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# False-Positive Results with Third-Generation Monoclonal Hepatitis B Surface Antigen Enzyme Immunoassay

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**Nonspecific hepatitis B surface antigen reactions with a third-generation enzyme immunoassay (Auszyme Monoclonal; Abbott Laboratories, North Chicago, Ill.) were investigated with 9,577 serum specimens in a clinical laboratory setting. Of the 196 serum specimens found reactive in Auszyme screen by the overnight procedure, 103 turned out to be true-positives, 71 were nonrepeatably reactive, and 22 were repeatably reactive but actually falsely positive (false-positive rate, 22 of 196, or 11.2%). Verification of the 196 screen reactives by the Auszyme 3-h incubation assay detected all but 4 true-positives, with a false-negative rate of 3.9% (4 of 103), and was negative for the rest. These observations reinforce the need for retesting all reactive specimens and confirming repeatedly reactive samples when the Auszyme Monoclonal test is used to detect hepatitis B surface antigen.**

Enzyme immunoassay is the preferred method of most diagnostic laboratories for detecting hepatitis B virus seromarkers (3). Although a number of manufacturers have been marketing hepatitis B enzyme immunoassay diagnostic kits, the products of Abbott Laboratories (North Chicago, Ill.) appear to have found wide acceptance in clinical laboratories. We became aware of nonspecific reactions with Auszyme Monoclonal, a qualitative third-generation hepatitis B surface antigen (HBsAg) enzyme immunoassay manufactured by Abbott Laboratories, and followed a verification protocol (Fig. 1) to rule out the nonspecific reactivity. The manufacturer recognizes the potential for nonspecific Auszyme Monoclonal reactions, offers explanations, and recommends retesting all reactive specimens and confirming repeatedly reactive samples (Auszyme Monoclonal package insert). Recently, Skurrie and Garland, from Australia, reported false positivity with Auszyme Monoclonal and cautioned clinical laboratories on the potential mislabeling of HBsAg status when this test is used (6). In this report, we present data gathered for a year to determine the extent of Auszyme Monoclonal nonspecific reactivity in a clinical laboratory setting.

Enzyme immunoassay technique was used to test for all hepatitis B virus seromarkers. The enzyme immunoassays kits were purchased from Abbott Laboratories, and the tests were carried out according to the instructions of the manufacturer by one experienced technologist.

All samples received for routine hepatitis B virus seroscreening or diagnosis were initially screened for HBsAg with Auszyme Monoclonal by the overnight procedure (incubation at room temperature for 16 h; procedure B), and the specimens were held at the ambient temperature until the results were read. All sera found screen reactive were retested by Auszyme Monoclonal procedure B and procedure A (incubation at 40°C for 3 h) within 24 h. Depending on the outcome, specimens were divided into three categories and analyzed further (Fig. 1). Sera showing positive or questionable Auszyme reactions were referred to the Hepatitis Reference Laboratory, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada, for further testing. At the reference laboratory, Ausria (HBsAg radioimmunoassay; Abbott Laboratories) was routinely carried out to confirm the Auszyme results. HBsAg proficiency panels were obtained from two sources (Laboratory Centre for Disease Control and Organon Teknika, West Hill, Ontario, Canada), and one of the panels consisted of samples with known concentrations of both ad and ay subtypes (Paul-Ehrlich Institute standard). These were tested blindly for determination of the Auszyme sensitivity levels by procedures A and B and for quality control purposes.

During the survey period, 9,577 serum specimens were screened for HBsAg. Of these, 196 serum specimens (2%) were found to be reactive by the overnight Auszyme screen. However, only 99 of these were confirmed to be true-positives for HBsAg by the verification protocol used (Fig. 1), i.e., reactive both by Auszyme procedures A and B and by Ausria and positive for anti-hepatitis B core (HBc) (Table 1). The pool 1 samples gave optical density readings mostly in the range of 2.000 (average range of cutoff value, 0.050 to 0.070), and the reactivity level remained about the same in

