

target and comprehensively controlling all steps of the procedure is necessary to achieve true quantitative immunohistochemistry.

Quantitative immunohistochemistry

Whether the ultimate goal is to find clinically meaningful cutoff levels or the accurate measurement of antigen molecules per cell, interlaboratory reproducibility of immunostains is fundamental.

At least two separate forms of quantitative immunohistochemistry are readily evident. In its simplest form, events are merely measured, with no attempt to assay for the quantity of analyte expressed. Examples of this type are counting micrometastases in bone marrow samples or measuring peritumoral blood vessels. In these examples, minor variations in the intensity of the immunoreactivity, attributable to the method of staining or fixation procedure, have little impact in the quantification itself. With good control of specimen preparation and staining procedures, these simple quantitative procedures are currently attainable by most laboratories.

Accurate conversion of immunoreactivity into levels of analyte per sample, is much more complex, requires special equipment and, given the vagaries of fixation and processing, is not currently possible on archival paraffin-embedded material. However, prospective studies may be possible with the use of specially designed control materials, as discussed below.

Controls in quantitative immunohistochemistry

Sections of tissue expressing the target molecule, as well as tissues known not to express it are currently used routinely in immunohistochemical procedures in virtually every laboratory. The problem with such control tissues is that, at best, they only serve to control the immunostaining procedure itself. Other sources of variation such as fixation and processing are not controlled. Moreover, the amount of target molecule present in the control tissues is, more often than not, unknown.

The ideal control for quantitative immunohistochemistry should apply to the entire procedure, from fixation to interpretation of results. A suggested possible approach is to suspend in a solid matrix, cultured cells – expressing known, and independently measured, quantities of the target molecule – and to place such artificial tissues within the tissue cassette alongside the specimen (5). By this means, both specimen and control are simultaneously subjected to fixation, processing, antigen retrieval, staining and interpretation.

This would be particularly useful in cases where the need for quantitative immunohistochemical assays is anticipated, as in the case of breast biopsies. Some progress along these lines has been recently reported using breast cancer cell lines that express known amount of hormone receptors (6). The addition of automated, computer-assisted microspectrophotometry, will facilitate conversion of intensity of immunoreactivity into actual expression of molecules. This then can lead to reports of quantitative immunostains in terms familiar to clinicians, with the added accuracy that immunohistological methods offer (4).

Perhaps of more immediate importance, such artificial tissues could provide a means of assuring comprehensive interlaboratory standardization of immunohistological methods, as convenient and reproducible check samples, readily scored by automated computerized microspectrophotometry.

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Automation in immunohistochemistry

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The purpose of this conference is to briefly discuss to the advantages and the disadvantages of the recent advances in automation of immunochemistry. The term "automation" must be understood as the set of processes involved in the preparation of stains in immunohistochemistry, including all the procedures, as well as the generation of the results and their interpretation, all requiring no direct manual intervention. Automation in immunohistochemistry offers the opportunity to improve our levels of sensitivity, reproducibility and standardization, while the working time is reduced and reagents are saved. Immunohistochemistry is a repetitive technique that consists of a cycle of washing with buffer, application of the reagent, then rewashing with buffer, which makes this a procedure which can be easily automated.

Problems of immunohistochemistry without automation

Three critical points in immunohistochemistry that depend directly on the human intervention can be delineated as follows:

- i) Appropriate handling of the specimen, its suitable fixation, its later inclusion in paraffin, as occurs with most of the samples, and the preparation of the sections are all vital in the final result of the stains.
- ii) Another critical step includes the preparation of reagents, antibodies and application of solutions, times of incubation, suitable dried washing and crystals. These activities are done manually and repetitively. To present, these activities have been object of automation and will be discussed in further detail.
- iii) The interpretation of the results is always the responsibility of an expert pathologist who is familiar with each one of the antibodies, its diagnostic possibilities and limitations. This is the most difficult aspect at the present time. An attempt is now being made to remove the human intervention from this process, but many years remain before automatic interpretation and quantification of stains can be applied to diagnostic routines.

A immunohistochemistry technician is able to handle 40-50 slides every 4 h, excluding the time taken for the previous process

of deparaffinization and antigenic recovery. The technician must also remain attentive, since every 5-15 mm some changes must be made. When multiple crystals are subjected to manual immunohistochemistry techniques, the time of incubation and the time that is required to apply the reagents are variable. This produces comparably unequal results, both within the same laboratory from one day to the next and from one laboratory to another.

