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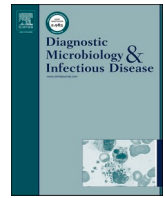
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Performance evaluation of the Access anti-HBc Total assay on the Dxi 9000 Access Immunoassay Analyzer

Simplice Dzamitika^a, Françoise Le Boulaire^a, Catherine Coignard^b, Claire Vincent^c, Jean-Christophe Plantier^d, Véronique Lemée^d, Sandrine Gréaume^e, Isabelle Voisin^e, Etienne Brochet^f, Yves-Edouard Herpe^f, Gaiane Demirdjian^g, Magali Karagueuzian^g, Derrick Afful^h, Rima Bayoudⁱ, Juliane Hey^{i,*}

^a Cerba Xpert, Saint-Ouen l'Aumône, France

^b Infectology, Specialized CoreLab Department, Eurofins Biomnis, Ivry-Sur-Seine, France

^c Biomnis Sample Library Department, Eurofins Biomnis, Ivry-Sur-Seine, France

^d Laboratoire de Virologie, Institut de Biologie Clinique, CHU Rouen, France

^e PLER Laboratory, Etablissement Français du Sang Haute-de-France – Normandie, Bois Guillaume, France

^f Centre de Ressources Biologiques Biobanque de Picardie, CHU Amiens-Picardie, France

^g R&D Department, Beckman Coulter, Immunotech, Marseille, France

^h Clinical Affairs Department, Beckman Coulter, Chaska, MN, United States

ⁱ Clinical Affairs Department, Beckman Coulter, Immunotech, Marseille, France

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ABSTRACT

This study evaluated the diagnostic and analytical performances of the Access anti-HBc Total assay on the Dxi 9000 Access Immunoassay System (Beckman Coulter Inc.). The multicenter study involved both prospective and retrospective sample collection from non-selected blood donors, hospitalized patients, or presumed anti-HBc Total positive individuals. Fresh/previously-frozen samples were tested with the Access and comparator assays to determine concordance; discrepant samples were tested with a second CE-marked assay. Among the 5983 non-selected fresh blood donor samples deemed anti-HBc Total negative, clinical specificity of the Access assay was 99.58% (95%CI: 99.38–99.72%). Clinical specificity was 99.27% (97.37–99.80%) among 273 anti-HBc Total negative hospitalized patient samples. Clinical sensitivity on 450 anti-HBc Total positive samples was 99.78% (98.75–99.96%). Evaluation in seroconversion panels revealed an average 1.4-day earlier detection versus a comparator assay. The Access assay demonstrated excellent clinical and analytical performances comparable to existing CE-marked anti-HBc Total assays. NCT04904835.

1. Introduction

Hepatitis B virus (HBV) infection is a global public health problem, with an estimated >2 billion people worldwide having evidence of past or present HBV infection [1]. The World Health Organization (WHO) estimated in 2019 that there were 296 chronic HBV carriers, 1.5 million new yearly infections, and 820,000 annual deaths due to the disease [2]. Classification of HBV infection requires the identification of several serological markers expressed during the acute, convalescent, and chronic phases of infection. These include the hepatitis B surface Antigen HBsAg, IgM and IgG antibodies to the hepatitis core Antigen

(anti-HBc IgM and anti-HBc IgG) which together make up total anti-HBc (anti-HBc Total), anti-HBsAg antibodies (anti-HBs), and hepatitis e Antigen (HBeAg) [3,4].

Anti-HBc Total antibodies appear in individuals infected with HBV, irrespective of the presence of symptoms, and the marker has been considered a useful aid in the diagnosis of HBV infection and as a screening test for donors [5]. The Centers for Disease Control and Prevention (CDC) currently recommends the use of a 'triple panel' comprising anti-HBc Total, HBsAg, and anti-HBs for HBV screening and testing among older adolescents and adults [6]. Other guidelines recommend testing for anti-HBc Total and HBsAg in all patients to be

All authors contributed equally to the manuscript

* Corresponding author at: Clinical Affairs Department, Beckman Coulter, Immunotech, 130 av de Lattre de Tassigny, 13009 Marseille, France.

E-mail address: jhey@beckman.com (J. Hey).

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treated with immunosuppressive therapy or chemotherapy that may induce HBV reactivation [7–10]. Notably, the observation of the “anti-HBc Total alone” status in some samples (anti-HBc Total positive but HBsAg and anti-HBs negative) has been suggested to imply occult HBV infection [11], making anti-HBc Total measurement key to detection of HBV infection in these individuals, especially for donor screening. In blood donors, high anti-HBs levels due to seroconversion are often interpreted as protection against HBV transmission to a recipient. However, given that HBV infections may still occur without detectable anti-HBs or with anti-HBs disappearance emanating from late-phase HBV reactivation, screening for anti-HBc Total could be useful for closing the diagnostic gap between HBsAg disappearance and anti-HBs appearance. It can also help detect the late phase of HBV infection where viral levels may be low [12].

