

Testing for HER2 Positive Breast Cancer

Challenge for Improvement of
Current Conditions and Practice

Assessment



Ludwig Boltzmann Institut
Health Technology Assessment

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Health Technology Assessment

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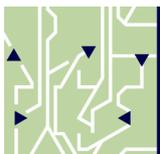
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Executive Summary

Background: Breast cancer is the most frequent cancer of women. In some breast cancers, the HER2 gene is amplified and over expressed. Patients with HER2 positive tumours have poorer prognoses than other types of breast cancer. The role of HER2 as a prognostic and therapeutic biomarker has been discussed for the past years. Extensive media coverage has publicized the availability of a targeted therapeutic antibody (trastuzumab) for HER2 positive tumours. In turn, public demand for this treatment has increased along with pressure for the reimbursement of costs associated with it.

The current use of trastuzumab for patients with metastatic cancer as well as those receiving adjuvant therapy highlights the importance of HER2 analysis. The ability to reliably identify patients who might benefit from trastuzumab treatment is not only important for clinical reasons such as positive clinical effects as well as the possibility of severe adverse events like grade 3 or 4 cardiotoxicity – but also for economic ones as well. The cost of treatment is currently – about 42,000.– Euros per treatment.

Whether HER2 expression can predict the response to antibody therapy, however, remains controversial and continues to be discussed in many studies. Recent estimates suggest that approximately 20% of current HER2 testing may be inaccurate.

Policy and Research question of assessment:

PQ: Are resources allocated to treat HER2 patients being used in the most efficient way?

RQ: What is the gold standard for diagnosing HER2 positive tumours? Which is the most accurate and reproducible method to identify candidates for potential therapy with monoclonal antibodies, and are the applied tests reliable for selecting HER2 positive patients? Is it necessary to look closer at specific areas of uncertainty – if so, which areas?

Method: This assessment report is based on a literature search in various databases (Medline, EmBase etc.) and includes studies (n = 75) published after the year 2000. The main focus was on the questions of validity, standardization and/or calibration of the two most commonly used methods IHC (immuno histochemistry) and FISH (fluorescence in situ hybridization), furthermore the inter-observer and the inter-laboratory concordance, and the role of the morphological variables and borderline test results.

Results: The review discusses – systematically – the HER2 testing results of more than 23,000 specimens, achieved in local, central or reference laboratories, explored by different testing methodologies at the DNA, RNA and protein level.

- ❖ Many studies are not comparable, because of differences in number of included specimen, the art of tissue extraction, histology of the specimen, and used test-methods.
- ❖ IHC results show much greater variability than FISH – test results, particularly for the FISH negative cases. The results of most presented studies indicate that high-level HER2 amplification and an IHC score of 3+ will identify HER2 positive breast carcinoma; the low-level amplification and/or IHC score of 2+ should be carefully interpreted.

HER2: prognostic & therapeutic marker became importance with availability of trastuzumab

reliable HER2 testing for accurately identifying patient who might benefit and for avoidance of false positive

identification of areas of uncertainty

literature searches, systematic analysis

data from 23,000 specimen but analysed by different testing methods

high-level HER2 amplification and an IHC score of 3+

challenges:	<ul style="list-style-type: none"> ❖ There is agreement that the most (cost-) effective testing strategy is to screen all patients with IHC, followed by FISH for IHC of 2+ (or of 2+ and 3+).
variabilities in 2+ interpretation	<ul style="list-style-type: none"> ❖ The challenge in routine practice is the differences that exist in the interpretations of probes. Adherence to strict guidelines and instructions for handling discordant results and validation with clinical results are needed.
uncertainty about clinical significance of low level amplification	<ul style="list-style-type: none"> ❖ Uncertainty exists on the clinical significance of low level gene amplification concerning the response to trastuzumab. The target for trastuzumab is the protein and the gene is only the surrogate marker of the true target.
inter-observer & inter-laboratory variabilities	<ul style="list-style-type: none"> ❖ Findings concerning different results from local/central laboratories point to moderate inter-observer and inter-laboratory reliability of test results. A volume/experience relationship is observed.
possible less HER2 positive than reported in clinical studies	<ul style="list-style-type: none"> ❖ Inter-laboratory comparisons and performance evaluations are important in overcoming test limitations. Based on the results of this assessment, there seem to be less HER2 positive women than generally reported in clinical treatment studies: not 20–30%, but rather 15–20% are amplifying HER2 positive in “real life” settings.
increased efforts in accurate testing will positively influence effects and costs for trastuzumab	<p>Conclusion: Although more than 23,000 patients (specimen) have been reviewed in the seventy-five studies published in recent years, many unsolved questions remain. From a societal perspective, the diagnostic performance of the test used to identify trastuzumab candidates has considerable influence on cost-effectiveness – independent of test cost – due to the high cost of treating patients with false-positive test results and the inability of patients with false-negative results to benefit from trastuzumab. Increased efforts in accurate testing results might lead to less trastuzumab treatments associated with clinical important adverse events: less might be more.</p> <p>Recommendation: The following recommendations are extrapolated from the results of this review:</p>
standardization, performance control, reference centers	<ul style="list-style-type: none"> ❖ Due to the high variability between the different IHC-tests, we recommend using only standardized and approved tests. ❖ Due to the consequential costs (non-monetary costs/side effects of therapy and monetary costs), we recommend to establish SOPs/ standard operating procedures. ❖ Due to high inter-laboratory variabilities, we recommend using a small number of reference centers rather than decentralised testing locations with lack of routine. ❖ Due to high inter-laboratory variabilities, we recommend national and international inter-laboratory exchange on results of diagnostic outcome.

Zusammenfassung

Hintergrund: Brustkrebs stellt die häufigste Form der bösartigen Erkrankungen der Frau dar. Bei einem Teil der Patientinnen wird das HER2 Gen amplifiziert und überexprimiert. HER2 positive Tumore haben eine schlechtere Prognose. Die Rolle des Transmembranrezeptors HER2 als prognostischer und therapeutischer Biomarker wurde in den letzten Jahren viel diskutiert. Die Verfügbarkeit einer zielgerichteten Antikörpertherapie (Trastuzumab) bei HER2 positiven Tumoren führte zur enormen Medienpräsenz und steigerte die allgemeine Nachfrage sowie den Rückerstattungsdruck.

Die Anwendungsmöglichkeiten von Trastuzumab macht klar, wie wichtig die HER2 Bestimmung ist. Die Möglichkeit, Patientinnen, die durch die Trastuzumab Behandlung profitieren könnten, verlässlich zu identifizieren, ist nicht nur aus klinischer Sicht wichtig – positive Beeinflussung des klinischen Verlaufs (begleitet allerdings von kardiotoxischen Nebenwirkungen Grad 3 oder 4) – sondern auch aus ökonomischer Sicht: hohe Behandlungskosten von ca 42.000.– Euro pro Behandlung sind zu veranschlagen.

Ob die HER2 Überexpression die Ansprechbarkeit auf die Antikörpertherapie definitiv voraussagen kann, bleibt strittig, und ist Diskussionsthema in zahlreichen Studien. Schätzungen zufolge dürften ca. 20 % der gängigen HER2 Testungen ungenau sein.

Gesundheitspolitische und Forschungsfrage des Assessment:

GF: Werden die Ressourcen, um HER2 Patientinnen zu behandeln, auf effiziente Art und Weise angewandt bzw. zur Verfügung gestellt?

FF: Was ist der Goldstandard bezüglich Diagnostik der HER2 positiven Tumoren? Was ist die sicherste und am besten reproduzierbare Methode, um Kandidatinnen für eine mögliche Therapie mit monoklonalen Antikörpern zu detektieren? Sind die angewandten Tests verlässlich bei der Selektion HER2 positiver Patientinnen? Ist es erforderlich, bestimmte Unsicherheitsareale genauer zu betrachten – und um welche handelt es sich hierbei?

Methode: Die vorliegende Arbeit basiert auf einer Literatursuche in verschiedenen Datenbanken (Medline, EmBase etc.) und beinhaltet Studien (n=75), die nach 2000 publiziert wurden. Das Hauptaugenmerk lag auf Studien zur Validität, Standardisierung und/oder Kallibration von den zwei am häufigsten angewandten Methoden IHC (Immunohistochemie) und FISH (Fluoreszenz In situ Hybridisierung), sowie auf Konkordanz zwischen Untersuchern und Labors, sowie der Rolle der einzelnen morphologischen Variablen und von „grenzwertigen“ Testergebnissen.

Ergebnisse: Die vorliegende Arbeit diskutiert systematisch die Resultate der HER2 Testung von mehr als 23.000 Proben, welche in lokalen, zentralen oder Referenz-Labors verarbeitet und untersucht wurden. Die analytischen Resultate basieren auf Testmethoden, welche auf DNA-, RNA- und Proteinebene durchgeführt wurden.

Die Vergleichbarkeit vieler Studien untereinander ist problematisch einerseits aufgrund von unterschiedlicher Anzahl der Proben, Art der Probenentnahme, oder der zugrunde liegenden Histologie, andererseits aufgrund der Verwendung verschiedener und/oder unterschiedlich durchgeführter Testmethoden.