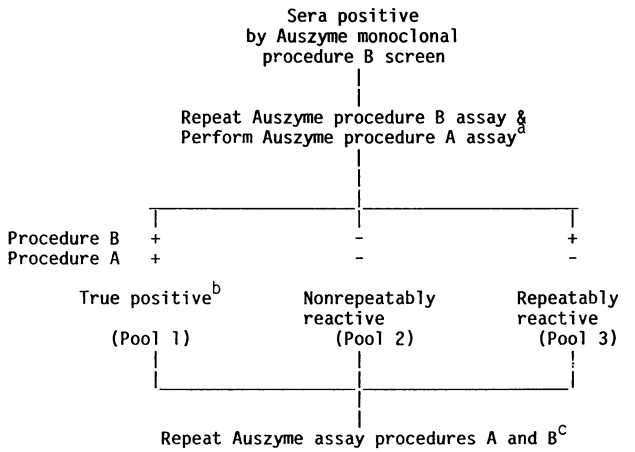


FIG. 1. HBsAg verification protocol. a, Samples held at ambient temperature and retested within 24 h of the initial screen; b, further testing by radioimmunoassay (Ausria) and for anti-HBc (Corzyme); c, samples stored at −20°C and retested a week to a few months later.

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TABLE 1. Results of HBsAg verification assays

| Category | Results of: | | | | | | | No. positive/total screen positive (%) |
|---|---|---|---|---|---|---|---|---|
| | Auszyme assay | | | | | Anti-HBc assay | HBsAg confirmation test (Ausria) | |
| | Initial[a] | | | Subsequent[b] | | | | |
| | Procedure B[c] | Repeat procedure B | Procedure A[d] | Procedure B | Procedure A | | | |
| True-positive (pool 1) | + | + | + | + | + | + | + | 99/196 (50.5) |
| Nonrepeatably reactive (pool 2) | + | − | − | − | − | − | − | 71/196 (36.2) |
| Repeatably reactive (pool 3) | + | + | − | +/− | − | − | − | 22/196 (11.2) |
| Repeatably reactive (pool 4)[e] | + | + | − | + | − | + | − | 4/196 (2.0) |

[a] Samples received and held at ambient temperature and all tests carried out within 30 h of receipt.
[b] Samples stored at −20°C and tested a week to a few months later.
[c] Incubation at room temperature for 16 h.
[d] Incubation at 40°C for 3 h.
[e] False-negative by procedure A and Ausria.

subsequent testings after storage (Table 2). Of the remaining 97 samples testing positive by the Auszyme screen, 71 were nonrepeatably reactive (Table 1); the pool 2 samples showed mostly lower levels of reactivity initially (borderline positivity to about 0.300) and tested negative subsequently by a repeated procedure B as well as by procedure A (Tables 1 and 2). There were 22 serum specimens that were repeatably reactive by Auszyme procedure B but negative by procedure A and Ausria and negative for anti-HBc (Table 1). The pool 3 samples gave optical density readings mostly in the range of about 0.200 to 0.700 (Tables 1 and 2). In this batch, subsequent follow-up samples tested negative for HBsAg by Auszyme procedure B, remained negative for anti-HBc and anti-HBc immunoglobulin M, and continued to test negative by Auszyme procedure A and Ausria. None of these 22 samples were from previously known HBsAg carriers or from those with a history of hepatitis. Therefore, these 22 were considered falsely positive by the Auszyme procedure B screen (false-positive rate, 22 of 196, or 11.2%). Finally, four serum samples tested repeatably reactive by Auszyme procedure B, negative by procedure A and Ausria, but positive for anti-HBc. Two of these were from a couple previously known to be HBsAg carriers, and the others were from two persons, both of whom had a history of hepatitis. In the first two cases, it was evident that the falling levels of HBsAg were still detectable by procedure B but not by procedure A or Ausria; in the next two cases, it was apparent that HBsAg had persisted at levels detectable by procedure B but not by procedure A or Ausria. Therefore, in these four instances, the Auszyme procedure B results were considered truly positive (Tables 1 and 2), thus increasing the true-positive total to 103. Conversely, the false-negative rate for procedure A Auszyme was 3.9% (4 of 103).

The assays of HBsAg proficiency panels gave accurate and consistent readings in all instances except one: a sample containing 0.1 U of ay subtype tested negative by Auszyme procedure A but positive by procedure B, indicating a higher sensitivity of the overnight incubation procedure. The lowest level of subtype ad measured was 0.1 U and was detected by both procedures.

Besides the overnight and 3-h Auszyme Monoclonal procedures, the manufacturer also recommends two other procedures for Auszyme, a 75-min incubation assay (procedure C) and a 2-h–1-h incubation assay (procedure D) specifically for samples containing sodium azide. We did not use these procedures in our study and do not know how they might compare with the two procedures we investigated.