Current anti-HBc Total serological tests utilize chemiluminescent microparticle immunoassay (CMIA) technology for the qualitative detection of total antibodies against the hepatitis B virus core antigen (anti-HBc Total) in human serum and plasma. These tests have >99% specificity for non-selected blood donors and are considered the most sensitive for donor screening and assessment of past HBV exposure [13]. It is crucial to emphasize that the implementation of anti-HBc Total blood screening is backed by various studies revealing that 5-10% of samples showing sole anti-HBc positivity contained detectable HBV DNA, with the frequency of detection increasing with the sensitivity of molecular tests [14–16]. Historically, anti-HBc assays were challenged by lower specificity, necessitating confirmation through alternative screening assays to ensure accuracy [17]. Another contributing factor to the strong performance of these tests has been their utilization with automated immunoassay systems, leading to increased sensitivity, specificity, and accuracy [18]. Given the importance of anti-HBc Total measurement in HBV screening and testing, the development of effective methods to complement existing ones remains an ongoing quest in the field.

The DxI 9000 Access Immunoassay Analyzer is a new, fully automated, high throughput system from Beckman Coulter, Inc. using CMIA technology [19]. The objective of this study was to evaluate the clinical and analytical performances of the Access anti-HBc Total assay on the DxI 9000 Access Immunoassay Analyzer as a diagnostic aid for HBV infection and as a screening tool for blood and plasma donors.

2. Material and methods

2.1. Study design and participants

This multicenter study involved both prospective and retrospective sample collection and was performed from February 2020 to September 2022 (sample collection) and June 2022 to October 2022 (sample testing) at four enrollment and four testing sites as provided in Supplemental Table S1. Eligible samples had sufficient volume to be tested and were from subjects aged ≥ 18 years who could be categorized as non-selected blood donors, hospitalized patients, or presumed anti-HBc Total positive subjects based on a CE-marked assay. Samples (EDTA plasma or serum) were either tested fresh or collected and stored frozen prior to testing. Samples were tested with both the Access anti-HBc Total assay (Access assay) and a first comparator assay to determine their non-reactive (NR), initially reactive (IR) or repeatedly reactive (RR) status. The Abbott ARCHITECT Anti-HBc II assay (Architect assay) was used as the first comparator assay for hospitalized patients and presumed positive samples [20]. For non-selected blood donor samples, the Roche cobas Elecsys Anti-HBc II assay was used as first comparator assay (Elecsys assay) [21]. As per their instructions for use (IFU), the Architect and Elecsys assays have diagnostic sensitivities of 100.0% among confirmed anti-HBc Total positive samples. The assays have claimed diagnostic specificities of 99.71% and 99.93%, respectively, among non-selected blood donors, as well as specificity of 100.0% each among hospitalized patients [20,21]. Consistent with the individual IFUs, external

studies have reported specificities of 99%-100% and sensitivities of 95%-100% for the Architect and Elecsys assays [18,22–24]. Samples yielding discrepant results between the Access and the first comparator assay were tested with a second anti-HBc Total comparator assay for consensus results. The SIEMENS – ADVIA Centaur HBc Total assay [25] was used as the second comparator assay for hospitalized and presumed positive samples; no duplicate testing was required for reactive samples per the IFU [26,27]. For non-selected blood donor samples, the second comparator assay was the Architect assay, with duplicate testing being required only for all IR samples per the IFU. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and the International Conference for Harmonization Guidelines for Good Clinical Practices.

2.2. Access anti-HBc total assay and comparator assays

The Access assay is a two-step competitive immunoassay (Figure S1). Paramagnetic particles coated with HBc antigen are mixed with a patient sample and anti-HBc antibodies in the sample bind to this antigen. After incubation, unbound materials are washed away using a magnetic field. An anti-HBc mouse monoclonal antibody alkaline phosphatase conjugate is added, which competes with patient antibodies to bind to the antigen. Following another incubation, a chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. If light production is below a certain threshold, the sample is deemed positive for the Access assay, with the result determined using stored calibration data. Table S2 summarizes the assay characteristics of the Access assay. Testing was done by the Access assay and the first comparator assay. All IR specimen results were retested in duplicate per the respective Assay's IFU. If discrepant results between the Access and first comparator assays after duplicate testing was observed, samples were tested on the second comparator assay.

2.3. Analytical performance studies

Analytical performance studies were performed at Beckman Coulter, Marseille, France. A total of 280 potential cross-reactant samples were tested and compared to Architect assay non-reactive status. Ten seroconversion panels were used to evaluate the sensitivity of the Access assay during the early phase of infection compared to Architect assay.

