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White Paper

Validation of the VERSANT kPCR Sample Preparation Module

Quality Nucleic Acid Extraction from a Wide Variety of Specimens

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Answers for life.

Successfully Automating the Molecular Laboratory

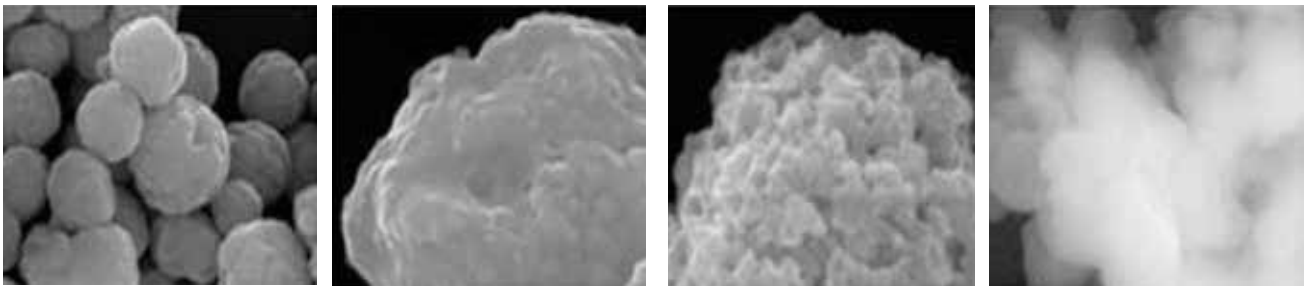
Validation of the VERSANT kPCR Sample Preparation Module for Extraction of Quality Nucleic Acids from a Wide Variety of Specimens

Executive Summary

Molecular diagnostics and life-science studies depend on the ability to effectively extract high-quality nucleic acids. Siemens Healthcare Diagnostics offers a high-throughput and fully automated system that can extract nucleic acids from 1 to 96 samples in a single batch run. The VERSANT® kPCR Sample Preparation (SP) Module and VERSANT Sample Preparation 1.0 Reagents (SP 1.0) and Sample Preparation 1.2 Reagents (SP 1.2) kits enable extraction of both DNA and RNA from a wide variety of specimen types.

Siemens magnetic nanobead technology enables exceptional RNA and DNA recovery, with sensitive and reproducible target detection in real-time PCR applications. In addition, the system offers the flexibility to combine different sample types in the same run and the versatility to use one sample for up to six PCR assays by splitting the eluate. Together, the VERSANT SP Module and IVD-labeled reagent kits provide an excellent solution for nucleic acid extraction for both Siemens- and laboratory-developed assays.

Figure 1. Electron microscopic pictures of different commercially available magnetic silica beads.



Siemens
250 nm*

Competitor A
(MagNA Pure LC Total NA Kit)
3200 nm*

Competitor B
(mSample Preparation System DNA)
1200 nm*

Competitor C
(QIAsymphony Virus and Bacterial Midi Kit)
4500 nm*

Magnetic Particle-based Isolation Technologies in Molecular Diagnostics

Nucleic acid isolation by in vitro diagnostic molecular testing has traditionally been labor-intensive, lacking robust reproducibility and sensitivity. For more than a decade, extraction technologies have employed magnetic particles coated with silica, which allowed the development of an automated extraction process. However, automated protocols have produced inconsistent results.[1]

A new methodology, developed by Siemens Healthcare Diagnostics, applies an entirely different approach to synthesizing silica-containing magnetic particles for nucleic acid isolation. Unlike most other magnetic beads, Siemens uses iron oxide rather than silica, along with a photolithographic toner as the base (Figure 1). These chemically pure iron oxide particles are further modified in a subsequent nanotechnology production step in which an ultrathin layer of silica is deposited onto the surface of the particles. The Siemens technology not only results in very small (<1 µm) and homogeneous particles for efficient washing steps, and therefore pure eluates, but also provides unique advantages, including pipetting reproducibility, rapid magnetization, and improved homogeneous suspension. The smooth surface area of the particles allows high purification yields, so that even small quantities of nucleic acids can be isolated from comparatively large matrix volumes (Figure 1).

*Indicated mean diameter evaluated by semiautomatic imaging processing routines from high-resolution scanning electron microscopy.