HER2 erlangte als prognostischer und therapeutischer Marker große Bedeutung seit der Verfügbarkeit von Trastuzumab

verlässliche HER2 Testung zur präzisen Identifizierung von potentiell profitierenden Patientinnen mit Vermeidung falsch positiver Ergebnisse

Identifikation der Bereiche mit hohem Unsicherheitsfaktor

Literatursuche; systematische Analyse

Daten von >23.000 Proben, mittels unterschiedlicher Methoden analysiert

„high-level“ HER2 Genamplifikation und ein IHC Wert von 3+	Die mittels IHC erlangten Ergebnisse zeigen viel größere Variabilität und somit mehr Interpretationsmöglichkeiten als die FISH Resultate – speziell wenn man die diskrepanten IHC Ergebnisse bei FISH negativen Fällen betrachtet. Die Ergebnisse der meisten vorliegenden Studien führen zur Auffassung, dass die „high-level“ HER2 Amplifikation und ein IHC Wert von 3+ HER2 positiven Tumore zu identifizieren vermag; die „low-level“ Amplifikation und/oder ein IHC Wert von 2+ müssen hingegen mit Vorsicht interpretiert werden.
Herausforderung	Übereinstimmung herrscht bezüglich der effektivsten und kostengünstigsten Teststrategie: screening aller Patientinnen mittels IHC, gefolgt von FISH für die IHC 2+ getesteten Proben (bzw. für jene mit 2+ und 3+ Auswertung).
Unterschiede in der 2+ Bewertung	Die wesentliche Herausforderung in der Routinetestung stellt die unterschiedliche Interpretationsmöglichkeit der Patientinnenergebnisse dar. Das Befolgen der strikten Anleitungen und Vorschriften für die Bewertung der diskordanten Ergebnisse und die Validierung mit klinischen Ergebnissen ist erforderlich.
unsicherheit bezüglich klinischer Signifikanz von „low-level“ Amplifikationen	Ungewissheit besteht hinsichtlich klinischer Signifikanz der „low-level“ Genamplifikation betreffend das Ansprechen auf Trastuzumab. Das Angriffsziel für Trastuzumab ist das Protein, und das Gen ist lediglich der Surrogatmarker des Angriffsziels.
inter-observer & inter-laboratory Variabilität	Untersuchungsergebnisse betreffend unterschiedliche Resultate aus lokalen/zentralen Labors weisen auf eine nur mäßige Übereinstimmung beim Vergleich der Bewerter und der Labors untereinander hinsichtlich Test-Verlässlichkeit hin. Beobachtet wurde eine diesbezügliche Beziehung zwischen untersuchter Anzahl/Mengen und Testerfahrung.
möglicherweise weniger HER2 positive Patientinnen als aus klinischen Studien hervorgeht	Vergleiche zwischen den Labors untereinander und Evaluierung der Testdurchführung sind von größter Wichtigkeit, um die Grenzbereiche der Testbewertung interpretierbar zu machen. Beruhend auf den Resultaten aus unserer Beobachtung scheint es weniger HER2 positive Patientinnen zu geben als allgemein aufgrund von klinischen Studienberichten angenommen: nicht 20 %-30 % sondern eher 15 %-20 % mittels Genamplifikation HER2 positiv getesteter Proben sind in „real life“ settings zu finden.

Zusammenfassung: Obwohl mehr als 23 000 Patientinnen (Proben) aus den 75 vorliegenden, in den letzten Jahren veröffentlichten Studien bewertet wurden, verbleiben zahlreiche ungelöste Fragen. Gesundheitspolitisch hat eine exakte Diagnosestellung in Hinblick auf die Identifizierung der Trastuzumab-Kandidatinnen beträchtlichen Einfluss auf die Kosten-Effektivität, und zwar unabhängig von den Test-Kosten selbst – nämlich infolge von hohen Behandlungskosten der falsch positiv getesteten Patientinnen. Große Bemühungen bezüglich verlässlicher Testresultate könnten zu weniger Trastuzumab-Behandlungen führen, verbunden mit weniger Nebenwirkungen: weniger könnte auch mehr bedeuten.

Empfehlungen: Die folgenden Empfehlungen sind aus den Resultaten dieses Überblicksbewertung abzuleiten:

- ❖ Wegen der hohen Variabilität unter den verschiedenen – kommerziell erhältlichen und individuell modifizierten – IHC-Tests empfehlen wir nur die Verwendung von standardisierten, zugelassenen Tests.
- ❖ Wegen der Folgekosten (nicht-monetäre Kosten wie Therapienebenwirkungen und real anfallende Kosten) empfehlen wir die Erstellung von SOP/Standard operating procedures.
- ❖ Wegen der großen Variabilität beim Laborvergleich empfehlen wir Bevorzugung der Zentrallabors/Referenzzentren entgegen der zu beobachtenden Dezentralisierungstendenz, speziell im Falle der FISH-Diagnostik.
- ❖ Wegen der großen Variabilität unter den testenden Laboreinheiten empfehlen wir verstärkten nationalen und internationalen Austausch über die Testergebnisse, sowie externe Evaluation bezüglich Testdurchführung.

zunehmende Bemühungen bezüglich verlässlicher Diagnostik werden die Effekte und Kosten für Trastuzumab positiv beeinflussen

Standardisierung, Durchführungskontrolle, Referenzzentren

1 Policy and Research Question

The Human Epidermal Growth Factor Receptor 2 Gene (“HER2”) encodes a protein expressed on the surface of breast cancer cells and makes those cells more sensitive to growth factors. An over-expression of HER2 can accelerate the genesis of tumours and the proliferation of cells. HER2 positive tumours have poorer prognoses. Trastuzumab (Herceptin), a recombinant, humanised monoclonal antibody that targets the HER2 protein, has been approved for metastatic and adjuvant treatment regimes in combination with chemotherapy. Having only been tested in animal research [1], however, scientists do not yet understand exactly how and why the antibody works.

Currently, trastuzumab is used for the treatment of advanced HER2 breast cancer and adjuvant treatment of primary tumours in their early stages. Diffusion of trastuzumab began on the basis of conference presentations before it was even officially approved by the EMEA. No critical appraisal of its efficacy and safety had been conducted prior to its broad diffusion under public pressure.

Table 1-1 and Table 1-2 give an overview of the current results of clinical trials on the efficacy and safety of trastuzumab for ARR/ absolute risk reduction (efficacy) with reduced mortality (Table 1-1) varying from 0.5% to 6.9% in the study with the least duration and the absolute risk increase for serious adverse events for patients treated with trastuzumab (Table 1-2) varying from 0% (again the study with the least duration) to 2.8%. A Belgian assessment by Huybrechts et al. from 2006 [1] provides a more detailed report of the results.

Table 1-1: Results of trastuzumab trials, comparison 1

Publ.	N	treatment duration H®	Follow-up (years)	mortality with H®	mortality without H®	NNT
NEJM 2005 [2]	3351	1 year	2	3.7%	5.5%	56
Abstr. 2005 [3]	2148	1 year	2	1.9%	3.4%	67
NEJM 2005 [4]	3387	1 year	1	1.7%	2.2%	200
Lancet 2007 [5]	3401	1 year	2	3.5%	5.3%	56
NEJM 2006 [6]	231	9 weeks	3	5.2%	12.1%	14

Source: presentation Felder-Puig 2007 [7]

Table 1-2: Results of trastuzumab trials, comparison 2

Publ.	N	treatment duration H®	Follow-up (years)	≥ grade cardial events with H®	≥ grade cardial events without H®	NNH
NEJM 2005 [2]	3351	1 year	2	3.1%	0.3%	36 (13)
Abstr. 2005 [3]	2148	1 year	2	2.3%	1.0%	77 (15)
NEJM 2005 [4]	3387	1 year	1	0.5%	0.1%	250 (18)
Lancet 2007 [5]	3401	1 year	2	0.6%	0.1%	200 (16)
NEJM 2006 [6]	231	9 weeks	3	0.0%	0.0%	– (8)

Source: presentation Felder-Puig 2007 [7]

The effects of trastuzumab in both the metastatic and the adjuvant setting – mostly reported upon in quoting the “reduced relative reduction” in mortality (ranging from 30% to 79%) has received extensive media coverage and increased the importance of ERBB2 analysis. Clinicians must base their treatment decisions on highly accurate information and precise measurements of gene expressions.

The proportion of HER2 breast cancers decreases with patient age [1] and is highest in the youngest patients. These clinical trials often may have had more younger than older patients and this could be why the 20–25% (and even more) of newly reported HER2 cancers cited in the clinical trials is higher than the amount reported in the “real world”. Only 16% of the patients in the HERA-trial [5] were 60 years or older [8]. Given the relatively wide range of possible HER2 positive receptor status in breast cancer patients, it is assumed that HER2 diagnostic testing is less sensitive and specific than generally anticipated and that a number of patients are recommended need-less treatments or not recommended necessary treatments because of it.

The ability to reliably identify patients who might benefit from trastuzumab treatment is not only important for clinical reasons (a high proportion of grade 3 or 4 cardiotoxicity) – but also for economic ones as well (high treatment costs of about 42,000.– Euros per treatment).

Using current methods, the ability of positive predictive values (PPV) of the receptor status to predict the benefit of trastuzumab treatments is modest (25%–40%) [9].

This therefore raises a *policy question*:

- ❖ Are resources allocated to treat HER2 patients being used in the most efficient way?

Related *research questions* include:

- ❖ What is the gold standard for diagnosing HER2 positive tumours?
- ❖ Are the applied tests reliable for selecting HER2 positive patients?
- ❖ Is it necessary to look closer at specific areas of uncertainty – in which areas?

2 Methods: Literature Search and Selection

The aim of the literature search was to identify relevant publications for answering the research questions on testing for HER2 positive breast cancer. The following databases were searched: Medline (via Ovid), Embase (via Ovid), Pascal Biomed (via Ovid), Cochrane CENTRAL (via Ovid) and Biosis Previews (via Ovid). The search strategies are included in Appendix 1.

literature search in various databases

Inclusion Criteria:

- ❖ Questions of validity, standardization and/or calibration of FISH or IHC
- ❖ General descriptions of tests: IHC & FISH
- ❖ Other HER2-test methods: serum, RT-PCR, CISH etc.
- ❖ Concordance FISH and IHC, $n = >80$, Sensitivity, specificity, predictive value, $n = >80$
- ❖ Inter-observer concordance, inter-laboratory concordance
- ❖ “Real-life” findings: central vs. small laboratories
- ❖ Tests with different materials (carcinoma in situ or invasive)
- ❖ Borderline HER2 expression, 2+ HER2 inconsistency & tools to evaluate
- ❖ Inter-observer reproducibility FISH
- ❖ Ring studies of FISH, standards
- ❖ Language English or German.

Exclusion Criteria:

- ❖ HER2 expression in tumours other than mamma
- ❖ Male mamma carcinoma
- ❖ Radiological diagnostics of carcinoma: HER2 vs. non-HER2
- ❖ Monitoring response to chemotherapeutic schemes
- ❖ Correlation between HER2 status and worse outcome
- ❖ Special subpopulations (pregnant)
- ❖ Testing primary vs. recurrent tissue
- ❖ Findings published before 2000
- ❖ Languages other than English or German.

