

# HBsAg blood screening and diagnosis: performance evaluation of the ARCHITECT HBsAg qualitative and ARCHITECT HBsAg qualitative confirmatory assays

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## Abstract

A low initial reactive rate for screening assays is important for time- and cost-effective infectious disease testing. Therefore, the new ARCHITECT HBsAg Qualitative screening assay, in conjunction with the new ARCHITECT HBsAg Qualitative Confirmatory assay, was introduced. As the role of hepatitis B surface antigen (HBsAg) as surrogate marker for HBV resolution and the monitoring of drug effectiveness are becoming increasingly important, the established ARCHITECT HBsAg Quantitative assay remains available on the market. Precision, sensitivity, and specificity of the newly developed screening assay were in the range of established HBsAg assays. Seroconversion sensitivity was slightly superior compared to other commercially available assays. An initial reactive rate of 0.2% (without HBsAg-confirmed positive samples of 0.17%) for the ARCHITECT HBsAg Qualitative assay was observed. As the new screening assay is a 1-step assay format, the “high-dose hook effect” was investigated to assess the risk of false-negative results, but even very high positive HBsAg samples obtained signals clearly above the cutoff.

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## 1. Introduction

Hepatitis B virus infection is one of the world's most prevalent infectious diseases and a serious global health problem (Lavanchy, 2004). Hepatitis B surface antigen (HBsAg) screening assays are used to identify persons infected with HBV and to prevent transmission of the virus by blood and blood products as well as to monitor the status of infected individuals in combination with other hepatitis B serologic markers. In most countries, testing for HBsAg is part of the antenatal screening program to identify HBV-infected mothers and to prevent perinatal HBV infection by subsequent immunization. HBsAg

quantitative determination has been discussed as a valuable tool in HBV disease management to determine the effectiveness of drug treatment (Kohmoto et al., 2005; Manesis et al., 2007; Zoulim and Perrillo, 2008). HBsAg as a surrogate marker for intrahepatic cccDNA clearance during drug treatment and therefore as a potential marker for HBV disease resolution (Chan et al., 2007; Wursthorn et al., 2006) requires an assay, like ARCHITECT HBsAg, that monitors HBsAg concentration by quantitation.

Meeting the requirements for screening as well for monitoring in one assay is a challenging prospect. A screening assay must be highly sensitive with a maximum specificity, while exhibiting a low initial reactive rate, which saves time and improves cost and effectiveness. This can be realized with an assay that exhibits a large interval between signals obtained from the sera of a representative negative population and the cutoff. The ARCHITECT HBsAg Qualitative assay was designed to meet this requirement.

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Testing various approaches resulted in the new ARCHITECT HBsAg Qualitative assay that uses a unique assay format called modified 1-step assay. In this study, an issue generally exhibited by 1-step assay known as “high-dose hook effect” (Fernando and Wilson, 1992; Hoofnagle and Wener, 2009) which increases the risk of false-negative results was evaluated.

In this publication, we report the performance data of the new ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays and discuss the potential risk of a high-dose hook effect.

## 2. Material and methods

### 2.1. Assay format

The ARCHITECT HBsAg Qualitative (1P97) assay (Abbott Ireland, Diagnostics Division, Sligo, Ireland) is a modified 1-step immunoassay for the qualitative detection of HBsAg in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology (Supplementary Fig. 1).

Samples, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pretrigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs) and is proportional to the bound antigen.

The ARCHITECT HBsAg Qualitative Confirmatory assay (Abbott Ireland, Diagnostics Division, Sligo, Ireland) (1P98) is a CMIA used for confirming the presence of HBsAg in human serum and plasma by means of specific antibody neutralization. The assay consists of 2 single tests that are both modified 1-step pretreatment immunoassays.

The sample and pretreatment 1 are combined in a reaction vessel (RV) and incubated. If HBsAg is present in the sample, it is neutralized by the antibody in pretreatment 1. An aliquot of the pretreated sample is added to the reaction mixture as explained above. Nonneutralized HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the RV and the sample is processed as explained above (Supplementary Fig. 2).

This sequence is repeated for the sample combined with pretreatment 2, which does not contain neutralizing antibodies (Supplementary Fig. 3). If the signal for the nonneutralized sample (incubated with pretreatment 2) result is greater than or equal to the cut-off ( $S/CO \geq 0.70$ ) and the RLU of the neutralized sample is reduced by at least 50% compared to the nonneutralized sample, the sample is considered confirmed for HBsAg.

