Sample-to-Sample Carryover Is Insignificant When Sharing Serum Samples Between ADVIA Chemistry Systems and ADVIA Centaur Immunoassay Systems

David Morris, Ph.D., Principle Staff Scientist
Ted Wright, M.S., Staff Scientist
Rodney Day, M.S., MT(ASCP), S.C., Director, Informatics and Consulting Services

Answers for life.

SIEMENS

Introduction

With laboratory systems, the potential for carryover between samples exists both within the analytical processes of a single instrument (internal carryover), or sample-to-sample carryover between the instrument sampling systems of multiple instruments. This type of carryover is defined as the inadvertent transfer of a small portion of one sample to the sample or samples immediately following it in the testing sequence of the analyzer. The incomplete washing of the sample aspiration/dispensing device in an instrument causes this type of carryover.

Most chemistry analyzers are equipped with permanent sampling systems that are washed between samples (e.g., the ADVIA® Chemistry Systems are equipped with a positive liquid displacement sample probe). Based on the sensitivity of the assay measurement technology, minimizing sample-to-sample carryover with chemistry-only systems has usually been considered less critical than with immunoassay systems. A number of newer immunoassay systems, such as the ADVIA Centaur® Immunoassay System, have been designed with disposable sampling tips to negate any potential sample-to-sample carryover related to the instrument sampling system. Within recent years, expansion of chemistry menus, incorporation of new measurement technology into chemistry systems, proliferation of laboratory automation systems, and improvements in chemistry system sampling devices have allowed numerous laboratories to reduce their aliquotting needs, and to share samples for testing between chemistry and immunoassay systems.

Additionally, some systems have been designed to perform a limited number of chemistry and immunoassay tests on a single analyzer platform without first performing sample aliquotting.

With infectious disease immunoassays, universally acceptable carryover limits have not been defined since the cutoffs used to distinguish between negative and positive samples for infectious disease testing varies between manufacturers. The significance of potential carryover with different tumor markers is also quite variable depending on the methods in use, and the populations tested. However, the National Research Laboratories in Australia (http://www.nrl.gov.au) in reviewing seven different Hepatitis B surface antigen (HBsAg) methods reported that a carryover of about 0.16 parts per million (ppm) from a high positive sample to a negative sample could have the potential of resulting in a false positive.

This study was undertaken to determine whether carryover exists between samples first tested on ADVIA Chemistry Systems, and then subsequently analyzed on ADVIA Centaur Immunoassay Systems, and if carryover between samples is found, to estimate its magnitude and potential clinical significance. Both the ADVIA WorkCell® and ADVIA® LabCell® Automation Systems allow the user flexibility in directing samples to either the chemistry systems or the immunoassay systems first, based on the sample's test requirements.

Methods

The test plan was to perform chemistry tests using various ADVIA® 1650 chemistry systems with selected samples (either negative samples or high positive samples for specific analytes) and then test the same sample tubes on the ADVIA Centaur system to determine if the presence or absence of detectable carryover from sample-to-sample could be documented.

Four analytes were selected for measuring potential sample-to-sample carryover: Hepatitis B surface antigen (HBsAg), antibody to human immunodeficiency virus (HIV 1/O/2)[1], antibody to Hepatitis C virus (HCV)1 and human chorionic gonadotropin (hCG). The concentrations of the high positive samples used for each analyte in the study are shown in Table 1.

Table 1: High Positive Samples Used in Study

Analyte	Concentration of High Positive
HBsAg	6,960,000 ng/mL
Anti-HIV	100,000 Index Value
Anti-HCV	1,800 Index Value
hCG	1,100,000 mIU/mL

The concentrations of these analytes are at or greater than the upper limit of what can be expected to be encountered in routine testing. The HBsAg concentration of 6,960,000 ng/mL (index value of ~ 70 million on the ADVIA Centaur system) was obtained by spiking purified HBsAg in the serum pool and is approximately 12 times greater than found in highly HBsAg-positive clinical samples. These artificially high samples were used to ensure that statistically significant levels of carryover could be determined. Any measured carryover was calculated as the difference of the measured value and expected value for negative samples. Carryover was considered statistically significant if the result was greater than 2.4 standard deviations of the negative sample mean, otherwise it is reported as non-detectable (within the imprecision of each assay). Each sample was measured for asparate aminotransferase (AST) on the ADVIA 1650. In this assay, 30 µL of sample is aspirated from the primary tube and placed into the instrument's dilution well. Because this assay uses a larger volume sample than other assays on the ADVIA 1650, it would have the greatest potential carryover at the sample probe.

