Screening for Interference in Immunoassays

To the Editor:

In their report in a recent issue of Clinical Chemistry, Emerson et al. (1) screened clinical samples for the presence of interference in four immunoassays. Three different techniques were applied, defining interference as (a) nonlinear assay responses with serial dilution, (b) discrepant assay results after pre-treatment with heterophile blocking reagent (HBR), or (c) positive reactions on a mouse-antibody-negative control reaction (Tandem ICON® ImmunoConcentration hCG). The percentage of interference-positive samples varied significantly by technique, and the authors therefore concluded that prescreening for interfering substances with these assays is not warranted.

False-positive results caused by assay interference could be detrimental if undetected, as shown in the hallmark report by Rotmensch and Cole (2). Interferences have been characterized or labeled as heterophile antibodies, human-anti-mammalian antibodies, human-anti-mammalian antibodies, or heterophile-specific matrix effects. This is, in our view, the most likely cause of the extremely high prevalence of interference (up to 80%) reported by Emerson et al. (1). Concerning the use of HBR, it would be expected that the blocking of false-positive responses caused by interfering heterophile antibodies would produce lower analyte values. We were surprised to notice mainly higher values for hCG after pretreatment with HBR as demonstrated by the authors, suggesting an unrealistically high prevalence of interference leading to false negatives.

We agree with the authors that screening all clinical samples for all types of possible interferences is not feasible in practice, and we alternatively directed our efforts to devising assays that would be less susceptible to interfering substances (3, 4). Assessment of the presence of interfering substances is done by use of nonfunctional antibody combinations in a sandwich ELISA format that should not give a true signal because it was designed against a nonexistent analyte (so-called nonsense format; see Fig. 1). We found that interference was particularly noticeable when we measured low-abundance analytes, necessitating low sample dilution factors. Thus, the impact of assay interference strongly depends on the amount of analyte in the sample.

We established a sandwich immunoassay format that applies avian antibodies in the preanalyte and mammalian antibodies in the postanalyte stage. This has been found to essentially preclude all interference in these assays (3, 4). This effect is obtained because interfering factors that would typically bridge between pre- and postanalyte antibodies in the absence of analyte, leading to false positives, do not bind to avian IgY antibodies. The occurrence of false negatives, induced by shielding of the preanalyte antibodies by the interference, is most likely also prevented by use of this format. Avian antibodies are easily obtained from the eggs of immunized chickens (5, 6).

Thus, the effective use of nonsense formats to assess the presence of interfering substances, and the fact that immunoassays can be devised that are much less susceptible to interference, might be of interest in this matter.

References

3. Grebenchtchikov N, Sweep CG, Geurts-Moespot A, Pifanelli A, Foekens JA, Benraad TJ. An ELISA avoiding interference by heterophilic antibodies in the measurement of components of the plas-
A hook effect is a common problem in immunoassays, where falsely low results below the cutoff are observed in assays using immunochromatography. As with the Cardiac Reader, there have been no reports regarding the frequency of a hook effect. We did not observe falsely low values below the cutoff (80 μg/L) in our studies. Other cardiac markers, such as creatine kinase, are probably abnormally high in most cases in which the hook effect of myoglobin occurs; therefore, the risk of making a misdiagnosis is probably limited. However, because blood myoglobin concentrations can become highly increased in some patients, falsely low results below the cutoff may occur with this method, although this was not the case in our study.

In conclusion, users of the Cardiac Reader should be aware of the possibility of the hook effect for myoglobin, especially when using immunochromatography methods. It is essential to consider the potential for falsely low results and adjust the assay parameters accordingly to ensure accurate diagnosis.