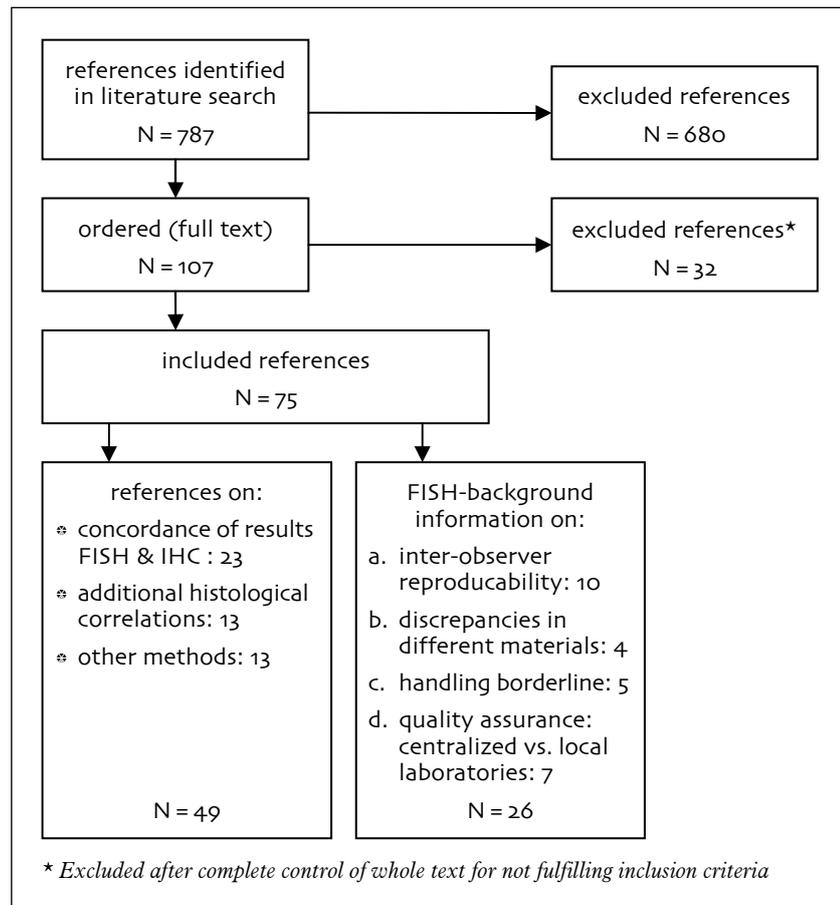


Figure 2-1: Literature selection

3 Introduction to HER2 Testing

3.1 Terminology & HER2 Mechanisms

ERBB2 (human epidermal growth factor receptor 2 gene) is the official name provided by the HUGO Gene Nomenclature Committee for the v-erb-b2 erythroblastic leukaemia viral oncogene homolog 2 gene that encodes a member of the epidermal growth factor receptor family of receptor tyrosine kinases [10].

Literature on the gene uses several other names, for example NEU, NGL, HER2, TKR1, HER2, c-erb B2, HER2/neu. This article refers to the gene by the commonly used term HER2.

The Human Epidermal Growth Factor Receptor 2 gene – ERBB2 – (commonly referred to as HER2) is a gene that helps to control how cells grow, divide and repair themselves. The HER2 gene provides the blueprint for manufacturing the HER2 protein. This protein, which is attached to a cell membrane, begins a signalling cascade that regulates normal cell growth. In a normal cell producing HER2, there are two copies of the gene and about 50,000 copies of the protein at the cell surface. The proto-oncogene HER2 gene resides on the long arm of chromosome 17. Normally, one HER2 gene exists for every chromosome 17. However, when a person develops cancer, the HER2 gene may create additional copies of itself and becomes “amplified”.

Many adult tissues, including those of the breast, endometrium, prostate and ovary, normally express low levels of a protein encoded with this gene [11]. The human HER2 gene encodes one member of a family of transmembrane tyrosine kinases (HER1-4), the prototype of which is the epidermal growth factor receptor (HER1, ERBB1) [12]. ER-positivity is inversely related to HER2 positivity [13]. Too many copies of the HER2 gene can result in the over-production, or over-expression, of the HER2 protein receptors found on the surface of tumour cells. HER2 over-expression is observed in invasive breast carcinomas with poor prognoses.

To what extent clinicians can use HER2 expression to predict the response to hormonal therapy, however, remains controversial and continues to be discussed in many studies [14].

human epidermal growth factor receptor 2 gene

commonly used term: HER2

function of HER2 gene

HER2 overexpression

3.2 Tumour Characteristics

Morphological variables such as tumour size, grade, and metastases to regional lymph nodes, are important prognostic indicators for breast cancer patients. In addition, amplification of HER2 proto-oncogene has been reported to occur in 10%–34% of invasive breast carcinomas [11], or in 20%–30% [15], 15%–30% [16, 17], 20%–25% [18, 19], or 20%–35% [14] of breast cancer tumours, respectively, as well as in up to 80% of DCIS (ductal carcinoma in situ) [16], and to be of both prognostic and therapeutic significance.

Molecular markers measured in the tumour or serum provide prognostic and/or predictive information that enables optimization of treatment selection for, and management of, breast cancer patients [20].

morphological variables as prognostic indicators

molecular markers

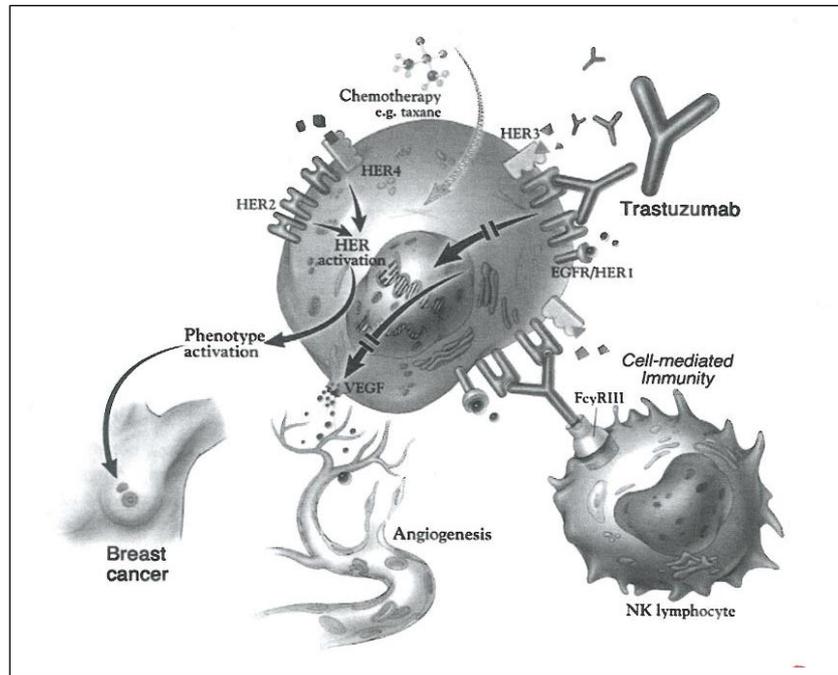


Figure 3-1: Proposed mechanism of action of trastuzumab
 Nahta et al. *Cancer Letters* 232 (2006)

**possible association
 between HER2
 amplification and
 histology and grade**

Scientists assume that a correlation exists between HER2 amplification and the histological type and grade of breast cancer. A review of the histological features and corresponding HER2 amplification results of 401 cases of invasive breast carcinoma support this as well [11]. Clinicians also assume a relationship between HER2 amplification and the type of breast cancer (ie, invasive ductal or invasive lobular) and the Scarff-Bloom-Richardson/SBR grade for invasive ductal carcinoma. Of the 401 cases, 388 were diagnosed as invasive ductal, invasive lobular or metastatic breast carcinoma. The remaining thirteen included “special type”- tumours, such as tubular, medullary, inflammatory, secretory and colloid carcinomas. Of the 388 cases, 300 were invasive ductal, 68 invasive lobular and 20 metastatic tumours.

**invasive ductal
 carcinomas more likely
 to show HER2
 amplification**

Overall, invasive ductal carcinomas were significantly more likely to show HER2 amplification than invasive lobular carcinomas. From the invasive ductal carcinomas more high grade carcinomas were HER2-positive as compared to low grade [11]. Hoff et al. found – concerning intra-tumoral heterogeneity in HER2 copy – a concordance of 100% between the invasive and intra-ductal components in all (14) carcinomas. Data from the study supports the association between HER2 amplification and both tumor type and histological grade of the invasive ductal carcinoma. The authors suggest, that if HER2 amplification is present in grade 1 (Scarff-Bloom-Richardson/SBR grading) invasive ductal or in invasive lobular carcinomas, a re-examination of the morphological features of the neoplasm should be performed to confirm tumour type and grade as a matter of quality assurance [11].

stability of HER2 status

When and where the oncogene HER2 may interfere in the development of in situ and invasive carcinomas remains unclear. Existing studies support stability over time – HER2 status would stay the same in the primary breast carcinoma and the following metastasis [14]. Andersson et al. [14] investigated intratumour HER2 heterogeneity within the invasive component and com-

pared invasive and in situ components of the same tumour. Seventy-eight consecutive invasive breast carcinoma specimens were prospectively evaluated. The material selected was primarily from small tumours. Five percent had a strong (3+) and nine percent a moderate (2+) HER2 over-expression.

When tests were performed using FISH (PathVision), 83% had no amplification, 6% a high grade (defined as >10 copies/nucleus) and 8% a low grade of amplification (defined as 6 to 10 copies/nucleus). Only one tumour (one percent) showed heterogeneity *within* the invasive component of the tumour with respect to HER2 status (as determined by IHC and FISH) within the invasive component.

Both in situ and invasive components existed in forty-eight tumours (62% of 78 tumours). Twelve of the 48 tumours (25% of those, but 15% of all 78) had at least two arbitrary units showing a different HER2 status analyzed by IHC. When eight of them were reanalyzed with FISH and IHC; the study found a discordance in amplification status between the in situ and the infiltration component (FISH negative) in five (6% of 78) of the reanalyzed tumours.

Andersson et al. [14] conclude that only the invasive component should be analyzed.

The most common combination of histological type in these tumours was invasive ductal carcinoma together with DCIS (ductal carcinoma in situ), followed by invasive lobular carcinoma with LCIS (lobular carcinoma in situ), tubular carcinoma/DCIS, tubular carcinoma/LCIS, lobular carcinoma/DCIS and mucinous carcinoma/DCIS.

Researchers generally agree that some problems exist regarding the correct diagnosis of HER2 status. HER2 expression is not seldomly heterogeneous in invasive compared with in situ components within a tumour. Up to 80% of DCIS over-express HER2 [16]. The observation that HER2 signalling pathway activation is more frequent in DCIS versus invasive carcinomas may indicate that HER2 amplification is an early event in the progression of human breast carcinoma. Therefore, only the invasive part of a tumour should be scored. Due to this, any technique that does not preserve the tissue architecture, such as fine-needle aspiration cytology or cytosol-based analyses, becomes a less favourable method for evaluation of HER2 status in primary tumours.

To avoid heterogeneity as a confounding factor in HER2 analyses, detection methods such as IHC and FISH, which can provide evaluation in a preserved tissue architecture, should be used [14].

