

---

## Leading article

---

### Testing for human epidermal growth factor receptor 2 in breast cancer; the revised ASO/CAP recommendations 2013

M Bilous

---

#### Introduction

Breast cancer is the most common malignancy affecting women, with an estimated 1.67 million new cases diagnosed in 2012 (1). Asia has experienced a more recent rise in the incidence of breast cancer and now contributes to around one third of the global disease burden (1,2). Breast cancer accounts for more than 522,000 deaths worldwide each year (1). A decline in breast cancer-associated mortality has been observed in countries such as the US, Canada and Australia, but this rate appears to be increasing in many Asian countries (3,4). Compared with their counterparts in developed nations, women with breast cancer in developing Asian countries can expect a poorer prognosis, in part due to a younger age and more advanced disease at presentation (2,5). Owing to poor awareness of the disease and a lack of organised breast screening programs, women with breast cancer in developing Asian countries continue to be diagnosed with late stage disease (2). The

pathology of breast cancers in young Asian women also differs from patients managed elsewhere in the world, with higher rates of oestrogen and progesterone receptor (ER,PR) negative disease and this contributes to the overall poorer outcomes (6).

Human epidermal growth factor receptor 2 (HER2) is a tyrosine kinase receptor that mediates signalling pathways involved in cell growth, division, motility and repair (7). A well-established biomarker and drug target in breast cancer, (8) overexpression of the HER2 protein is associated with aggressive disease, and shortened disease-free and overall survival (9,10). Trastuzumab was the first monoclonal antibody-based therapy developed to target HER2. Numerous studies have demonstrated improved survival in women with HER2-positive breast cancer who received treatment with trastuzumab, both in early (11-15) and metastatic disease (16-19). Accurate determination of HER2 status is critical

---

*University of Western Sydney and Healthscope Pathology, Sydney, Australia.*

*Correspondence: Michael Bilous, University of Western Sydney and Healthscope Pathology, Sydney, Australia. Email: Michael.Bilous@healthscope.com.au*

---

to ensure appropriate selection of patients who are most likely to derive benefit from trastuzumab and other anti-HER2 therapies. Routine evaluation of HER2 status is performed through analysis of breast cancer tissue using immunohistochemistry (IHC) for HER2 protein assessment and/or in situ hybridisation (ISH) for HER2 gene amplification. Interlaboratory variation is a challenge for both assay types and central retesting in the context of therapy trials has revealed a lack of reproducibility among laboratories performing and interpreting HER2 tests (20;21).

Overexpression of the HER2 protein or amplification of the HER2 gene is reported in approximately 13–25% of early and metastatic breast cancers (22-25). These data are primarily based on studies performed in the US, Europe and Australia, and may not reflect the incidence of HER2-positive breast cancer among women in Asia. While some reports have suggested that ethnically Asian women with breast cancer living outside of Asia are more likely to have HER2-positive disease (26-28), there are limited data on the incidence of HER2-positive breast cancer among Asian women living in Asia. In contrast to developed nations such as Australia, where universal testing for HER2 in breast cancer has recently been established, (29) diagnostic testing for breast cancer in Asia does not routinely include determination of HER2

status (3). Patient access to HER2 testing, as well as testing quality and reproducibility issues associated with IHC and ISH assays, are barriers to accurately determining the true incidence of HER2-positive breast cancer in countries in this region (3,22).

