INTRODUCTION OF AN AUTO-DILUTION FOR ARCHITECT HBsAg QUANTITATIVE ASSAY

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2012 Asia Pacific Association for the Study of the Liver Taipei Conference • February 2012

Abstract

Background: Monitoring of HBsAg concentration can provide an indicator of treatment efficacy. 90% of positive samples which require monitoring return concentrations greater than 250 IU/ml. These samples require dilution to fall within the dynamic range of the Abbott ARCHITECT HBsAg assay (LN 6C36). The aim of the project is to provide a quantitative HBsAg assay which will provide an on-board dilution at 1:500 for samples greater than 250 IU/mL and to allow customers to pre-select whether a sample will be run in neat or diluted mode.

Methods: A new assay file was developed for the Abbott ARCHITECT HBsAg assay to allow the user to select an option to provide an on-board dilution of 1:500. Using the new assay file a comparison was completed on a sample set evaluated with both the new automated dilution and a manual dilution using Manual Diluent as recommended by the assay package insert. A total of (n = 344) samples were tested on two ARCHITECT instruments and the values for each sample were compared to assess the correlation between manual and automated dilution methods across the range of samples tested.

Results: A strong correlation was observed between both methods with a plot of Automated vs. Manual data yielding a slope values of 0.96 and 0.97 using Least Squares and Passing Bablok regressions respectively.

Conclusions: The introduction of a 1 :500 automated on-board dilution for the Abbott ARCHITECT HBsAg assay is proceeding based on the data collected. The associated project is ongoing with a projected launch date in the first quarter of 2012.

Introduction

Hepatitis B virus (HBV) is the most prevalent global viral infection and results in greater than one million deaths per year. Approximately 350 million people worldwide are chronic carriers of the virus. (1),(2), (3) and 2 billion people have been infected. (4) Hepatitis B surface antigen (HBsAg) is recognized as a key serological marker of acute and chronic HBV infection.(5) and can be detected in the serum several weeks before the onset of disease. The marker may also be detected through the acute and chronic stages of infection. Detection of HBsAg in a sample indicates that the individual is probably infectious and the level of antigen present may be correlated with the relative level of infection and the severity of disease. (1), (2) and (6).

Enzyme immunoassays for the detection of HBsAg were first described by Engvall and Perlmann (7-9) and Van Weemen and Schuurs (10) in 1971. In 1976 and 1977, solid phase "sandwich" enzyme immunoassays were developed in which HBsAg was captured on a solid phase coated with polyclonal antibodies against HBsAg (anti-HBs) and then detected with anti-HBs conjugated to an enzyme (11-13). In the early 1980's, monoclonal anti-HBs based assays were developed for the detection of HBsAg (14-19). ARCHITECT HBsAg is a chemiluminescent microparticle immunoassay (CMIA) which uses microparticles coated with monoclonal anti-HBs for the detection of HBsAg. HBsAg assays are routinely used to aid in the diagnosis of suspected hepatitis B viral (HBV) infection and to monitor the status of infected individuals, *i.e.,* whether the patient's infection has resolved or the patient has become a chronic carrier of the virus (20).

Methods & Procedures

Serum and Plasma samples positive for HBsAg (>250 IU/mL) were sourced from Promeddx. Samples were diluted using the manual dilution process, as recommended in the current ARCHITECT HBsAg Package Insert (List 6C36). The same samples were diluted using new assay files to enable an autodilution to 1:150 and 1:500. All samples were diluted by a factor of five hundred manually and by factors of five hundred and one hundred and fifty through automated dilutions.

All assays were performed using Abbott Diagnostics ARCHITECT reagents (List 6C36) with an additional diluent added to the reagent configuration. The additional reagent was composed primarily of negative human plasma and was manufactured as part of the assessment of the new dilution protocol.

Assessments were performed initially to assess the feasibility of two dilution protocols and subsequently to confirm the effectivity of one proposed protocol through design verification. A total of 244 samples were assessed throughout the study.

Satistical Analysis

Data was analyzed using MS Excel 2007 (Microsoft Inc, Seattle, WA, USA) and SAS Version 9.1, to assess the correlation between the manual and auto dilutions performed on the sample sets. In order to be considered effective the following criteria were required to be met.

The slope of the correlation curve of the manual versus auto dilution results should be 1.0 +/- 0.1 and no individual sample should differ betweeen manual and autodilution by more than 30%.

Results

- All sample sets, tested through both feasibility and design verification stage met the outlined requirements to demonstrate the effectivity of the proposed autodilution for the ARCHITECT HBsAg assay.
- The initial samples set of 241 samples showed slope values of 0.9 and 1.0 for the 1:150 and 1:500 autodilutions respectively in correlation plots of manual versus auto-dilution (Table 1).
- The subsequent sample set of 103 samples was tested on the manual and 1:500 dilutions only and showed slope values of 0.96 and 0.97 in the correlation plot of manual versus auto-dilution through Least Squares and Passing Bablok regression respectively (Table 2).