The time necessary to offer the results of the immunostains is a minimum of 24 h, which delays the emission of the report by at least one day. The complexity of the manual procedure causes inadequate errors through changing of the correct sequence, errors in the times of incubation, washing, etc.. The consumption of reagents is also an important factor.

Some automatic systems in immunohistochemistry

The market has provided different answers to the problem of the automation in immunohistochemistry. There are several types of equipment now available on the market. Two major groups can be distinguished: those that use the principle of capillarity as the basis of the design of the instrument, and those that have slides in a horizontal position and, therefore, provide the reagent either by pulverization or by dripping it onto the slide. All the equipment has a reaction chamber with various capacities for the slides, a dispensation system for the reagents and a computer-run system that controls all the steps. Table 1 details the better characteristics of some of this equipment. Neel *et al.* recently undertook a comparative study to evaluate five instruments for several factors, including: the analytical flexibility, *i.e.*, the number of protocols and number of antibodies by run and the ability to transfer manual immunohistochemistry techniques to automatic instruments; the feasibility, *i.e.*, the ability of each instrument to selfregulate incubation temperature; the productivity, measuring mainly the number of slides/time dedicated; and, finally, the cost of the reagents and the consumable ones for each of the instruments. In my opinion, independent of the conclusions obtained by the authors of this study, each laboratory must obtain its own conclusions regarding the advantages that some of these instruments offer, and adapting them to the concrete needs of each one of the laboratories where they are going to be installed.

Effects of automation of immunohistochemistry

The most important consequences of automation in immunohistochemistry are noticed more by the personnel who work in the laboratory. Automation simplifies the 16-step manual method down to another maximum five-step method, which more or less reduces

the time per slide from 11 mm to 1 mm. The number of slides that can be examined in one work day is increased remarkably. With some equipment, the stains can be ready in less even than 1 h, with quick results included in the final report. It is the responsibility of the pathologist to combine the information obtained in a slide dyed with hematoxylin and eosin with the results of the new immunohistochemistry techniques. In an acceptable time frame, automated immunohistochemistry provides the pathologist with trustworthy results in standardized conditions that will not vary from one day to the next. On the other hand, if a cost analysis is undertaken in which we include the cost of the apparatus, one of the consumable reagents, maintenance, the study, and the response time, we will observe that this cost will always be much less than the amount saved with the working time of the laboratory technicians. Having trustworthy and fast results provides for greater speed of diagnosis, which in turn can shorten the period of hospitalization, thus resulting in important savings. The automated immunohistochemistry guarantees constant quality and discharge of stains and improves the standardization and optimization of the different techniques. The use of computer systems that run this equipment makes possible the exchange of programs containing different protocols for staining, and, therefore, the use of same the protocols with similar instruments allows for standardization from one laboratory to another.

Requirements of automation in immunohistochemistry

Analytical flexibility is necessary. To achieve this, an open system with which all types of reagents and protocols can be used is indispensable. The instruments must be able to process many antibodies, many slides, as well as many protocols – ideally as many as there are antibodies.

The productivity must be high, meaning therefore that the time dedicated to each stain must be little; ideally in less than 1 h it must be possible to dye more than 40 slides. The different instruments must be able to function without great time spent by technical personnel. The cost per test with an automatic system, including the cost of the antibodies and reagents, would have to be same as that for the manual method. The use of closed systems would favor biosecurity in every laboratory.

Conclusion

Automation contributes decisively to the standardization of immunohistochemical techniques. In addition, it also allows laboratories without technicians with great abilities to have highly reliable immunohistochemical techniques. Automation improves the confi-

Table 1. Comparison of automated immunostainers.

| Features | Techmate | Horizon | Nexes | Ventana ES | Leica | Optimax | Cadenza |
|------------------------------------|-------------|-------------|--------------|------------|----------|---------|----------|
| Volume of reagent/slide (p1) | 150 | 150 | 100 | 100 | Variable | 100-400 | Variable |
| Individual time control for slides | No | No | Yes | Yes | No | No | No |
| Programming | Complex | Simple | Complex | Complex | Simple | Complex | Simple |
| Enclosed processing | No | No | Yes | Yes | No | No | No |
| Temperature control | No | No | Yes | Yes | Yes | No | No |
| Specialized slides | Yes | Yes | No | No | No | No | No |
| Slide numbers | 120 | 40 | 20 by module | 40 | 20 | 40 | 20 |
| System machine | Capillarity | Capillarity | Spraying | Spraying | Spraying | Drop | Drop |

dence of the pathologist in immunohistochemistry and, therefore, it allows him or her to decisively integrate the results of the immunohistochemical techniques in the diagnosis.

