Hepatitis B virus (HBV) infection occurs worldwide, with the prevalence of hepatitis B surface antigen (HBsAg) varying according to countries, reaching more than 8% in Africa, Asia, and the Middle East (Lok and McMahon, 2007). HBV infection can resolve spontaneously in adults, but chronicity occurs in 5–10% of cases resulting in chronic hepatitis with serious liver damage such as fibrosis, cirrhosis, and hepatocellular carcinoma (Barazani et al., 2007). Recovery from hepatitis B infection is associated with the loss of HBsAg in blood, while the viral genome can persist for extended period of time, particularly in hepatocytes and mononuclear cells (Murakami et al., 2004; Pawlotsky, 2006). It is therefore important to obtain accurate diagnosis of HBV infection for long term follow-up of HBsAg carriers, to avoid new infections (Arraes et al., 2003; Barcena et al., 2006; Behzad-Behbahani et al., 2006) and to prevent the risk of reactivation of HBV notably in immunocompromised patients (Calabrese et al., 2006; Coiffier, 2006; Lalazar et al., 2007).

The diagnosis of HBV usually relies on tests of three main serological markers, namely hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs) and antibody to hepatitis B core antigen (anti-HBc). In addition, detection of hepatitis B e antigen (HBeAg), antibody to HBeAg (anti-HBe), anti-HBc IgM and HBV DNA can be helpful for accurate diagnosis of HBV infection (Trepo et al., 1993; Chevaliez and Pawlotsky, 2005).

Anti-HBc antibodies are of primary importance since they represent a long lasting serological marker of HBV infection. They appear in the acute phase of the infection, generally persist lifelong, and indicate HBV infection independently of the stage of infection (acute, chronic or recovered). Furthermore, individuals with a serological anti-HBc alone pattern can potentially transmit the hepatitis B virus (Grob et al., 2000; Weber et al., 2001).

Efficacy and reliability of screening methods for anti-HBc are thus critical for routine diagnosis of infection with HBV. Since most anti-HBc tests are highly sensitive, results close to the cut-off value may occur frequently. These borderline values may be difficult to distinguish between non-specific reactions and very low levels of circulating anti-HBc antibodies (e.g., in immunocompromised patients or remote past HBV infection). In general, concomitant testing for additional HBV markers and the availability of previous biological and clinical data constitute an invaluable help for interpretation. Nevertheless, in some situations, and especially for the anti-HBc alone pattern, an accurate diagnosis of HBV infection remains difficult to establish.

The Department of Virology of the hospital of Nice acquired recently an automated ARCHITECT® anti-HBc assay (Abbott Lab-
Fig. 1. Results of 107 serum samples analysed with three anti-HBc immunoassays. 107 ARCHITECT® borderline ratios ranging from 0.70 to 2.70 are presented on abcissa axis. For each serum, the histogram indicates result by the AxSYM® assay (percentage of inhibition), and the rhombus represents result by the VIDAS® assay (index depending on intensity of fluorescence). The greyed rectangles note the grey zone of these two tests.

oratories, IL, USA) as a convenient instrument choice allowing for a large volume, fast and low cost automated testing of sera. However, the generation of frequent borderline values that are difficult to interpret limits its use.

The aim of the present study was to test further samples with borderline values using the ARCHITECT® test, in order to investigate whether another anti-HBc detection test would be helpful and whether it would be of value to define the grey zone for the ARCHITECT® anti-HBc assay.

To this end, a serological confirmation approach was used, as reported frequently (Weber et al., 1998; Taylor et al., 2004; Chen et al., 2005; Kafatos et al., 2007). Available sera with borderline results when tested by the ARCHITECT® anti-HBc assay, a two step Chemiluminescent Microparticle Immunoassay (CMIA), were analysed using two other methods. One method was developed by the same manufacturer (Abbott Laboratories, IL, USA) but is based on a different principle: the AxSYM® Core™ is a competitive Microparticle Enzyme Immunoassay (MEIA); the second method is provided by another manufacturer (bioMérieux, Lyon, France) to avoid potential common non-specific reactivity: the VIDAS® Anti-HBc Total II assay is an enzyme linked fluorescent assay (ELFA) based on an inhibition principle.