2.3. Statistical analysis

Determination of final anti-HBc Total status for tested samples was done per the algorithm in Table S3. Specificity and sensitivity analyses were done per CLSI EP12-A2 guidelines [28]. Point estimates in percentage, event counts, and 2-sided 95% Wilson Score confidence intervals were calculated. Observed frequencies in NR, IR, RR Access assay results versus anti-HBc Total status of sub-populations of interest were tabulated. Demographic summary tables were reported in descriptive statistics. All statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, NC, USA) and Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3. Results

3.1. Demographic characteristics

A total of 6797 samples from non-selected blood donors, hospitalized patients, and presumed anti-HBc Total positive individuals were collected, of which 6779 were eligible following application of the eligibility criteria (Fig. 1). Among these eligible samples, 6758 (non-selected blood donors, $n=6000$; hospitalized patients, $n=458$ and presumed anti-HBc Total positive, $n=300$) were tested. After a total of 35 samples were excluded from the collection, 6744 final samples were eligible for statistical analysis (non-selected blood donors, $n=5998$;

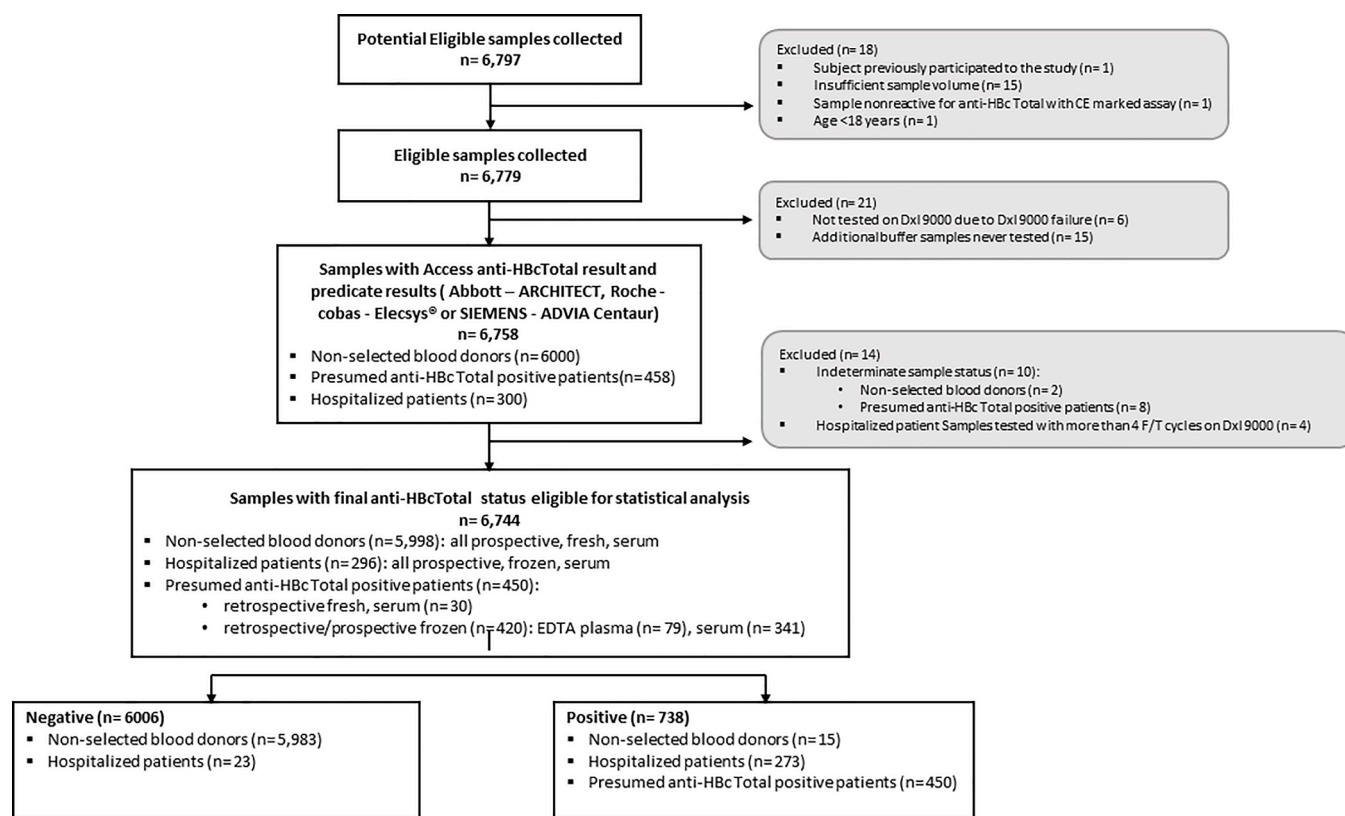


Fig. 1. Non-selected blood donor, hospitalized patient and presumed anti-HBc Total positive samples collected, tested, and analyzed.