The results of our study indicate that the following prac-

TABLE 2. Representative Auszyme Monoclonal optical density values for different categories of HBsAg reactions

| Category | Sample no. | Optical density by Auszyme assay | | | | | Anti-HBc assay | HBsAg confirmation (Ausria) |
|---|---|---|---|---|---|---|---|---|
| | | Initial[a] | | | Subsequent[b] | | | |
| | | Procedure B[c] | Repeat procedure B | Procedure A[d] | Procedure B | Procedure A | | |
| True positive (pool 1) | 123 | 2.000 | 2.000 | 2.000 | 2.000 | 2.000 | Reactive | Reactive |
| | 141 | 0.127 | 0.110 | 0.137 | 0.140 | 0.138 | Reactive | Reactive |
| Nonrepeatably reactive (pool 2) | 228 | 0.077 | 0.025 | 0.004 | 0.017 | 0.010 | Nonreactive | Nonreactive |
| | 213 | 0.140 | 0.008 | 0.004 | 0.010 | 0.007 | Nonreactive | Nonreactive |
| Repeatably reactive (pool 3) | 313 | 0.269 | 0.187 | 0.010 | 0.009 | 0.006 | Nonreactive | Nonreactive |
| | 307 | 1.323 | 1.182 | 0.022 | 1.056 | 0.009 | Nonreactive | Nonreactive |
| Repeatably reactive (pool 4)[e] | 447 | 0.076 | 0.109 | 0.036 | 0.073 | 0.036 | Reactive | Nonreactive |
| | 469 | 0.118 | 0.096 | 0.007 | 0.081 | 0.029 | Reactive | Nonreactive |

[a] Samples received and held at ambient temperature and all tests carried out within 30 h of receipt.
[b] Samples stored at −20°C and tested a week to a few months later.
[c] Incubation at room temperature for 16 h.
[d] Incubation at 40°C for 3 h.
[e] False-negative by procedure A and Ausria.

tical points may be worth considering when the Auszyme Monoclonal test is used to detect HBsAg. (i) The overnight Auszyme Monoclonal assay (procedure B) is overly sensitive and can give rise to false-positive reactions. In our study, the overall false-positive rate was 0.2% (22 of 9,577), similar to that (0.3%) reported by Skurrie and Garland (6), but it rose to 11.2% when expressed in terms of the total Auszyme screen reactives (22 of 196). However, the Auszyme procedure B has a presumed negative predictive value of 100%, and therefore it is ideally suited as a screen test. (ii) The nonrepeatably reactive results could well be due to technical errors (Ausyzme package insert); this is usually corrected by repeated testing. Also, the nonspecific reactions appear to be lost upon storage. Thus, repeated testing after a brief storage might be helpful to a great extent in overcoming the Auszyme Monoclonal false positivity. (iii) The 3-h incubation Auszyme Monoclonal assay (procedure A) is more specific in detecting HBsAg than the overnight procedure, and therefore it is ideally suited as a verification procedure for the overnight screen reactives. However, the 3-h incubation assay is less sensitive than the overnight procedure, with a false-negative rate of about 4%.

When used as a supplementary test to HBsAg assay, the anti-HBc test had 100% positive and negative predictive values. Thus, in our limited series, anti-HBc was a reliable adjunct to HBsAg testing and helpful in detecting false Auszyme reactions. However, it is common knowledge that early in the acute stage of hepatitis B virus infection, tests for anti-HBc may be negative, and this negative rate could be as high as 8% (4). Therefore, negative anti-HBc tests cannot be entirely relied upon without a follow-up specimen tested subsequently for HBsAg and anti-HBc. Nevertheless, the maximum false-negative rate after both Auszyme procedure A and anti-HBc tests are used to verify the Auszyme procedure B screen reactives is expected to be the product of the individual false-negative rates, i.e., $4\% \times 8\% = 0.32\%$, an extremely lower rate. Also in this context, the occurrence of a variant of hepatitis B virus (1, 2, 5, 7) is a reason for caution against disregarding low-level HBsAg-only positivity as being nonspecific. Those infected with the variant virus test positive for HBsAg, and HBsAg positivity is not accompanied by the presence of anti-HBc; according to one report, most of those infected have normal transaminases and a low titer of HBsAg activity, with 97% losing HBsAg subsequently (7).

In conclusion, our observations confirm those of Skurrie and Garland (6) and emphasize, as recommended by the manufacturer, that all reactive specimens should be retested and repeatably reactive samples should be confirmed when the Auszyme Monoclonal test is used to detect HBsAg. Our results also emphasize the importance of patient history and follow-up laboratory tests in the accurate interpretation of test results when discrepancies are encountered.

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