Sources of interference. According to Rosano (personal communication), blood samples from lipemic or nonfattening subjects are acceptable for use in this assay, but hemolyzed samples do not give reliable results. Consistent with these observations, we found that supplementing nonlipemic human serum standard with an equal volume of lipemic serum standard (Lipid Control-E Elevated; Sigma Chemical Co., St. Louis, MO) before precipitation of the egulobulin fraction did not affect Clq quantification, in that the color yield of supplemented samples was 99% that of non-supplemented samples. To test the effect of lysed erythrocytes on the color yield, we isolated erythrocytes from fresh human blood on a Porell gradient (4), washed them twice with 9 g/L NaCl, lysed the pellet in water, and added an aliquot of the lysate containing 7.5 μmol of hemoglobin (as indicated by absorbance at 412 nm) to 250-μL aliquots of human serum standard before the egulobulin precipitation step. Color yield was depressed about 3%, compared with the same serum sample analyzed without the addition of erythrocyte lysate.

We obtained additional evidence that hemolysis depresses color yield by collecting a blood sample from a subject (who had given informed consent) and hemolyzing an aliquot by stirring it with an applicator stick. Serum was separated by centrifugation and analyzed for Clq. Hemolysis depressed the apparent Clq content from 87.1 to 56.3 mg/L.

In summary, we found that the method of Rosano et al. (1) was highly satisfactory when modified as described above. The modified method permitted the convenient analysis of 46 samples per day. In our hands, the method was highly reproducible: values for aliquots of the same serum sample analyzed four times over six weeks agreed within 1.1%.

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References

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Serum Index Identifies Lipemic Samples Causing Interference with Bilirubin Assay on Hitachi 717

To the Editor:
Recently, while reviewing data from patients, we noticed a striking correlation between the triglyceride concentration and the bilirubin result obtained with the BMC Bilirubin DPD assay (Boehringer Mannheim Corp., Indianapolis, IN 46250) and a Hitachi 717 analyzer for samples from one patient:

<table>
<thead>
<tr>
<th>Sample date</th>
<th>Triglyceride</th>
<th>Bilirubin mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/24</td>
<td>1360</td>
<td>5.0</td>
</tr>
<tr>
<td>11/15</td>
<td>7030</td>
<td>37.0</td>
</tr>
<tr>
<td>11/19</td>
<td>4750</td>
<td>23.0</td>
</tr>
<tr>
<td>11/29</td>
<td>2040</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Consequently, we prepared a series of samples with known amounts of Intralipid, using the protocol of Glick and Ryder (1), and measured the apparent bilirubin concentration. Subsequently, BMC published Customer Bulletin 91-07E, revising a previously published Intralipid interferogram on the Bilirubin DPD method. According to the Bulletin, Intralipid at 240 mg/L would raise the bilirubin result by 2.0 mg/L. This agrees with our results (not shown).

Because we do not have an alternative bilirubin method that will handle our workload, we sought a mechanism to identify samples that had a significant amount of interference from lipemia. We defined significant interference as the amount of lipemia that would increase the bilirubin result by 2.0 mg/L. From our Intralipid study, this was equivalent to an Intralipid concentration of 290 mg/L.

Glick et al. (2) described a procedure for identifying samples with lipemia by using Hitachi instruments. In brief,
degrees of potential interference caused by lipemia are measured by taking readings at two wavelengths for diluted serum samples. A scaling factor is used so that the result, the Lipemia Index, corresponds to the concentration of Intralipid. To evaluate the effectiveness of using this procedure for identifying samples with interference, we measured the Lipemia Index of all samples during a one-month period. Forty-six samples with a Lipemia Index > 200 (equivalent to an Intralipid concentration of 290 mg/L) and a bilirubin < 30 mg/L were centrifuged in an Airfuge (Beckman Instruments, Inc., Brea, CA 92821) for 5 min and the floating lipid material was removed. The submatant material was mixed well, and the bilirubin and the Lipemia Index were remeasured.

Bilirubin and Lipemia Index results obtained for the original sample were compared with bilirubin and Lipemia Index results measured for the centrifuged sample (Figure 1). Using least-squares regression, we calculated that a change of 220 in the Lipemia Index corresponds to a change of 2.0 mg/L in bilirubin concentration (~25% less than the 290 predicted from the Intralipid interference study described earlier).

We conclude that the Lipemia Index is useful for identifying samples that may have falsely increased bilirubin results caused by interfering lipemia. We conclude further that the difference between the predicted and observed concentrations of Intralipid that corresponds to a 2.0 mg/L change in bilirubin concentration may represent an error in the scaling factor used to calculate the Lipemia Index; alternatively, this difference may indicate that Intralipid underestimates the amount of interference caused by sample lipemia in the DPD bilirubin method.

It is important for each laboratory to establish its own policies for identifying and processing samples that may contain significant amounts of interfering substances. In our laboratory, we routinely centrifuge all samples that have a Lipemia Index > 220 and a bilirubin concentration between 10 and 30 mg/L. If there is insufficient sample to centrifuge, or if the Lipemia Index is still > 220, we add to the report a comment stating that the sample is lipemic and that the bilirubin result may be falsely increased.

References

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A Caveat for Molecular Biology Diagnosis of Papillomavirus

To the Editor:

The incidence of adenocarcinoma of the cervix is apparently increasing (1), and characteristics of the disease continue to need clarification. Human papillomavirus (HPV) has been implicated, and may be important in oncogenesis (2). In particular, HPV type 18 and, to a lesser degree, type 16 have been associated with aggressive cervical neoplasia (3). Because of the nature of HPV infections, detection and type discrimination rely heavily on nucleic acid methods. However, caution must be exercised in making the molecular biology-based diagnoses.

We have examined 11 paraffin-embedded early endocervical glandular neoplastic lesions by RNA–RNA in situ hybridization and polymerase chain reaction (PCR) methods with use of a variety of primers. Included in the PCR assays were the consensus primers of Manos et al. (4), which have been suggested for widespread screening of HPV (5). Although nine of 10 adequate samples gave clear positive results for HPV types 16 or 18 with the in situ method, screening with the consensus PCR primers showed no HPV involvement. The samples proved unsuitable for classic Southern blot analyses, as well. Although fixation procedures can diminish the size of sequences amplifiable in paraffin-embeddeds tissues (6), in this case, amplification of the KM-19 sequence, which has an amplimer size > 1.0 kb, was readily achieved from the same samples. In additional studies, confirmation was not seen with other PCR primers, including the E5 open-reading frame and upstream regulatory regions for HPV types 16 and 18. Best agreement was seen when using primers from type-specific E6 and E7 regions. The amplimers in these systems are the smallest used, <200 bp, compared with a 450-bp amplimer from the consensus region.

Although ultimately an agreement of 90% was seen between the sum of all positive findings in PCR trials and the in situ method, our experience indicates that individual PCR amplifications may miss HPV involvement in adenocarcinoma of the cervix. With the information we can generate from these materials, we are unable to conclude whether the disparity results from differential efficiencies of the PCR systems, or actual deletion of the consensus region from the HPV DNA present in this disease. This inability is particularly unfortunate because such deletions have been proposed to associate with the aggressiveness of the lesions (7).

References