The ARCHITECT HBsAg (6C36) assay (Abbott Ireland, Diagnostics Division, Sligo, Ireland) is a quantitative 2-step assay in which the sample and the microparticle are incubated in a first step. After washing, the conjugate is added in a second step (Deguchi et al., 2004). ARCHITECT HBsAg assay was used to determine the concentration of HBsAg in a positive specimen during the high-dose hook study.

### 2.2. Precision

Precision was assessed for the new screening assay on a panel consisting of 1 human plasma sample positive for HBsAg and 3 lots of the positive and negative control. Internally, a 20-day precision study based on guidance from the CLSI document (Clinical and Laboratory Standards Institute) EP5-A2 was performed across 3 different reagent lots, 3 different calibrator lots, and 2 different instruments. Each panel member was tested in 2 replicates. At the 2 external evaluation sites, a 5-day precision study based on guidance from the CLSI document EP15-A2 was performed across 3 different reagent lots, 1 calibrator lot, and 1 instrument per site. Each panel member was tested in 3 replicates. For both precision studies 1 control lot was used to validate the calibrations.

### 2.3. Specimens for specificity assessment

A total of 5895 randomly selected serum and plasma samples were collected from volunteer blood donors at 5 blood-donation centers in Germany, Italy, and Portugal.

In addition, 619 randomly selected clinical specimens were collected from hospitalized patients at one hospital in Germany and Portugal.

### 2.4. Specimens for sensitivity assessment

Sensitivity was assessed on 506 samples from HBsAg-positive patients including samples from patients with chronic hepatitis B infection and samples with different HBsAg subtypes. Additionally, 33 seroconversion panels from different commercial suppliers (ZeptoMetrix, Buffalo, NY; Boston Biomedica, West Bridgewater, MA) and the WHO Reference Panel (National Institute for Biological Standards and Control [NIBSC] code 03/262, traceable to the Second International Standard for HBsAg, subtype adw2, genotype A, NIBSC code 00/588) were tested.

### 2.5. HBsAg mutant detection

A panel consisting of 9 different recombinant HBsAg mutant samples was tested. The mutant panel consisted of the following mutations: Thr 126 to Ser; Gln 129 to His; Met 133 to Leu; Asp 144 to Ala; Gly 145 to Arg; Thr 126 to Ser + Gly 145 to Arg; Pro 142 to Leu + Gly 145 to Arg; Pro 142 to Ser + Gly 145 to Arg; Asp 144 to Ala + Gly 145 to Arg.

Table 1  
Precision

Study	Member	n	Mean S/CO	Intra-run		Inter-run	
				SD	%CV	SD	%CV
20-day precision	NC	1440	0.29	0.041	13.8	0.042	14.3
	PC	1440	3.74	0.071	1.9	0.098	2.6
	Plasma	480	1.77	0.053	3.0	0.068	3.8
5-day precision	NC	270	0.35	0.072	20.8	0.080	22.9
	PC	270	3.85	0.090	2.3	0.109	2.8
	Plasma	90	1.89	0.047	2.5	0.074	3.9

Assay reproducibility was assessed for ARCHITECT HBsAg Qualitative on a panel consisting of 1 human plasma sample positive for HBsAg and 3 lots of the positive and negative control. Twenty-day precision study was performed internally across 3 different reagent lots and 2 different instruments. Each panel member was tested in 2 replicates. Five-day precision study was performed at external sites across 3 different reagent lots and 1 instrument per site. Each panel member was tested in 3 replicates.

### 2.6. Potential interfering substances

Potential interference was assessed on 261 HBsAg-negative and unspiked specimens (259 for ARCHITECT HBsAg Qualitative Confirmatory assay) and on a total of 258 specimens spiked with HBsAg-positive material. Serum samples from the following categories were tested: viral infection (*HTLV-I*, *HSV*, *CMV*, *HCV*, *EBV*, *HIV-1*, *HIV-2*); fungal/yeast/protozoal/bacterial infection (*Treponema pallidum*, *Toxoplasma gondii*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*); autoimmunity (rheumatoid factor, antinuclear antibodies [ANA]); other conditions (pregnant females all trimesters, multiparous females, immunoglobulin from monoclonal and polyclonal gammopathy for IgG and IgM or multiple myeloma, influenza vaccine recipients, hemodialysis patients, hemophiliacs, multiple transfusion recipients, human anti-mouse antibody).

