Performance Evaluation of an Improved Anti-Streptolysin O Method (ASO_2) on the ADVIA Chemistry Systems

Datta P, Losapio M, Dai J

Answers for life.

SIEMENS

Abstract

Objective:

The quantitative determination of antistreptolysin O (ASO) antibodies in human serum or plasma is often used by clinicians to establish the degree of infection due to group A hemolytic streptococci. Increased ASO levels are also associated with rheumatic fever and poststreptococcal glomerulonephritis. Recently, Siemens Healthcare Diagnostics released an improved ASO assay (ASO_2) for use on the automated, random access ADVIA® 1650, ADVIA® 1800, ADVIA® 2400, and ADVIA® 1200 Chemistry Systems. The results of an evaluation of this method are presented here.

Methods:

All ADVIA Chemistry Systems can use the same reagent packs, calibrators, and controls. In this assay, ASO in the sample is reacted with a latex reagent (latex particles coated with streptolysin O antigen), resulting in agglutination and increased turbidity. This turbidity is measured by the ADVIA Chemistry Systems at 571 nm. The ASO concentration in the sample is read off a linear two-point standard curve.

Results:

The imprecision of the new method (ASO_2) with three-level commercial serum protein controls and two serum pools (ranging from 130 to 900 IU/mL) on all three ADVIA Chemistry Systems was ≤2.5% (within-run CV) and ≤3.9% (total CV) (n = 40). The analytical range/linearity of the new methods on all ADVIA systems was 25–1000 IU/mL. A twofold autodilution for samples with ASO > 1000 IU/mL extends the assay's upper range to 2000 IU/mL. The ADVIA 1650/1800 method (y) correlated well with both OLYMPUS ASO on AU600 and ROCHE ASLO on HITACHI 717: y = 1.00 (OLYMPUS) – 13.3 (r = 0.98; n = 50; range: 51–693 IU/mL); y = 0.95 (ROCHE) + 12.7 (r = 0.96; n = 49; range: 21–485 IU/mL). The ADVIA 2400 and ADVIA 1200 ASO_2 methods, in turn, agreed with the ADVIA 1650/1800 ASO_2 method: ADVIA 2400 ASO_2 = 1.00 (ADVIA 1650/1800 ASO_2) + 7.1 (r = 0.99; n = 61; range: 31.9–921 IU/mL) and ADVIA 1200 ASO_2 = 0.99 (ADVIA 1650/1800 ASO_2) + 0.8 (r = 0.99; n = 60; range: 31.9–645.5 IU/mL). The equivalency of serum and plasma (both K_2EDTA and Li-heparin) with the ASO_2 method was shown on all ADVIA systems (regression slopes 0.99–1.01).

No prozone effect was observed for the highest ASO concentration tested (up to 8500 IU/mL). The ASO_2 method showed no interference with unconjugated or conjugated bilirubin (60 mg/dL), hemoglobin (1000 mg/dL), Intralipid® or avian triglycerides (1000 mg/dL), and rheumatoid factor (400 IU/mL) on all ADVIA systems. The method has a minimum of 60 days of on-system stability and calibration frequency on all systems.

Conclusion:

The improved ASO_2 method, when used on any ADVIA Chemistry System, can measure serum or plasma ASO concentrations precisely and accurately over a broad range for routine clinical chemistry laboratory use.

Background

The group A hemolytic streptococci cause widespread disease. Such infections may start with a sore throat and general malaise, but may end up with more serious conditions of rheumatic fever and glomerulonephritis. Identifying the root cause is important in diagnosis and management of such diseases. The antistreptolysin O (ASO) test is used for such purposes.

Streptolysin O is one of the several toxic immunogenic exozymes produced by the streptococci. Thus, an increase in ASO titer is usually an indication of a recent streptococcal infection. Approximately 80 to 85 percent of individuals with a current streptococcal infection demonstrate an elevated ASO titer.[1] The ASO concentration may, however, decrease by the time a patient presents with acute rheumatic fever, so a negative ASO result can not rule out streptococcal infection.

