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Editorial

The last issue of Applied Cancer Research brought up for discussion the timely and interesting subject of quality and the importance of optimized protocols for immunohistochemical exams, especially in what we call today the "panel of breast cancer molecular types definition."

Immunohistochemical technique had its implantation in the decade of the 70s and its use applied to diagnostic pathology disseminated in the following years. At that time, the number of available antibodies was limited and the reaction revelation techniques were not very sensitive, and it goes without saying that the prices for these reactions were towering. Despite this, its popularity grew quickly and has turned into one of the most important instruments in diagnostic pathology in the twentieth century. Since that time, and especially in the last five years, we have been in the midst of a tremendous technical advancement. The development of polymeric revelation systems and the appearance of a new generation of monoclonal antibodies produced in rabbits have changed the way we can use and interpret immunohistochemical tests.

Besides these incontestable technological advancements, there is still a long way to go so that we have quality control, reproducibility and confidence, especially when the result of the test changes medical decision or gives information of the clinical behavior of the tumor.

One of the most problematic aspects, whether in daily practice or scientific investigation, is the pre-analytic process. Although this is much simpler and less expensive than technological development, little or no attention has been given to the process of adequate fixation, time of fixation, time of cold ischemia and the transport of material to the immunohistochemical laboratory. Today, the great problems of reproducibility and the comparison of results are tied to the low commitment of pathologists with the pre-analytic process. It is not uncommon that we encounter materials of low quality, which leads to serious problems of diagnosis that compromises good quality service.

In spite of these problems, the immunoexpression analysis of markers is now in a new era. It was only a few years ago, with the development of molecular pathology, pathologists had many doubts of the future of immunohistochemistry and even what path surgical pathology would take. However, against overwhelming odds, immunohistochemistry experienced a great rebirth and the curve of its use and importance has seen a positive inflection in the direction of growth. The necessity of validation of molecular findings resulting from experiments with gene expression has made immunohistochemistry one of the fundamental tools in molecular pathology.

We still have much work ahead for immunohistochemistry to have the appropriate development so that complete development is reached. The principal path of this process passes through the standardization of methods and materials. This methodologi-

cal standardization in several laboratories is urgent and mandatory. Today, more often than not, if an investigator or surgical pathologist needs to demonstrate the presence or absence of a determined protein simply, they simply acquire a flask of antibody (preferably from a reputable company) and conduct their test. The urgency of time means that we do not make the necessary tests. In this sense, the study of Rocha and collaborators gains a unique importance. In this study, the best amplification systems are tested for one of the most important tests in surgical pathology, the expression of estrogen receptors in breast carcinoma, and moreover, the reading of the reaction is used in an automated manner. The literature referring to the technical aspects and validation of several antibodies are rare, especially when compared to studies that use the technique to prove a hypothesis. Rarer still are the scientific articles testing the technical aspects common to all the reactions done in the laboratory. Standardization, the development of validation tests, quality control and the comparison between different antibodies and revelation systems is imperative for all who use immunohistochemistry as a diagnostic or research instrument. The use of automation, controlled pre-analytic processes, standard operational procedures and quality measurement tests are mandatory for laboratories that want to have immunohistochemistry revealing what takes place in tumor cells. We need to encourage that work in our laboratories is done with scientific rigor in the test and search for the ideal antibody choice, adapting more to the question that it aims to answer, and to overall laboratory standardization.

Finally, it is appropriate to observe the post-analytic phase that the study of Rocha *et al.* also suggests for discussion. In that study, the comparison used an automated system. Today, we have on the market several types of machines that carry out automated immunohistochemical analysis. These optical density readers bring another of the most significant advancements of the last several years. It is widely known that immunohistochemical reaction is not a stoichiometric reaction, where intensity can have a direct relation with protein quantity. Reaction to reaction, many factors can influence the intensity of the chromogen deposit and it should not be interpreted directly. However, it is fundamental that we establish acceptance levels to a reaction to classify it as positive and give this result a biological meaning. We cannot think of an optical density number as absolute, but we can when comparing tissues, thus, this objective analysis can be extremely useful. Accordingly, studies using these machines are very promising and should, with economy, substitute our subjective analysis.

In summary, this method, which has gained a new impulse recently with the validation of gene expression, and especially in therapeutic and prognostic indication, must be perfected and studies as this should undoubtedly be stimulated so that we have a scientifically sustained evolution.

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