Reliable Measurement of Circulating Immune Complex Depends on Stable, Accurate Reference Material

To the Editor:

Accurate quantification of circulating immune complexes (CIC) in serum is essential for the diagnosis, estimation of prognosis, and monitoring of antigen–antibody–complement-mediated diseases (1). At least 40 different assays for quantifying CIC have been reported in the past 20 years, but no single one can be used exclusively because results generally correlate poorly with the disease state (2). Attempting to standardize these assays of CIC, an expert panel under the auspices of the IUUIS/WHO investigated the suitability of two international reference preparations (IRP), based on either heat-aggregated human IgG (hA-IgG) or solubilized tetanus toxoid:anti-tetanus (TeaTe) complexes (3). They suggested that TeaTe be used as the IRP for standardization, but some manufacturers (e.g., Sigma Diagnostics, Cytotech, and Immuno-medics) continue to use the IRP hA-IgG.

At Hartford Hospital, we have complied with the recommendations of the IUUIS/WHO by offering two different assays to assist in the diagnosis and monitoring of immune complex related diseases: the Raji cell RIA (performed by Nichols Laboratory, San Juan Capistrano, CA 92675), and the CIC-C1q solid-phase EIA (performed in-house with a kit from Cytotech, San Diego, CA 92121). Evaluation of the CIC-C1q EIA and our six months of experience with it have been satisfactory, with results that supported the patients’ clinical presentations and substantial agreement with Raji cell RIA results, i.e., 65% agreement in results for 51 patients. This agreement increased to 74.5% when we used the Cytotech confirmatory procedure (4) on samples with above-normal results by the CIC-C1q EIA, but normal by Raji cell assay (n = 5), finding all such increases to be attributable to nonspecific factors (5). Confirmatory procedures do not exist for the Raji cell assay, so we cannot investigate nonspecific increases when results are normal by CIC-C1q EIA but above-normal by the Raji cell assay (n = 12).

A recent discrepancy in the CIC-C1q EIA values for our two in-house control materials ("RG", 2.60 mg Eq/L, and "32", 10.70 mg Eq/L) led us to re-evaluate the assay. The CVs for within-run and day-to-day runs—15.9% and 37.0% for RG, and 7.2% and 21.5% for the 32 controls—were within the manufacturer’s specifications and are representative of the present state of the art in CIC assays. However, an examination of the CVs for inter-lot assays gave 194.5% and 39.0% for the RG and 32 controls, respectively. As Figure 1 shows, the curves for concentration vs absorbance for the different standards in each lot depart from linearity for lots A, E, and F, although the regression analyses still met the manufacturer’s stipulation for acceptable slope and intercept. This nonlinearity of the standard curves caused negative or positive biases in CIC-C1q EIA results for test samples.

This discrepancy was attributable to incorrect value assignment to the standards, because use of standards from lot B in the assays of lots E or F gave us acceptable linear curves. Evidence that accuracy and an acceptable reference standard material are still major problems in CIC assays. The instability of the materials in the longitudinal study by Nydegger and Svehag (3) had also been previously noted by other investigators (6). We therefore urge the IUUIS/WHO to re-evaluate the problem of international Reference Material for CIC assays. All forms of reasonable materials should be included: heat-treated, sodium sulfate-precipitated, alkali-treated, or glutaraldehyde-polymerized human IgG, and the solubilized TeaTe complexes. The effects of lyophilization and long-term storage conditions should also be thoroughly addressed.

References

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The "Fecatwin Sensitive" Test for Fecal Occult Blood Is Not Intended for Use without Testing Positives with the FECA-EIA Assay

To the Editor:

Recently a latex-agglutination test for immunological detection of fecal occult blood was described in this journal (1). We believe that the comparison of this test with three guaiaec tests as carried out by the investigators does not give a true picture of the performance of the various tests. Furthermore, the article contains a few minor errors, deserving comment because they could mislead the readers.

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