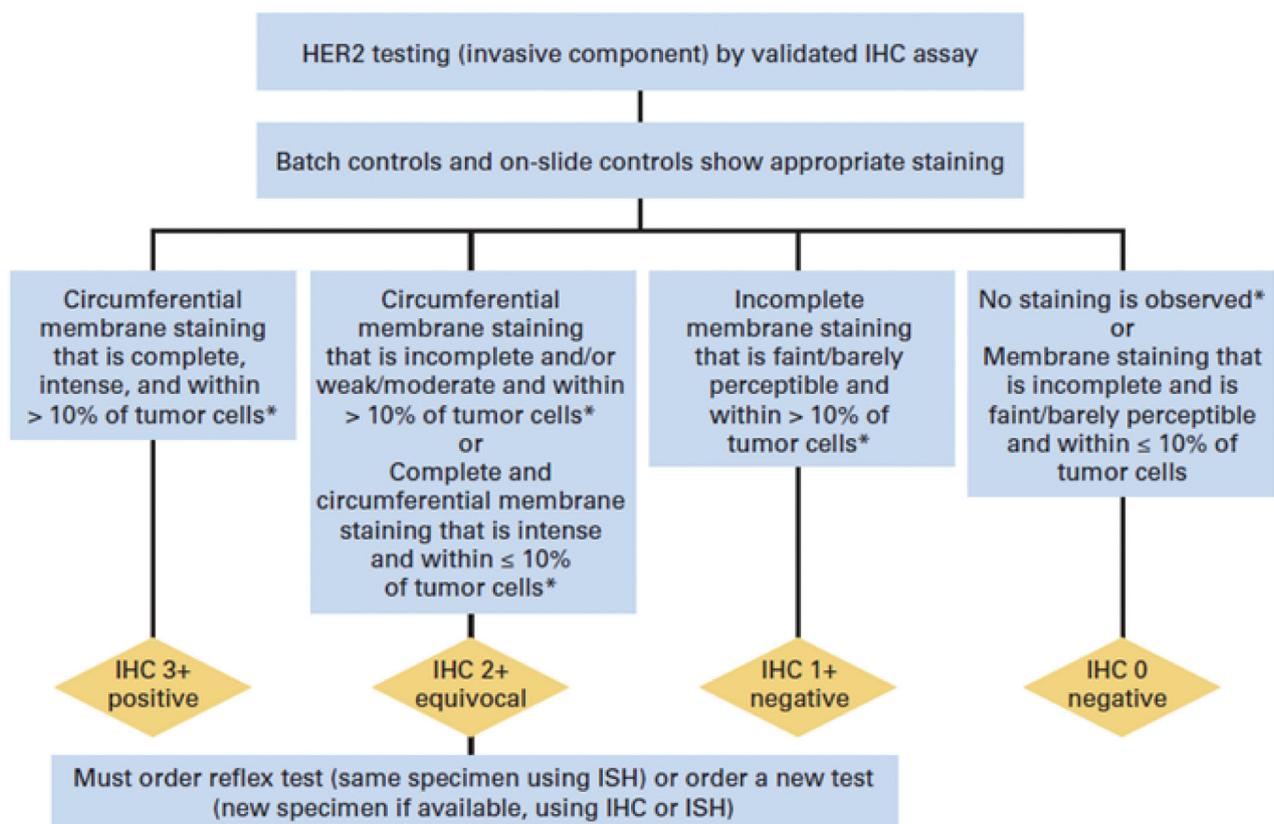
To ensure accuracy and reproducibility of HER2 testing, it is necessary to establish, validate and subsequently control all aspects of the test from removal of the tissue specimen from the patient to signing out of the final report. In 2007 The American Society of Clinical Oncology and the College of American Pathologists issued guidelines for the performance and interpretation of the test (30). There is evidence to suggest that since the publication of these guidelines testing accuracy improved with a reduction in false positive and negative cases (31). However in the intervening 5 or 6 years as HER2 testing became more widespread, experience and publications accumulated and new tests emerged, it was necessary to update the guidelines. The guidelines were revised in 2013 following extensive review of the medical literature by an international panel of pathologists and oncologists (32). The aim was to update the earlier guidelines in the light of increasing experience of the tests. Also driving some of the changes is data suggesting that testing accuracy has improved since the 2007 Recommendations were published in January 2007. Included in the

revised recommendations are more details on pre-analytical and analytical test performance, the addition of new tests such as brightfield ISH and the interpretation of problematic cases. A data supplement was included to address the more controversial and/or challenging case scenarios and the publications have been made freely available on both the ASCO and CAP web sites.

**Summary of recommendations**

HER2 should be assessed for all breast cancer patients, early, metastatic and recurrent and the result expressed as HER2 positive or negative on the basis of one or more tests. In rare cases a result of “indeterminate” may

be given. This category is used for cases in which technical or interpretive issues prevent a result being given. In this situation another specimen should be requested and/or a alternative test employed. IHC and ISH are both recommended test types as endorsed in the 2007 Recommendations. Immunohistochemistry to assess protein overexpression is the most commonly used first line test with ISH used to retest equivocal cases. However ISH may also be used as a first line test. Before commencing routine testing, laboratories should demonstrate high concordance with a representative set of validated cases. All equivocal tests must be retested by an alternative test whichever first line test is used.

