We hopefully all agree that the CLSI could abandon its recommendation for drawing a discard tube using standard venipuncture techniques.

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The Authors’ Reply
We appreciated the comments from Drs Favaloro and Lippi on our study. We are glad that the findings in our report are in line with those mentioned in their letter.1-3 Together, these articles strengthen the conclusion that drawing a discard tube can be abandoned for coagulation testing.

The hypothesis of our study was that when using today’s collection systems and reagents, a discard tube is not necessary with regard to possible activation of released tissue thromboplastin during venipuncture. Our statement regarding CLSI guidelines and discard tubes is applicable only to the method used in our study. We agree with Drs Favaloro and Lippi that the preanalytic problems of intravenous catheters (ie, heparin contamination) and winged sets (underfilling) could lead to erroneous results and still require a discard tube, but these were not our topics under investigation.

References

Immunohistochemical Staining of Inflammatory Cells in Liver Biopsy Specimens of Patients With Autoimmune Hepatitis, Primary Biliary Cirrhosis, and Overlap Syndromes

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To the Editor
I read with great interest the study by Lee et al1 evaluating IgG and IgM immunohistochemical staining of inflammatory cells in liver biopsy specimens of patients with autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), and overlap syndromes. The authors report an IgG-predominant infiltrate in all cases of AIH. However, a large proportion (12/26) of PBC cases also showed a predominantly IgG+ infiltrate, which would potentially decrease the usefulness of IgM and IgG immunohistochemical staining in this setting.

In contrast with these results, Daniels et al2 reported IgG-predominant infiltrates in all cases of AIH (n = 38) and IgM-predominant infiltrates in the vast majority (16/18 [89%]) of PBC cases. Similarly, our group recently published a study3 evaluating IgM and IgG immunohistochemical staining in various forms of liver disease, including AIH, PBC, primary sclerosing cholangitis (PSC), and hepatitis C. In our study, an IgM/IgG ratio of 1 or more accurately distinguished PBC from AIH in 90.9% of cases, PBC from AIH or PSC in 87.8% of cases, and PBC from all other groups in 90.9% of cases. The latter 2 studies indicate that AIH and other forms of chronic liver disease typically show IgG+ plasma cells, while a predominantly IgM+ infiltrate represents a fairly sensitive and specific finding for PBC.

Some aspects of the study by Lee et al1 may have contributed to their discrepant results. First, several of the selected patients had received treatment for AIH, PBC, or both. Immunosuppression is known to affect the concentration of plasma cells in liver biopsy specimens in AIH,4 whereas ursodeoxycholic acid therapy has been associated with decreased portal inflammatory infiltrate in PBC.5 Therefore, we cannot exclude an influence of treatment in the distribution and phenotype of inflammatory cells in AIH or PBC. All PBC and AIH biopsy specimens included in our study were from untreated patients undergoing initial diagnostic evaluation because it is currently unclear whether any results obtained from analyses of treated patients are applicable to untreated cases.

Second, the use of tissue microarray may have significantly limited the amount of tissue for analysis. In several of our cases, assessment of numerous portal tracts was necessary...
dilutions, a fairly “clean” background can be obtained, allowing quantitative analysis or back-to-back comparison of immunohistochemical slides. Lee et al\(^1\) correctly point out, however, that with dilution of reagents according to routine, validated protocols for the aforementioned stains will often yield suboptimal slides. To circumvent this problem, we suggest a “liver protocol” dilution of IgG and IgM of 1:20,000 to 1:25,000.

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The Authors’ Reply

We thank Dr Moreira for his insightful comments about our article.\(^1\)

We acknowledged the limitations of our study in the “Discussion” portion of our article regarding the use of tissue microarrays and the background staining for IgG and IgM immunostains. The latter had some background staining, but we thought that it was not sufficient to interfere with interpretation.

In regard to treatment effect, our study involved a combination of pretreatment and posttreatment biopsy specimens. Of patients with PBC, 62.5% were untreated before liver biopsy and 37.5% received pretreatment with ursodiol. Of the patients with AIH, 58% were untreated before liver biopsy and 42% received pretreatment with one or multiple immunosuppressant agents. Within our overlap syndrome group, 54.5% were untreated before liver biopsy and 45.5% had received pretreatment (ursodiol, 1 patient; mycophenolate mofetil, 1 patient; azathioprine, 1 patient; hydroxychloroquine, 1 patient; and corticosteroids, 3 patients).

Overall, 41% of the entire cohort received treatment before the liver biopsies, and this may explain the discrepancy between our results and those of Daniels et al\(^2\) and Moreira et al.\(^3\)

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