Miniature tissue microarrays for HercepTest® standardisation and analysis
C Gulmann, P Loring, A O’Grady, E Kay

**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 x 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). The immunohistochemical staining was consistent throughout the time period.

**Abbreviations:** TMA, tissue microarray
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,7 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)
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.

Authors’ affiliations
C Gulmann, P Loring, A O’Grady, E Kay, Department of Pathology, Beaumont Hospital and Royal College of Surgeons in Ireland, Dublin 9, Ireland

Correspondence to: Dr C Gulmann, Department of Pathology, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland; cgulmann@rcsi.ie

Accepted for publication 19 May 2004

REFERENCES