To obtain borderline reactive samples, a series of unselected sera sent to the hospital laboratory for the diagnosis of infection with HBV was used, thus containing a large number of samples from individuals at risk for HBV infection. Over a 2-month period, 3540 serum samples from patients hospitalized to various medico-surgical services, or from hospital workers, were collected and submitted for routine diagnosis of markers of HBV infection (HBsAg, anti-HBs and anti-HBc using the fully automated ARCHITECT® i2000SR from Abbott Laboratories) at the laboratory of Virology (Archet Hospital, Nice, France). Reactivity to HBV core antigen was observed frequently, with a prevalence of 15.4% exceeding the French anti-HBc prevalence of 8.18% (Zarski, 2006). Anti-HBc antibodies were detected in 545 specimens from patients with acute hepatitis (2), chronic hepatitis (66), or presenting a pattern of recovered infection (477).

Out of the 3540 sera, emphasis was placed on the study of samples with borderline values, negative or positive, around 1.00. Usually, the ARCHITECT® qualitative result depends on a sample to cut-off (S/CO) ratio, and specimens with a ratio greater than 1.00 are considered as reactive. A definition of a grey zone is not recommended by the manufacturer, but a note in the package insert indicates the possible configuration of the ARCHITECT® System to use a grey zone interpretations. Therefore, in the laboratory, sera with a ratio between 0.90 and 1.10 are defined as grey zone reactive, centrifuged once again, tested in duplicate, and then classified as inconclusive if the ratio is reproducible within this range. Nevertheless, a better definition of the grey zone for this assay is desirable, and therefore sera with a ratio falling in a wider interval, ranging from 0.70 to 2.70, were tested by the two others anti-HBc immunoassays.

In the present study, 184 of the 3540 sera had an anti-HBc ratio by the ARCHITECT® assay between 0.70 and 2.70. Among these, 107 had sufficient volume for additional testing. They were distributed as follows: 25 were non-reactive (ratio of 0.70–0.89), 19 inconclusive (ratio between 0.90 and 1.10), and 63 slightly reactive (ratio of 1.1–2.70). All samples were HBsAg negative, of which 56 had anti-HBc alone and 51 had anti-HBs. Independently of anti-HBs, the anti-HBc profile of these 107 samples was examined

Table 1

Analysis of anti-HBc results carried out with AxSYM® and the VIDAS® assays for 107 HBsAg negative sera found to have ARCHITECT® anti-HBc borderline ratios, with or without anti-HBs.

<table>
<thead>
<tr>
<th>Results by the ARCHITECT® anti-HBc assay</th>
<th>Results by the AxSYM® Core™ and the VIDAS® Anti-HBc total immunoassays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples (Anti-HBs negative, anti-HBs positive)</td>
<td>Both negative</td>
</tr>
<tr>
<td>Non-reactive (ratio 0.70–0.89)</td>
<td>25 (13, 12)</td>
</tr>
<tr>
<td>Grey zone (ratio 0.90–1.10)</td>
<td>19 (11, 8)</td>
</tr>
<tr>
<td>Reactive (ratio 1.1–2.70)</td>
<td>63 (32, 31)</td>
</tr>
</tbody>
</table>

a Of which 30 with ratio <1.80.
b Of which 12 with ratio <1.80.
using the VIDAS® and the AxSYM® anti-HBc immunoassays. These results are shown in Fig. 1.

As summarized in Table 1, agreement between the three assays was demonstrated for only 30 samples. Indeed, 16 of the 25 non-reactive sera by the ARCHITECT® assay were found negative by the AxSYM® and the VIDAS® assays, and only 14 of the 63 slightly reactive samples were confirmed as positive. In addition, among the 19 ARCHITECT® inconclusive sera, supplementary concordant testing allowed interpretation for 16 samples: 13 were negative and 3 were positive. Therefore, a total of 46 (30 + 16) out of the 107 borderline results by the ARCHITECT® assay were validated using the AxSYM® and the VIDAS® tests which gave a clear and concordant positive or negative result (Fig. 2a).

It is concluded that, when required, the use of a second assay is desirable in order to increase accuracy of the ARCHITECT® anti-HBc assay and to secure the diagnosis of infection with HBV. The concordance of the AxSYM®/VIDAS® results allowed the choice of a second assay depending on criteria other than technical performance.