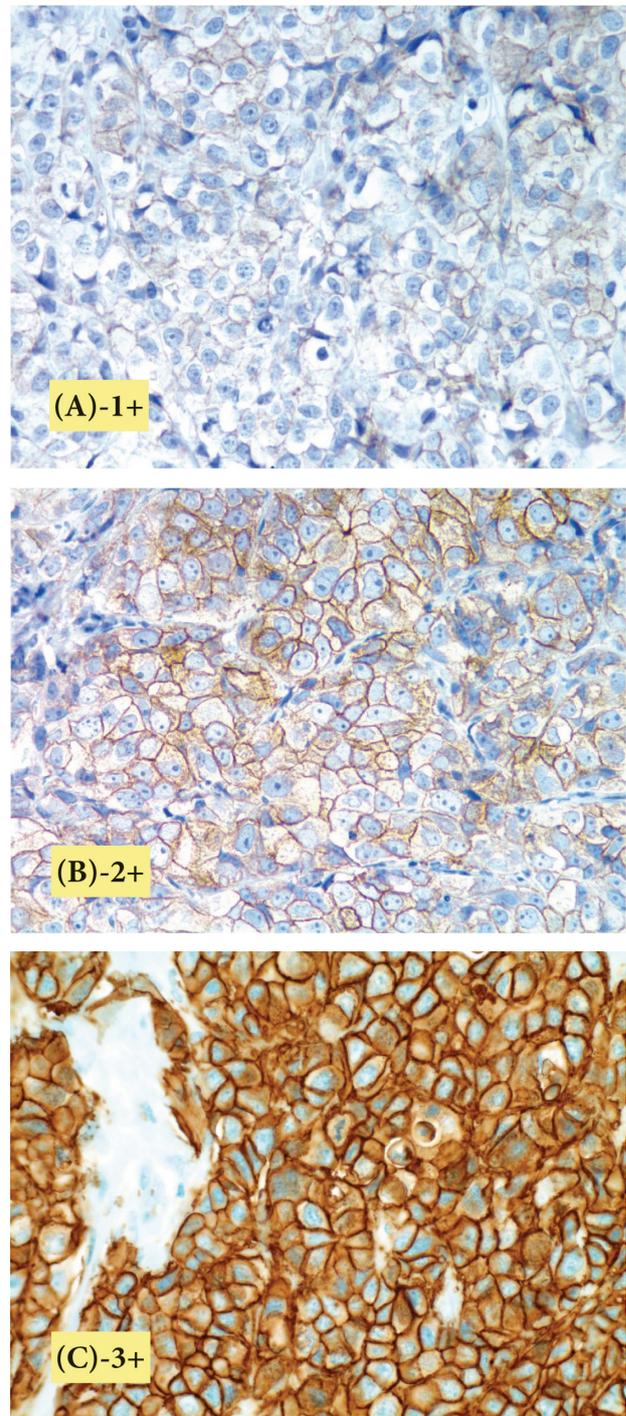


**Fig. 1a.** HER2 testing algorithm by immunohistochemistry - Wolff AC et al. JCO 2013

Great emphasis is placed upon concordance between histological findings and the HER2 result and repeat testing should be considered when discordance is found. In addition there is also emphasis placed upon multidisciplinary communication particularly between the pathologist and oncologist so that the information from the written report is fully explained and this is not only confined to difficult cases or an unusual result. Testing should only be performed in laboratories that have been accredited by a recognised international, national or other relevant authority. Membership of an external Quality Assurance Program is strongly recommended.

Figure 1b shows the different staining patterns that may be seen in HER2 testing. A positive HER2 status (3+) is achieved when complete, intense, circumferential staining is seen in >10% of the cancer cells; revised from 30% which was the threshold in the 2007 Recommendations. The reason for this change is that in the original pivotal clinical trials of trastuzumab in patients with metastatic breast cancer, 10% was the threshold. It was increased to 30% in the 2007 Recommendations as a result of concern that the number of false positive IHC 3+ results was high. Current evidence suggests that this number has fallen and reversion to a threshold for which clinical evidence exists is now justified. An equivocal

result (2+) is achieved when >10% of the cancer cells show circumferential membrane staining which is weak or moderate intensity. Included



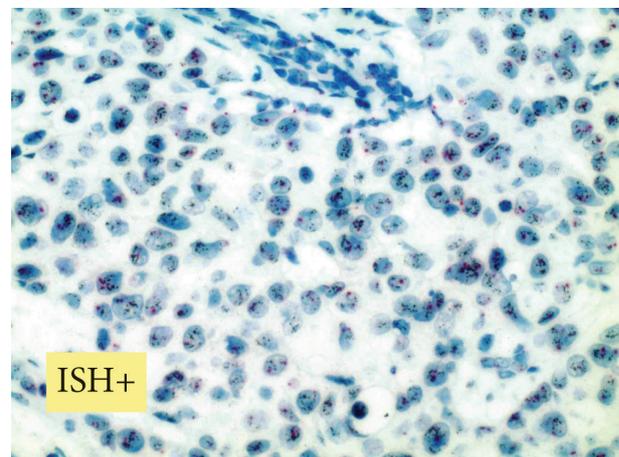
**Figure 1b.** Different staining patterns seen in HER2 testing by immunohistochemistry (HER2 x400)

