CPAL
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Laboratory

Technical Bulletin
No. 125

May 29, 2014

Hepatitis B Surface Antibody
- Testing System Change –

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Affected Tests:

Mnemonics:	**HBSAB QNT**
Test Name:	Hepatitis B: HBsAb Quant
Test Number:	**1750042**
Specimen:	0.5 mL Serum, 2-8º C 7 days

Effective Date: June 16, 2014

Performed: Monday through Saturday

Method Change: HBsAb testing will be moved from the Siemens Immulite 2000 analyzer to the Abbott Architect i2000 analyzer. This will eliminate the need for split samples when other hepatitis or HIV testing are on the same order.

Background:
The Architect AUSAB assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of antibody to Hepatitis B surface antigen (anti-HBs) in human serum and plasma. It is intended for quantitative measurement of antibody response following hepatitis B virus (HBV) vaccination, determination of HBV immune status, and for the laboratory diagnosis of HBV disease associated with HBV infection when used in conjunction with other laboratory results and clinical information.

Hepatitis B virus (HBV) is a major cause of liver disease and is endemic worldwide. The virus can be transmitted through direct contact with blood and body fluids including sexual contact. The incubation period for HBV infection can range from 1 to 6 months averaging around 6 to 8 weeks. Typical acute clinical symptoms of HBV hepatitis include malaise, jaundice, gastroenteritis, and fever. However, HBV infection can also result in subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. Although most adult patients with HBV infection completely recover from acute illness and clear the virus, 5 to 10% of patients with HBV may become chronic carriers. It is estimated that over 300 million people worldwide are chronic carriers of the virus. Chronic HBV infection is associated with the development of hepatocellular carcinoma. In HBV infected neonates, approximately 90% develop chronic hepatitis B infection.

Anti-HBs assays are often used to determine the success of hepatitis B vaccination. The presence of anti-HBs has been shown to be important in protection against HBV infection. Numerous studies have demonstrated the

effectiveness of the hepatitis B vaccine to stimulate the immune system to produce anti-HBs and to prevent HBV infection.

Assays for anti-HBs are also used to monitor the convalescence and recovery of hepatitis B infected individuals. The presence of anti-HBs after acute HBV infection and loss of hepatitis B virus surface antigen (HBsAg) can be a useful indicator of disease resolution. Detection of anti-HBs in an asymptomatic individual may indicate previous exposure to HBV or HBV vaccination.

Principle of Test:
The Architect AUSAB assay is a two-step immunoassay for the quantitative determination of HBsAb in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. In the first step, sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. Anti-HBs present in the sample binds to the rHBsAg coated microparticles. After washing, acridinium-labeled rHBsAg conjugate is added in the second step. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of anti-HBs in the sample and the RLUs detected by the Architect *i* System optics. The concentration of anti-HBs in the sample is determined using an active Architect AUSAB calibration curve.

Results Interpretation:
The Architect HBsAb test is interpreted as Reactive, GrayZone, or Nonreactive based upon the interpretive criteria presented in Table 1 below.

Table 1	**Architect AUSAB Interpretation**		
Initial Result	**Retest Result**	**Result**	**Interpretation**
<8.00 mIU/mL	No retest required	Nonreactive	Individual is considered not immune to HBV infection
≥ 8.00 mIU/mL to <12.00 mIU/mL	Both of the retest results are < 8.00 mIU/mL	Nonreactive	Individual is considered not immune to HBV infection
	One or both of the retest results are ≥ 8.00 mIU/mL to < 12.00mIU/mL	Grayzone Reported as Indeterminate	The immune status of the individual should be further assessed by considering other factors such as clinical status, follow up testing, associated risk factors, and the use of additional diagnostic information.
	Both of the retest results are ≥ 12.00 mIU/mL	Reactive	Individual is considered to be immune to HBV infection
≥ 12.00 mIU/mL	No retest required	Reactive	Individual is considered to be immune to HBV infection

Limitations:
1. For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.
2. A nonreactive test result does not exclude the possibility of exposure to hepatitis B virus.
3. Results obtained with the ARCHITECT assay for HBsAb may not be used interchangeably with values obtained with different manufacturers' assay methods.
4. Results from immunosuppressed patients should be interpreted with caution.
5. Assay does not differentiate between vaccines and natural infections.
6. Performance characteristics have not been established for therapeutic monitoring.
7. A reactive anti-HBs result does not exclude co-infection by another hepatitis virus.