Materials and Methods

The ADVIA Chemistry ASO_2 method (Siemens Healthcare Diagnostics, Tarrytown, NY) determines ASO concentration in serum or plasma by latex microparticle–associated immunoturbidimetry. In the assay, suspended uniform latex particles coated with streptolysin O react with patient sample containing antibodies, to produce an increase in turbidity. A quantitative value for the concentration of antistreptolysin O present in the sample is obtained by comparison with a standard.

In this method, as run on the ADVIA 1650, ADVIA 1800, ADVIA 2400, or ADVIA 1200 system, sample is added to the reagent (R1), which contains a buffer. After 5 minutes, the second reagent, R2, containing latex microparticles coupled to streptolysin O antigen, is added. After an additional 5 minutes' incubation at 37˚C, the reaction absorbance is measured (endpoint).
All ADVIA Chemistry ASO_2 methods use two-point calibration: blank (water) and a single calibrator (ADVIA Chemistry Liquid Specific Protein Calibrator, Level 6). Of the ADVIA Chemistry systems used in our studies, the ADVIA 1650, 1800 and 2400 use a 5× sample predilution. From the diluted sample, multiple assays can be run. Except for the ADVIA 1200 system, which uses undiluted sample, the ADVIA Chemistry systems automatically perform the dilution using the system diluent (saline).

All ADVIA Chemistry systems use the same reagent packs, calibrator, and controls. A single calibrator value and control ranges are used across all platforms. The assay has a minimum of 60 days' on-system stability on all systems. The calibration is stable for 60 days (on the ADVIA 1650, 1800 and 1200 systems) and 30 days (on the ADVIA 2400 systems).

The OLYMPUS ASO (on AU600) and ROCHE ASLO (on HITACHI 717) methods were run according to the manufacturers' protocols.

Results

Precision

Precision estimates were obtained using three-level commercial controls and two patient serum pools. Each sample was assayed two times per run, two runs per day, for ten days (20 replicates/sample). Precision estimates were computed according to CLSI document EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline—Second Edition (Table 1).

Table 1. Imprecision of ADVIA Chemistry ASO_2 method. Concentrations are expressed in IU/mL.

		Within-Run		Total	
	Mean	SD	CV (%)	SD	CV (%)
ADVIA 1200					
Control 1	132.3	3.10	2.3	5.16	3.9
Control 2	287.4	2.81	1.0	8.12	2.8
Control 3	444.0	8.37	1.9	14.69	3.3
Low Pool	280.6	4.58	1.6	10.03	3.6
High Pool	882.3	10.79	1.2	23.42	2.7
ADVIA 1650/1800					
Control 1	135.6	2.95	2.2	4.15	3.1
Control 2	289.3	4.52	1.6	5.36	1.9
Control 3	453.0	8.37	1.8	11.18	2.5
Low Pool	285.2	2.25	0.8	4.62	1.6
High Pool	919.1	4.35	0.5	9.73	1.1
ADVIA 2400					
Control 1	130.6	2.03	1.6	3.30	2.5
Control 2	282.8	7.18	2.5	7.21	2.5
Control 3	445.5	6.66	1.5	8.46	1.9
Low Pool	281.4	4.09	1.5	5.95	2.1
High Pool	897.8	22.46	2.5	25.82	2.9

Linearity

The linearity and analytical range of the new method was determined by assaying nine-level dilutions of a high serum pool with saline on the ADVIA Chemistry systems. The observed ASO_2 concentrations were compared with expected values, and the linear regression parameters (slope, intercept, and correlation coefficient, r) are presented in Figures 1–3.

Figures 1–3. Linearities of ADVIA Chemistry 1200, 1650, 1800, and 2400 ASO_2 methods.
The line of identity appears as a dashed line in each figure.