in the equivocal category are cases which show incomplete circumferential staining that is weak or moderate, visible at low power microscopy and present in >10% of cancer cells. This change from the previous recommendations has been included to avoid the risk of a false negative result and to highlight the fact that some rare breast cancer subtypes may include HER2 positive cells that show “U” or cup-shaped membrane staining reflecting the absence of luminal staining in these cells. The addition of advice concerning the microscope objective follows the algorithm recommended for gastric and gastro-oesophageal junction cancer described by Ruschof et al (33). An equivocal result is also reported if there is complete intense circumferential staining in ≤10% of cancer cells. In all these situations reflex ISH or a new test must be requested to achieve a definite result

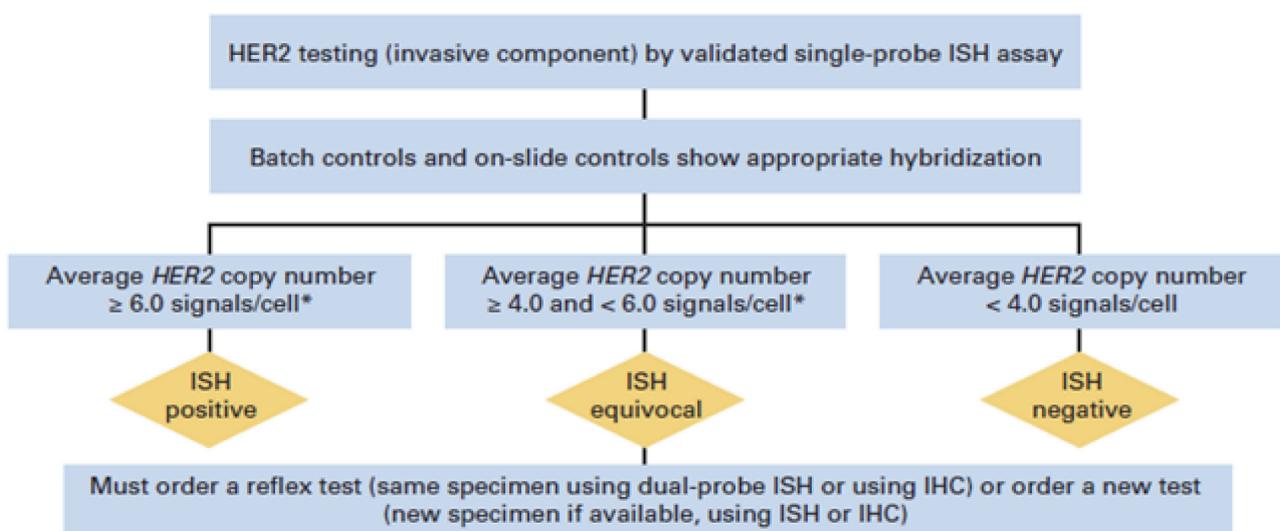
and “2+ Equivocal” should not be reported as the final result.