hospitalized patients, $n=296$; presumed anti-HBc Total patients, $n=450$). This comprised a comparable proportion of females (50.5%; $n=3405$) and males (49.5%; $n=3339$), with median age being 45 (range, 18–106) years. Following application of the sample status algorithm, ~7% of samples ($n=488$) were judged to have final positive status for anti-HBc Total (non-selected blood donors, $n=15$; hospitalized patients, $n=23$; presumed HbC Total positive patients, $n=450$), with 6256 samples having final negative status for anti-HBc Total comprising 5983 non-selected blood donors and 273 hospitalized patients.

3.2. Specificity in non-selected blood donors and hospitalized patients

Of the initial 6000 fresh non-selected blood donor samples evaluated at least once with both the Access and one of the comparator assays, 2 samples were found to be NR for the Access assay, RR for the Elecsys assay and NR for the Architect assay; these were adjudged an indeterminate sample status and excluded from the performance analysis (Table 1). All 15 RR samples in the comparator assay were not counted in the specificity analysis. Among the remaining 5983 non-selected blood donor samples that were NR with the reference Elecsys assay, 5958 were found to be NR with the Access assay. The resulting overall diagnostic specificity of the Access assay on the non-selected fresh blood donor samples with adjudged anti-HBc Total negative status was 99.58% (95%CI: 99.38–99.72%). Of the 25 discordant samples, 11 were from vaccinated patients (i.e., positive for anti-HBs and negative for both HBsAg and anti-HBc Total) and 14 were from patients susceptible to HBV infection who were never infected and had no evidence of immunization (i.e., negative for HBsAg, anti-HBs and anti-HBc Total) (Table 2).

A total of 296 frozen serum hospitalized patient samples were tested at least once with the Access and one of the comparator assays. Twenty-three samples had RR status in both assays and so were not included in the evaluation of diagnostic specificity. Of the 273 hospitalized patient samples that were NR per the comparator assays, 271 were concordant

NR per the Access assay while 2 samples were RR in the Access assay but NR in both the Architect and ADVIA Centaur HbC Total assays (Table 1). One sample was from a patient with early acute infection serological profile (i.e., positive for HBsAg and negative for both anti-HBc Total, anti-HBc IgM and anti-HBs). The other sample was from a patient with an indetermined disease status (i.e. positive for both HBsAg and anti-HBs, and negative for both anti-HBc Total and anti-HBc IgM) (Table 2). Diagnostic specificity of the Access anti-HBc Total assay on the 273 hospitalized patient samples adjudged negative status for anti-HBc Total was 99.27% (95%CI: 97.37–99.80%).

Combining the non-selected blood donor ($n=5983$) and hospitalized patient ($n=273$) samples included in the specificity calculations, the overall diagnostic specificity of the Access assay on samples with negative anti-HBc Total status was 99.57% (95%CI: 99.37–99.70%).

Of note, among the 15 blood donor samples found to be RR with the first comparator assay, 14 samples were also found to be RR with the Access assay (Table 1). One sample was found to be NR with the test assay (1.10 S/CO) but reactive in both the Architect (0.48 S/CO, 0.50 S/CO, 0.52 S/CO) and ADVIA Centaur HbC Total assays (1.32 S/CO, 1.29 S/CO, 1.28 S/CO). This sample was from an immune patient due to natural infection as per CDC classification (Table 2).

3.3. Diagnostic sensitivity on presumed HbC total positive patients

A total of 458 presumed anti-HBc Total positive patient samples were tested at least once with the Access and comparator assays, including 427 frozen and 31 fresh samples. Among the fresh samples, 1 sample was adjudged to have an indeterminate status and was excluded from the analysis. All remaining 30 samples were RR in both the Access and Architect assays. Among the 427 frozen samples, 7 were considered indeterminate and excluded from the analysis (Table 1). Of the 420 remaining presumed positive samples, 419 were found to be RR for both Access and Architect assays while 1 was NR in the Access assay but weakly reactive in both the Architect and ADVIA Centaur HbC Total

Table 1

Summary of results for non-selected blood donors, hospitalized patients, and presumed anti-HBc Total positive samples.