Figure 1

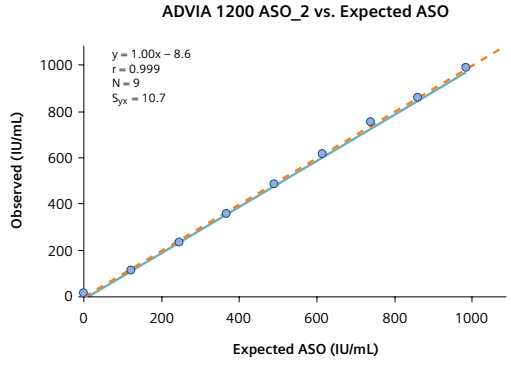


Figure 2

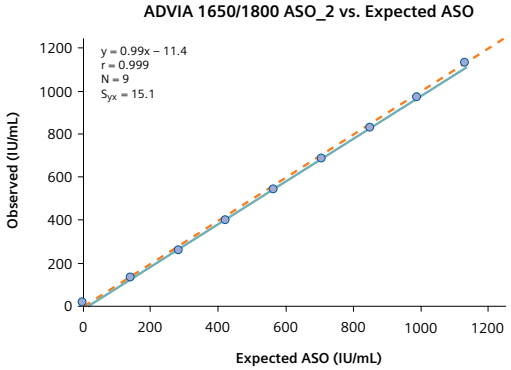
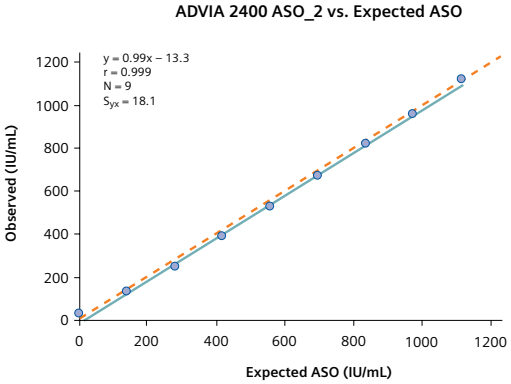


Figure 3



Extended Assay Range by Auto-rerun

The ADVIA Chemistry systems allow flagging and auto-rerun of the ASO_2 method, with 2-fold increased dilution, for samples containing ASO_2 levels higher than the upper assay range (1000 IU/mL), thus extending the reportable assay upper range to 2000 IU/mL. The accuracy and precision of the auto-rerun feature were assessed with two samples containing ~760 and 679 IU/mL ASO. The samples were assayed without and then with auto-rerun after 2-fold dilution performed automatically on the system (both in five replicates, on all three systems). The precision and means of both sets of results were comparable (Table 2).

Table 2.
Auto-rerun of samples assayed by the ASO_2 method on the ADVIA 1200, 1650/1800, and 2400 systems. Concentrations are expressed in IU/mL.

ADVIA 1200			ADVIA 1650/1800			ADVIA 2400		
Original	Rerun	Recov.	Original	Rerun	Recov.	Original	Rerun	Recov.
769.0	751.0	98%	906.0	817.6	90%	906.8	818.9	90%
678.9	609.9	90%	732.8	665.7	91%	771.4	696.8	90%

Method Comparison

We compared the ADVIA 1650/1800 ASO_2 method with two commercial ASO assays—the ROCHE ASLO assay run on the HITACHI 717 system, and the OLYMPUS ASO method—by assaying 49 and 50 serum samples, respectively. As shown in Figures 4 and 5, the ADVIA 1650/1800 ASO_2 method (y) showed equivalency with both comparison ASO methods (x). Furthermore, we compared the ASO_2 method on serum samples run on all the ADVIA Chemistry systems (Figures 6 and 7). All linear regression data for the method comparisons are presented in Table 3.

Table 3.
Regression summary for ASO_2 method comparisons (Figures 4–7).
Concentrations are expressed in IU/mL.

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.

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
Telephone: +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-0701332-GC2-4A00 | 09-2011 |

Figures 4–7. Correlation plots. The line of identity appears as a dashed line in each figure.