High Sensitivity for RNA Extraction

Method Comparison

The Siemens VERSANT kPCR SP Module and SP 1.0 reagents deliver increased RNA extraction recovery and provide better protection from sample interference compared to other commercially available sample preparation platforms.

Figure 2 shows a comparison of the VERSANT kPCR SP Module with SP 1.0 reagents versus four other commercially available methods in the extraction and detection of norovirus RNA from 39 stool samples obtained from norovirus-infected patients.

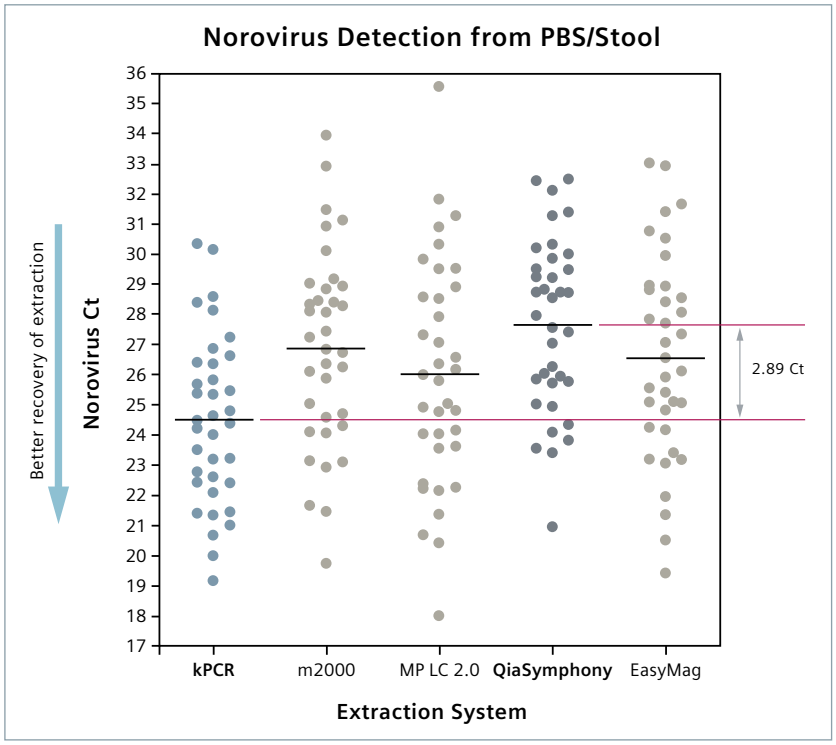
The five commercially available automated platforms included:

- EasyMAG (bioMerieux)
- m2000sp (Abbott)
- MagNA Pure LC 2.0 (Roche)
- QIAsymphony (Qiagen)

VERSANT kPCR SP Module extraction efficiency was objectively compared among platforms by using (1) similar sample input and output volumes and (2) an identical real-time PCR cycler (VERSANT kPCR AD unit) and CE-marked assay for amplification and detection of norovirus RNA (AnDiaTec). RNA was extracted on each platform using identical stool aliquots for each patient sample and the extraction reagents recommended for the individual systems.

Overall, the Siemens method provided up to a 7-fold (maximum 2.89 Ct mean difference between VERSANT SP and QIAsymphony; Figure 2)[1] more sensitive extraction recovery for norovirus RNA from stool samples than the other methods. In contrast to the other commercial platforms, all samples tested with the VERSANT SP platform and SP 1.0 reagents tested positive for both the target RNA and the spiked RNA-based internal controls.

Figure 2. Comparison of the VERSANT kPCR SP Module with four commercially available automated extraction platforms.



High Sensitivity for DNA Extraction

Method Comparison

The Siemens VERSANT kPCR SP Module and SP 1.2 reagents deliver higher DNA yield compared to other commercially available preparation platforms.

Five whole-blood specimens from cytomegalovirus (CMV)†-infected patients were processed on three different sample preparation platforms—QIAsymphony (Qiagen), MagNA Pure LC 2.0 (Roche Diagnostics), and VERSANT SP Module—with their respective whole-blood sample preparation reagent kits.

CMV viral load was measured and calibrated to copies/mL by external quantification standards in the DNA eluates obtained from these platforms using the CMV R-gene kit (Argene/bioMerieux) on the VERSANT AD module of the VERSANT kPCR Molecular System‡ (Siemens Healthcare Diagnostics). Sample input and eluate output amounts were equivalent across all three platforms. Table 1 compares the extraction performance of the three commercial systems after quantification into copies/mL for the same five aliquoted whole-blood patient samples.