Ridolfi et al. [21] state that when compared to invasive carcinomas, a higher percentage of DCIS cases (up to 55% of high grade DCIS) are HER2 positive. A small percentage of invasive breast carcinomas demonstrate HER2 amplification, but no detectable over-expression. Almost all cases with mixed DCIS/invasive elements exhibited concordant HER2 IHC expression. In four cases, the DCIS component showed 3+ membrane staining and the invasive component was negative. In two of the cases, the FISH findings were concordant with IHC but in the other two, both the DCIS and invasive elements were amplified by FISH. A discordance in over-expression was apparent without a comparable discordance in amplification by FISH. Dressler et al. [22] mention in the overview on the measurement of HER2 status that protein over-expression occurs in up to two-thirds of in situ carcinomas.

Since HER2 over-expression is very common in high-grade, comedo-type DCIS, the HER2 status may be useful in determining the invasive potential in patients [23].

**14% (IHC)–16% (FISH)
of invasive tumours are
HER2 positive**

**invasive component
more reliable**

**HER2 amplification:
early event in the
progression of breast
carcinoma**

**for correct diagnosis of
HER2 status: invasive
part of tumour &
preserved tissue**

**about 2/3 DCIS HER2
overexpression**

3.3 Relevance of Testing HER2 Status

availability of a targeted monoclonal antibody therapy

The availability of a targeted monoclonal antibody therapy for HER2 positive breast cancer has focused interest on the accurate detection of HER2 status. HER2 positivity – amplification of the HER2 and/or overexpression of its protein – is required for the selection of patients for the therapy. However, researchers do not agree on the best method for determining HER2 status.

estimated inaccuracy of HER2 testing in 20% of patients

Technical validation of HER2 testing means that the testing assay is sensitive, specific, reproducible, can be interpreted in a relatively uniform manner among laboratories and, importantly, that the technical validation has been calibrated to clinical outcome [20]. Recent estimates suggest that approximately 20% of current HER2 testing may be inaccurate [10].

The definition of optimal algorithm for HER2 testing depends on laboratory documentation:

- ❖ Internal validation procedure (25-100 samples tested by alternative validated method (in the same lab or in another lab))
- ❖ Internal quality assurance procedure (use of standardised operating procedures including routine use of control materials)
- ❖ External proficiency assessment (participation in testing program)
- ❖ Laboratory accreditation.

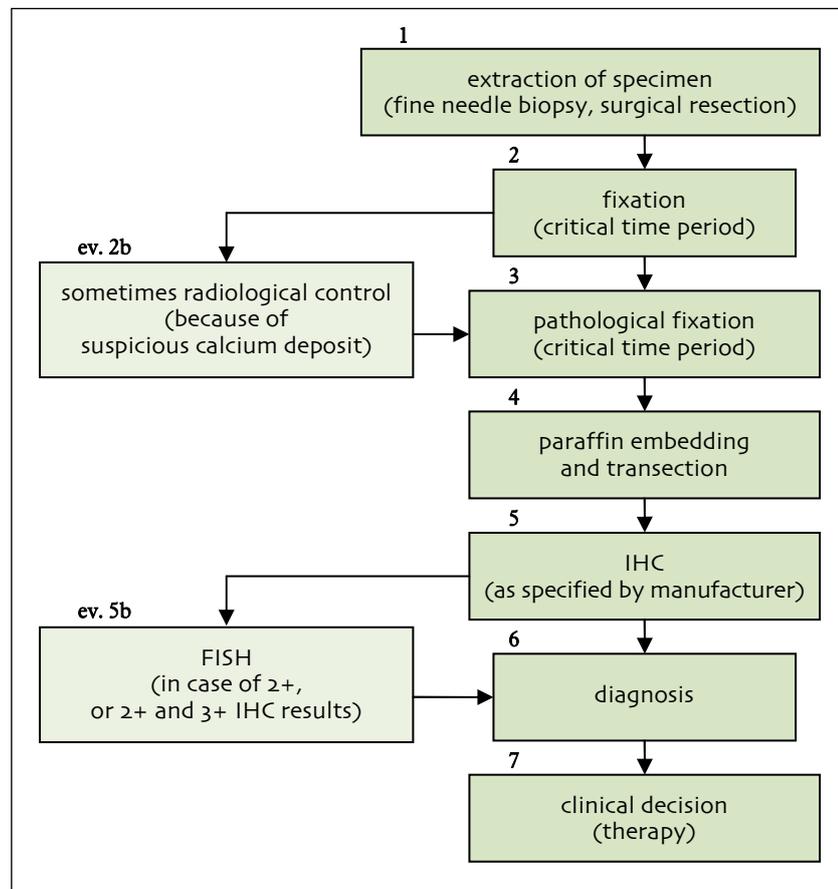


Figure 3-2: Procedure for Determining HER2 Status (own presentation)

Although specific fixation protocols vary, commonly all specimens (from needle core biopsies, open or lumpectomy excisions, mastectomies, metastatic lesions or unspecified samples) undergo fixation in neutral buffered formalin.

fixation protocols vary

For inclusion in different studies, the following criteria for specimens should be met: previously molecular characterisation for HER2 gene amplification (either Southern hybridization or slot/dot blot hybridization using DNA extracted from breast cancer specimens) and HER2 over-expression assessed by either solid matrix blotting of RNA or protein extracted from breast cancer specimens (Northern hybridization, dot blot hybridization, and Western immunoblot) or IHC. The analyses of amplification and expression should show agreement between amplification and expression status. However, the use of a wide variety of analytical methodologies for assessing HER2 status has complicated interpretation of results.

solid matrix blotting techniques

3.4 HER2 Testing Methods

HER2 testing methodologies measure HER2 over-expression[20]:

- ✿ At the DNA level by
 - ✿ FISH
 - ✿ Southern Blot
 - ✿ PCR – Competitive
 - Differential
 - Real-time
- ✿ At the RNA level by
 - ✿ Northern Blot
- ✿ At the protein level by
 - ✿ ELISA
 - ✿ Western Blot
 - ✿ IHC.

To determine clinical HER2, tissue-based methods, such as IHC analysis and FISH have replaced whole-tissue extraction methods, such as Southern Blot analysis, enzyme-linked immunosorbent assay, and PCR, which may require fresh tissue or suffer dilution owing to admixing of tumour and normal cells.

most widely applied techniques for assessing HER2 status

A variety of methods are available to assess tissue HER2 status, but for clinical and research purposes, the most widely applied techniques are:

- ✿ ImmunoHistoChemistry (IHC) and
- ✿ Fluorescence in Situ Hybridization (FISH).

IHC and FISH are performed on sections cut from formalin-fixed, paraffin-embedded specimens and have emerged as the most frequently employed techniques in clinical practice and clinical research [20]. In surgical laboratories they easily can be performed according to manufacturer's specifications. The tissue results of the recommended IHC and FISH are not reliable to correlate with the serum HER2 (SHER2) levels.

3.4.1 Immunohistochemistry (IHC)

protein-based test	A protein-based test is used to determine the total amount of HER2 protein receptors on the surface of the cell. To do this, the surface of the cell is “stained” with an antibody. Over thirty anti-HER2 mouse monoclonal or rabbit/goat polyclonal antibodies are available. Scientific literature reports more accurate results for testing with R60 Polyclonal Antibody, 10H8 Monoclonal Antibody or CB11 Monoclonal Antibody, when previously molecularly characterized. These antibodies are directed towards either the extracellular or intramembraneous domains of the HER2 receptor[20]. A pathologist has to judge the degree of colour change in the cell to determine a HER2 protein measurement level of 0, 1+, 2+ or 3+.
many different testing antibodies	
IHC scoring method	IHC scoring method [24]: 0 (negative): no staining is seen, or membrane staining in less than 10% of tumour cells; 1+ (negative): a faint/barely perceptible membrane staining is detected in more than 10% of tumour cells. The cells are only stained in part of the membrane; 2+ (borderline): weak to moderate complete membrane staining is seen in more than 10% of tumour cells; 3+ (positive): strong complete membrane staining is seen in more than 10% of tumour cells.
IHC performed on formalin-fixed, paraffin-embedded tissue	IHC can be performed easily on formalin-fixed, paraffin-embedded tissue – the form typically preserved by clinical trials – as well as on frozen samples [20]. Control slides (to control signal positivity) from cell lines with predetermined HER2 expression levels are included and this method is less expensive than FISH.
disadvantages of IHC: not standardized, high variabilities	However some disadvantages exist: Tissue fixation and processing, as well as differences in scoring criteria, controls and inter-observer variability in interpreting results can all contribute to variability [20]. Fixation protocols, antibodies used and staining procedures influence the success of IHC [25]. Pre-analytical variables such as tissue-handling and fixation can affect immunoreactivity. Researchers studying IHC results using 28 different anti-HER2 antibodies found marked variability with positive results ranging from 2% to 30% in the same group of specimens[26].
manufacturers	<p>Pathway (Ventana Medical systems, Tuscon, AZ) Monoclonal antibody CB-11 is used</p> <p>HercepTest (DAKO) Rabbit polyclonal antibodies are used.</p> <p>The Food and Drug Administration (FDA) was the first to approve the HercepTest IHC kit (Dako). Since then, the kit has achieved wide usage and offers a comprehensive protocol, standardized reagents and control slides [20]. Later, the FDA also approved Pathway. For both, a final score of 3+ is considered positive.</p> <p>IHC does not seem to be a reproducible technique as long as there are no standardizations of procedure, for example, using the primary antibody NCL-CB11, because of different methods of immunostaining as well as different scoring systems [27].</p>
accuracy of HER2 testing	Researchers compared CBE356 (not approved) to HercepTest in 167 FISH characterised breast carcinoma [28]. IHC was performed using CBE356 mouse monoclonal antibody, and HercepTest which is based on polyclonal antibody. Sensitivity, specificity, predictive values, and overall accuracy were calculated for both IHC methods using gene amplification by FISH as the endpoint.

Results showed positive IHC staining for HER2 using CBE356 to be more accurate and 23% more sensitive at predicting HER2 gene amplification than the other method.

