Heteroantibody: Phantom of the Immunoassay

The heterophilic antibody, or heteroantibody, has been haunting immunoassays for many years. Interference by heteroantibodies in human sera can either increase or decrease the results of an immunoassay. For instance, by binding to a site other than the analyte-binding site, the heteroantibodies are capable of cross-linking the signal antibody with the capture antibody, thereby generating a false assay response. Such analytical errors may lead to mistakes in diagnosis and even to unnecessary surgical procedures (1).

Estimates of the prevalence of heteroantibody interference vary considerably, although as many as 40% of individuals may have one or more heteroantibodies that could interfere with some immunoassays (2). Many of the ever-expanding number of sensitive immunoassays containing murine monoclonal antibodies are using undiluted serum as the specimen for analysis, which increases the risk of heteroantibody interference in the assay. The increasing clinical application of mouse monoclonal antibodies for targeted imaging and immunotherapy also underscores the necessity for handling the heteroantibody problem in the best possible manner.

The standard approach for reducing heteroantibody interference is to include in the assay excess nonspecific immunoglobulin from the animal whose antibody is used in the immunoassay. For example, in the case of human anti-mouse heteroantibody, including mouse sera or nonspecific mouse immunoglobulin in the immunoassay can, in most cases, eliminate human anti-mouse heteroantibody interference.

However, two papers in this issue of Clinical Chemistry demonstrate that the phantom of heteroantibody interference refuses to vanish from the immunoassay. Boerman et al. (3) uncovered heteroantibody interference in an in-house immunofluorometric assay for the CA-125 ovarian cancer marker. Discordant results between this assay and a commercially available immunoradiometric CA-125 assay became concordant by including normal mouse sera in the immunofluorometric reagent. In addition, as they noted, the heteroantibody interference was best minimized when added serum and reagent antibody were from the same mouse strain. Kricka et al. (4) studied heteroantibody interference in an enzyme immunoassay of hepatitis B surface antigen in two patients receiving mouse monoclonal immunoglobulins. In one case, the interference was blocked by incubation with nonspecific mouse immunoglobulin at room temperature. However, in the second case, only prolonged incubation with a high concentration of the specific therapeutic mouse monoclonal antibody at 4 °C was able to block the heteroantibody interference.

Heteroantibody interference needs to be considered by the clinical chemists who develop and manufacture immunoassay kits. Inclusion in the assay of nonspecific mouse immunoglobulin that is allotypically and isotypically matched to the reagent antibody would appear to be a good standard practice. Even so, the possibility of an idiotypic specificity by the heteroantibody may result in assay interferences in some cases. The clinical chemist confronted with an immunoassay result that does not appear to fit the clinical picture, and perhaps also confronted by an alarmed physician, should consider the possibility of a phantom in the immunoassay.

References

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