The VERSANT kPCR SP Module and SP 1.2 reagents consistently produced higher viral load results on the same set of patient samples (Table 1).[2] Higher quantification in copies/mL originated from lower detected Ct values between the three platforms, which correlate with better DNA extraction sensitivity.

Table 1. CMV viral load measurement of patient samples processed using different sample preparation platforms.

Quantitation (copies/mL)			
Clinical Sample Number	**MagNA Pure LC 2.0**	**QIAsymphony**	**kPCR**
1	2467	5219	17,250
2	618	1713	2471
3	372	1094	22,650
4	167	164	1539
5	6666	6601	13,350
P-value** Against kPCR	0.011	0.019	–

***P*-value of less than 0.05 means that the VERSANT SP 1.2 Reagents kit—along with the VERSANT kPCR SP module—yielded significantly higher quantitations than the other methods. A two-sample paired t-test of log quantitation was used.

†CMV is in development. Not for Sale in the U.S.
‡Not available for sale in the U.S. Product availability may vary by country.

A Universal Extraction Solution for Diverse Sample Types

Method Comparison

VERSANT Sample Preparation Reagents kits (SP 1.0 and SP 1.2) provide high recovery of DNA and RNA from a wide variety of clinical specimen types using the VERSANT kPCR Sample Preparation Module.

Recovery of nucleic acids from clinical samples was evaluated using the VERSANT SP Module with both the VERSANT Sample Preparation 1.0 Reagents (optimized for RNA, but also amenable for DNA) and the VERSANT Sample Preparation 1.2 Reagents (optimized for DNA) kits. Each sample type was spiked with 10 ng of human breast adenocarcinoma total RNA (MCF-7) or human genomic DNA (gDNA). Seven to ten independent samples of each sample type were analyzed. In-house, real-time PCR assays targeting housekeeping genes RPL37A for MCF-7 RNA and PAEP for gDNA were used for amplification and quantification of RNA or DNA. Recovery was evaluated by comparing the concentration of MCF-7 RNA or gDNA in each sample (minus endogenous nucleic acid) to the same amount of MCF-7 RNA or gDNA amplified without going through the sample extraction process. When necessary, samples were pretreated with lysis buffer or PBS (VERSANT Sample Preparation 1.0 Reagents kit) or pretreatment buffer (VERSANT Sample Preparation 1.2 Reagents kit).

Recovery of bacterial DNA was evaluated using *Chlamydia trachomatis* (CT) cells spiked into ThinPrep and SurePath culture media (commercially available collection media for vaginal swabs) at a concentration of $10,^{6}$ $10,^{5}$ and 10^{4} copies/mL run in triplicate. Samples were extracted using both the VERSANT Sample Preparation 1.0 and 1.2

Table 2. DNA/RNA mean recovery in clinical samples.

Sample	Pretreatment Required	SP 1.0 Average RNA Recovery (%)	SP 1.0 Average DNA Recovery (%)	SP 1.2 Average DNA Recovery (%)
Amniotic fluid	Yes	78	29	72
Ascites	Yes	66	36	95
BAL	Yes	66	53	140
Breast milk	Yes	97	36	57
Buccal swab	Yes	66	37	47
Cells in culture media	Yes	65	57	68
CSF	Yes	81	39	71
Eye swab	Yes	64	34	86
NP swabs	Yes	79	40	116
PBMC	Yes	71	85	78
Plasma	No	56	15	70
Semen††	Yes	36	>100	45
Serum	No	45	15	118
Sputum	Yes	57	82	93
Stool	Yes	67	42	60
Urine‡‡	Yes	Not tested	Not tested	132
Vaginal swabs‡‡	Yes	Not tested	Not tested	76
Whole blood‡	Yes	Not tested	Not tested	94

††These samples have high background DNA.
‡‡These sample types were not evaluated with SP 1.0 Reagents in this study, as they had previously been validated for use in a CE-marked assay.
‡For use with VERSANT Sample Preparation 1.2 Reagents only.