Because IHC testing lacks accuracy and reproducibility (use of different antibodies, differences in tissue fixation), a FISH test is often performed to confirm the results. The main advantage of the IHC assay over FISH is that it is faster and more economic to use [29]. Automated cellular imaging (ACIS)TM has been able to improve accuracy and reproducibility in IHC scoring, the concordance rate between IHC-ACIS (>2.2) and FISH (≥2.0) by PathVysion was 94% (247 primary breast cancer cases were studied) [30].

advantages of IHC over FISH

3.4.2 Fluorescence in Situ Hybridization (FISH)

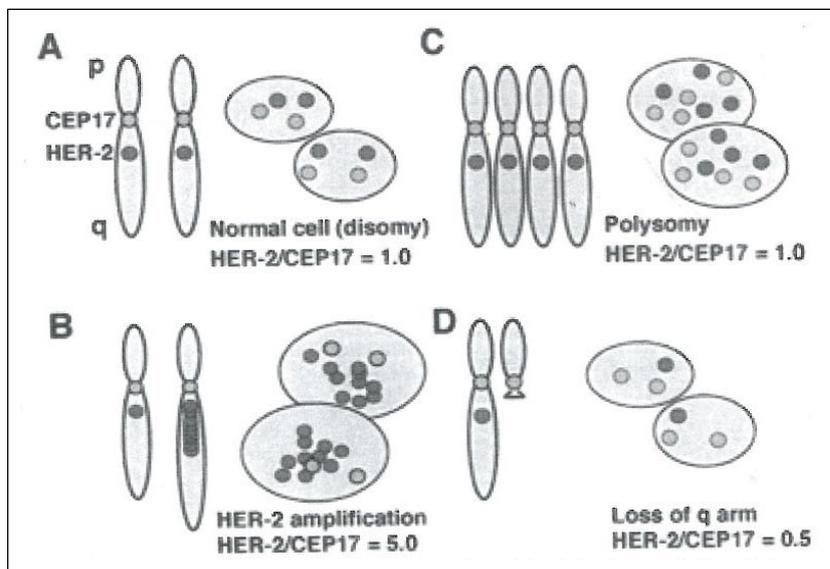


Figure 3-3: Patterns of chromosome 17 alterations detected by FISH (PathVision)
Breast cancer Vol. 13 No 2006

FISH detects amplification of the HER2 gene. It is a gene-based test that measures the number of HER2 genes in a cell. The FISH test “highlights” the HER2 genes inside the cell, making them appear fluorescent so they may be accurately counted. If a patient has more than two genes per cell, the tissue is considered HER2 positive. If the test shows a normal gene count, it is considered HER2 negative.

gene-based test

The FISH is a type of hybridization in which a DNA “probe” is labelled with fluorescent molecules so that it can be seen with a microscope. The word “in situ” means that the hybridization occurs “in place”, within the nucleus of specimen cells that have been fixed to a microscope slide. To conduct a FISH analysis, one warms fixed cells mounted on a microscope slide to unwind their chromosomal DNA and allow access of the DNA probe. After adding the probe, the specimen cells are then cooled to allow the DNA probe to hybridize with its complementary target DNA. Once hybridized, the fluorescent molecules on the probe will show precisely where their target DNA lies along a chromosome. Depending upon the design of the probe DNA, one can detect many types of genetic changes.

procedure

pro's and con's of FISH testing

The procedure is not dependent on the subjective interpretation of the intensity of staining. Although, it is less susceptible to problems associated with tissue handling and fixation, the technique is not always feasible in routine practice (fluorescence microscopy). Another disadvantage is the need for specialist equipment, time and skills for interpretation and costs. In a small fraction of cases HER2 – FISH fails to produce interpretable results [31], only 3.9% of 405 FISH non-informative tumours showed a 3+ IHC result as compared with 12.6% of those with successful FISH. Unspecified tissue damage that is impairing the FISH analysis is probably reducing IHC staining.

manufacturers

Path Vision (Vyses), distributed by Abbott, uses two DNA probes; one specifically recognizes the HER2 gene and the other is specific for the chromosome 17 centromere. Gene and centromere signals are determined in 60 nuclei and the HER2 gene: chromosome 17 centromere ratio is calculated. A ratio of ≥ 2 indicates HER2 amplification [20].

Inform HER2 (Ventana) uses a single probe to measure the absolute number of HER2 gene copies. The key difference is the inclusion of a probe for chromosome 17 in the Path Vision assay but not in the Ventana assay [32]. A dual probe FISH assay should be used because it can distinguish HER2 gene amplification from chromosome 17 polysomys [12]. The standard FISH method not only analyzes whether the HER2 gene is amplified but also assesses the degree of amplification. The gene dosage phenomenon could have a significant role in the spectrum of therapeutic responses [32].

3.4.3 IHC and FISH

2 most commonly used methods

The two most commonly used methods are IHC and FISH. The current practice is to consider

- ❖ an IHC score of 0 or 1+ as HER2 negative
- ❖ an IHC score of 2+ as ambiguous
- ❖ an IHC score of 3+ as HER2 positive.

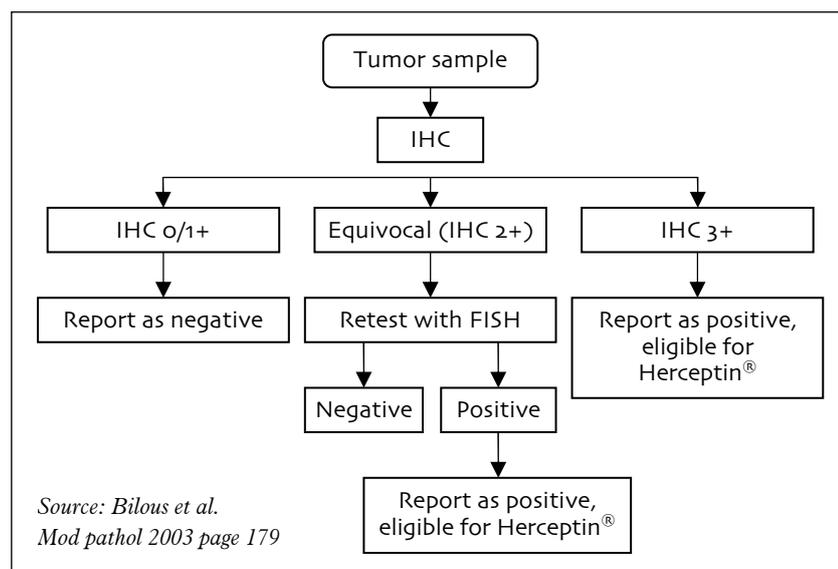


Figure 3-4: Algorithm for determining HER2 status

The IHC 2+ category is re-tested by FISH:

- ❖ PathVision (Vyses):
FISH ratio <2 – non amplified (negative)
FISH ratio ≥2 – amplified (positive)
- ❖ Inform HER2 (Ventana): Gene amplification is noted as none (1–5 copies), low-level (6–10 copies) or high level (>10 copies).

3.4.4 Fluorescence Immuno Histo Chemistry (FIHC)

A simultaneous detection of HER2/neu gene amplification and protein expression has been established. Based on four paraffin-embedded breast cancer cell lines, a combined fluorescent immunostaining (FIHC) and FISH method has been developed by using the PathVision HER2 DNA Probe Kit (Vysis) and the polyclonal antibody from the HercepTest (Dako). As a reference for both gene amplification and protein expression, researchers used four breast cancer cell lines. By combining and optimizing individual procedures as a multiparametric approach, the new method could be useful in predicting outcome of treatment in patients with discordant IHC/FISH results [25].

simultaneous detection of HER2 amplification and protein expression

3.4.5 Chromogenic In Situ Hybridization (CISH)

The assay target is the HER2 gene. CISH offers the ability to view morphological features of the cells analyzed using the traditional brightfield microscopy. However, HER2 and centromer 17 are not simultaneously visible using CISH. Furthermore, researchers have demonstrated that a small number of cases displaying low level amplification by CISH contained chromosome 17 polysomy by FISH.

viewing morphological cell features using brightfield microscopy

The concordance of CISH and FISH is 96% – 100% in different reported series [33]. Another study [34] found a concordance between CISH and FISH of 95% in 174 assessable cases. Considering FISH as the gold standard, CISH has a sensitivity of 97.5% and a specificity of 94%. Reagent costs of CISH are about half of those of FISH, but CISH's superiority has yet to be definitively demonstrated.

concordance between CISH and FISH is high

3.4.6 Polymerase Chain Reaction (PCR)

PCR is a technique under investigation for the testing of HER2 status, either to complement or to substitute other tests. In practice, PCR can fail for various reasons. Therefore a number of techniques and procedures have been developed for optimizing PCR conditions. Quantitative real-time PCR, for example, is a homogenous method that includes both amplification and analysis.

Garcia et al. [33] presented a study where concordance rates between real-time PCR and IHC and FISH were 91% and 92%, respectively. Because PCR requires micro-dissection, the author theorizes that any technique not preserving tissue architecture is less favourable in evaluating HER2 status.

quantitative real-time PCR includes both amplification and analysis

quantitative competitive PCR, quantitative differential PCR	<p>The quantitative competitive PCR adds a level of control using an internally amplified competitor [35]. A quantitative differential PCR targets the HER2 gene and a reference gene, enabling calculation of a ratio [36]. Dressler et al. [37] discuss the HER2 results of a study comparing IHC, FISH and D-PCR. They found only a moderate level of concordance among the three methods in assays from 524, 523 and 491 cases, respectively. It seems that none of the methods is clearly superior.</p> <p>In conclusion, for confirmatory HER2 testing, PCR could be used for indeterminate cases.</p>
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3.4.7 Enzyme-Linked Immunoabsorbent Assay (ELISA)

	<p>The HER2 gene product is composed of a cytoplasmic domain with tyrosine kinase activity, a transmembrane domain and an extracellular domain (ECD). The HER2 ECD levels can be detected by ELISA.</p>
advantage of ELISA	<p>The advantage of this method is that blood is relatively easy to collect. Real-time monitoring of changes in HER2 status in response to HER2 targeted therapies is possible. But ECD levels do not always correlate with the tumour load.</p>
correlation between HER2 ECD and tissue methods	<p>Many studies show a good correlation between serum levels of HER2 ECD and other methods on tissue (IHC and FISH) in metastatic breast cancer [38]. Significant higher concentrations of serum HER2 were found in FISH positive tumours [39]. However, in another study [40], in spite of concordance with tissue, HER2 overexpression (85% and 95% respectively) and FISH (83%), fifty percent of patients whose disease did not show gene amplification, had elevated baseline HER2 ECD levels. The study found no correlation between baseline ECD level and likelihood of response to therapy.</p> <p>Otherwise, a significant reduction in serum levels predicts improved response rates and time to progression [19].</p> <p>The method is FDA-approved to monitor response to therapy.</p>

4 HER2 Testing: Validity and Reliability

4.1 Critical Appraisal of Meta-analysis on IHC & FISH

Accurate testing for HER2 in breast cancer can be achieved using IHC with complementary FISH testing.