**In-Situ Hybridisation**

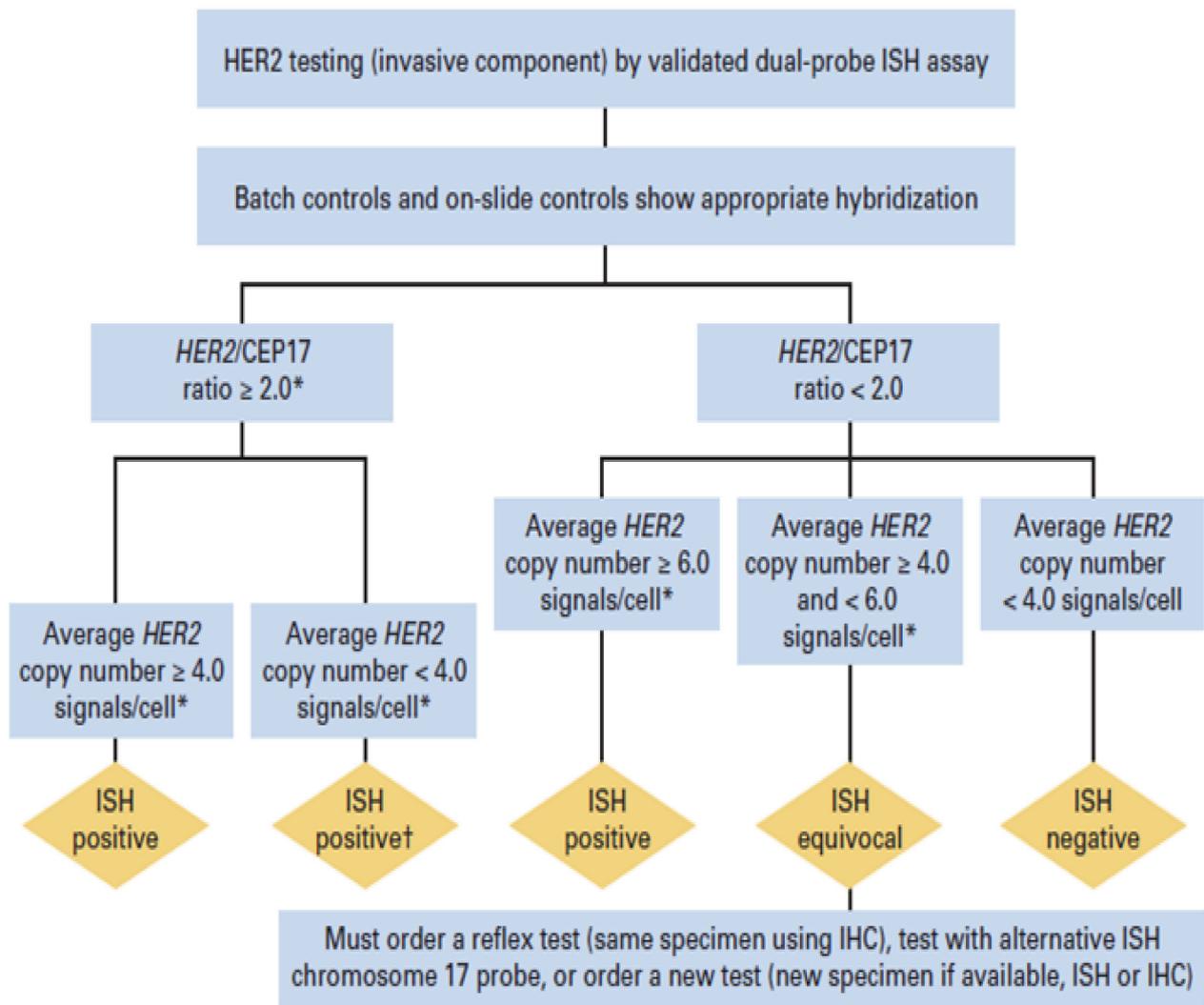
Both fluorescence and brightfield ISH are now recommended test types. A positive result is one in which the HER2 gene copy number is ≥6 signals per cancer cell after counting at least 20 contiguous cells within an area representing >10% of the tumour (Figure 2).



**Fig. 2.** A positive result in In-Situ Hybridisation (ISH)



**Fig. 3a.** HER2 testing algorithm by single probe in situ hybridisation Wolff AC et al JCO 2013



**Fig. 3b.** HER2 testing algorithm by dual probe in situ hybridisation Wolff AC et al JCO 2013

A HER2/CEP17 ratio of  $\geq 2$  is also considered a positive result. This is a change from the 2007 Recommendations which had raised the threshold to  $\geq 2.2$  to avoid a false positive result. Various combinations of HER2 gene copy number and HER2/CEP17 ratio may be seen, the emphasis being on considering both before a final result is given (Figures 3a and 3b). There is inevitably a lack of strong clinical data to support some aspects of the algorithm

and some combinations of HER2 copy number and HER2/CEP ratio. The recommendations are based on the best available evidence from subgroup analysis primarily of the trastuzumab adjuvant therapy trials and the desire to not withhold anti-HER2 treatment from any patient who may benefit from it.

**Histopathological Concordance**

**Fig.4.** Histopathologic features suggestive of possible HER2 test discordance Wolff et al JCO 2013

New HER2 test should not be ordered if the following histopathologic findings occur and the initial HER2 test was negative:
Histologic grade 1 carcinoma of the following types:
* Infiltrating ductal or lobular carcinoma, ER and PgR positive
* Tubular (at least 90% pure)
* Mucinous (at least 90% pure)
* Cribriform (at least 90% pure)
* Adenoid cystic carcinoma (90% pure) and often triple negative
Similarly, a new HER2 test should be ordered if the following histopathologic findings occur and the initial HER2 test was positive:
Histologic grade 1 carcinoma of the following types:
* Infiltrating ductal or lobular carcinoma, ER and PgR positive
* Tubular (at least 90% pure)
* Mucinous (at least 90% pure)
* Cribriform (at least 90% pure)
* Adenoid cystic carcinoma (90% pure) and often triple negative
If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test must be ordered on the excision specimen if one of the following is observed:
* Tumor is grade 3
* Amount of invasive tumor in the core biopsy is small
* Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core
* Core biopsy result is equivocal for HER2 after testing by both ISH and IHC
* There is doubt about the specimen handling of the core biopsy (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