Group	N (%) of samples	Access anti-HBc Total result	1st anti-HBc Total comparator assay result ¹	2nd anti-HBc Total comparator assay result, if needed ²	Final anti-HBc Total sample status
Non-selected Blood Donors	5958 (99.30)	NR	NR	NA	NEG ³
	14 (0.23)	RR	RR	NA	POS ⁴
	25 (0.42)	RR	NR	NR	NEG ³
	1 (0.02)	NR	RR	RR	POS ⁴
Hospitalized Patients	271 (91.55)	NR	NR	NA	NEG ⁵
	23 (7.77)	RR	RR	NA	POS ⁴
	2 (0.68)	RR	NR	NR	NEG ⁵
Presumed anti-HBc Total positive fresh samples	30 (96.77)	RR	RR	NA	POS ⁶
Presumed anti-HBc Total positive frozen samples	419 (98.13)	RR	RR	NA	POS ⁶
	1 (0.23)	NR	RR	R	POS ⁶

¹ Abbott – ARCHITECT Anti-HBc II assay for hospitalized and presumed positive samples and Roche Cobas Elecsys Anti-HBc II assay for non-selected blood donors.² SIEMENS - ADVIA Centaur HBc Total assay for hospitalized and presumed positive samples and Abbott – ARCHITECT Anti-HBc II assay for non-selected blood donors.³ Included for specificity on non-selected blood donors and overall specificity.⁴ Included for overall sensitivity.⁵ Included for specificity on hospitalized patients and overall specificity.⁶ Included for sensitivity based on anti-HBc Total positive patients confirmed positive by testing algorithm and overall sensitivity.

assays (Tables 1-2). This sample was from a patient immunized following a natural infection according to the CDC classification based on the interpretation of hepatitis B serological test results (Table 2). Therefore, diagnostic sensitivity of the Access anti-HBc Total assay among these 450 confirmed positive samples (30 fresh and 420 frozen) was 99.78% (95%CI: 98.75–99.96%). The sensitivity of the Access assay was stratified according to HBV disease stages available from medical records registered in the electronic case report form at enrolment. The sensitivity was 100.0% in patients with acute HBV infection, patients with chronic HBV infection, and those with unknown disease stage, while it was 99.64% among patients recovered from HBV infection (Table S4).

3.4. Overall sensitivity on anti-HBc total positive samples

Overall diagnostic sensitivity of the Access assay on 488 total anti-HBc Total positive samples, from anti-HBc Total positives confirmed positive by the sample status algorithm (n=450), non-selected blood donors (n=15) and hospitalized patients (n=23), was 99.59% (95%CI: 98.52–99.89%). Sensitivity according to subclassification per the CDC, based on interpretation of Hepatitis B serological test results, are shown in Table 3. Sensitivity of 100.0% was obtained for patients with acute HBV infections (11/11), patients with chronic HBV infections (90/90) as well as for patients among the unclear serological interpretation group having only HBc Total marker as positive (94/94). For immune patients due to natural HBV infection, diagnostic sensitivity was 99.30% (283/285).

3.5. Analytical performance of access assay

Specificity of the Access assay on potentially cross-reacting samples was evaluated using 280 samples which were tested for cross-reactivity to 28 different categories of potential cross-reactants. The specificity was 99.64% (95%CI: 98.01–99.94%) compared to samples found to have non-reactive status per the Architect assay.

The performance of the Access assay on seroconversion panels was also evaluated. Seven anti-HBc Total seroconversion panels were tested with the Access and Architect assays, including a total of 167 bleeds. The Access assay detected the first reactive bleed on the same bleed day as the comparator method for four panels. Results from the remaining three panels showed earlier detection of the first reactive bleed per the Access assay compared to the comparator method, including a panel

which never seroconverted from a nonreactive status to a reactive status per the comparator method. The difference of mean first day of detection across the 7 seroconversion panels was 1.4 days between the Access and Architect assays, with earlier detection observed with the Access assay (Table 4).

4. Discussion

Anti-HBc is considered a long-lasting serological marker of HBV infection owing to its appearance in the acute phase of infection, lifelong persistence, and ability to indicate HBV infection regardless of disease stage [5]. Unsurprisingly, the CDC recommends testing for total anti-HBc in addition to HBsAg and anti-HBs for HBV screening, while guidelines from other bodies such as the AGA, EASL, APASL, and AASLD recommend testing for anti-HBc together with HBsAg in all patients about to receive immunosuppressive therapy or chemotherapy that may induce HBV reactivation [13].

The necessity for confirmatory testing in anti-HBc screening, particularly within the realm of blood donor screening, is paramount given the implications of a reactive result, which typically leads to the exclusion of a donor. Over three decades of literature on anti-HBc have pinpointed specificity as a pivotal issue, emphasizing that inaccuracies in test results can lead to significant consequences, including the unwarranted deferral of healthy donors [29–31]. The emphasis on specificity is crucial because it directly influences the reliability of screening assays in identifying true exposure to hepatitis B virus, thereby safeguarding the blood supply while ensuring that eligible donors are not unnecessarily excluded. Hence, specificity in anti-HBc testing is of critical importance in transfusion medicine and assays measuring this marker are expected to exhibit strong performance in this regard.