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External quality assessment of immunocytochemistry: The experience of the UK National External Quality Assessment Scheme

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Introduction

The UK National External Quality Assessment Scheme (UK NEQAS) for Immunocytochemistry has been established since the mid-1980s. Besides monitoring and reporting on standards, the Scheme also has an educational and supportive role. The activity, while subscription funded, is nonprofit making.

Today the Scheme offers assessment of immunocytochemistry employed in five diagnostic areas: general histopathology, neuropathology, hematopathology, breast cancer and non-gynae cytology.

Operating Procedure

Participating laboratories are asked to submit immunostains for specified antigens on sections provided by the Scheme and on 'in-house' control sections together with a completed questionnaire on the methodology employed. All slides are coded to ensure anonymity.

Assessment is carried out by four independent assessors using a multihead microscope. The assessment panel usually includes one histopathologist and three biomedical scientists. Each assessor scores up to 5 points and the scores are then totalled. Scores of less than 10 indicate poor quality immunocytochemistry; 10-12 points (inclusive) are given to slides with suboptimal immunostaining where a little improvement is required. Scores greater than 12 indicate that the immunostaining is of the expected standard. Participants receive reports on the scores they have achieved together with some comments from the assessors.

All participating laboratories also receive assessment review booklets that include the following information: an outline of the assessment criteria employed for the antigens in question; photographs of best examples of immunostaining; examples of methods achieving scores in the region of 18-20/20; and graphical illustrations comparing scores achieved with reagents/methods and instruments employed.

Example of assessment run for estrogen receptor protein in breast cancer

Estrogen receptor immunostaining in breast cancer has gained clinical importance in recent years and as a result, external assessment of this protein now occurs on a regular basis. During 1998, UK NEQAS provided participants with sections from a composite block comprising three different breast cancers. The levels of estrogen receptor protein in each tumor was confirmed by immunostaining in several different laboratories and was as follows: high expressor, medium expressor or low expressor.

Assessment criteria

In summary, the scores were awarded as follows:

- i) The demonstration of expected levels of estrogen receptor in all three cases attracted scores of 13/20 or more (passed).

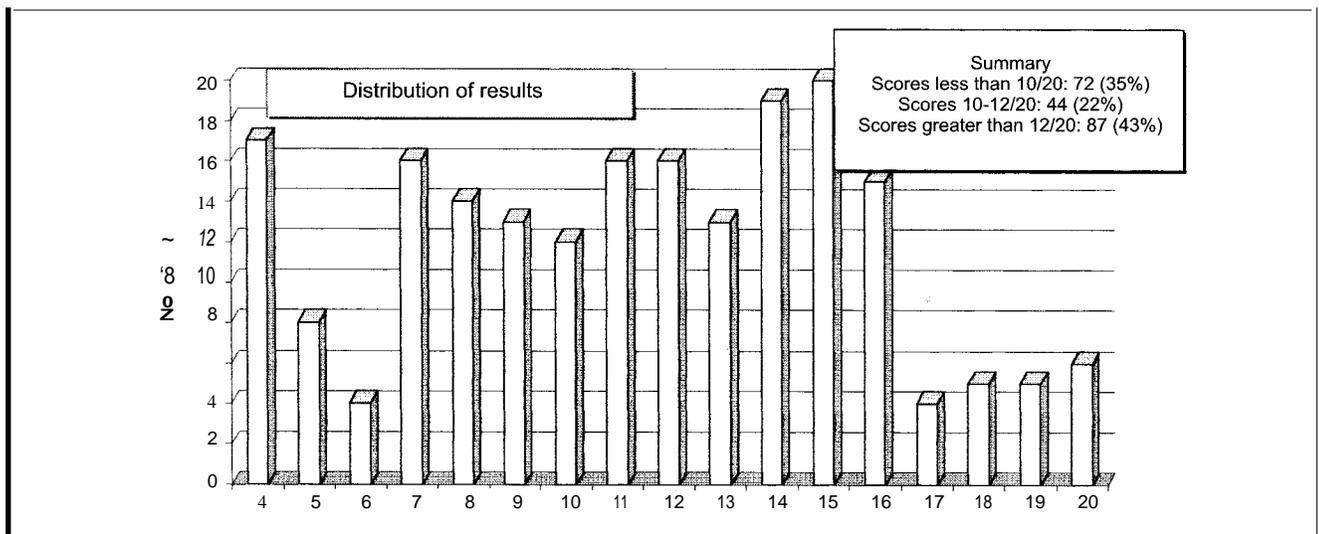


Figure 1. Assessment run 42E estrogen receptors (UK NEQAS sections).