SHORT REPORT

Miniature tissue microarrays for HercepTest® standardisation and analysis

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J Clin Pathol 2004;**57**:1229–1231. doi: 10.1136/jcp.2004.018689

Aims: To assess the practicality of using a miniature tissue microarray (TMA) with several examples of each HercepTest® score from 0 to 3+ as a control for routine HercepTest immunohistochemistry.
Methods: A TMA was constructed from in house cases of breast cancer where HercepTest on the whole sections showed scores 0, 1+, 2+, or 3+. The TMA, which measured 5 × 5 mm, was designed with four rows (each representing scores 0, 1+, 2+, and 3+), with five 0.6 mm cores from separate cases. In all, 20 individual cases were represented and the TMA took less than one hour to construct. Fifty sequential 4 μm sections were cut from the TMA to maximise the number of available sections. They were stored at 4˚C for 1–270 days and when a case needed HercepTest staining the section was added to the TMA tissue control slide.
Results: All slides contained tissue spots and immunohistochemical staining was consistent throughout the time period.
Conclusions: The miniature TMA with examples of all HercepTest scores described here is an ideal tissue control and can be used as a visual reference for scoring a case. Slides stored at 4˚C could be used for up to 270 days.

Investigation of HER-2/neu expression in breast tumours is rapidly becoming a standard test in most pathology laboratories. It is usually assessed by means of immunohistochemistry and most laboratories use the HercepTest® (DakoCytomation, Ely, Cambridgeshire, UK).[1] The immunoslides are scored semiquantitatively according to HercepTest guidelines as 0, 1+, 2+, and 3+. Zero (0) and 1+ are considered negative and 3+ positive. Cases that score 2+ are borderline and require further evaluation by in situ hybridisation for Her-2/neu amplification. There are two common problems with the HercepTest. (1) The HercepTest is sensitive to variations in antigen retrieval (antigen retrieval buffer temperature and incubation time) used and requires appropriate positive and negative controls with each run. Ideally, this should include borderline cases, but often these are not performed except for in external quality assurance test sets.[1 2] (2) The guidelines for scoring the HercepTest provided by DakoCytomation are complex and many pathologists are aided by the light micrograph examples included (DakoCytomation HercepTest® scoring manual). In our experience these guidelines are best kept beside the microscope for consultation in each case, in particular for 1+ and 2+ cases.

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Tissue microarrays (TMAs) are constructed by taking cylindrical core biopsies from ''donor'' blocks with subsequent precise arraying into a new ''recipient'' paraffin wax block using a commercial precision instrument. Traditionally, the TMA technique is considered to be a research tool for the examination of large numbers of cases.[3] However, smaller arrays use minimal amounts of tissue, are quick to construct, and can be used as controls present on stained glass slides.

It was the aim of our study to explore the possibility of constructing a miniature TMA with several examples of each of the scores from 0 to 3+. Sections of this miniature TMA could be used as controls on sections of current tumours requiring HercepTest evaluation. The advantage of such a TMA would be twofold: first it would be an important internal control that would be readily available for review (being present on the actual relevant slide) and second it would be a useful visual reference for the pathologist scoring the case.

METHODS

Routine cases of breast carcinoma were reviewed for their HER-2/neu score and specimens that were homogeneous and typical for a particular score (0, 1+, 2+, and 3+) were picked and specific areas marked on the glass slide. Cases with the 2+ score were further evaluated by fluorescent in situ hybridisation using the Vysis PathVysion (Abbott UK, Queensborough, Kent, UK) HER-2 assay. One 0.6 mm core was taken from each case using a precision instrument (Beecher Instruments, Silver Spring, Maryland, USA). In this way, 20 cases were included with five cores for each of the scores 0, 1+, 2+, and 3+. A marker core of kidney tissue was used for orientation. Fifty sections (4 μm thick) were cut from the TMA and mounted at the bottom of adhesive slides (SurgiPath) (fig 1). The first and 50th TMA sections were stained to confirm the original HercepTest whole section scores. The remaining sections were stored at 4˚C in a sealed slide box and stained one to 270 days after being sectioned. Each time the HercepTest was requested in the laboratory, a section of the tumour for staining was mounted above the TMA control section. Both sections were then dried overnight at 55˚C before staining.

Immunohistochemistry

The assessment of HER-2/neu expression was carried out using the HercepTest kit according to the manufacturer's instructions. After dewaxing in xylene and rehydration through graded alcohols to distilled water, the section was subjected to heat induced epitope retrieval by immersion in preheated epitope retrieval solution (95–99˚C) in a waterbath for 40 minutes. The sections were then allowed to cool down for 20 minutes at room temperature. Endogenous peroxidase activity was blocked by a five minute treatment with peroxidase blocking agent. Sections were then incubated