The Access assay demonstrated a considerable ability to accurately target HBc Total antibodies in samples, as expected for such an assay. Among 5983 non-selected anti-HBc Total negative blood donors, diagnostic specificity of the Access assay was 99.58% (95%CI: 99.38–99.72%), which compares with that of currently marketed anti-HBc Total assays. As claimed in their respective IFUs, the Architect and Elecsys Anti-HBc II assays have specificities of 99.71% (95%CI: 99.52–99.84%) and 99.93% (95%CI: 99.84–99.97%), respectively, among non-selected blood donors [20,28]. Among 273 anti-HBc Total negative hospitalized samples, the Access assay had a specificity of 99.27% (95%CI: 97.37–99.80%); in the Architect and Elecsys assays,

Table 2

Summary of results for discordant specimens included in statistical analysis.

Group	Access anti-HBc Total assay		1st anti-HBc Total comparator assay result ¹		2nd anti-HBc Total comparator assay result ²		Final anti-HBc Total sample status	Stage of infection based on CDC classification
	S/CO	Interpretation	S/CO	Interpretation	S/CO	Interpretation		
Presumed anti-HBc Total positive patients	1.01	NR	1.04, 1.09, 1.08	IR, RR, RR	0.6	R	Positive	Immune due to the natural infection
Hospitalized patients	0.97, 0.97, 0.96	IR, RR, RR	0.36	NR	0.28	NR	Negative	Early acute infection
	0.84, 0.81, 0.83	IR, RR, RR	0.29	NR	0.13	NR	Negative	Indetermined disease status
Non-selected blood donors	1.1	NR	0.48, 0.50, 0.52	IR, RR, RR	1.32, 1.29, 1.28	IR, RR, RR	Positive	Immune due to the natural infection ³
	0.86, 0.81, 0.80	IR, RR, RR	2.21	NR	0.22	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.97, 0.99, 0.97	IR, RR, RR	2.26	NR	0.21	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.75, 0.72, 0.72	IR, RR, RR	2.34	NR	0.25	NR	Negative	Immune due to hepatitis B vaccination
	0.95, 0.93, 0.92	IR, RR, RR	2.28	NR	0.23	NR	Negative	Immune due to hepatitis B vaccination
	0.72, 0.66, 0.68	IR, RR, RR	2.24	NR	0.23	NR	Negative	Immune due to hepatitis B vaccination
	0.90, 0.89, 0.86	IR, RR, RR	2.16	NR	0.16	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.43, 0.44, 0.44	IR, RR, RR	2.22	NR	0.12	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.72, 0.73, 0.68	IR, RR, RR	2.19	NR	0.25	NR	Negative	Immune due to hepatitis B vaccination
	0.99, 0.99, 0.98	IR, RR, RR	2.16	NR	0.18	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.42, 0.45, 0.41	IR, RR, RR	2.04	NR	0.33	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.60, 0.57, 0.56	IR, RR, RR	2.18	NR	0.42	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.90, 0.82, 0.88	IR, RR, RR	2.24	NR	0.2	NR	Negative	Immune due to hepatitis B vaccination
	0.84, 0.77, 0.71	IR, RR, RR	2.31	NR	0.08	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.91, 0.84, 0.88	IR, RR, RR	2.36	NR	0.22	NR	Negative	Immune due to hepatitis B vaccination
	0.62, 0.66, 0.66	IR, RR, RR	2.37	NR	0.11	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.97, 0.91, 0.94	IR, RR, RR	2.15	NR	0.11	NR	Negative	Immune due to hepatitis B vaccination
	0.51, 0.52, 0.54	IR, RR, RR	2.27	NR	0.62	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.95, 0.96, 0.97	IR, RR, RR	2.19	NR	0.17	NR	Negative	Susceptible: never infected and no evidence of immunization
	0.73, 0.76, 0.74	IR, RR, RR	2.26	NR	0.08	NR	Negative	Susceptible: never infected and no evidence of immunization
0.86, 0.86, 0.87	IR, RR, RR	2.21	NR	0.11	NR	Negative	Immune due to hepatitis B vaccination	
0.79, 0.76, 0.79	IR, RR, RR	2.06	NR	0.11	NR	Negative	Immune due to hepatitis B vaccination	
0.87, 0.84, 0.83	IR, RR, RR	2.2	NR	0.19	NR	Negative	Immune due to hepatitis B vaccination	
0.97, 1.02, 0.98	IR, RR, RR	2.32	NR	0.29	NR	Negative	Susceptible: never infected and no evidence of immunization	
0.91, 0.89, 0.86	IR, RR, RR	2.27	NR	0.19	NR	Negative	Immune due to hepatitis B vaccination	
0.90, 0.91, 0.96	IR, RR, RR	1.36	NR	0.07	NR	Negative	Susceptible: never infected and no evidence of immunization	

¹ Abbott – ARCHITECT Anti-HBc II assay for hospitalized and presumed positive samples and Roche cobas Elecsys Anti-HBc II assay for non-selected blood donors.² SIEMENS - ADVIA Centaur HBc Total assay for hospitalized and presumed positive samples and Abbott – ARCHITECT Anti-HBc II assay for non-selected blood donors.³ Based on interpretation of Hepatitis B serologic test results (i.e., positive for both anti-HBs and anti-HBc Total and negative for HBsAg).