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Results (cont'd)

Dilution factor	Result	Instrument 1	Instrument 2	Pooled data over 2 instruments
150	r^2 value of correlation	0.934	0.958	0.961
	Slope of correlation	0.9	0.9	0.9
500	r^2 value of correlation	0.982	0.987	0.989
	Slope of correlation	0.9	1	1

Table 1: Feasibility results from correlation slopes of auto and manual dilutions

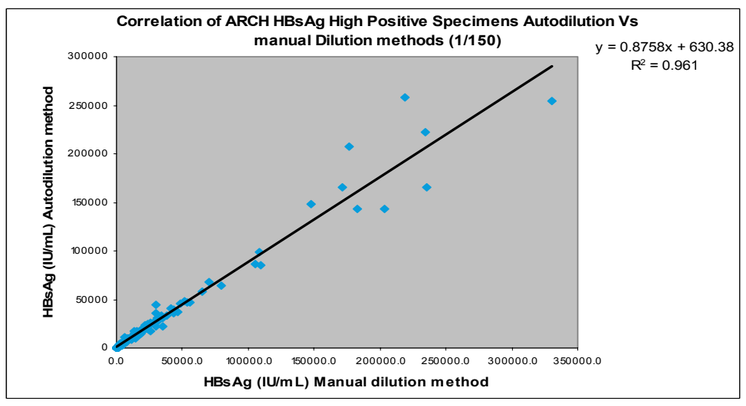


Figure 1: Correlation of manual dilutions versus 1/150 auto dilution.

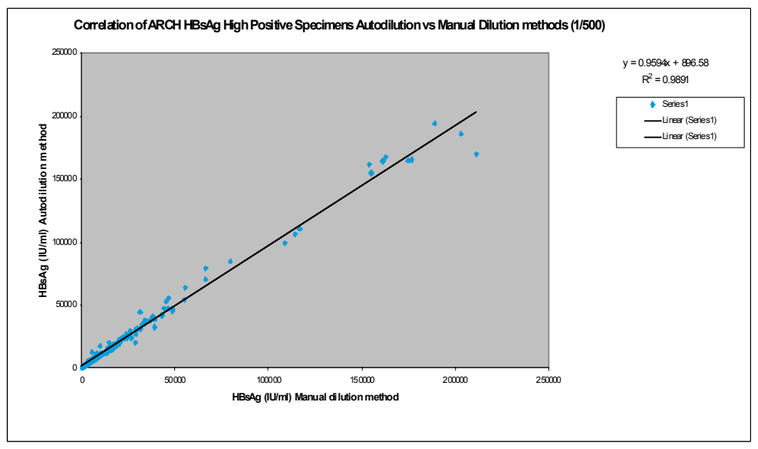
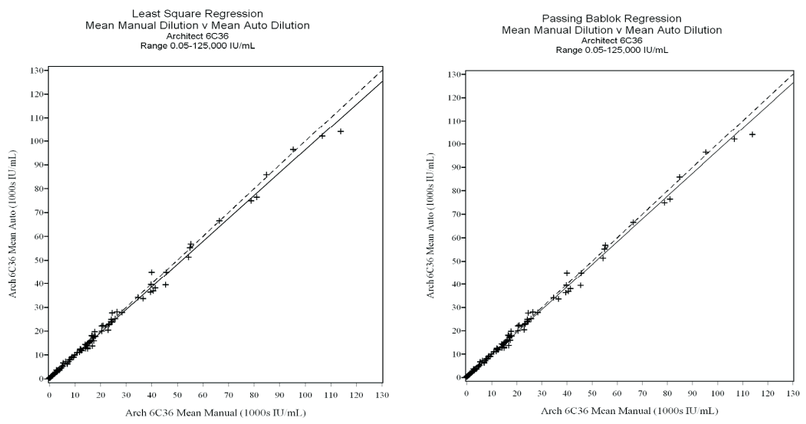


Figure 2: Correlation of manual dilutions versus 1/500 auto dilution.

Regression analysis method	Slope Reorted	Acceptance
Passing Bablok	0.96	1.0 +/- 0.1
Least Squares	0.97	1.0 +/- 0.1

Table 2: Design Verification results from correlation slope of auto and manual dilutions



Summary Statistics

n = 103			
r = 1.00	(95% CI =	1.00, 1.00)	
Slope = 0.96	(95% CI =	0.95, 0.97)	
Intercept = 0.14	(95% CI =	-0.26, 0.53)	
Arch 6C36 Mean Manual (1000s IU/mL)	(Min, Max =	0, 114)	
Arch 6C36 Mean Auto (1000s IU/mL)	(Min, Max =	0, 104)	

Figure 3: Design Verification Least Square Regression of manual dilutions versus 1/500 auto dilution.