In 2006, researchers from the McGill University Health Centre [41] presented a literature review on the validity and reliability of two commonly used HER2 testing methods: IHC and FISH. The studies included in the meta-analysis show enormous variability.

Table 4-1: Studies in meta-analysis taken from Dendukuri

Studies with results reported jointly for IHC 0 and 1+ categories								
Author (Year)	N	Distribution of IHC results (%)			FISH positivity rate (%) By IHC score			
		0.1+	2+	3+	0.1+	2+	3+	
1. Lottner (2005)	215	78.1	11.6	10.2	2.4	72.0	100.0	
2. Loring (2005)	110	56.4	15.5	28.2	0.0	0.0	87.1	
3. Dowset (2003)	426	63.4	12.7	23.9	0.7	48.1	94.1	
4. Press (2002)	117	74.4	11.1	14.5	14.9	100.0	100.0	
5. Bartlett (2001)	210	85.2	10.0	4.8	6.7	90.5	90.0	
6. Hoang (2000)	100	74.0	2.0	24.0	0.0	0.0	70.8	
7. Kakar (2000)	112	70.5	15.2	14.3	1.3	3.5	87.5	
8. Mrozowskiak (2004) †	360	2.8	87.5	9.7	0	20.3	91.4	
9. Yaziji (2004) †	2913	49.0	39.5	11.5	2.8	17.0	91.6	

Studies with results reported separately for IHC 0 and 1+ categories									
Author (Year)	N	Distribution of IHC results (%)				FISH positivity rate (%) By IHC score			
		0	1+	2+	3+	0	1+	2+	3+
10. Lal (2004)	2279	44.6	31.4	13.7	10.3	1.1	3.1	26.5	89.7
						2.1	5.7	24.6	90.6
11. Ogura (2003)	110	24.6	47.3	9.1	18.2	3.7	3.8	10.0	100.0
12. Tsuda (2001)	101	41.6	34.7	5.9	17.8	2.4	2.9	0.0	83.3
13. McCormick (2002) †	198	26.8	29.8	22.7	20.7	3.8	8.5	3.5	87.5
14. Roche (2002) **, †	119	7.6	8.4	10.1	73.9	0	0	0	89.8
15. Press (2005) **, †	842	36.5	17.8	36.5	9.3	3.6	5.3	26.5	66.4
16. Dolan (2005) †	129	1.6	16.3	72.1	10.1	0	0	8.1	62.5

** HercepTest™ conducted at various laboratories compared with FISH at a central facility.

† Distribution of sample test scores not representative of population.

The objective of the meta-analysis was to critically assess the literature concerning validity and reliability of IHC and FISH as a basis for a testing strategy, and to provide a cost-effectiveness analysis. Although the inclusion criteria seem to be rather strict, the presented studies differ in many points.

objective of the meta-analysis: cost-effectiveness analysis

Lottner et al., 2005: 97.7% concordance IHC & FIHC	Lottner et al. [25] present a new form of HER2 testing by means of FIHC (fluorescence IHC, a simultaneous measurement of protein over expression and gene-amplification as a multiparameter approach) in 215 primary breast carcinoma (node-positive, node-negative, primary tumour or axillary nodes not assessed). The study found 97.7% concordance between conventional IHC and FIHC. Cases showing discordance at DNA and protein levels could be identified and analyzed in more detail by this simultaneous method. The data defined in Table 2 only refer to the conventional IHC and FIHC results.
Loring et al., 2005: 99% concordance	Loring et al. [42] compare FISH and CISH in TMA (tissue microarrays) from 119 archival breast resection cases. The authors conclude that re-testing is possible by either FISH or CISH (concordance between FISH (PathVision) and CISH (Zymed) was 99%). Intratumoral heterogeneity did not affect a patient's HER2 status (21 IHC 2+ scored cases).
Dowsett et al., 2003: 7-19% variances in 2+ between laboratories	Dowsett et al. [16] compared local and reference FISH testing in 426 metastatic breast carcinomas (sent in from 37 hospitals and tested in three reference centres). The correlation between two analyses (IHC by HercepTest, FISH by PathVysion) was calculated. They found very few cases of discordance in the 0, 1+ and 3+ samples. The proportion of 2+ varied from seven percent in centre 2 to nineteen percent in centre 1. Overall, approximately 50% (30%/40%/82% in the three centres) of the IHC 2+ tumours were found to be FISH positive. For a between-laboratory IHC reproducibility study a total of 60 tumours were re-scored; in all cases the discrepancies were scored 2+.
Press et al., 2002: accuracy for different IHC and FISH tests	This study presents [43] an overview of four different IHC tests (R60 Polyclonal Antibody, 10H8 Monoclonal Antibody, HercepTest (Dako) and CB11 Monoclonal Antibody) and two different FISH (Inform HER2 (Ventana) and PathVysion (Vysis)). One hundred and seventeen breast cancer specimen were previously molecularly characterized for HER2 gene amplification (Southern hybridization or slot/blot hybridization – using DNA) and overexpression (Northern hybridization or dot/blot hybridization or Western immunoblot – assessed by RNA or protein) using solid matrix blotting techniques. Following accuracy rates were calculated: FISH: 97.4% for Vysis and 95.7% for Ventana. IHC: 96.6% for R60, 95.7% for 10H8, 89.7% for CB11, and 88.9% for HercepTest.

Table 4-2: Sensitivity and specificity of different IHC and FISH tests, taken from Press et al., 2002

Assay	No. Positive/ No. Negative	Sensitivity	95% CI	Specificity	95% CI	Kappa	95% CI	Accuracy	95% CI
FISH, Vysis	42/75	41/43=0.954	0.842–0.994	73/74=0.986	0.927–1.0	0.945	0.883–1.0	0.974	0.927–0.995
FISH, Ventana	44/73	41/43=0.954	0.842–0.994	71/74=0.960	0.886–0.992	0.909	0.830–0.987	0.957	0.903–0.986
IHC-R60	39/78	39/43=0.907	0.779–0.974	74/74=1.00	0.951–1.0	0.925	0.853–0.997	0.966	0.915–0.991
IHC-10H8	38/79	38/43=0.884	0.749–0.961	74/74=1.00	0.951–1.0	0.906	0.825–0.986	0.957	0.903–0.986
IHC-CB11	31/86	31/43=0.721	0.563–0.847*	74/74=1.00	0.951–1.0	0.766	0.644–0.888	0.897	0.828–0.946*
IHC-DAKO	30/87	30/43=0.698	0.539–0.828	74/74=1.00	0.951–1.0	0.745	0.618–0.871	0.889	0.817–0.939*

Table 4-3: Results on IHC and FISH sensitivity and specificity by Press et al., 2002

	Sensitivity	Specificity
FISH (pos if HER:CEP 17≥2)	95.4%	98.6%
IHC (pos if ≥2)	69.8%	100%
IHC (pos if ≥3)	39.5%	100%

The authors concluded that the Vysis FISH assay was the most accurate, followed by R60, Ventana, 10H8, CB11 and HercepTest. IHC assays had perfect specificity, but lower sensitivity. The in-house IHC assays were not significantly different from the FISH assays for identification of overexpression (although less sensitive).

Bartlett et al. [44] compared commercial IHC assays and FISH to a more accurate standard Q-IHC (quantitative radio-immunohistochemistry) on 191 frozen and formalin fixed breast carcinoma sections. HER2 was assessed retrospectively by IHC with HercepTest (Dako), by monoclonal antibody CB11 (Ventana) and by FISH (PathVision). The accuracy for CB11 IHC was lower than for Herceptest or FISH; FISH predicted HER2 overexpression better than CB11 IHC or HercepTest. Technical differences among the tests arise from strategies to circumvent the effects of tissue fixation and processing. Fixation covalently links proteins and nucleic acids, which preserves morphology but “masks” epitopes and decreases tissue permeability to antibodies and DNA probes. IHC-based tests were more susceptible to inter-observer variation than FISH.

Bartlett et al., 2002:
tissue fixation and processing lead to inter-observer variation

Table 4-4: Results on IHC and FISH sensitivity and specificity by Bartlett et al., 2001 in Dendukuri

	Sensitivity	Specificity
FISH (pos if HER: CEP 17≥2)	84.1%	94.6%
IHC (pos if ≥2)	61.9%	98.6%

A study by Hoang et al. [45] analyzed the concordance between FISH and IHC (two different tests) in 100 consecutive cases resulting??? in 91% for HercepTest and 76% for e2-4001 monoclonal antibody test. Four pathologists completely agreed to IHC scoring (e2-4001 and HercepTest) in 75 and 85 cases, respectively.

Hoang et al., 2000:
inter-pathologist variation

Kakar et al. [26] presented the results of a comparison of IHC and FISH in 112 consecutive unselected patients. The concordance was 88% overall (88%/98 of 112 cases among cases with 3+, 35%/6 of 17 cases among the 2+ cases and 99%/78 of 79 cases among cases with 0 and 1+ staining). Survival data of 65 patients (stratified into 2 groups: survival >50 months and <50 months) revealed that patients with IHC results 3+ and FISH signal ratio >4.0, had shorter survival rates. However, the numbers in the groups were too small for exact interpretations. Costs and turnaround time were greater for FISH.

Kakar et al., 2000:
low concordance for IHC 2+

Mrozkowiak et al. [46] tested 360 invasive breast cancer specimen, comparing HercepTest and FISH with Oncor-QBiogene. The number of IHC 2+ scored samples was much higher compared with other studies presented in the meta-analysis. FISH results were divided into three categories: no/low/high amplification. The results are not representative for comparison with other studies from the meta-analysis because different methods were used.

Mrozkowiak et al., 2004:
results not comparable

For the 2913 breast cancer specimen in the study of Yaziji et al. [18], both FISH and IHC results were available. The concordance rate was 64.9% when scores of 2+ and 3+ positive tumour sections were grouped together. The sensitivity of IHC, including tumour sections with scores of 2+ and 3+, was 92.6% and the specificity of IHC tests with scores of 3+ was 98.8%.

Yaziji et al., 2004:
low concordance for IHC 2+

Lal et al., 2004:
low concordance for
IHC 2+/25%

Lal et al researched the effect of dual-color (D-FISH) versus single-color FISH (S-FISH) scoring in 2279 cases of invasive breast carcinoma [32]. The study considered a ratio for HER2/chromosome 17 \geq 2 (D-FISH) or an absolute HER2 copy number per nucleus \geq 4 (S-FISH) as positive gene amplification. Overall concordance in HER2 status with IHC was 87% for D-FISH and 86% for S-FISH. The researchers found concordance among groups scored by IHC as 0, 1+ and 3+, but not so good in the group scored 2+. Here only about a quarter was FISH positive. D-FISH and S-FISH scoring results were discordant in 4% (89 tumours), of which 9% (8) had 3+ IHC staining and none showed high-level HER2 amplification.