Table 3
Sub-classification among all confirmed anti-HBc Total positive samples, based on CDC classification.

CDC Classification ¹	HBV Profile	Presumed anti-HBc Total positives confirmed positive N of samples	Hospitalized patients N of samples	Non-selected blood donors N of samples	Overall Sensitivity of Access anti-HBc Total assay % (95%CI)
Patients with acute HBV infection	HBsAg +	11	0	0	100.0%
	Anti-HBc Total +				
	Anti-HBc IgM + Anti-HBs -				
Patients with chronic HBV infection	HBsAg +	88	2	0	100.0%
	Anti-HBc Total +				
	Anti-HBc IgM - Anti-HBs -				
Patients immune due to natural HBV infection	HBsAg -	258 ³	16	11 ⁴	99.30%
	Anti-HBc Total +				
	Anti-HBs +				
Unclear serological interpretation ²	HBsAg -	90	4	4	100.0%
	Anti-HBc Total +				
	Anti-HBs -				
Disease status indetermined	Other HBV serological profiles	3	1	0	100.0%
	Total	450	23	15	99.59% (98.52 - 99.89%)

¹ Based on interpretation of Hepatitis B serologic test results and required HBsAg, anti-HBs, anti-HBc Total and anti-HBc IgM assay results on the same draw.

² Four (4) possibilities: 1. Resolved infection (most common), 2. False-positive anti-HBc, 3. "Low level" chronic infection, 4. Resolving acute infection.

³ Included one (1) false negative sample for Access anti-HBc Total assay.

⁴ Included one (1) false negative sample for Access anti-HBc Total assay.

this was 100.0% (95%CI: 98.42–100.0%) and 100.0% (95%CI: 99.60–100.0%), respectively [20,21].

Discordant results seen among non-selected blood donors in this study were from either susceptible individuals who had never been infected with HBV and had no evidence of immunization (14/25 individuals) or those who were likely immune due to hepatitis B vaccination (11/25 individuals). Blood donors in the study were close to a healthy population as individuals with prior infection were excluded. Considering that achieving specificity in vaccinated individuals is a challenge for Hbc assays [32], the observation of nearly similar false positivity rates among the prior-vaccinated and unvaccinated groups may be indicative of good assay specificity, especially given that the vaccination rate among blood donors is nearly 50%. Most of the discordant non-selected blood donor samples (20/25) were also low positive samples (>0.70S/CO). Some reports have indicated that anti-HBc assays typically demonstrate low positive predictive value in low-prevalence populations, especially with low-level anti-HBc-reactive samples [17,33,34]. The observed false positive rate among vaccinated individuals is also not unexpected. In a study, the false positive rate for HBsAg following recent hepatitis B vaccination was 2.56% [35]. Among hospitalized patients, one of the discordant results was from a patient with early acute HBV infection serological profile. Such an occurrence may be attributed to a transient state of resolved HBV infection right before appearance of anti-HBs [29].

Cross-reactivity among infectious disease antibody assays is well documented [36]. Tested on potentially cross-reacting samples, the Access assay displayed a high specificity of 99.64% (95%CI: 98.01–99.94%) with only one discordant result compared to samples that had non-reactive status per the Architect assay.

Testing of Hbc Total antibodies is important in identifying individuals with "anti-HBc alone" status (i.e., HBsAg negative, anti-HBs negative, but Hbc Total positive). These individuals comprise up to 20% of all people with HBV serological markers and mostly located in low prevalence regions such as Europe and the US. The "anti-HBc alone" status can result from past HBV infections associated with a risk for reactivation in the context of immunosuppression [11,37–40]. Consequently, these individuals may have occult HBV infections and can potentially transmit the disease within a window before appearance of anti-HBs antibodies or HBsAg mutant infections and hence not detectable by HBsAg test assays. Therefore, development of sensitive Hbc Total immunoassays remains a major need in the field. Among the 450 presumed anti-HBc Total positive patient samples, diagnostic sensitivity of the Access assay was 99.78% (95%CI: 98.75–96.96%), which is comparable to that of the Architect (100.0%) and the Elecsys assays (100.0%), per their respective IFUs [20,21].