### 2.7. Specificity and sensitivity calculation

Specificity was defined as the percentage of HBsAg-negative specimens correctly identified as nonreactive and

was calculated as:  $[\text{true negative}/(\text{true negative} + \text{false positive})] \times 100$ .

Sensitivity was defined as the percentage of HBsAg-positive specimens correctly identified as reactive and was calculated as follows:  $[\text{True Positive}/(\text{True Positive} + \text{False Negative})] \times 100$ .

### 2.8. Supplemental testing

Specimens that were initial and repeat reactive (S/CO  $\geq 1.00$ ) with the ARCHITECT HBsAg Qualitative assay were confirmed with the ARCHITECT HBsAg Qualitative Confirmatory assay and the PRISM HBsAg (Abbott, Wiesbaden, Germany) and PRISM HBsAg Confirmatory assay (Abbott, Wiesbaden, Germany).

### 2.9. HBsAg concentration determination of high positive samples

Due to quantification limitations of available HBsAg immunoassays, the determination of HBsAg concentration was performed by diluting the neat HBsAg samples and testing the diluted samples with the ARCHITECT HBsAg assay. The HBsAg concentration was recalculated by multiplying with the dilution factor.

## 3. Results

### 3.1. Precision

The inter-run precision for the 5- and for the 20-day precision studies was 2.8% and 2.6% for the positive control, respectively, and 3.9% and 3.8% for a weak positive sample, respectively (Table 1).

### 3.2. Specificity

The specificity of the ARCHITECT HBsAg Qualitative assay was estimated in low-prevalence blood donor populations (Table 2). Specificity was 99.97% for the ARCHITECT HBsAg Qualitative assay. Two blood donor samples which were initial and repeat reactive were confirmed by the respective confirmation assay and by the PRISM HBsAg

Table 2

Comparison of assay specificity based on data from seronegative blood donor and hospitalized/ diagnostic specimens

Type	n	ARCHITECT HBsAg Qualitative					
		Mean S/CO	SD	SD to cutoff <sup>a</sup>	IR (%)	RR (%)	Specificity (CI)
Overall blood donors	5895	0.31	0.067	10.3	12 (0.20)	4 (0.07)	99.97% (99.88–100%)
Blood donor plasma	3110	0.31	0.061	11.2	9 (0.29)	3 <sup>a</sup> (0.10)	99.97% (99.82–100%)
Blood donor serum	2785	0.30	0.072	9.7	3 (0.11)	1 (0.04)	99.96% (99.80–100%)
HP/Diagnostics	619	0.30	0.057	12.2	3 (0.48)	0	100% (99.41–100%)

Specificity was tested on 5895 randomly selected serum and plasma samples that were obtained from volunteer blood donors at 5 blood donor centers in Germany, Italy, and Portugal. Additional 619 randomly selected serum clinical specimens were collected from hospitalized patients at one hospital in Germany and Portugal. The separation of the population from the cutoff (S/CO = 1.00 for ARCHITECT HBsAg Qualitative) value is determined by the following formula:  $\text{SD to cutoff} = (1 - \text{mean S/CO})/\text{SD}$ .

<sup>a</sup> In 2 of 3 samples, presence of HBsAg was confirmed with PRISM HBsAg, PRISM HBsAg Confirmatory, and ARCHITECT HBsAg Qualitative Confirmatory assays. S/CO = Sample to cutoff, IR = initial reactives, RR = repeat reactives, SD = standard deviation, CI = confidence interval.

### Blood Donors

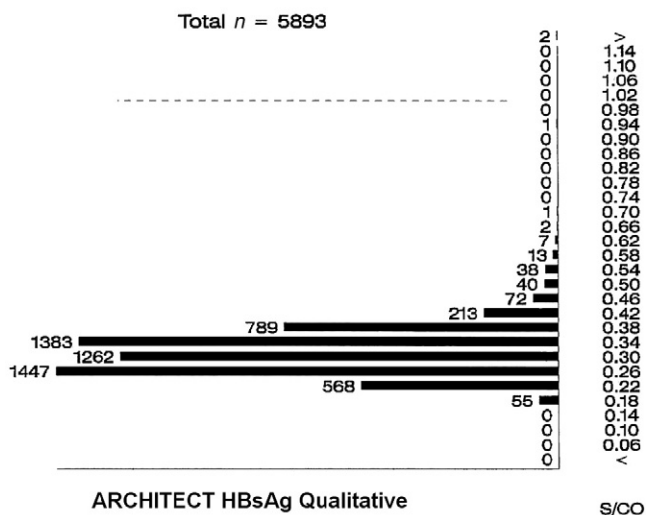


Fig. 1. Distribution of random blood donor population. Specificity distribution of a random blood donor population tested on the ARCHITECT HBsAg Qualitative assay. In total 5893 samples were tested; 2 repeat reactive and confirmed positive samples were excluded from this calculation (dotted line represents the cutoff; S/CO = sample to cutoff).

and PRISM HBsAg Confirmatory assay, resulting in an initial reactive rate of 0.20% on the screening assay on the blood donor population. The new screening assay demonstrated a narrow distribution of S/CO values for the blood donor population (Fig. 1). The cutoff value was 10.3 standard deviations (SD) above the mean of the blood donor population.