Validation Data:

Precision

Manufacturer's criteria for precision are within run and between run CV<10% for results within the grayzone and reactive ranges. No claims are made for results in the nonreactive range. Laboratory evaluation criteria for negative results were 100% interpretive agreement. All precision criteria were met (Tables 2 and 3).

Table 2 Within Run Precision

mIU/mL Interp	% Agreement	mIU/mL Interp	% CV % Agreement
0.0410 Nonreactive	100%	15.035 Reactive	4.3% 100%

Table 3 Between Run Precision

mIU/mL Interp	% Agreement	mIU/mL Interp	% CV % Agreement
0.0285 Nonreactive	100%	14.396 Reactive	4.9% 100%

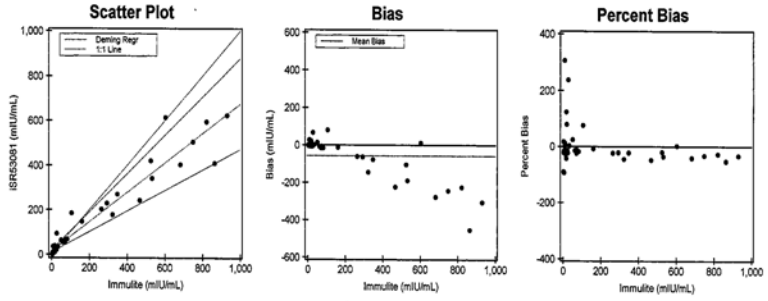
Linearity

The linear range of the assay was tested using six standards that span the measurement range of 0.0 – 1000 mIU/mL. Regression analysis yielded a slope of 0.980, intercept of 0.016 and total error of 2.6%.

Method Comparison

A total of 41 specimens were split and analyzed utilizing Siemens Immulite 2000 and Abbott Architect i2000 assays. Architect results were compared to Immulite results. Quantitative analysis yielded a Correlation Coefficient (R) of 0.9612 and average bias of -31.256%. (Figure 1)

Figure 1: HBsAb Quantitative Method Comparison Architect i2000 vs Immulite 2000



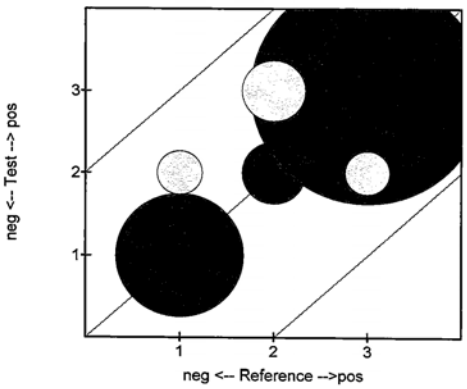
Regression Analysis

	Deming	Regular
Slope	0.661 (0.599 to 0.723)	0.645 (0.583 to 0.707)
Intercept	14.598 (-7.336 to 36.531)	17.960 (-3.894 to 39.813)
Std Err Est	54.208	54.009

95% Confidence Intervals are shown in parentheses

Semi-Quantitative analysis was also performed to determine level of agreement and symmetry. Out of 41 specimens, there were four that did not have perfect agreement. For each of these, one instrument showed results in the grayzone, while the other showed either reactive or nonreactive interpretations. The tests for agreement and symmetry passed. (Figure 2)

Figure 2: **HBsAb Semi-Quantitative Method Comparison. Architect i2000 vs Immulite 2000**



Statistical Analysis
(Comparison of two Laboratory Methods)

Agreement 90.2% (77.5 to 96.1%)
95% confidence interval calculated by the "Score" method.

McNemar Test for Symmetry:
Test < Reference 1 (2.4%)
Test > Reference 3 (7.3%)
Symmetry test PASSES p = 0.625 (Exact Test)
A value of p<0.05 suggests that one method is consistently "larger".

Cohen's Kappa 79.0% (59.5 to 98.6%)
Kappa is the proportion of agreement above what's expected by chance. Rule of thumb is Kappa>75% indicates "high" agreement. We would like to see VERY high (close to 100%) agreement.

Statistical Summary

Test	Reference 1	2	3	Total
1	8	--	--	8
2	1	2	1	4
3	--	2	27	29
Total	9	4	28	41

Number excluded or missing: 0

Legend

	Reference	Test
1	NonReactiv (N)	NonReactiv (N)
2	GrayZone (GZ)	GrayZone (GZ)
3	Reactive (R)	Reactive (R)

References:

1. Architect System AUSAB package insert; 6/06.