Overall sensitivity of the Access assay among all confirmed anti-HBc Total positive samples was 99.59% (95%CI: 98.52–99.89%), with the highest sensitivity (100.0%) observed among patients with acute or chronic HBV infections. Sensitivity among those with unclear serological interpretation, characterized by HBsAg negative, anti-HBs negative, and anti-HBc Total positive status, was also 100.0%. The serological profile of these groups of patients, who made up nearly 20% of all the anti-HBc Total positive samples, are consistent with the "anti-HBc alone" serological status [11,29,39]. The incidence rate observed is consistent with the reported rate ranging between 1%–32% depending on the population [11,37–39,41]. Observation of the "anti-HBc alone" status has been attributed primarily to false positivity [11]. However, in the case of this study's RR results, potential contributing factors that have been suggested include immunocompromised status (HIV or HCV co-infection), resolved infection, chronic infection, and passive transfer, among others [11,29,42].

When tested on seroconversion panels, the difference in the mean first day of detection across 7 seroconversion panels was 1.4 days between the Access and Architect assays, with the Access assay detecting seroconversion earlier. This suggests that the Access assay may be more discriminatory for negative versus positive samples during early acute

Table 4

Seroconversion panels results: First Detection Day / Difference in First Detection Day.

Panel ID	Access anti-HBc Total assay (days)	Abbott ARCHITECT Anti-HBc II assay (days)	Difference in First Detection Day: Access anti-HBc Total assay vs Abbott ARCHITECT Anti-HBc II assay
HBV-6281	41	41	0
HBV-9092	85	85	0
HBV-9093	49	49	0
HBV-9072	159	>159*	≤ -1*
HBV-001	29	29	0
HBV-002	56	59	-3
HBV-004	65	71	-6

Panels were obtained from Biomex GmbH, Heidelberg Germany [n=3] and Zeptomatrix, Buffalo, NY, USA [n=4]. * The panel never seroconverted from a nonreactive status to a reactive status with the Abbott ARCHITECT Anti-HBc II assay.

HBV infection. This characteristic, combined with the desirable precision and strong specificity and selectivity observed reveals the Access assay as powerful immunoassay for measuring anti-HBc Total antibodies.

This study did have some limitations. Due to being a cross-sectional testing study with no additional clinical information or follow-up available on patients, additional information or follow-up samples would have been helpful for resolving discrepant samples. Further investigations were also precluded by the limited sample volume. Further, as shown by independent performance studies, the real-life performances can be slightly lower than those seen during initial evaluation done in a controlled setting. Therefore, independent assessment of our findings would be beneficial to confirm the strong performances observed for the Access assay.

To summarize, the newly developed Access anti-HBc Total assay from Beckman Coulter, Inc. for use on the Dxl 9000 Access Immunoassay Analyzer demonstrated strong diagnostic and analytical performance which was comparable to those claimed by currently commercialized anti-HBc Total assays.

CRediT authorship contribution statement

Simplice Dzamitika: Writing – review & editing, Writing – original draft, Investigation. **Françoise Le Boulaire:** Writing – review & editing, Writing – original draft. **Catherine Coignard:** Writing – review & editing, Writing – original draft, Investigation. **Claire Vincent:** Writing – review & editing, Writing – original draft. **Jean-Christophe Plantier:** Writing – review & editing, Writing – original draft, Investigation. **Véronique Lemée:** Writing – review & editing, Writing – original draft, Investigation. **Sandrine Gréaume:** Writing – review & editing, Writing – original draft. **Isabelle Voisin:** Writing – review & editing, Writing – original draft. **Etienne Brochot:** Writing – review & editing, Writing – original draft, Investigation. **Yves-Edouard Herpe:** Writing – review & editing, Writing – original draft. **Gaiane Demirdjian:** Writing – review & editing, Writing – original draft. **Magali Karagueuzian:** Writing – review & editing, Writing – original draft. **Derrick Afful:** Writing – review & editing, Writing – original draft. **Rima Bayoud:** Writing – review & editing, Writing – original draft. **Juliane Hey:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Rima Bayoud, Juliane Hey, Gaiane Demirdjian, Magali

Karagueuzian, and Derrick Afful are Beckman Coulter employees.

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Ethical Approval

Research involving human subjects complied with all relevant national regulations and institutional policies and is in accordance with the tenets of the Helsinki Declaration. Research using anonymized, leftover specimens does not require specific ethical approval in France as it falls outside of the Loi Jardé (n°2012-300 of 5 March 2012; amended by ordinance n°2016-800 of 16 June 2016).

The Dxl 9000 Access Immunoassay Analyzer is CE marked and FDA cleared but not currently for sale or distribution in all markets.

Author Statement

All authors contributed equally to the manuscript

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.diagmicrobio.2024.116303](https://doi.org/10.1016/j.diagmicrobio.2024.116303).

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