ARCHITECT Hepatitis B Surface Antigen Assay: Sensitivity and Mutant Detection Improvements*

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Abstract

Hepatitis B virus (HBV) infection continues to be a global public health problem. Efficient diagnosis of HBV surface antigen (HBsAg) is critical for identification of infection, treatment and prevention of transfusion transmitted viral infections. Improvement in HBsAg assay sensitivity is essential in order to reduce the early window period during the acute phase of the disease. In addition, the assay must detect HBsAg mutants that are continually being generated due to vaccine escape, immune selection and an error prone reverse transcriptase enzyme. A prototype HBsAg assay with enhanced sensitivity and mutant detection was developed for the fully automated ARCHITECT platform.

The current ARCHITECT HBsAg assay utilizes monoclonal antibody coated magnetic microparticles for capture of HBsAg and an acridinium (ACR)-labeled goat polyclonal conjugate for detection. To improve sensitivity for detection of HBsAg and mutants, a large panel of monoclonal antibodies was evaluated and new antibodies binding to specific epitopes were selected for use in the prototype assay. In addition, modifications to the assay protocol were implemented. The sensitivity of the ARCHITECT HBsAg assay was improved over 5 fold as compared to the current assay. The improvement in sensitivity resulted in detection of additional acute phase samples as evident by performance on 21 seroconversion panels. The ARCHITECT HBsAg prototype assay detected earlier bleeds in over the half of the panels compared to the current assay. The improved prototype assay was also evaluated using a panel of HBsAg mutants. All the mutants were efficiently detected by the improved prototype assay with high signal to cutoff ratio. The sensitivity improvement did not compromise the assay specificity. In summary, the selection of new monoclonal antibodies for HBsAg capture on microparticles as well as the conjugate and modification of the assay protocol resulted in development of an improved assay with high sensitivity and enhanced mutant detection.

Introduction

The current ARCHITECT HBsAg assay is a 2-step immunoassay, using Chemiluminescent Microparticle Immunoassay (CMIA) technology for the detection of HBsAg in human serum and plasma on the ARCHITECT *i*2000 and *i*2000SR instruments.

The ARCHITECT HBsAg assay improvement goals:

(1) Sensitivity equal to the current best in class Abbott PRISM HBsAg assay.

(2) Enhanced sensitivity for mutant detection.

Methods and Procedures

Method: ARCHITECT Prototype Assay Reagents

HBsAg Assay	Current ARCHITECT	*ARCHITECT Prototype
Solid Phase	Monoclonals	Monoclonals
Conjugate	Goat Polyclonal-ACR	Goat Polyclonal-ACR
		Monoclonals

* In development

- Evaluated monoclonal antibodies for inclusion on capture and detection phase
- Analytical sensitivity determined by Abbott sensitivity panels and linear regression analysis
- Evaluated mutant detection with Abbott recombinant HBsAg panel
- Specificity determined by testing 2000 normal blood donor samples
- Seroconversion sensitivity evaluated with 21 HBV seroconversion panels

Results

Analytical Sensitivity

Sensitivity Panel Series	ARCHITECT Assay	*ARCHITECT Prototype Assay	PRISM Assay
Abbott HBsAg Sensitivity Panel (ad subtype)	0.15 ng/mL	0.051 ng/mL	0.100 ng/mL
Abbott HBsAg Sensitivity Panel (ay subtype)	0.13 ng/mL	0.031 ng/mL	0.080 ng/mL

* In development

The ARCHITECT prototype assay is ~ 2-3 fold for ad subtype and ~ 4-6 fold for ay subtype more sensitive than current ARCHITECT and PRISM HBsAg assays.

HBsAg Mutant Detection

HBsAg Recombinants	Current ARCHITECT Assay	ARCHITECT Prototype Assay
Mutant Panel	S/CO (Positive S/CO >1)	S/CO (Positive S/CO >1)
Gln-129-His	19.4	79.9
Met-133-Leu	15.9	56.3
Asp-144-Ala	9.8	57.9
Gly-145-Arg	12.0	17.7
Pro-142-Leu + Gly-145-Arg	8.8	13.0
Pro-142-Ser + Gly-145-Arg	8.4	12.1
Thr-123-Ala	7.1	66.9
122 Asn Thr Insertion	4.6	5.3
122 Arg Ala Insertion	5.6	7.4
Negative Control	**0.5**	**0.2**
Positive Control	23.4	77.6

The ARCHITECT prototype assay detects all mutants with a higher signal to cutoff ratio than the current ARCHITECT assay.

Results (cont.)

Seroconversion Sensitivity: ARCHITECT Prototype Assay vs PRISM Assay

	Number of Bleeds Detected from Each Panel	
Panel ID	PRISM Assay	ARCHITECT Prototype Assay
902	8	8
907	5	6
914	5	5
915	12	12
916	3	3
922	9	10
923	2	2
924	4	4
926	6	6
928	5	5
929	5	6
931	6	6
932	9	9
6271	4	4
6272	7	8
6273	4	3
6274	7	7
6281	7	7
6283	5	5
6288	6	6
6291	3	3
Total Number of Bleeds Detected	**122**	**125**

The ARCHITECT Prototype assay sensitivity was comparable to the PRISM assay for seroconversion detection.

Seroconversion Sensitivity: ARCHITECT Prototype vs Current ARCHITECT Assay

	Number of Bleeds Detected from Each Panel	
Panel ID	Current ARCHITECT Assay	ARCHITECT Prototype Assay
902	6	8
907	5	6
914	4	5
915	5	12
916	2	3
922	7	10
923	2	2
6271	2	4
6272	5	8
6273	2	3
6274	6	7
Total Number of Bleeds Detected	**46**	**68**

The ARCHITECT prototype assay was more sensitive than the current ARCHITECT assay for seroconversion detection.

Summary: ARCHITECT HBsAg Assay Sensitivity and Mutant Detection Improvement

HBsAg Assay	Current ARCHITECT	ARCHITECT Prototype
Analytical Sensitivity	ad = 0.15, ay = 0.13 ng/ml	ad = 0.051, ay = 0.031 ng/ml
Seroconversion Sensitivity	Good	Comparable to PRISM assay
Specificity	I R = 0.46% R R = 0.16% 99.87%	I R = 0.30% R R = 0.15% 99.85%
Mutant Detection	Broad	Broad & More Sensitive

I R = Initial Reactive; R R = Repeat Reactive

Conclusions

- The ARCHITECT HBsAg prototype assay is ~ 3-4 fold more sensitive than the current ARCHITECT HBsAg assay without compromising specificity.
- The ARCHITECT HBsAg prototype assay sensitivity improvements were demonstrated based on the performance of the assay in detection of HBsAg mutants and seroconversion panels.
- The ARCHITECT HBsAg prototype assay offers high-throughput automation for diagnosis and management of HBV infection.