Additionally, the specificity of the ARCHITECT HBsAg Qualitative assay was calculated by examining a diagnostic population (Table 3). Based on randomly selected hospital patient specimens, the specificity for the assay was 100%. The cutoff value for the ARCHITECT HBsAg Qualitative assay was 12.2 SD above the mean of the diagnostic population (Fig. 2).

### 3.3. Sensitivity

Evaluation of sensitivity was performed on 506 HBsAg-positive specimens. The HBsAg Qualitative assay showed

Table 3  
Comparison of assay sensitivity on precharacterized HBsAg-positive specimens

Specimen	n	ARCHITECT HBsAg Qualitative		
		INR (%)	RNR (%)	Sensitivity (%)
HBsAg Positive	461	1 (0.22)	1 (0.22)	99.78 (460/461)
Chronic HBsAg	45	0	0	100 (45/45)
Total	506	1 (0.20)	1 (0.20)	99.80 (505/506)

Sensitivity was assessed on 506 samples from HBsAg-positive patients including samples from patients with chronic hepatitis B infection and samples with different HBsAg subtypes. INR = Initial nonreactives; RNR = repeat nonreactives.

99.80% sensitivity (Table 3). One sample which was initial and repeat nonreactive was tested reactive on PRISM HBsAg (1.43 S/CO, 2.58 S/CO, and 1.74 S/CO) and confirmed with the PRISM HBsAg Confirmatory assay (1.89 S/CO, neutralization: 103%). This specimen was also tested low reactive (1.06 S/CO, 1.05 S/CO, 1.04 S/CO; neutralization: 102%; 100 copies/mL) by the specimen supplier.

### 3.4. Seroconversion sensitivity

Thirty-three commercially available seroconversion panels were tested (data of 25 panels not shown). To classify the performance of the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays, the results were compared to data of 8 recently published seroconversion panels (Muhlbacher et al., 2008) by utilizing the calculation model of time delays (Table 4).

Mean number of delayed days to seroconversion detection was 5.38 for commercial assay 1 and commercial assay 2 (total number of days = 43) compared to 3.13 days (total number of days = 25) for the ARCHITECT HBsAg Qualitative assay. All reactive samples were confirmed by the ARCHITECT HBsAg Qualitative Confirmatory assay. Detailed results are shown in Supplementary Table 1.

### 3.5. Analytical sensitivity

The analytical sensitivity of the ARCHITECT HBsAg Qualitative assay was estimated by testing the WHO Reference Panel NIBSC code 03/262 (traceable to the Second International Standard for HBsAg, subtype adw2,

### Diagnostic / Hospitalized Donors

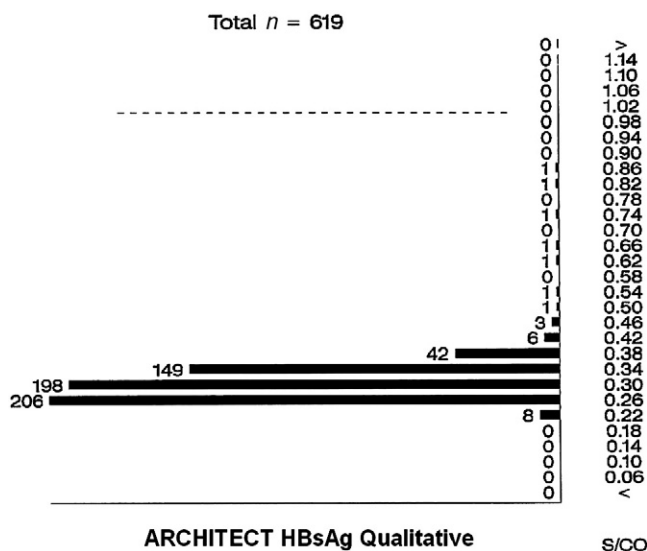


Fig. 2. Distribution of overall hospitalized/diagnostic specimens. Specificity distribution of a random hospitalized/diagnostic specimen population tested on the ARCHITECT HBsAg Qualitative assay. In total 619 samples were tested (no repeat reactive samples were observed). Dotted line represents the cutoff; S/CO = sample to cutoff.