Table 4-5: Correlation D-FISH/S-FISH, Lal et al., 2000

S-FISH	D-FISH	
	positive	negative
Positive	294	57
Negative	32	1896

**47% of FISH positive
borderline tumours**

The discrepancy was apparently due to polysomy 17 in tumours that were negative by D-FISH and positive by S-FISH and monosomy 17 with concurrent duplication of the HER2 gene on the remaining chromosome 17 in tumours that were positive by D-FISH and negative by S-FISH. Among all FISH positive tumours, 10% were negative by IHC and almost half (47%) showed borderline to low HER2 amplification. This might explain instances where there was no response to therapy.

**association with low
response to
trastuzumab**

Even among the FISH-positive tumours scored immunohistochemically as 2+, the degree of HER2 amplification was significantly lower than that of 3+ tumours, but rather similar to that of FISH-positive tumours scored immunohistochemically as negative for HER2. The authors suggest that a relatively low level of HER2 amplification observed in the 2+ tumours also might contribute to the lower response rate to Trastuzumab-based therapy = gene dosage phenomenon. The present study, however, does not permit a determination of which FISH assay is better for clinical testing.

Table 4-6: HER2 amplification of 2+, Lal et al., 2004

Immunohistochemical Score	Total No. (%) of Cases	Ratio <2.0		Ratio \geq 2.0		
		No. (%)	Mean (Range)	No. (%)	Mean (Range)	Media
0	1,017 (44.6)	1,006 (98.9)	1.1 (1-1.9)	11 (1.1)	3.4 (2.0-6.8)	2.4
1+	715 (31.4)	693 (96.9)	1.2 (1-1.9)	22 (3.1)	3.5 (2.0-5.9)	2.9
2+	313 (13.7)	230 (73.5)	1.2 (1-1.9)	83 (26.5)	3.6 (2.0-10.5)	2.8
3+	234 (10.3)	24 (10.3)	1.2 (1-1.7)	210 (89.7)	5.1† (2.1-18.6)	4.7
Total	2,279 (100.0)	1,953 (85.7)	—	326 (14.3)	—	—

Ogura et al., 2004:
90% discordance with
IHC 2+

Ogura et al. [47] analyzed 110 specimen of 113 consecutive patients (age: range from 27 to 82 years; 42 pre-, 68 postmenopausal) with invasive ductal carcinoma (IHC performed by HercepTest, FISH by PathVision). They conclude that an IHC score of 3+ is FISH positive, while an IHC score of 2+ is not equivalent to FISH positive because 90% of the samples analyzed having an IHC score of 2+ had discordant FISH results.

Table 4-7: IHC/FISH sensitivity taken from Ogura et al., 2004

	IHC			
	0	+	++	+++
FISH				
Neg	26	51	9	0
Pos	1	2	1	20
FISH pos rate	4%	4%	10%	100%

Tsuda et al. [48] conducted a study of two independent observers performing FISH testing (by PathVision). The study found a good reliability in intratumor heterogeneity (there was a concordance of 100% between the invasive and intraductal components in all 14 carcinomas). HER2 amplification detected by FISH was found to be compatible with the Southern Blot analysis, HercepTest and the own polyclonal antibody Test (Nichirei). The concordance was 91% for 2+/3+ IHC results and FISH, with a sensitivity of 70% and specificity of 97%.

Tsuda et al., 2002:
high concordance for
combined 2+/3+

McCormick et al. [12] analyzed 215 samples from different hospitals by IHC (HercepTest) and FISH (PathVision). The HER2 status concordance was high for IHC 0, 1+ and 3+ tumours but poor for 2+ tumours. Two pathologists independently scored slides as 0, 1+, 2+ or 3+. Cytoplasmic staining was ignored; only invasive tumour was scored. Pathologist concordance was 78% overall and 95%, 62%, 75% and 83% for the 3+, 2+, 1+ and 0 IHC group, respectively. FISH slides were scored by one observer. When samples were grouped as IHC positive (2+, 3+) or IHC negative (0, 1+) the concordance was 92% and 96%, respectively. One postulated advantage of IHC analysis over FISH for HER2 determination is the potential to detect protein overexpression in HER2-nonamplified tumours.

McCormick et al., 2005 :
inter-pathologist
variation highest
with 2+

The authors mention that such overexpression-positive/amplification-negative tumours occur in 3% to 8% of breast cancers in most series but were as high as 29% and 31% in comparison of FISH (PathVision) with IHC (HercepTest) and the Genentech CTA, respectively.

**1/3 overexpression-
positive/amplification-
negative tumours**

In a study performed by Roche et al. [49], tumour specimens from the first 119 patients enrolled in the Breast Intergroup Trial N9831 were centrally tested (IHC with HercepTest, FISH with PathVision). HER2 status at enrolment was determined in one of 65 local laboratories (IHC: 50% by HercepTest and 42% by other methods, and FISH: 7% by PathVision and 1% by Ventana). The study found poor concordance (74%) between local and central testing for HER2 status.

Roche et al., 2002:
poor concordance
between local & central
laboratories

In another study [50], 2600 women were prospectively evaluated by FISH for entry in clinical trials. The study found that 26% of the participants had HER2 gene amplification. Overall, the HER2 alteration status determined by local IHC showed 79% agreement rate with central performed FISH. The agreement rate of central and local performed IHC was 77.5%, and for central and local performed FISH 92%. Concerning inter-/intra-laboratory reproducibility, better agreement between outside and central tests was found for FISH as compared to HercepTest.

Press et al., 2005:
poor IHC concordance
between local & central
laboratories,
good for FISH

In the study of Dolan et al. [51] IHC (by HercepTest) data were available for 129 specimens. The study found a discordance between IHC and FISH of ap-

Dolan et al., 2005:
concordance increases
with increase of tumour
grade

proximately 92% at IHC scores of 2+ when the data was tested with FISH (PathVision). With regard to specimen type (core biopsy cases and excision/excisional biopsy cases: 79% and 62%, respectively) and tumour grade (the rates of discordance decreased with increasing tumour grade), a slightly varying concordance at FISH was found, the tumour type having no statistically significant affect on concordance rates.

4.2 Conclusions from Meta-Analysis

The meta-analysis was carried out to estimate the distribution of IHC scores and to determine IHC validity (FISH was considered the gold standard). Table 4-1 summarizes the results from studies evaluating the concordance between IHC and FISH.

Table 4-8: Results of Meta-Analysis (%)

IHC 0	36.1%	FISH: 1.7% pos
IHC +	35.5%	FISH: 3.4% pos
IHC ++	12.2%	FISH: 29.9% pos
IHC +++	16.2%	FISH: 91.9% pos

FISH is golden standard,
non-approved IHC-tests
better accuracy than
HerceptTest

Some trials use different tissues (eg TMA) for testing, showing new options for clinical practice, and demonstrate new methods that differ from conventional IHC/FISH ([25]). In two studies ([44] and [43]), authors compared IHC and FISH with a third reference standard, the Q-IHC (quantitative radio-immunohistochemistry) and solid matrix blotting. The latter methods are considered to be more accurate but impossible to use in a clinical setting. The researchers found that FISH had a higher sensitivity than IHC and that three other IHC assays using different antibodies (R 60 Polyclonal antibody, 10H8 Monoclonal Antibody, CB11 Monoclonal Antibody) had better overall accuracy than HerceptTest.

current strategy:
FISH after IHC 2+
low inter-observer/
laboratory concordance
for IHC, high for FISH

The currently pursued strategy of confirmatory testing for IHC 2+ cases alone is expected to correctly diagnose 96.9% women.

In general, study results of reliability between/within observers/laboratories, demonstrated that the reliability of FISH was better than for IHC [16]. The relatively subjective nature of the interpretation of the IHC data may mean that less experienced centres are less likely to reach the same level of concordance described in some studies.

The strategy of performing FISH on all cases is supposed to comprise the highest incremental cost-effectiveness ratio (ICER), the strategy of confirmatory testing for IHC 2+ and 3+ cases the lowest.

Overall, the McGill University Health Centre meta-analysis [41] presents results of HER2 testing gained by different methods in local or central departments. Based on the interpretation of all the results, the authors recommend the confirmation of the positive HER2 status of IHC 2+ and 3+ scored specimen by FISH, and an IHC test for patients with a positive FISH test alone, performed in a local laboratory.

5 HER 2 Testing: Key Issues

The majority of published reports examining the impact of HER2 status on clinical outcome and response to therapy employed retrospective HER2 testing, primarily by IHC. A major problem when interpreting published studies of HER2 in the clinical setting is that different methods have been used to evaluate HER2 alterations. These methodological differences likely contribute substantially to conflicting results and, therefore, conflicting conclusions [43]. Further, nearly all studies discussed – besides the results achieved by different methods used for HER2 testing – also the morphological patterns, and tried to show future possibilities in enhancement.

many different testing methodologies contribute to conflicting test results

Mann et al. [52] discussed the results of 100 consecutive patients, having had both core and surgical specimen (no therapy in between). Two pathologists examined each slide independently. To assess whether there was a systematic tendency for core biopsies to show a positive result more often than the corresponding surgical tissue sample, the authors used a log-linear model to test the symmetry of the comparison. They noted an occasional disparity between assay results on core biopsy and surgical specimens.

problem of tissue acquisition

Gong et al. [9] discussed different methods of tissue acquisition yield samples with different cellular compositions. Fine needle aspiration (FNA) samples mainly contain neoplastic cells and a few infiltrating leucocytes; whereas tissue samples contain variable amounts of neoplastic cells, connective tissue, and benign breast epithelium. In this study mRNA-based oestrogen receptor and HER2 determinations had similar results on both types of samples. Three of 43 tumours with an IHC score of 3+ were not amplified by FISH (PathVysion) with a discordant rate of seven percent.