Abbreviations: TMA, tissue microarray

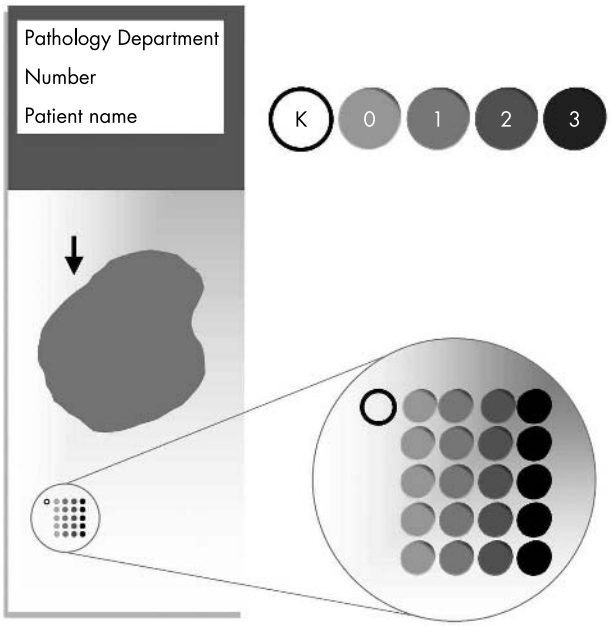

Figure 1 Schematic drawing of a glass slide with a tissue sample (single arrow) and the tissue microarray (ring). A close up of the ringed tissue microarray is shown on the right hand side (lower). Key to array cores upper right: K, kidney control core; 0–3, HercepTest score.

with the anti-HER-2 polyclonal antibody for 30 minutes at room temperature, followed by incubation with a visualisation reagent (labelled streptavidin–biotin–immunoperoxidase). The antigen–antibody reaction was visualised using 3-3′-diaminobenzidine as chromogen and counterstaining was performed with haematoxylin. Suitable negative and positive control slides were treated in a similar manner to ensure appropriate staining.

Immunohistochemical assessment

Formal assessment was carried out by a single pathologist (CG) when all the slides had been stained (that is, after 270 days) using the DakoCytomation HercepTest scoring manual. Figure 2 shows examples of cores showing HercepTest scores 0 to 3+. As described above, all the TMA sections were used as controls for routine cases and were therefore also checked independently by other pathologists within the department.

RESULTS

In total, 50 sections were examined one to 270 days after sectioning. All slides contained tissue spots and the small size of the array prevented the problem of fitting current sections on to the glass slides. All TMA tissue spots adhered well to the slides regardless of time after initial sectioning. Formal assessment of the slides showed that the staining was consistent throughout this period of time: there was no attenuation in the immunohistochemical signal and all cores showed preservation of the original score. There were no discrepancies noted between the formal assessment of the TMA sections and the more informal assessment by the other pathologists in the department when used in a routine setting.

DISCUSSION

Our report describes a miniature TMA with cores representing all possible HercepTest scores. Fifty sections from this TMA were cut sequentially and stored on glass slides in a fridge for up to 270 days. When a current case needed HercepTest staining the sections were added to the TMA tissue control slide.

The TMA control was present on the same slide as the test case and included borderline cases (2+), in addition to negative (0 and 1+) and positive (3+) cases. Therefore, small variations in staining intensity could be detected within each run of immunohistochemical staining. Another advantage was that this system provided a visual aid to the pathologist scoring the case, because the TMA was present on the same slide as the case and also because the TMA provided depth of focus, which is lost in photomicrographs

As recommended by Ellis *et al*,[4] tissue based controls from breast cancer cases are a valuable adjunct to the cell line control provided with the HercepTest. Furthermore, the use of in house tissue controls, which have been subjected to similar/identical fixation, processing, and embedding procedures as the test cases can act as ''total assay'' controls encompassing all the laboratory processes that will impact on the interpretation of HER-2/neu expression. The fact that the TMA control is on the same slide as the test case cuts down on slide handling, and may lead to significant time and cost savings in a reference laboratory carrying out hundreds of HercepTest assays each year.

To maximise the number of slides available from the TMA, all 50 sections were cut at baseline and stored in a fridge on glass slides. After 270 days there was no decrease in the immunohistochemical signal. Furthermore, because all cores showed preservation of the original score, tumour heterogeneity did not appear to play an important role.

The use of multitissue blocks as controls for immunohistochemical assays is not new,[5] and multitissue TMAs have also been used successfully as general controls.[6 7] Our current study specifically looks at the use of control TMAs in

Take home messages

- We have developed a miniature tissue microarray for better and efficient internal HercepTest quality control and easier, more accurate scoring
- Slides stored at 4˚C could be used for up to 270 days
- This approach could be extended to other immunohistochemical assays (especially if they have very complex scoring systems, like the HercepTest)

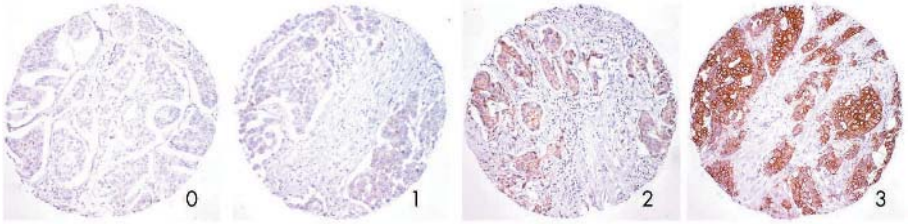

Figure 2 Light micrographs of examples of cores showing HercepTest scores 0 to 3+. Original magnification, ×100.

HercepTest immunohistochemical assays. Given the importance of the accurate assessment of HER-2/neu status this is of considerable interest. A similar approach to other immunohistochemical assays (especially if they, in line with the HercepTest, have very complex scoring systems) could be envisaged.

In summary, we describe a miniature TMA for better and efficient internal HercepTest quality control and easier, more accurate scoring.

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Accepted for publication 19 May 2004

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