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Editorial

Immunoassay interference: a relatively rare but still important problem

Immunoassay is a beautiful analytical technique. The method uses the extraordinary specificity of a bioanalytical reagent, the antibody, and a labeling system based on absorbance, fluorescence, luminescence, etc., to come up with measurements of incredible sensitivity and specificity. Routinely, this method can quantify minute amounts of diverse analytes present in highly complex biological fluids such as serum. Serum total protein concentration (comprising hundreds of thousands of different molecules) is in the range of 10^{11} ng/l and a good immunoassay can detect down to 1 ng/l. To compare with the total population on earth (6×10^9), it can be concluded that this technique can identify a single person with relative ease.

Despite its extraordinary power, the method is not perfect. This is, from one point of view, desirable for Clinical Chemists. Many of us are employed to optimize immunoassay methodology at industrial settings, or work in hospitals to delineate analytical problems which complicate the interpretation of data and may lead to wrong diagnoses and inappropriate treatments. There are many reasons for an immunoassay to fail [1]. Some reasons are clearly pre-analytical and may not be due to immunoassay failure per se. For example, inappropriate collection or storage of a specimen may lead to degradation of the analyte of interest (e.g., adrenocorticotropic hormone) and to an erroneously low result, despite optimal performance of the immunoassay used. On other occasions, the immunoassay may produce erroneous results due to contamination of the sample with analyte (e.g., carry-over of human choriogonadotropin from a serum of a pregnant woman to a serum that was originally negative for the analyte due to ineffective washing of the pipettor of an instrument). On other occasions, erroneous results for anlaytes may be reported due to sample mix-up, mislabeling, etc. Also, sample quality may affect some immunoassay results (e.g., presence of overt hemolysis, hyperbiliorubimia, lipemia, etc.).

Other general problems of immunoassay are related to antibody specificity and cross-reactivity. Sometimes, the antibody may recognize closely related molecules (e.g., cortisol and cortisone). Cross-reactivity problems are well known to immunoassay kit manufacturers and the package inserts of each immunoassay include known cross-reactants. Other problems of immunoassays have been recognized for many years [1,2]. For example, it is well documented that the presence of autoantibodies in diseases such as rheumatoid arthritis, systemic lupus erythematosus, scleroderma, chronic active hepatitis, etc., may lead to erroneous results for some analytes [3,4]. On other occasions, endogenous hormone-binding proteins (present in excess or absent) may also complicate immunoassay performance. Endogenous autoantibodies to the antigens of interest (e.g., against thyroid hormones, thyroglobulin, etc.) may further complicate the analysis of serum of certain groups of patients. By far though, the most common problems that are seen in immunoassays are related to the presence of heterophilic antibodies in patients' sera or to the presence of huge amounts of analytes, leading to the so-called high-dose hook effect [5–8]. These problems have been reviewed in the past and there is no need to elaborate on them in detail. But what are "heterophilic antibodies"? These are antibodies present in human serum, recognizing animal immunoglobulins, usually employed in commercial immunoassays (e.g., those derived from mice or rabbits). In sandwich-type immunoassay formats, these antibodies have the ability to link the capture and detection antibody without presence of antigen, thus leading to false-positive results. This problem has been recognized for many years [5,6] and there are now ways of eliminating most (but sometimes not all) of the interference. This is achieved by addition in the reagent mixture of animal immunoglobulins such as rabbit, goat or mouse immunoglobulins, or proprietary reagents developed by companies. One would thus expect that interference by heterophilic antibodies in commercial immunoassays should be a relatively rare event.

In this issue of *Clinical Biochemistry*, Cole and Khanlian report false-positive human choriogonadotropin (hCG) results, identified over many years, and pinpoint to the fact that most of these false-positives are related to a specific product, the Abbott AxSym Total Beta-hCG Assay [9]. The authors propose a simple method to eliminate this interference by sample dilution with a diluent containing goat immunoglobulins. Human choriogonadotropin is an important biochemical test, used to confirm pregnancy, identify gestational trophoblastic diseases, including neoplasms and testicular germ malignancies in men. Since hCG is such a powerful cancer biomarker, its apparent presence in serum of men and non-pregnant women is a strong indication of a disease process, even in the absence of clinical symptoms. The authors have documented that the vast majority of patients with falsely elevated hCG were treated with inva-

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sive procedures, including dilation and curettage, laparoscopy, chemotherapy, hysterectomy, salpingo-oophorectomy, and throracotomy.

There are some clear messages from this paper. One is that despite immunoassay being a wonderful analytical technique, we still see false, and potentially misleading results. Such data, as shown in this paper, can be produced by kits from diverse manufacturers. It is up to the intelligence, competence, and experience of the Clinician to contrast such data with the clinical situation and make a decision on how to proceed further. In any case, suspect data should be brought to the attention of the Clinical Chemist who will then explore, and likely offer solutions by repeat analysis, testing on different platforms, etc. What we always teach our Clinical Chemistry residents and Medical students is that every biochemical test must be interpreted in the context of the whole clinical picture and never in isolation.

Of particular interest in this case is as to why a product from a major manufacturer (Abbott) appears to demonstrate, according to Cole and Khanlian [9], such a poor performance, in comparison to other manufacturers of the same test. Since there is a simple solution to this problem, I hope that the manufacturer already has, or will take measures to rectify it at the earliest possible time.

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