Figure 4

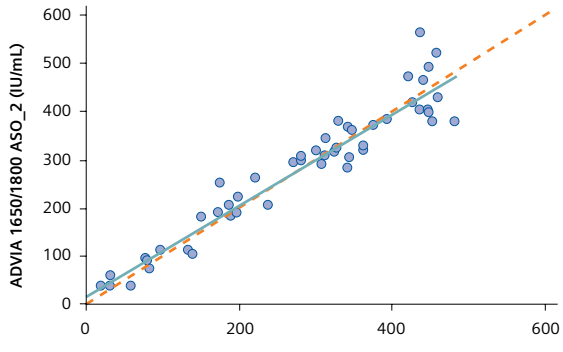


Figure 5

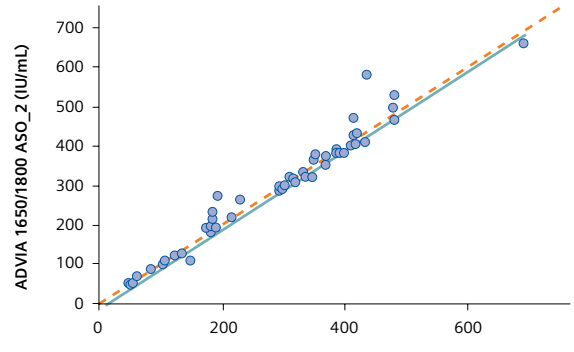


Figure 6

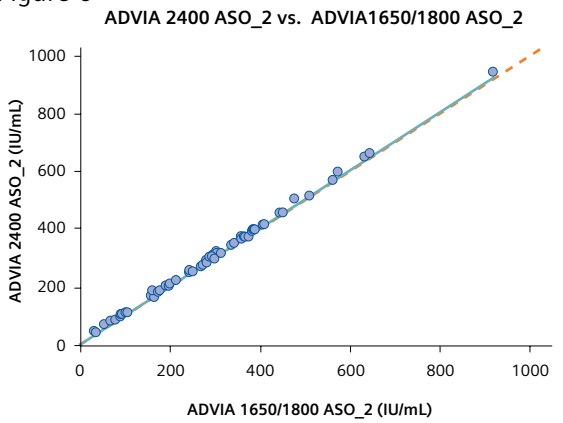
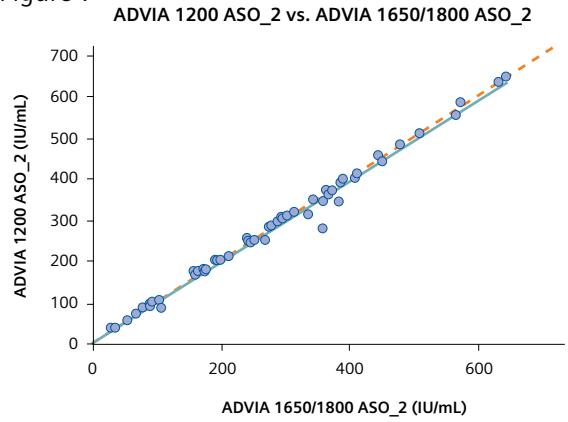


Figure 7



Serum vs. Plasma Comparison

Matched Li-heparin or K_2EDTA plasma (y) and serum (x) samples were analyzed with the new method on all ADVIA Chemistry systems. The following regression data were obtained (Table 4).

Table 4.
Regression summary: serum vs. plasma, ASO_2.

ADVIA Platform	y	x	Slope	Intercept	S_{yx}	r	n	Range (IU/mL)
1650/1800	Plasma, Li-heparin	Serum	1.01	–2.2	4.4	0.99	40	32.0–306.9
1650/1800	Plasma, EDTA	Serum	0.99	–1.7	3.2	0.99	40	32.0–306.9
2400	Plasma, Li-heparin	Serum	1.00	–1.1	4.7	0.99	29	30.0–318.4
2400	Plasma, EDTA	Serum	0.99	–1.8	4.7	0.99	29	30.0–318.4
1200	Plasma, Li-heparin	Serum	1.01	–3.0	4.3	0.99	29	28.8–343.0
1200	Plasma, EDTA	Serum	0.99	–1.7	3.9	0.99	29	28.8–343.0

Prozone Effect

A high ASO serum sample and its dilutions were run using undiluted sampling in the assay (routine ASO_2 measurements use 1:5 prediluted samples), thus testing up to 5× the analyte concentrations in the samples. In Figures 8–10, theoretical ASO concentrations in the samples (x) are plotted against the absorbances observed in the assay; the dotted lines represent the absorbance for the upper limit of the assay (1000 IU/mL). No prozone effect (absorbance signal falling into the assay calibration range) was observed for all the samples tested: up to 8500 IU/mL ASO concentration (on all ADVIA Chemistry systems).