Table 4  
Seroconversion sensitivity

Seroconversion panel	HBV-DNA PCR	Commercial assay 1	Commercial assay 2	ARCHITECT HBsAg Qualitative	ARCHITECT HBsAg Qualitative Confirmatory
PHM 903	0	7	7	7	7
PHM 904	0	1	1	1	1
PHM 906	0	0	0	0	0
PHM 909	0	8	8	8	8
PHM 910	0	18	18	1	1
PHM 928	0	7	7	6	6
PHM 932	0	0	0	1	1
PHM 933	0	2	2	1	1
Total number of days	0	43	43	25	25
Mean number of days	0.00	5.38	5.38	3.13	3.13

Eight of 33 evaluated seroconversion panels corresponded to seroconversion panels previously tested and published (Muhlbacher et al., 2008). Results of the 8 seroconversion panels as published are shown and compared to results obtained from the ARCHITECT HBsAg Qualitative and ARCHITECT Qualitative Confirmatory assays.

The day of the blood donation with the first positive result (last negative + 1 day) in comparison to the most sensitive assay has been calculated. The first positive bleed of the most sensitive comparator assay (HBV-DNA PCR) is considered as day 0.

genotype A, NIBSC code 00/588) consisting of 4 different dilution members. Sensitivity was assessed by linear regression analysis across 3 different reagent lots and ranged from 0.041 to 0.049 IU/mL.

### 3.6. Mutant detection

All 9 samples representing different HBsAg mutants were tested reactive across 3 different ARCHITECT HBsAg Qualitative reagent lots (Table 5).

### 3.7. Potentially interfering substances

Two specimens (1 ANA and 1 flu vaccine recipient) of the 261 unspiked specimens tested as initial and repeat reactive on the ARCHITECT HBsAg Qualitative assay and on the ARCHITECT HBsAg Qualitative Confirmatory assay could also be detected by PRISM HBsAg assay and confirmed by PRISM HBsAg Confirmatory assay. The resolved specificity on the remaining 259 unspiked samples was assessed to be 100% (259/259) (95% confidence interval 98.59–100%). The resolved sensitivity on the 258 spiked positive specimens and the 2 naturally reactive samples was

estimated to be 100% (260/260) (95% confidence interval 98.59–100%).

### 3.8. High-dose hook effect

Eighty-one natural HBsAg-positive samples with concentrations ranging from 10 to 178,000 IU/mL were assessed. The highest positive sample still revealed an S/CO of 418. We could define that, for the ARCHITECT HBsAg Qualitative assay, the hook effect starts at approximately 8,000 IU/mL. After spiking a negative sample with recombinant HBsAg to a concentration of  $2.05 \times 10^6$  and  $4.1 \times 10^6$  IU/mL, ARCHITECT HBsAg Qualitative assay could clearly detect this sample with S/COs of 154 and 90, respectively (Fig. 3).

## 4. Discussion

We here presented a comprehensive study to investigate the performance of the new ARCHITECT HBsAg Qualitative screening assay, which was introduced in conjunction with a new ARCHITECT HBsAg Qualitative Confirmatory assay.

Table 5  
Mutant sensitivity

Mutation	Architect HBsAg Qualitative Lot 1 S/CO	Architect HBsAg Qualitative Lot 2 S/CO	Architect HBsAg Qualitative Lot 3 S/CO
Thr 126 to Ser	16.76	15.39	15.42
Gln 129 to His	18.31	16.35	16.90
Met 133 to Leu	16.14	15.33	15.19
Asp 144 to Ala	10.05	9.37	9.64
Gly 145 to Arg	11.56	11.37	10.24
Thr 126 to Ser + Gly 145 to Arg	8.75	8.31	8.09
Pro 142 to Leu + Gly 145 to Arg	9.23	8.87	8.68
Pro 142 to Ser + Gly 145 to Arg	10.69	10.93	9.93
Asp 144 to Ala + Gly 145 to Arg	5.20	5.41	4.75

The mutant sensitivity of the ARCHITECT HBsAg Qualitative assay was evaluated across 3 different reagent lots. Testing was performed internally.