It is important that the result of the HER2 test is concordant with other features of the cancer. Figure 4 lists features that suggest

possible discordance and which should lead to consideration of retesting. Most of these would be acknowledged by pathologists routinely

reporting breast cancer cases. For example a histological grade 1 carcinoma which is a tubular carcinoma should not be HER2 positive. If a positive result is reported then a new test should be ordered. Perhaps more controversial and questioned by some reviewers of the Recommendations (34), is the advice that if a grade 3 carcinoma is found to be HER2 negative in a core biopsy specimen, then the test must be repeated on the excision specimen. The aim of this and other possible discordant categories in which re-testing is suggested, is to avoid withholding anti-HER2 treatment from patients who may benefit and also to emphasise the need to correlate all the features of a cancer, including grade, type and hormone receptor status in providing comprehensive information about the tumour.

This will inevitably entail duplicate testing of cases which has significant financial implications. It also raises logistical problems for patients for whom core biopsy and surgical excision are performed at different sites with different laboratories performing the tests and the result of a core biopsy not known to the pathologist reporting the excision specimen. However the emphasis on this correlation step is applicable to all molecular testing of histopathology specimens from tumours at any site and ensures that there is integration of comprehensive and reliable information about

the tumour and molecular testing results are not seen in isolation from the pathology findings. It perhaps should be noted that similar rigorous attention to the other pathology features including grade and hormone receptor status are assumed to be in place for this correlation to be relevant.

### **Pre-analytical factors and the data supplement**

The Data Supplement is a comprehensive document providing information about the background information used in the Recommendations, offering guidance on the interpretation and reporting of heterogeneity and polysomy and outlining the rationale used to develop the testing algorithms, and evidence is provided to support these. Further information on the identification and reporting of heterogeneity and polysomy is also available in a separate publication (35). The Data Supplement also has sample report formats for use by laboratories.

These recommendations highlight the importance of pre-analytical factors in determining the accuracy of the result, in much greater detail than those published in 2007. Poor control of pre-analytical factors remains the greatest threat to accurate testing. Specifically, the time to commencement of fixation and total time of fixation are recognised as being of paramount importance. The time from removal

of the specimen to commencement of fixation should be no more than 1 hour. Fixation time should be between 6 and 72 hours. In laboratories globally but especially in the Asia-Pacific region these times are often poorly controlled and not documented. With the rapid increase in molecular testing as an adjunct to standard pathology reporting of tumours from many sites, recording cold ischaemic time and total fixation time have become essential. An issue again particularly in the Asia-Pacific region, is the use of reliably constituted 10% neutral buffered formalin; the only fixative recommended for HER2 testing. For centralised HER2 testing with laboratories referring paraffin blocks or unstained slides to a reference laboratory often at some distance from where they were processed or where there is some distance between the operating theatre and the laboratory, this information may be difficult to control and subsequently to collect, but mechanisms should be put in place to ensure that this data is recorded and forwarded to the testing laboratory.

### Future Directions

The Review Committee considered other tests for HER2 including DNA expression by microarray and mRNA expression by rtPCR. Evidence in support of introducing these into clinical practice is currently lacking but as further studies are reported there is support for introducing these into laboratories provided

that appropriate validation testing has been performed.

### Conclusion

The revised recommendations provide a comprehensive set of guidelines for HER2 testing from specimen acquisition to report sign-off. Where strong evidence is lacking advice has been offered based upon best available information. The challenges facing pathology laboratories particularly in the Asia Pacific region include control of the pre-analytical phases of the test and managing the financial impact of the testing program. HER2 testing can be considered a prototype for the likely increase in demand for molecular tests on a variety of tumour types many of increasing complexity compared to HER2. Putting in place robust measures for HER2 testing will inevitably help in managing future tests as they expand the reach of personalised medicine.