Figure 8. Prozone curve for ADVIA 1200 ASO_2.

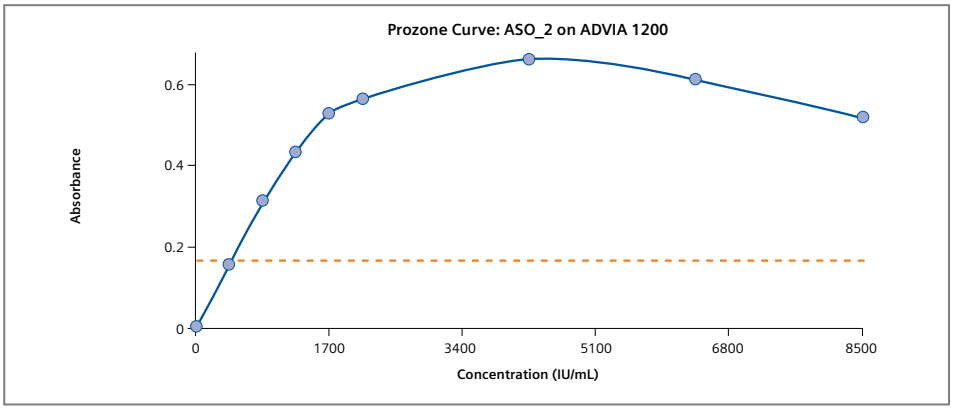


Figure 9. Prozone curve for ADVIA 1650/1800 ASO_2.

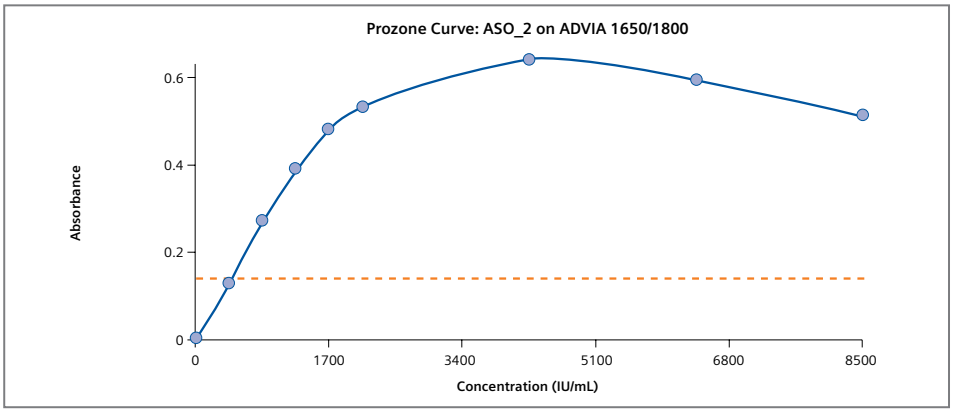
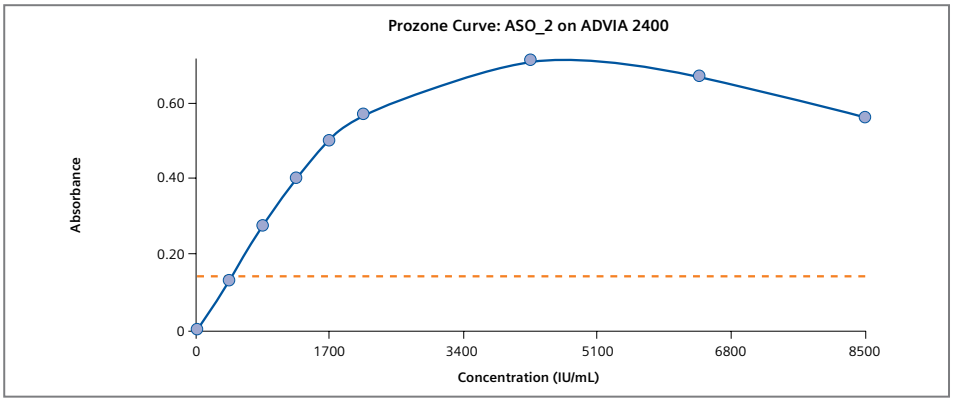


Figure 10. Prozone curve for ADVIA 2400 ASO_2.