Summary Statistics

n = 103			
r = 1.00	(95% CI =	1.00, 1.00)	
Slope = 0.97	(95% CI =	0.96, 0.99)	
Intercept = -0.01	(95% CI =	-0.08, 0.05)	
Arch 6C36 Mean Manual (1000s IU/mL)	(Min, Max =	0, 114)	
Arch 6C36 Mean Auto (1000s IU/mL)	(Min, Max =	0, 104)	

Figure 4: Design Verification Passing Bablok Regression of manual dilutions versus 1/500 auto dilution.

Precision Comparison

				Within-run		Between-run		Between-day		Within-Laboratory (Total)			
sample	Dilution	N	Mean (IU/mL)	SD	%CV	SD	%CV	SD	%CV	SD	SD Upper 95% CL	%CV	%CV Upper 95% CL
A	Auto	40	2957.47	101.39	3.43	10.239	0.35	44.509	1.5	111.202	141.617	3.76	4.79
B	Auto	40	1521.1	45.182	2.97	0	0	0	0	45.182	55.699	2.97	3.66
C	Auto	40	770.41	21.008	2.73	3.696	0.48	4.862	0.63	21.878	27.155	2.84	3.52
A	Manual	40	2872.65	195.431	6.8	152.533	5.31	83.417	2.9	261.568	350.633	9.11	12.21
B	Manual	40	1416.7	119.916	8.46	93.338	6.59	0	0	151.96	198.174	10.73	13.99
C	Manual	40	716.34	50.782	7.09	0	0	40.275	5.62	64.814	91.667	9.05	12.8

Table 4: Precision Comparison between auto and manual dilution on ARCHITECT *i*1000 and *i*2000 platforms

- Precision of three representative samples was assessed on the ARCHITECT *i*1000 and *i*2000 platforms.
- Table 4 shows that the precision values (%CV) obtained for the automated dilution are better than for those obtained using a manual dilution process.

Discussion

The ultimate goal of HBV therapy is the maximum reduction or loss of HBsAg (21) with but not necessarily including seroconversion to anti-HBs. Prolonged suppression of HBV DNA has been shown to decrease the risk of the development of cirrhosis and hepatocellular carcinoma (22). Quantitation of HBV has a growing clinical utility in the monitoring of therapy in the case of chronic Hepatitis B (23). Therapy in these cases may include treatment by pegylated interferon or with nucleos(t)ide analogues (23). Studies have suggested the use of HBsAg as a biomarker for the prognosis and response to therapy in cases of chronic Hepatitis B (24). It has been shown that HBsAg titers can correlate with Serum HBV DNA and intrahepatic cccDNA levels, with some variation in the different disease phases (21, 24, 25, 26).

Quantitation of serum HBsAg may also be utilised to distinguish between different phases of chronic Hepatitis B infection (26) and serum HBsAg may act as a marker for the identification of inactive carriers (27).

The results presented indicate that an autodilution can be introduced to the current ARCHITECT HBsAg assay and will be facilitated by the introduction of new size codes (6C36-41 and 6C36-42) which will have an associated new assay file and an on-board diluent as part of the reagent kit.

Launch of the new size codes for the 6C36 assay is scheduled for the first quarter of 2012.

Conclusion

- The data presented supports the introduction of a new autodilution option for the ARCHITECT HBsAg Assay (6C36).
- Preparation for launch is in process in the ABOTT Diagnostics manufacturing plant in Sligo, Ireland to facilitate the release of two kit sizes, 6C36-41 which will provide a 1x 100 test kit and 6C36-42 which will provide a 1x 400 test kit.
- New ARCHITECT HBsAg Reagents, LN 6C36-41 (1 X100T) and LN 6C36-42 (4x100T) will provide a 1:500 automated dilution for patient samples greater than 250IU/mL. This will provide the option of running the sample undiluted or pre-selecting Automated Dilution which allows default dilution to be set to 1:500.
- The new ARCHITECT HBsAg Reagent will require the use of a new assay file HBsAg Auto (707_002).
- The new ARCHITECT HBsAg reagent kit will contain an additional reagent bottle, ARCHITECT HBsAg Assay Diluent (6C36J) to facilitate the auto dilution function to be utilized. No changes were made to the existing reagent components.
- The option to manually dilute a sample using the ARCHITECT HBsAg Manual Diluent (6C36-40) will still exist.

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