

Low-quality manufactured immunoenzymatic assay kits as a source of confusion in science

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To the Editor Manufactured kits using immunoenzymatic methods are a key research tool to measure the concentrations of various peptides and proteins in biological fluids. Specificity of polyclonal or monoclonal antibodies used by a manufacturer is critical to the quality of the assay. The process of antibody production before obtaining a patent protection does not require experiments to confirm specificity of antigen recognition in biological fluids. Some manufacturers show data of cross-reactivity with selected peptides and proteins. However, in biological fluids, not only peptides in full length, but also those arising from the degradation, are present. They are frequently not biologically active but may be detected together with the active peptide or protein. Therefore, a scientist, as the user of a kit, frequently does not know what exactly he or she measures in the specimens.

The enthusiasm associated with the launch of a new tool on the market, which creates a chance for new discoveries, marginalizes the need to validate the immunoenzymatic kit against reference methods. The specificity of antibodies can be validated in functional assays, such as western blot, immunohistochemistry, and immunofluorescence.¹ For proteins or peptides in biological fluids, the most appropriate validation test is western blot, as it enables determination of antibody specificity against target protein based on molecular weight as well as lack of binding with other proteins in the blood.

We would like to describe a case of the use of a nonvalidated immunoenzymatic assay, namely, the visfatin/NAMPT EIA kit, developed by Phoenix Pharmaceuticals Inc. (Burlingame, California, United States). It was introduced to the market in 2005 as the first immunoenzymatic assay kit for C-terminal visfatin (NAMPT). The first concern about the quality of the kit was raised by Körner et al² in 2007. They found no correlations between serum visfatin levels obtained by the enzyme immunoassay (EIA) and radioimmunoassay or between the EIA and enzyme-linked

immunosorbent assay (ELISA), and reported a discrepancy between the mean levels of 20 ng/dl. Furthermore, they found that the EIA detected a single distinct peak of high molecular mass more than 500 kDa, but no product close to the molecular mass of visfatin (55 kDa) or its dimer (110 kDa). Therefore, studies that have applied this kit show data for an undefined protein with a molecular weight of ~500 kDa, wrongly considered as visfatin/NAMPT. ELISA kits are more specific for the assessment of visfatin/NAMPT.² They give significantly lower values (more than 10 times) in a plasma or serum sample.³ The study by Körner et al² went unnoticed by Phoenix Pharmaceuticals and numerous investigators who still use their EIA.^{4,5}

We decided to inform scientists about the questionable quality of some EIA kits to increase their awareness about the issue. The choice they make is important and the quality of kits is crucial to obtain reliable results from research. On the other hand, manufacturers should consider performing control studies with western blot to validate the specificity of antibodies used for EIA, ELISA, or electrochemiluminescence immunoassay before making the product available to users. Such information should be included in a product manual. Moreover, companies should monitor the use of their products in published studies. An introduction of such new customs, including validation of antibodies specificity against reference method and monitoring of the studies utilizing the kit are expected for products labeled “only for research”.

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