Three ADVIA 1650 systems (to test any variability in sample carryover from instrument to instrument) and one ADVIA Centaur system were used. The samples were first tested on the ADVIA 1650 systems and the sample tubes were then transferred to the ADVIA Centaur System.

For each analyte of interest, the sample work list shown in Table 2 was performed on each ADVIA 1650 system. A separate series of samples was used for each analyte and for each ADVIA 1650 system. The negative samples were human base pools free of the analyte(s) of interest. Negative samples were placed immediately after one, two, or five previously sampled high concentration or highly positive samples. Additionally, multiple negative samples were placed at the beginning of each run, and were used to determine the negative sample mean for each analyte. Sample placement used on the ADVIA 1650 system is illustrated at Figure 1.

A single AST test was performed per sample on each ADVIA 1650 system. All of the negative samples were then tested on the ADVIA Centaur system.

Results

The results of our measurements for sample-to-sample carryover are presented in Table 3. Carryover measurements for the first negative sample following the high concentration or highly reactive samples are presented (samples 5, 10, 18 from the worklist).

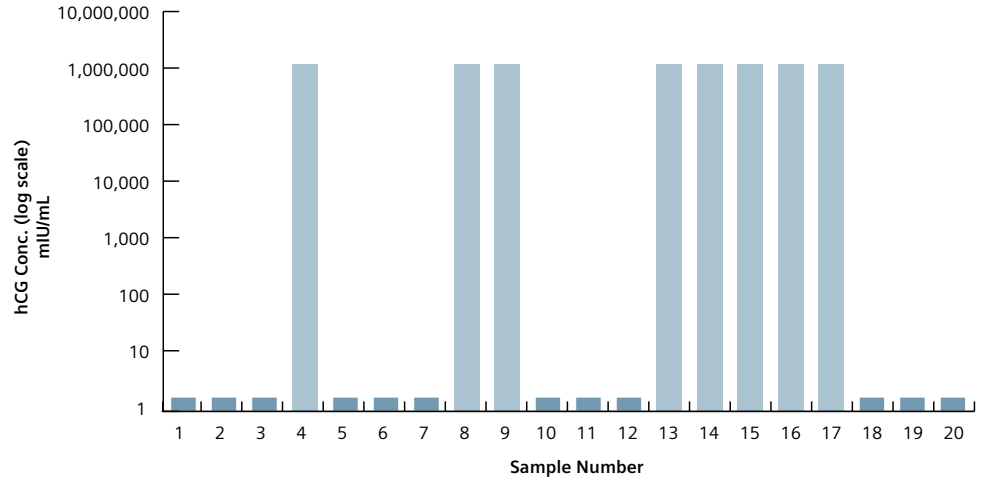
These results demonstrate that no carryover was detectable in the first negative sample following a highly positive sample for hCG, HCV or HIV. Clinically insignificant carryover was measured with the artificially spiked HBsAg samples after sampling on ADVIA 1650 system No. 2 and ADVIA 1650 system No. 3, but was not detectable after sampling on ADVIA 1650 system No. 1. These measurements ranged from non-detectable to 0.025 parts per million (ppm).

Table 2: ADVIA 1650 Work List

Sample Sequence	Analyte Concentration
1	Negative
2	Negative
3	Negative
4	**High Positive***
5	**Negative**
6	Negative
7	Negative
8	**High Positive**
9	**High Positive**
10	**Negative**
11	Negative
12	Negative
13	**High Positive**
14	**High Positive**
15	**High Positive**
16	**High Positive**
17	**High Positive**
18	**Negative**
19	Negative
20	Negative

*Concentrations are as detailed in Table 1.

Figure 1: Sequence of hCG-containing samples used on the ADVIA 1650 systems



Discussion

In these experiments, when using high positive samples at concentrations at or greater than the upper limit of the clinical range, HIV, HCV and hCG were not found to have any detectable carryover on the ADVIA Centaur system after testing these same samples for AST on a variety of the ADVIA 1650 systems. In the case of HBsAg, the high positive samples are at an artificially high concentration created by spiking antigen into the base pool. These artificially high levels of HBsAg were used to see if we could document any carryover in these studies, and whether there is variability in the washing efficiency between different ADVIA 1650 chemistry systems. With these artificially high HBsAg samples, we obtained an average sample-to-sample carryover of 0.013 ppm (range: ND to 0.025 ppm), but carryover was undetectable on using the samples from one of the three ADVIA 1650 systems tested.

