shape. As the composition of these particles is essentially chemically-pure iron oxide, and as there is little to no silica present, these “naked” particles are not suited to binding nucleic acids. Modifying the beads in a subsequent nanotechnology production step adds an ultrathin layer of silica, less than 1 nm thick and corresponding to only a few molecular layers of Si-O entities, that gets deposited onto the surface of the particles. This processing step has no detectable effects on the bulk composition of the particles, their overall iron content, or their shape or size distribution (Figure 2). The bead technology approach confers very unique advantages to the Siemens method of isolating nucleic acids. The homogenous size produces high pipetting reproducibility, and the high iron content allows for quick magnetization, while the small particle size provides good suspension behavior. The large surface area of the particles provides for high purification yield so that even minor quantities of nucleic acids can be isolated from comparatively large matrix volumes.

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Figure 1.

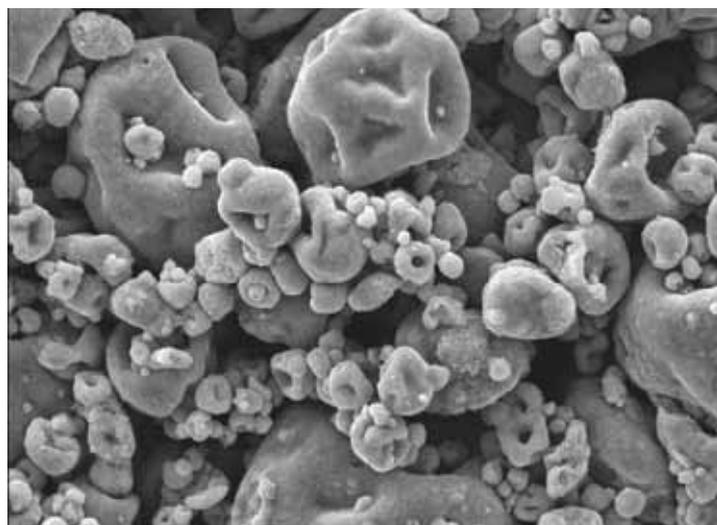


Figure 1. Electron microscopic picture of commercial magnetic silica beads

Figure 2.

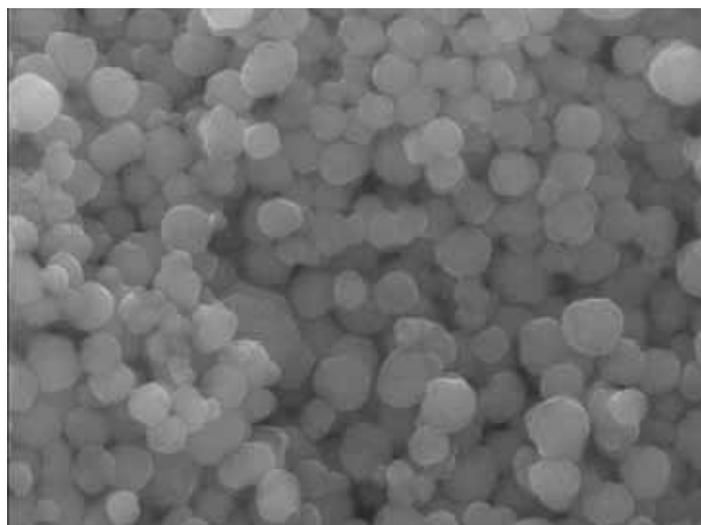


Figure 2. Electron microscopic picture of Siemens' magnetic iron oxide beads coated with a nanolayer of silica

Extraction Method Eases Influenza's Impact

Jens Verheyen, MD, a leading virologist, reports on how improved extraction methods enabled his laboratory to handle surging test volume in the face of the H1N1 influenza pandemic. *Perspectives* asked Dr. Verheyen to share details.



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What is the type and size of your laboratory?

The Institute of Virology is part of the University of Cologne. We perform mainly routine virological diagnostics for patients of the university hospital. Samples from patients of other hospitals are sent to our institute for testing in specific assays as well.

How did H1N1 impact your community and your laboratory?

During the first wave of the novel influenza (H1N1 2009) pandemic, diagnostic tests were performed as part of national surveillance programs to limit virus transmission. During the onset of the pandemic, the focus moved to the identification of patients infected with the pandemic (H1N1 2009) influenza virus who were considered to be at risk of severe complications. The number of samples arriving at our institute for influenza diagnosis was above forty samples per day during the most intensive weeks of 2009.

How has turnaround time changed with the implementation of this nucleic acid isolation methodology?

At the beginning, only a few samples were analysed several times a day. Then, these individualized diagnostic settings were changed due to the increasing number of samples arriving at our institute. The implementation of the VERSANT® kPCR Sample Prep Analyzer led to the ability to process up to 96 samples per run, extracting nucleic acids for influenza diagnostics. Even though influenza was the main focus of interest, the simultaneous extraction of RNA and DNA also allowed the lab to directly proceed with the analysis of other respiratory viruses including adenoviruses, rhinoviruses, parainfluenza viruses, respiratory-syncytial viruses and human metapneumoviruses in specific clinical settings.

What has been the impact on workflow?

The automated workflow and the processing time of less than two hours for 48 samples and three hours for 96 samples, respectively, allowed the integration of the excessive number of influenza diagnostics into the daily routine at our institute.

Do you foresee a change in the volume of this type of testing with the capabilities conferred by the new technology?

With automation and throughput that allows us up to 96 samples at the same time, in combination with a processing time of less than three hours the VERSANT kPCR Sample Prep system was our best option for the isolation of nucleic acids from nasopharyngeal swab samples during the influenza pandemic. Furthermore, the ability to simultaneously extract RNA and DNA from the same sample with the same reagents provided us with outstanding capabilities to handle respiratory viral testing at our institute, despite the overwhelming volume which arose as a result of the (H1N1) pandemic. It made a very real difference in the way the hospital could diagnose and treat patients. Now, we're certainly relieved that the pandemic has subsided, but the technology will continue to be a cornerstone of our molecular lab.

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