### References

- (1) Globocan. Breast Cancer. Estimated Incidence, Mortality and Prevalence Worldwide in 2012. [http://globocan.iarc.fr/Pages/fact\\_sheets\\_cancer.aspx](http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx) 2014
- (2) Agarwal G, Pradeep PV, Aggarwal V, Yip CH, Cheung PS. Spectrum of breast cancer in Asian women. *World Journal of Surgery* 2007 May;31(5):1031-40

- (3) Green M, Raina V. Epidemiology, screening and diagnosis of breast cancer in the Asia-Pacific region: Current perspectives and important considerations. *Asia-Pacific Journal of Clinical Oncology* 4, S5-S13. 2008. Ref Type: Journal (Full)
- (4) Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, Sandelin K, Derossis A, Cody H, Foulkes WD. Is breast cancer the same disease in Asian and Western countries? *World Journal of Surgery* 2010 Oct;34(10):2308-24.
- (5) Lim SE, Back M, Quek E, Iau P, Putti T, Wong JE. Clinical observations from a breast cancer registry in Asian women. *World Journal of Surgery* 2007 Jul;31(7):1387-92.
- (6) Chu KC, Anderson WF, Fritz A, Ries LA, Brawley OW. Frequency distributions of breast cancer characteristics classified by estrogen receptor and progesterone receptor status for eight racial/ethnic groups. *Cancer* 2001 Jul 1;92(1):37-45.
- (7) Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nature Reviews and Molecular Cell Biology* 2001 Feb;2(2):127-37.
- (8) Weigel MT, Dowsett M. Current and emerging biomarkers in breast cancer: prognosis and prediction. *Endocrine Related Cancer* 2010 Dec;17(4):R245-R262.
- (9) Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987 Jan 9;235(4785):177-82.
- (10) Press MF, Pike MC, Chazin VR, Hung G, Udove JA, Markowicz M, Danyluk J, Godolphin W, Sliwkowski M, Akita R, . Her-2/neu expression in node-negative breast cancer: direct tissue quantitation by computerized image analysis and association of overexpression with increased risk of recurrent disease. *Cancer Research* 1993 Oct 15;53(20):4960-70.
- (11) Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I, Gianni L, Baselga J, Bell R, Jackisch C, Cameron D, Dowsett M, Barrios CH, Steger G, Huang CS, Andersson M, Inbar M, Lichinitser M, Lang I, Nitz U, Iwata H, Thomssen C, Lohrisch C, Suter TM, Ruschoff J, Suto T, Greatorex V, Ward C, Strahle C, McFadden E, Dolci MS, Gelber RD. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *New England Journal of Medicine* 2005 Oct 20;353(16):1659-72.
- (12) Slamon D, Eiermann W, Robert N, Pienkowski T, Martin M, Press M, Mackey J, Glaspy J, Chan A, Pawlicki M, Pinter T, Valero V, Liu MC, Sauter G, von MG, Visco F, Bee V, Buyse M, Bendaahmane B, Tabah-Fisch I, Lindsay MA, Riva A, Crown J. Adjuvant trastuzumab in HER2-positive breast cancer. *New England Journal of Medicine* 2011 Oct 6;365(14):1273-83.
- (13) Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Jr., Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *New England Journal of Medicine* 2005 Oct 20;353(16):1673-84.

- (14) Perez EA, Romond EH, Suman VJ, Jeong JH, Davidson NE, Geyer CE, Jr., Martino S, Mamounas EP, Kaufman PA, Wolmark N. Four-year follow-up of trastuzumab plus adjuvant chemotherapy for operable human epidermal growth factor receptor 2-positive breast cancer: joint analysis of data from NCCTG N9831 and NSABP B-31. *J Clinical Oncology* 2011 Sep 1;29(25):3366-73.
- (15) Smith I, Procter M, Gelber RD, Guillaume S, Feyereislova A, Dowsett M, Goldhirsch A, Untch M, Mariani G, Baselga J, Kaufmann M, Cameron D, Bell R, Bergh J, Coleman R, Wardley A, Harbeck N, Lopez RI, Mallmann P, Gelmon K, Wilcken N, Wist E, Sanchez RP, Piccart-Gebhart MJ. 2-year follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer: a randomised controlled trial. *Lancet* 2007 Jan 6;369(9555):29-36.
- (16) Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *New England Journal of Medicine* 2001 Mar 15;344(11):783-92.
- (17) Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L, Wolter JM, Paton V, Shak S, Lieberman G, Slamon DJ. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *Journal of Clinical Oncology* 1999 Sep;17(9):2639-48.
- (18) Kaufman B, Mackey JR, Clemens MR, Bapsy PP, Vaid A, Wardley A, Tjulandin S, Jahn M, Lehle M, Feyereislova A, Revil C, Jones A. Trastuzumab plus anastrozole versus anastrozole alone for the treatment of postmenopausal women with human epidermal growth factor receptor 2-positive, hormone receptor-positive metastatic breast cancer: results from the randomized phase III Tandem study. *Journal of Clinical Oncology* 2009 Nov 20;27(33):5529-37.
- (19) Marty M, Cognetti F, Maraninchi D, Snyder R, Mauriac L, Tubiana-Hulin M, Chan S, Grimes D, Anton A, Lluch A, Kennedy J, O'Byrne K, Conte P, Green M, Ward C, Mayne K, Extra JM. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *Journal of Clinical Oncology* 2005 Jul 1;23(19):4265-74.
- (20) Paik S, Bryant J, Tan-Chiu E, Romond E, Hiller W, Park K, Brown A, Yothers G, Anderson S, Smith R, Wickerham DL, Wolmark N. Real-world performance of HER2 testing--National Surgical Adjuvant Breast and Bowel Project experience. *Journal of the National Cancer Institute* 2002 Jun 5;94(11):852-4.
- (21) Roche PC, Suman VJ, Jenkins RB, Davidson NE, Martino S, Kaufman PA, Addo FK, Murphy B, Ingle JN, Perez EA. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *Journal of the National Cancer Institute* 2002 Jun 5;94(11):855-7.