This small amount of sample-to-sample carryover measured would not be detected for a typical high positive clinical sample in a laboratory. Literature has reported that levels of HBsAg can reach about 500,000 ng/mL (Hoofnagle, Ann. Rev. Med. 1981:32: 1 – 11). This level corresponds to an index of five million. Even assuming a maximum potential carryover of 0.025 ppm when testing on the ADVIA 1650 system and ADVIA Centaur system combination, the maximum sample-to-sample carryover on the sample following this high positive would be 0.125 index units, which is significantly less than the standard deviation of the negative mean of HBsAg samples (over 30% less than 1 S.D.). To have potential significance with HBsAg test results, the observed sample-to-sample carryover would need to be at greater than 0.10 ppm (at least four times what was found in these experiments).

Conclusion

This study demonstrates that clinically significant sample-to-sample carryover is not detectable between ADVIA Chemistry Systems and ADVIA® Immunoassay Systems using samples with artificially elevated concentrations of hCG, HIV, HBsAg or HCV. The ADVIA 1650 Chemistry System's positive displacement sampling system with the integrated analyzer washing system is efficient in preventing sample-to-sample carryover in samples with high levels of infectious disease markers or tumor markers.

With these highly sensitive assays, and when using samples in concentrations that would normally be found in clinical laboratories, significant sample-to-sample carryover is non-detectable on the ADVIA Centaur immunoassay tests subsequently performed after sampling on the ADVIA 1650 Chemistry System.

These findings of insignificant carryover with clinical specimens will allow the laboratory to safely share samples between ADVIA Chemistry Systems and Siemens Healthcare Diagnostics' immunoassay systems in the laboratory without making separate aliquots for each system. Additionally, the laboratory can have the choice of whether to run the samples first on the ADVIA Centaur system, first on the ADVIA 1650 system, or a mixture of both methods, depending on the needs and turn-around requirements for each laboratory. With our laboratory automation solutions, the ADVIA WorkCell or the ADVIA LabCell, each site can set different priorities by test in the software tables.

Table 3: ADVIA Centaur Results of Sample Carryover Study
Sample-to-Sample Carryover (PPM) Results*

System	Analyte Tested on ADVIA Centaur			
	HBsAg (ppm)	HIV (ppm)	HCV (ppm)	hCG (ppm)
ADVIA 1650 - No. 1	ND	ND	ND	ND
ADVIA 1650 - No. 2	0.013	ND	ND	ND
ADVIA 1650 - No. 3	0.025	ND	ND	ND
High Positive Conc.	69,600,000	100,000	1,800	1,100,000
Units	Index	Index	Index	mIU/mL
Negative Sample Mean	0.41	0.31	0.00	1.50
St. Dev. Negative Sample Mean	0.185	0.012	0.00	0.12
Minimum Carryover Detection Limit (ppm)	0.0064	0.2880	0.0000	0.2618

***Note:** ND=Not Detected (<2.4 SD from the Negative Sample Mean). Results are expressed in parts per million (ppm).

Siemens Healthcare Diagnostics, a global leader in clinical diagnostics, provides healthcare professionals in hospital, reference, and physician office laboratories and point-of-care settings with the vital information required to accurately diagnose, treat, and monitor patients. Our innovative portfolio of performance-driven solutions and personalized customer care combine to streamline workflow, enhance operational efficiency, and support improved patient outcomes.

ADVIA, ADVIA Centaur, ADVIA WorkCell, LabCell, and all associated marks are trademarks of Siemens Healthcare Diagnostics Inc. All other trademarks and brands are the property of their respective owners.

The ADVIA Centaur HCV and HIV assays are developed, manufactured and sold by Siemens for Ortho-Clinical Diagnostics, Inc. Assay availability subject to local regulatory requirements. Not available in the U.S.

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

Global Siemens Headquarters

Siemens AG
Wittelsbacherplatz 2
80333 Muenchen
Germany

Global Siemens Healthcare Headquarters

Siemens AG
Healthcare Sector
Henkestrasse 127
91052 Erlangen, Germany
Phone: +49 9131 84 - 0
www.siemens.com/healthcare

Global Division

Siemens Healthcare Diagnostics Inc.
511 Benedict Avenue
Tarrytown, NY 10591-5005
USA
www.siemens.com/diagnostics

www.siemens.com/diagnostics

Order No. A91DX-0700188-GC3-4A00 | 09-2011 | All rights reserved | © 2011 Siemens Healthcare Diagnostics Inc.