P2211 INTRODUCTION OF AN AUTOMATIC INSTRUMENTAL DILUTION FOR HIGH TITER SPECIMENS IN HBsAg QUANTITATIVE ASSAY

DiaSorin The Diagnostic Specialist

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OBJECTIVE

LIAISON® XL murex HBsAg Quant assay is designed for a sensitive detection of acute HBV infections including HBsAg escaping mutants, moreover its quantitation supports the role of HBsAg as a predictive marker for the anti-HBV treatment response. Quantitation of HBsAg has an increasing clinical utility as a biomarker for the prognosis and response in therapy in cases of chronic Hepatitis B.
A new assay protocol for an auto-dilution of out of range specimens was developed and then assessed.

METHODS

A new assay protocol was developed for the **LIAISON® XL murex HBsAg Quant** assay to allow the user to select on board dilution of specimens containing HBsAg concentrations above the assay range (>150 IU/mL). These samples can be automatically diluted using the specific Specimen Diluent loaded in the ancillary reagent area of the LIAISON® XL instrument.
The recommended dilution factor is 1:400. Assay range is set from 0.030 to 150 IU/mL, where best precision performance can be achieved. Depending on the laboratory populations, about 60-80% of positive HBsAg samples are expected to show concentrations greater than 150 IU/mL.
Using the new assay protocol and the Specimen Diluent as recommended by Instructions for Use of the assay, we performed a comparison on a panel of 40 samples, evaluated both with automated dilution and with manual dilution 1:400. Linearity was then also verified on a set of 17 samples serially auto-diluted 1:200 – 1:400 – 1:800. Precision comparison on five replicates between manual and auto-dilution was also performed.

Statistical Analysis

The value of each diluted sample was compared using Analyze-it Software (version 2.21 Excel12+) to assess the correlation between manual and instrumental dilution.
Correlation was considered adequate if

- linear regression slope between manual and auto-dilution is within 0.90 ÷ 1.10 with an R2 > 0.9;
- a % bias of 75% ÷ 125% (± 25%) is obtained between manual and auto-dilution for all samples;
- precision of auto-dilution should be consistent with data obtained using manual dilution, with CV% below 5%.

RESULTS

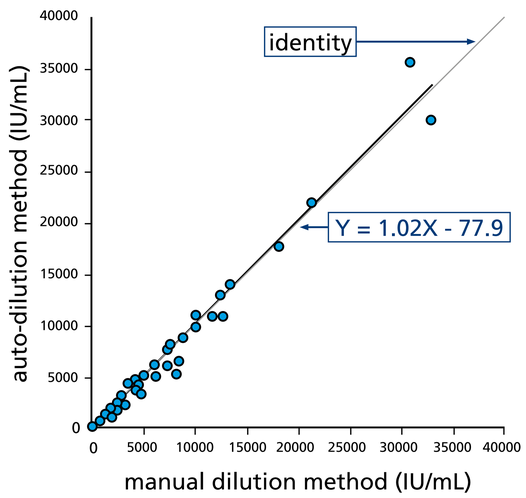
ANALYSIS OF MANUAL VERSUS AUTO-DILUTION

The linear regression slope on the first panel of 40 samples was within 0.90 ÷ 1.10 with an R2 > 0.95. A polynomial regression fit of the sera data yielded statistically not significant at second and third terms.

Table1:
Results from correlation slope of auto and manual dilutions

Term	Obtained	95%C.I.
Slope	1,02	0.97-1.08
R^2	0,98	-
% bias	2.6 (mean)	-

Figure1:
Scattered plot with Passing & Bablok Fit



The calculated % bias between manual and auto-dilution for all samples is within the range of 75 -125%.
Based on the above linear regression data we concluded that LIAISON® XL murex HBsAg Quant assay exhibits acceptable linearity of auto versus manual-dilution.

AUTO-DILUTION ACCURACY

The linear regression slope on a set of 17 samples serially diluted 1:200 – 1:400 – 1:800 was obtained.

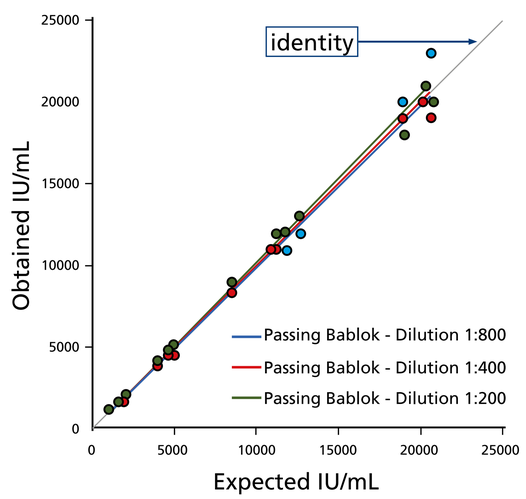
Table 2:
Results from correlation slopes of auto-dilutions and expected value (manual dilution)

Term	Obtained	95%C.I.
Slope 1:200	1,02	0.96-1.04
Slope 1:400	1,00	0.97-1.01
Slope 1:800	0,98	0.96-1.03

Table 3:
Analysis of variance for repeated measures among tested auto-dilutions *(ANOVA – C.I.95%)*

number of group	3
P value	0.545
F	0.619
Are means signif. different? (P < 0.05)	NO

Figure 2:
Scattered plot with Passing & Bablok Fit



Based on the above linear regression data and the Analysis of variance for repeated measures among conditions, we concluded that no significant trends between tested auto-dilutions occurred.

AUTO-DILUTION PRECISION

Precision on five replicates between manual and auto-dilution was obtained.
CV% values obtained for the automated dilution ranged from 1.3% to 4.8%, thus demonstrating the high precision of the new assay protocol for the auto-dilution.

Table 4:

	CV%									CV% (mean)
	S-1	S-2	S-3	S-4	S-5	S-6	S-7	S-8	S-9	
Auto-dilution	4.8%	2.6%	3.6%	2.3%	1.6%	1.9%	1.3%	2.1%	3.6%	2.6%
Manual-dilution	1.7%	1.3%	0.9%	0.6%	1.3%	2.0%	1.3%	1.7%	2.9%	1.5%

DISCUSSION

- As a result of DiaSorin's commitment to quality and continuous improvement, updates have been introduced to the LIAISON® XL murex HBsAg Quant assay (Catalog Number 310250). The LIAISON® XL murex HBsAg Quant Specimen Diluent (Catalog Number 310252) can be used as ancillary reagent managed automatically by the LIAISON® XL instrument.
- The data supports the adequacy of the auto-dilution choice in the LIAISON® XL murex HBsAg Quant system for patient samples higher than 150 IU/mL. Recently, quantitation of HBV has a growing clinical utility in the monitoring of therapy in the case of chronic Hepatitis B, therefore it has been suggested the use of HBsAg as a biomarker for the prognosis and response to therapy in cases of chronic Hepatitis B.
- The option of manually dilute the sample is still feasible in the LIAISON® XL murex HBsAg Quant system.

CONCLUSIONS

On board dilution with dedicated Specimen Diluent improves accuracy by reducing the potential errors due to manual handling of samples. The LIAISON® XL murex HBsAg Quant direct two-step sandwich CLIA assay ensures reliable data showing high analytical performance in the quantitative determination of HBsAg in serum/plasma specimens. The same kit can be used for monitoring the therapy and to screen hospitalized patients and donors population, with clear cost benefit for the laboratory and reduction of turn-around time.

23TH ANNUAL MEETING OF THE EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASE, BERLIN, 27-30TH APRIL 2013