- (22) Choritz H, Busche G, Kreipe H. Quality assessment of HER2 testing by monitoring of positivity rates. *Virchows Archive* 2011 Sep;459(3):283-9.
- (23) Gown AM, Goldstein LC, Barry TS, Kussick SJ, Kandalaf PL, Kim PM, Tse CC. High concordance between immunohistochemistry and fluorescence in situ hybridization testing for HER2 status in breast cancer requires a normalized IHC scoring system. *Modern Pathology* 2008 Oct;21(10):1271-7.
- (24) Owens MA, Horten BC, Da Silva MM. HER2 amplification ratios by fluorescence in situ hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clinical Breast Cancer* 2004 Apr;5(1):63-9.
- (25) Morey AL, Farshid G, Fox SB, Francis GD, McCue G, von Neumann-Cosel V, Bilous M. Determining HER2 (ERBB2) amplification status in women with breast cancer: final results from the Australian in situ hybridization program. *TBC* 2014
- (26) Clarke CA, Keegan TH, Yang J, Press DJ, Kurian AW, Patel AH, Lacey JV, Jr. Age-specific incidence of breast cancer subtypes: understanding the black-white crossover. *Journal of the National Cancer Institute* 2012 Jul 18;104(14):1094-101.
- (27) Parise CA, Bauer KR, Caggiano V. Variation in breast cancer subtypes with age and race/ethnicity. *Critical Reviews in Oncology/ Hematology* 2010 Oct;76(1):44-52.
- (28) Telli ML, Chang ET, Kurian AW, Keegan TH, McClure LA, Lichtensztajn D, Ford JM, Gomez SL. Asian ethnicity and breast cancer subtypes: a study from the California Cancer Registry. *Breast Cancer Research and Treatment* 2011 Jun;127(2):471-8.
- (29) Pathmanathan N, Provan PJ, Mahajan H, Hall G, Byth K, Bilous AM, Balleine RL. Characteristics of HER2-positive breast cancer diagnosed following the introduction of universal HER2 testing. *Breast* 2012 Dec;21(6):724-9.
- (30) Wolff AC, Hammond ME, Schwartz JN, Hagerty KL, Allred DC, Cote RJ, Dowsett M, Fitzgibbons PL, Hanna WM, Langer A, McShane LM, Paik S, Pegram MD, Perez EA, Press MF, Rhodes A, Sturgeon C, Taube SE, Tubbs R, Vance GH, van d, V, Wheeler TM, Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Journal of Clinical Oncology* 2007 Jan 1;25(1):118-45.
- (31) Middleton LP, Price KM, Puig P et al. Implementation of ASCO/CAP HER2 Guideline Recommendations in a tertiary care facility increases HER2 immunohistochemistry and fluorescence hybridization concordance and decreases the number of inconclusive cases. *Archives in Pathology and Laboratory Medicine* 2009;133; 755-780.

- (32) Wolff AC, Hammond ME, Hicks DG, Dowsett M, McShane MC, Allison KH, Allred DC, Bartlett JM, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu P, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G, Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Journal of Clinical Oncology* 2013; 31; 3997-4013.
- (33) Ruschoff J, Hanna W, Bilous M, et al HER2 testing in gastric cancer: a practical approach. *Modern Pathology* 2012;25;637-650.
- (34) Rakha EA, Staczynski J, Lee AHS, Ellis IO. The updated ASCO/CAP guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. *Histopathology* 2014;64;609-615.
- (35) Hanna W, Ruschoff J, Bilous M, et al. HER2 in situ hybridisation in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. *Modern Pathology* 2013; 1;4-18.