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A Response to the Response:

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The purpose of my previous article was to identify many of the potential sources of error to be considered when evaluating quantitative data from immunohistochemistry (IHC) and histochemical reactions on sections.

Factors that may affect the evaluation of quantitative IHC data were listed but not necessarily placed in their order or importance. This would be difficult to do, as the relative importance of factors may differ for different antigens and histochemical procedures.

As far as can be determined, there appear to be no definitive articles that unequivocally show that IHC reactions are stoichiometric. In most laboratories, it is impractical to accurately determine section thickness, and furthermore many of the image analysis systems used may be somewhat rudimentary. Accurate determination of section thickness may therefore be somewhat of a moot point. One important point to note is that usually only a small number of sections are examined, and this often limits or prevents the generation of meaningful statistics.

In the USA, the increasing use of quantitation of IHC may be due, not to the need for greater reproducibility and accuracy, but to the need to justify the pathologist's decision in difficult (borderline) cases. It is questionable whether this can be accomplished to the degree that is always required – due to the limited number of sections customarily used and other factors.

Is the measurement of nuclear antigens useful? This is a difficult question to answer. The majority of papers in which nuclear antigens have been accurately quantitated have been electron microscopy rather than light microscopy based, and have used IHC prior to processing.

For many years the tendency has been (at the light microscopic level) to use the thinnest possible sections to obtain the optimal resolution and clear nuclear detail. This has increased the possibility of variation in section thickness and also the detachment and loss of portions of nuclei from the surrounding tissues. It should be noted that this problem of detachment can occur not only with nuclei but also with other structures including nonattached cells such as erythrocytes.

Standardization of fixation is difficult due to the large number of variables including prefixation time, amount of free blood, volume of fixing solution, size and composition of tissue, and so on. The routine fixation steps carried out in most pathology laboratories essentially result in only a partial fixation, and even with standard sized blocks of the same tissue there can be considerable variation.

There is no absolute answer as to whether quantitation is a useful tool in IHC. The question that should be asked is what significance will the pathologist place on the IHC quantitative data? Pathologists use their considerable expertise, and in most cases base their diagnosis on many factors, of which the IHC quantitation may play a minor or maior role depending on individual circumstances. ■