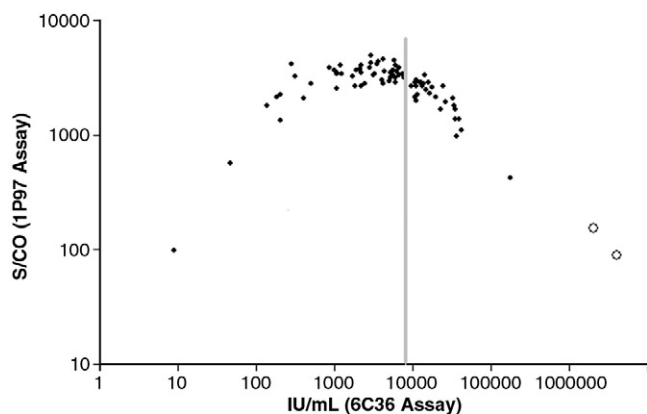


Fig. 3. Hook effect. A total of 83 HBsAg-positive samples were tested on ARCHITECT HBsAg (6C36) and ARCHITECT HBsAg Qualitative (1P97) assay. Samples with a concentration of at least 250 IU/mL were diluted, and the results were recalculated by multiplying with the dilution factor. The line indicates the starting point of the hook effect. Filled circles = Natural HBsAg positive samples; hollow circles = negative samples spiked with recombinant HBsAg.

The initial reactive rate of ARCHITECT HBsAg Qualitative assay of the blood donor population was evaluated at 0.2%. Two of the 12 initial reactive tested blood donors were confirmed positive. Considering these confirmed positive samples the initial reactive rate can be calculated at 0.17%. This is in agreement with previous studies where initial reactive rates were found in the range of 0.16% to 0.32% (Louisirochanakul et al., 2010). In laboratories, high initial reactive rates lead to increased costs (Acar et al., 2010) and the reporting of unclear results to sample donors may cause psychological distress and confusion (Jonas et al., 2005). A low initial reactive rate therefore is a benefit for donors/patients and users.

Testing of precharacterized HBsAg-positive samples revealed a sensitivity of 99.8% for the new screening assay. The discrepant sample showed signals that were very close to the cutoff, and also the supplier information stated an extremely low reactive signal in terms of antigen concentration and PCR results.

The analytical sensitivity for the WHO HBsAg Reference Panel (traceable to the Second International Standard for HBsAg) was confirmed to be in the range of current commercially available HBsAg assays (La'ulu and Roberts, 2006; Nick and Scheiblaue, 2007).

Previously, the seroconversion sensitivity of different commercially available HBsAg screening assays had been published (Muhlbacher et al., 2008). Eight of these panels corresponded with panels used to determine seroconversion sensitivity for the present study. To classify the performance of the ARCHITECT HBsAg Qualitative and ARCHITECT HBsAg Qualitative Confirmatory assays the results of the above mentioned panels were compared to those of the comparator assays. The ARCHITECT HBsAg Qualitative assay was able to detect seroconversion 2.25 days earlier than the comparator assays 1 and 2. These data suggest that

the seroconversion sensitivity of the ARCHITECT assays is slightly superior to that of the comparator assays.

One risk in utilizing assays based on the 1-step principle is the high-dose hook effect (Hoofnagle and Wener, 2009). The hook effect is independent of the analyte and has been reported for HBsAg (Ch'ng et al., 1987; Liu and Green, 1985; Palomaki, 1991) and other 1-step immunoassays as reviewed elsewhere (Frieze et al., 2002). Challenging the new ARCHITECT HBsAg Qualitative screening assay with a panel of natural high-positive HBsAg samples up to 178,000 IU/mL and by spiking a negative sample with recombinant HBsAg to 4,100,000 IU/mL demonstrated that the assay detects the samples with S/COs clearly above the cutoff. During the acute phase of hepatitis infections, the HBsAg concentrations can reach peak levels of 10,000 to 100,000 PEI Units (Gerlich and Kann, 1998) equaling approximately 23,000 to 230,000 IU/mL. We therefore consider the risk of missing a very high positive HBsAg sample as extremely low.

The presented data show that the new ARCHITECT HBsAg Qualitative screening assay exhibits a low initial reactive rate, while displaying a sensitivity and a specificity that are comparable or even superior to well-established commercially available assays (La'ulu and Roberts, 2006; Muhlbacher et al., 2008; Nick and Scheiblaue, 2007). In conjunction with the new ARCHITECT HBsAg Qualitative Confirmatory assay, HBV infections can be reliably detected and confirmed by the neutralization method.

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