Regarding to 63/107 weakly reactive sera by the ARCHITECT® test, 32 were negative by the two other anti-HBc tests (Table 1). This absence of concordance, shown in Fig. 2b and c, makes the interpretation of the results more difficult. Indeed, this pattern of results could be: either an ARCHITECT® false positive result or an ARCHITECT® true positive result with a titer of anti-HBc antibodies under the sensitivity level of the AxSYM® and the VIDAS® assays. For these 32 ARCHITECT® positive sera, using additional informations such as the clinical history of the patients and the available results of markers of HBV infection on previous sera, 11 samples were classified as ARCHITECT® false positives, one was from a patient with a remote HBV infection, and 20 remained equivocal with no follow-up data and the impossibility for supplementary testing, particularly HBV DNA detection. Therefore, among a total of 32 samples (11 + 1 + 20) with a ratio between 1.11 and 2.70 by the ARCHITECT® assay and negative by both the AxSYM® and the VIDAS® assays, nearly one third (11/32) were classified as false positive illustrating an impaired specificity of the ARCHITECT® assay. Nevertheless, one sample, from a patient with a remote HBV infection, was positive by the ARCHITECT® test but non-reactive by the other two assays demonstrating in some situations the higher sensitivity of the ARCHITECT® test.

Twenty nine (9 + 3 + 17) of the 107 ARCHITECT® borderline reactive sera remained with indeterminate status despite testing by the three assays because of inconclusive or discrepant results by the AxSYM® and the VIDAS® assays (Table 1). Of note, 12 of these 29 samples were coming from patients with a known history of remote infection with HBV, characterized by decreasing levels of antibodies over time. Among these 12 samples, two had a ratio by the ARCHITECT® test between 0.70 and 0.89; three between 0.90 and 1.10 and seven between 1.11 and 2.70, while results by the VIDAS® assay were negative but close to the grey zone or inconclusive in the grey zone interval and results by the AxSYM® assay were inconclusive or positive. Such data gave advantage to the ARCHITECT® sensitivity as compared to the VIDAS® one in detecting remote past infection.

Therefore, our analysis shows that results by the ARCHITECT® immunoassay have to be interpreted cautiously for samples with a borderline ratio around 1.00. Actually, the manufacturers do not give recommendations about the use of a grey zone with the ARCHITECT® immunoassay, the positive and negative interpretation being determined only by comparing the ratio to 1.00. Nevertheless, our study demonstrates a clear need for a grey zone definition to increase the accuracy of the results. In order to improve the interpretation of ARCHITECT® borderline results, we extended our predefined grey zone, which was 0.90–1.10, to a widened interval, ranging from 0.90 to 1.80 around the cut-off index. Indeed, most of the discrepant samples identified in the present study had a ratio by the ARCHITECT® test comprised between 1.10 and 1.80 (see details in Table 1 and Fig. 1) justifying an extension of the upper limit of the interval from 1.10 to 1.80. To date, as there is no confirmatory assay for anti-HBc, we recommend strongly other laboratories to use a grey zone for the ARCHITECT® assay, as defined in the present study. In particular, results should be interpreted in regard to past infections with HBV when available and we recommend the use of an additional immunoassay in order to check for the anti-HBc serological status. Indeed, there are precedents for using a second assay: confirmatory testing is often used for HIV and HCV testing algorithms. Certainly, this approach does not resolve all problems in interpreting borderline values, but the use of the grey zone we have just established has the advantage to maintain enough sensitivity to detect low titers of anti-HBc antibodies while...
preventing false positive results. Moreover, this allows for continuing routine testing while waiting for the new ARCHITECT® assay with an improved specificity to reach the market (Wiesner et al., 2007).

In summary, performance characteristics of the ARCHITECT® Anti-HBc assay meet the requirements of current clinical laboratory practices for its high throughput and time cost-effectiveness. It seems like a suitable choice for a first intention assay in laboratories with a need for urgent serology testing and an ever increasing workload. Nevertheless, reliance on a single assay for anti-HBc detection is sometimes unwise and one cannot avoid borderline values, especially in a hospital laboratory as shown in the present study. Therefore, we redefined a grey zone for the ARCHITECT® assay and implemented a routine second phase of screening. Since the AxSYM® Core™ assay, used widely in routine diagnosis in European laboratories (Kafatos et al., 2007), is provided by the same manufacturer, the VIDAS® Anti-HBc Total II assay was chosen because it is easy to use despite its operating mode in small series.

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References