Reagents kits, then amplified using the VERSANT CT/CG DNA 1.0 assay (kPCR). To evaluate extraction recovery, CT reference standard DNA was amplified without sample extraction and compared to CT cells amplified with extraction at the same starting concentration.

DNA and RNA were successfully purified from all clinical specimen types tested (Table 2).[3] The VERSANT SP 1.0 Reagents are optimal for extraction of RNA only and combined DNA and RNA from one sample; the VERSANT SP 1.2 Reagents are optimal for extraction of DNA only.

CT bacterial DNA was recovered in both ThinPrep and SurePath collection media at all levels tested, indicating that bacterial DNA in these sample types can be isolated with both VERSANT SP 1.0 and SP 1.2 Reagents with similar efficiency (Table 3).[4]

Table 3. Bacterial DNA mean recovery in cell transport media.

Culture Medium	CT DNA Spike (copies/mL)	SP 1.0 Reagents (Average Ct)	SP 1.2 Reagents (Average Ct)
ThinPrep	1,000,000	23.1	22.3
	100,000	26.4	25.8
	10,000	29.9	29.0
SurePath	1,000,000	23.5	23.8
	100,000	26.8	26.8
	10,000	30.0	30.0

Flexibility and Workflow Consolidation

The VERSANT kPCR SP Module is flexible allowing the extraction of a variable batch size of 1 to 96 samples in about 3 hours. Furthermore, sample types may be mixed in an individual batch run. There are nine open-channel extraction protocols that allow for adjustment of different sample input and eluate output volumes.

The VERSANT kPCR SP Module with the VERSANT MiPLX Software Solution provides an innovative approach to improving workflow and consolidation in the molecular laboratory. Furthermore, it enables flexible automation of Siemens assays as well as laboratory-developed and third-party assays on the VERSANT kPCR Molecular System.

The VERSANT MiPLX Software Solution introduces dynamic protocols that:

- Automate sample extraction from a wide variety of sample types in one run.
- Automate PCR assay setup for a more efficient workflow.
- Enable menu expansion through integration of the Siemens menu with laboratory-developed and third-party assays.
- Provide the ability to get more information out of a single sample extraction by splitting the eluate instead of the sample.
- Allow one, two, four, or six assays to be run from one extracted sample on a single PCR plate through multiplexing capabilities.

Summary

The Siemens VERSANT kPCR Sample Preparation Module includes features that enhance the quality and recovery of nucleic acids from a large variety of sample types while maximizing the laboratory's efficiency and productivity.

- A consolidated platform, with universal protocol and reagents for the isolation of DNA and RNA from diverse clinical specimens, minimizes equipment needs, inventory requirements, and QC management.
- Iron oxide beads (<1 µm in size) coated with a nanolayer of silica improve reproducibility, recovery, and quality and result in enhanced assay performance.
- Two IVD-labeled VERSANT Sample Preparation Reagents kits provide extraction of DNA and RNA from a large variety of sample types.
- The VERSANT kPCR SP Module features fast, easy setup and walkaway operation.
- The module provides high throughput, with up to 96 samples extracted per run in less than 3 hours.
- Enhanced versatility supports accurate quantitative and qualitative detection for a wide range of molecular diagnostic applications, including sequencing, end-point PCR, hybridization assays, and real-time PCR.
- Nine extraction protocols provide flexibility for sample input and eluate output volumes.
- Dynamic protocols allow automated real-time PCR assay setup (lab-developed and third party), including analysis of up to six assays on one sample eluate in one run.

1. Verheyen, et al. Extraction of viral nucleic acids: Comparison of five automated nucleic acid extraction platforms. Journal of Clinical Virology. 2012;54(3):255-259.
2. Wang, et al. Evaluation of an automated sample preparation system for detection of human cytomegalovirus in whole blood (poster ECCMID 2012).
3. Wagner, et al. Manual and automated nucleic acid extraction from multiple clinical specimen types using Siemens VERSANT Sample Preparation Reagents kits (poster AMP 2013).
4. Wagner, et al. A fully automated method for extraction of nucleic acid from multiple clinical specimen types using the Siemens VERSANT Sample Preparation Reagent kits (poster ASM 2014).

This work was supported in part by Siemens Healthcare Diagnostics.

Product availability may vary from country to country and is subject to varying regulatory requirements. Please contact your local representative for availability.

Order No. A91DX-MM-141068-XC1-4A00

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