Interference

Interference was evaluated by spiking serum pools containing a clinically relevant ASO_2 concentration (~160 to 230 IU/mL) with hemoglobin (from lysed human red blood cells), unconjugated bilirubin, conjugated bilirubin, a triglyceride lipid (Intralipid and avian triglyceride concentrate), and rheumatoid factor. Multiple levels of interfering substances were tested on all ADVIA Chemistry platforms. Representative results at the highest concentration of interfering substance tested are summarized in Table 5.

On-system Stability and Calibration Frequency

The on-system stability and calibration frequency of the new method was studied on all three ADVIA Chemistry platforms by calibrating the assays on day 0, leaving the reagent wedges on the respective systems, and assaying the calibrator and 3-level commercial controls (LSP-1, LSP-2 and LSP-3) on days 22, 35, 46, 56 and 61 (Figures 11 through 13). The percent recoveries of all four samples were compared with respect to their day 0 results. All recoveries were within the 90% to 110% range (except on ADVIA 2400, where the day 56 recoveries were 88% to 90%, though day 61 recoveries were 98% to 107%). These data indicate that the new method has an on-system stability and calibration frequency of at least 60 days.

Table 5. Interference summary for ADVIA 1650/1800, 2400 and 1200 ASO_2.

ADVIA 1200			ADVIA 1650/1800			ADVIA 2400		
	ASO_2 (IU/mL)	% of Control		ASO_2 (IU/mL)	% of Control		ASO_2 (IU/mL)	% of Control
Hb (mg/dL)			**Hb (mg/dL)**			**Hb (mg/dL)**		
0	190	100.0	0	181	100.0	0	191	100.0
250	191	100.7	250	179	98.8	250	182	95.3
500	189	99.6	500	174	96.4	500	181	94.7
750	186	98.2	750	173	95.9	750	177	92.6
1000	184	97.1	1000	170	94.1	1000	173	90.5
Bilirubin, Free (mg/dL)			**Bilirubin, Free (mg/dL)**			**Bilirubin, Free (mg/dL)**		
0	180	100.0	0	167	100.0	0	175	100.0
15	187	103.9	15	169	101.4	15	176	100.5
30	182	101.0	30	172	102.9	30	172	98.1
45	182	101.0	45	170	101.7	45	173	98.5
60	179	99.4	60	173	103.9	60	173	98.4
Bilirubin, Conj. (mg/dL)			**Bilirubin, Conj. (mg/dL)**			**Bilirubin, Conj. (mg/dL)**		
0	182	100.0	0	174	100.0	0	178	100.0
15	178	97.8	15	175	100.6	15	178	100.1
30	181	99.3	30	171	97.9	30	177	99.4
45	181	99.8	45	168	96.5	45	173	96.9
60	180	98.8	60	170	97.2	60	176	99.1
TRIG (Intralipid) (mg/dL)			**TRIG (Intralipid) (mg/dL)**			**TRIG (Intralipid) (mg/dL)**		
0	192	100.0	0	175	100.0	0	181	100.0
250	190	98.9	250	173	98.5	250	179	98.8
500	185	96.2	500	178	101.4	500	178	98.3
750	185	95.9	750	173	98.8	750	181	100.3
1000	187	97.1	1000	176	100.5	1000	180	99.8
RF (IU/mL)			**RF (IU/mL)**			**RF (IU/mL)**		
0	198	100.0	0	225	100.0	0	229	100.0
100	201	101.5	100	231	102.7	100	234	102.2
200	201	101.5	200	233	103.6	200	237	103.5
300	211	106.6	300	237	105.3	300	250	109.2
400	208	105.1	400	242	107.6	400	244	106.6

Figures 11–13. On-system stability.

