

HER2 testing

Guidelines for HER2 testing in the UK

E D Hsi, R R Tubbs

The need to update in a new era of immunohistochemistry testing

For decades anatomical pathologists have established and managed diagnostic immunohistochemistry (IHC) laboratories to assist in accurate tissue diagnosis. Although test validation, quality control (QC), and quality assurance (QA) procedures have always been a part of routine operations, test results were always viewed in the context of clinical, morphological, and other IHC test results. However, there has been a shift in the use of these tests. Now, targeted treatments are being determined based on the results of a stand alone IHC test. Specifically, eligibility for trastuzumab is determined by IHC testing (in the UK) or by IHC and/or fluorescent in situ hybridisation (FISH) testing in the USA. Because this represents a fundamental change in the use of IHC in the clinical laboratory, additional attention to the performance and interpretation of this IHC test is warranted. In the current issue of the *Journal of Clinical Pathology*, Ellis and colleagues detail guidelines for HER2 testing in the UK.¹

The guidelines can be briefly summarised as follows:

(1) Formalin fixation should be used. In the USA, testing is only approved by the Federal Drug Administration for formalin fixed tissue. Other fixatives such as Bouin's type fixatives and, in our experience, some of the newer formalin alternative fixatives preclude FISH testing.

(2) A relatively high case load is recommended because this is correlated with better performance.

(3) A two tiered system is recommended in which clear IHC results (either 0-1+ or 3+) can be accepted. An equivocal result (2+) should be arbitrated by FISH testing.

(4) IHC methods should be standardised, validated within a laboratory using cases with known FISH results, controlled, and scored in a uniform manner based on the HercepTest system.

(5) IHC QA must include external programmes, such as the well developed United Kingdom National External Quality Assessment Scheme for Immunocytochemistry (UKNEQAS-ICC) programme.

(6) FISH testing must also be standardised, use validated control tissues, and have a uniform scoring system that includes a chromosome 17 centromeric probe to avoid false positives caused by aneusomy.

Perhaps because of the centralised nature of health care in the UK, compared with the USA, the UK QA/QC programmes seem to be more developed than is currently the case in the USA. The guidelines set forth by Ellis *et al* appear reasonable and, for the most part, attainable in most laboratories, regardless of their location. However, they bring up several areas that need to be emphasised.

(1) Is there a minimum case load under which laboratories should be discouraged from performing HER-2 testing? As a result of comparing local and centralised testing from a national breast cancer trial, significant discordance was noted and found to be related to test volume.² Although volume of testing seems to be related to accuracy, clearly there are laboratories capable of performing quality testing with lower volumes, and a high test volume does not ensure an accurate test result. We would maintain that if an individual laboratory can properly validate an assay and perform acceptably in an external validation, then it should be permitted to offer the test.

(2) Given the call for standardisation of testing methods, how far can we actually go in standardising methods? Currently there are several antibody reagents available for use (as mentioned by Ellis and colleagues¹), including clone CB11, clone TAB250, and AO485 polyclonal antiserum. Previous studies have shown variability of performance based on the primary antibody reagent used.³ Detection systems are also varied, depending on the assay vendor. For example, a non-biotin containing, polymer based system is used by one company (Dako, Ely, Cambridgeshire, UK), whereas a streptavidin-biotin system is used by another (Ventana Medical Systems, Tucson, Arizona, USA). Epitope retrieval is also variable from one laboratory to another, parti-

cularly if microwaving is used because microwave machines can vary in power output and the effect is also dependent on the volume of material to be heated. Complete automation, including on line antigen retrieval controlled by a microprocessor, is available in one platform (Ventana), and offers the best hope of standardisation. Because of vendor dependent methodological variability and market forces, it is unlikely that only one method will be used by all laboratories offering HER2 testing by IHC. This puts even more importance on monitoring the end result of the assay (internal QC), on strict validation procedures using tissues with known FISH results, and on participation in external QA programmes.

(3) Regarding the logical conclusion of this last point, the UKNEQAS-ICC programme is a well developed programme that uses well characterised cell lines as controls and tests the technical performance of the IHC stain through referee scoring of the participant's stained slide. It must be pointed out that this is only one component of a total commitment of an individual laboratory towards performing an accurate HER2 test, and is not a substitute for the required prior internal validation and ongoing routine controls when new assays are developed.

(4) Observer scoring of IHC slides is another underestimated source of variability.^{4,5} Although fixed images of scoring systems are helpful, they are suboptimal for fine scoring exercises that require plane of focus and interpretation over a large field of tissue. The type of scoring required for HER2 testing is unlike the dichotomous scoring for other stains that pathologists are accustomed to interpreting. A goal of future programmes might be to include the evaluation of prestained multitumour array slides with an educational rather than punitive bias to improve test interpretation. New image analysis systems may play an as yet undefined role in assisting with stain interpretation in the future, but these systems are still in their infancy for clinical testing. Inclusion of these types of data in prospective clinical trials will be the best way to prove clinical usefulness. One need only remember the unrealised promises of image based DNA ploidy analysis in previous clinical applications to keep a level head regarding expectations for this technology.

FISH is considered the gold standard for HER2 assessment and correlates best with outcome and response to trastuzumab treatment.⁶ Many laboratories in the USA use this technology as the primary method for assessing HER2 status. However, the same principles of

test validation, ongoing QC, and participation in external QC programmes should apply. Unfortunately, well developed external programmes such as the CYH interphase FISH College of American Pathologists survey programme are yet to be developed for FISH in the UK, and less formal arrangements with colleagues for external test validation and QA may need to be in place for the immediate future. Again, as for IHC, because multiple platforms may exist—each with different procedures—careful attention to the test result and internal controls (such as endogenous signals in normal cells) is required. Automation promises to help reduce technical variation in the many carefully temperature controlled conditioning, hybridisation, and washing steps required for in situ hybridisation. New bright field assays using chromogenic or autometallographic in situ hybridisation may ultimately replace HER2 clinical laboratory testing by IHC and FISH.^{7 8}

In summary, Ellis and colleagues put forth guidelines for HER2 testing that should be assimilated by all those who do this type of testing. Hopefully, none of this comes as a surprise to that audience. However, these guidelines emphasise the evolving state of HER2 testing procedures. This type of testing,

in which the result cannot be easily predicted from the morphological appearance, and which determines target specific treatment, will only become more common. Already, other targets are being assessed (c-kit, epidermal growth factor receptor, CD20, CD25, CD33, and CD117 to name a few) and the list is rapidly expanding.⁹ HER2 serves as a model and should bring home to the reader that we are now in a new era of quantitative IHC testing, which requires heightened attention to both the technical and interpretive details of our assays.

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Authors' affiliations

E D Hsi, R R Tubbs, Department of Clinical Pathology, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195, USA

Correspondence to: Dr E D Hsi, Department of Clinical Pathology, L-11, Cleveland Clinic Foundation, 9500 Euclid Ave, Cleveland, OH 44195, USA; hsie@ccf.org

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