Figure 11

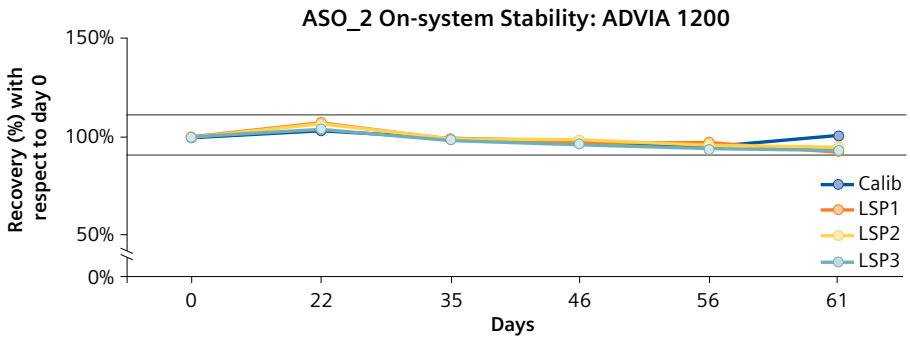


Figure 12

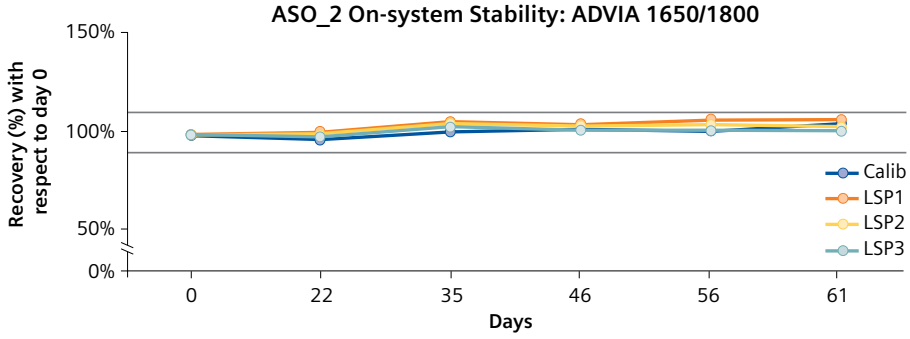
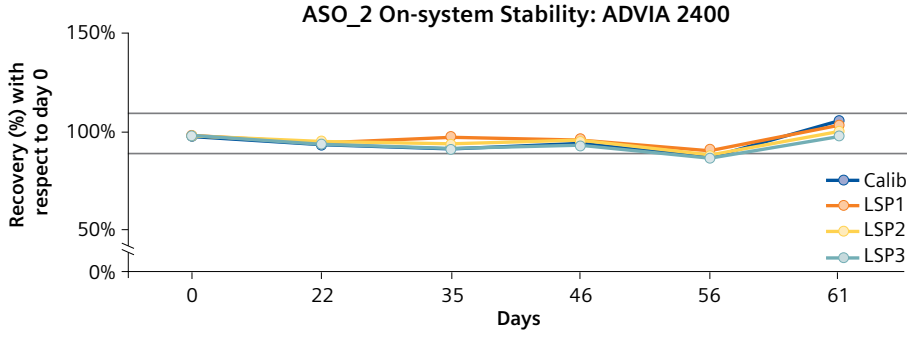


Figure 13



Conclusions

The results of our evaluation of the ADVIA Chemistry ASO_2 method on the ADVIA Chemistry 1200, 1650, 1800, and 2400 systems show that the method has acceptable performance data to be used in clinical laboratories for routine determination of serum or plasma ASO across a relatively wide range of concentrations.

References

1. Thomas L. Streptococcus pyrogenes infection. In: Thomas L. ed. Clinical Laboratory Diagnosis.
   Use and assessment of Clinical Laboratory results. 1st Edition (1998). TH-Books, Frankfurt/Main, Germany.