These discrepancies are expected because DNA, mRNA and protein degrade at different rates and pre-analytical factors (ie, fixation length) affect results. When analyzing the results, it is important to be aware that the handling and processing of tissue samples before the HER2 assay can affect the results (type of specimen, time from excision to fixation, specimen slicing before fixation). Therefore, researchers need to standardize the steps and procedures involved [53].

problem of pre-analytic time

The fixation time should be between 6 and 48 hours [10]. The staining of the tissue – elapsed time between fixation and staining is important. Prolonged storage of unstained slides can result in significant loss of immunostaining intensity for some antigens/antibodies [20]. HercepTest relies on samples being fixed by a defined protocol (kit insert). Fixation preserves morphology but decreases tissue permeability to antibodies and DNA probes [44]. Overfixation can produce negative results because formalin suppresses the antigenicity [54]. Formalin fixation for four days or longer might produce inaccurate data by FISH, and formalin fixation for one week or longer might result in inaccurate IHC results.

problem of fixation and prolonged storage

The circumstances that take place prior to analysis, such as the time between removal of the tissue (tissue acquisition) by fine-needle aspiration, core biopsy, surgical resection and embedding in formalin (fixation), are important to the results.

false-positive, false-negative

This time period should be minimized to be as short as possible. That could be a problem in hospitals, where surgery departments are not in physical proximity to pathological laboratories.

pro & contra (de-)centralization

potential for false-negative	The antibodies are developed from animals immunized with native, nonfixed HER2 protein as antigen. In some formalin-fixed breast cancer specimens HER2 is not recognized because none of the HER2 antibodies were produced using formalin-fixed HER2 protein as antigen [43].
no completely standardized procedure for IHC	The IHC test is not performed consistently across laboratories and every country has its own favoured methods. By comparing studies in which the authors comment on the interpreting and scoring of IHC-stained slides, the percentage of positively staining cells, the intensity and location (cytoplasmic versus membraneous, and ductal carcinoma in situ versus invasive component) of staining, and the positivity cut-off used is to be considered [20]. Few of the laboratories have validated their immunohistochemical staining assessments using standardized specimens with known molecular changes. A large number of pathology laboratories use in-house reagents and protocols with widely varying sensitivity, specificity, reproducibility and practicability [20]. The identification of patients with high HER2 expression by Q-IHC is unsuitable for routine diagnostics. Many studies refer to accuracy of the HercepTest. Vincent-Salomon et al. [55] used different IHC tests for HER2 at a multicenter level, and achieved accuracy of 93% and 95% for the HercepTest.
problem of polysomy 17 in FISH testing	<p>One study [56] investigated the effect of chromosome 17 copy number in determining HER2 status in breast cancer. When a ratio ≥ 2 was considered as criterion for FISH positivity, 49.3% of cases showed amplification, 56.2% by using a net HER2 gene copy number >4 as an alternative criterion. A net increase in HER2 gene copy number consecutive to polysomy 17 in the absence of specific gene amplification might lead to a strong protein overexpression in a small subset of breast carcinomas.</p> <p>Polysomy 17 is responsible for discordance in HER2 status as defined by different FISH scoring systems [15]. Best concordance was found with the HER2/CEP17 ratio and HER2>6 scoring. An alternative for the clinical practice could be the correction for chromosome17: HER2 >6, but not HER2 >4 as alternative. Varsheney et al. [57] found the incidence of chromosome 17 polysomy in 2+ non-amplified cases to be similar to the incidence of polysomy of 17 in negative cases. The findings indicate that weak overexpression (2+) of HER2 without gene amplification is not secondary to chromosome 17 polysomy.</p>
problem of borderline results	<p>A parallel trend was observed between progressively higher levels of FISH signals and ascending IHC staining categories [58]. Both FISH (corrected or uncorrected for chromosome 17 polysomy) and IHC findings were found to be independent predictors of poor survival in multivariate analysis.</p> <p>Researchers have established the 2+ category of IHC as the main source of discrepancy between IHC and FISH results [32]. The variability in definition and use of a borderline interpretation may be partly the result of different interpretations of the FISH-PathVision kit insert (ratio of 1.9 should be reported as no amplification, of 2.1 as amplification, and near the cutoff of 1.8–2.2 should be interpreted with caution).</p> <p>Aneusomy (additions or deletions of entire chromosomes) seems to be related to a particular subset of 3+ cases whose biology should be further investigated [59]. Cases with 2+ and 3+ scores are associated with a weakly/moderately and intensely positive membrane in more than ten percent of total tumour cells. Among all FISH positive tumours, 10% were negative by IHC; almost half (47%) showed borderline to low HER2 amplification. This could explain instances where there was no response to therapy [32]. Lewis</p>

et al. [60] used tumor-bearing blocks from 20 invasive breast carcinomas with IHC scores of 2+ *with* gene amplification and from eighteen cases with IHC scores of 2+ *without* gene amplification.

They found a significant degree of intratumoural heterogeneity with respect to HER2 protein expression. Overall, 68% of the 2+ cases would have had a different immunohistochemical score if another slide(s) had been examined. They observed that staining heterogeneity, within a slide and in different blocks of a tumour, is a feature of 2+ staining tumours.

**intratumoural
heterogeneity**

Many studies found poor correlation between weak positive IHC scored specimen and the evaluated FISH results (ie, [61]). Killeen et al. [62] compared IHC and FISH results with tumour morphology and found that borderline HER2 tumours are a unique tumour type and do not represent laboratory imprecision. Whether borderline amplification is correlated with sensitivity to therapy is unknown [54].

*Table 5-1: Tumor characteristics and HER-2 status
Killeen et al., 2006, page 101*

	HER-2 neg	HER-2 borderline	HER-2 pos	<i>p</i>	Difference
Age, mean	60.40	61.30	56.86	0.029	Pos < Neg or Bord
Tumor grade (mean score)	1.75	1.89	2.57	<0.001	Pos > Neg or Bord
Nuclear mean score	1.91	2.04	2.54	<0.001	Pos > Neg or Bord
Tubule mean score	2.38	2.57	2.83	<0.001	Pos > Neg or Bord
Mitotic mean score	1.47	1.55	2.20	<0.001	Pos > Neg or Bord
Ki-67 mean %	15.73	15.60	21.23	NS	
ER, % positive cases	86.00	84.00	42.00	<0.001	Pos < Neg or Bord
PR, % positive cases	72.00	61.00	28.00	<0.001	Pos < Neg or Bord
HER-2 copy # (mean)	2.24	4.10	13.57	<0.001	Pos > Neg > Bord

For tumours with low-level or equivocal amplification, future surveys will have to face additional challenges to more accurately define interlaboratory variability in HER2 FISH analysis near the critical cut-off range. Breast tumours with low and with high copy numbers at HER2-amplification have excellent reproducibility. However, there still needs to be a consensus on the use of a separate equivocal/borderline interpretative category for standardization of cutoff values used to define interpretative categories. McCormick et al. [12] found a high concordance between two pathologists (only invasive tumour tissue was scored). The authors of another study reported high agreement levels for tumours with IHC scores of 0, 1+ and 3+, but poor for tumours with IHC score of 2+ by 6 specialized pathologists [54].

**problem of inter-
laboratory variability for
borderline tumours**

Many studies present the problem of unsatisfying concordance IHC/FISH and IHC/IHC (from different manufacturers). One study [33] showed HER2 overexpression determined by IHC (HercepTest) in 19%. FISH (PathVysion) performed on equivocal cases demonstrated HER2 amplification in 18%. IHC and FISH together showed HER2 overexpression/gene amplification in 21% of breast invasive carcinoma. Another study [28] compared IHC using CBE356 with the HercepTest in formalin fixed, paraffin wax embedded blocks of 167 carcinoma, previously analyzed by FISH (PathVision). Overall CBE 356 antibody was a more accurate predictor of HER2 gene amplification by FISH than the HercepTest.

**problem of insufficient
concordance IHC & FISH**

Table 5-2: Comparison of two IHC Tests (2+, 3+ positive cases)
Ainsworth et al., 2005 [28]

	Accuracy	Sensitivity	Specificity	PPV	NPV
CBE56	94%	89%	95%	84%	97%
HercepTest	91%	66%	98%	92%	91%

**problem of discrepancies
between protein
expression and gene
amplification**

In another study of Dybdal et al. [63] the overall concordance between FISH and IHC results was 82% (in 529 fixed breast cancer tissue specimens).

Birner and al. [64] found a good correlation between FISH (PathVysion) and four different IHC tests. The study population consisted of 303 unselected cases of invasive breast cancer, 207 cases were analyzed. The authors of another study [59] discussed results of IHC and FISH on 81 archival formalin-fixed and paraffin embedded specimens. Data described in the literature for 3+ carcinomas showed a 3%–10% discrepancy between protein expression and gene amplification. In their study the difference was up to 22%. Reddy et al. [65] presented data that show a higher concordance between HER2 IHC and FISH when both tests are performed at a central laboratory. Researchers analyzed 923 breast cancer patient samples using IHC (HercepTest and a test with the R60 antibody) and FISH (one test with total number of cells, the other using the ratio) [66]. Sensitivity and specificity of FISH were superior to IHC. HercepTest showed more false-negative results.

**problem of uncertain
histology**

The authors of one study [48] found a concordant amplification status between the invasive and intraductal components. Hoff et al. [11] presented data from 401 invasive carcinomas. Invasive ductal carcinomas were more likely to demonstrate HER2 amplification than invasive lobular carcinomas, and among the invasive ductal carcinomas higher grade invasive more than lower grade. Researchers suggest re-examination of tumours diagnosed as SBRgrade 1 invasive ductal carcinomas or lobular carcinomas. Ductal of no special type was the predominant cancer type (81.5%) in a study discussing the prediction of HER2 status in 1540 specimen [67], followed by ten percent of infiltrating lobular carcinomas of classical variant. Researchers have found a strong correlation between HER2 3+ and infiltrating ductal carcinoma, grade 2 and 3, suggesting a predominant HER2 positive phenotype.

**problem of variations in
sensitivity and specificity
of tests**

Variability in sensitivity exists because formalin-fixation and paraffin-embedding adversely effect antigenicity and immunostaining of the vast majority of protein antigens, and the ability of antibodies to recognize their epitope varies from antibody to antibody [43]. The sensitivity of IHC, including tumour sections with scores of 2+ and 3+, was 92.6% and the specificity of IHC tests with scores of 3+ was 98.8% [18]. In 101 cases [48], the overall concordance of 93% was found between FISH (PathVision) and DNA amplification by Southern Blot: 93%, with a sensitivity of 76% and a specificity of 96%. Variation in assay sensitivity was studied in twenty-six laboratories [29]. Laboratories using HercepTest had the highest level of reproducibility in assay sensitivity and evaluation. The reported sensitivity of the HercepTest varied among centres.

**problem of inter- and
intra-observer reliability**

In some studies, two pathologists independently estimate the same specimen. The subjective nature of interpreting the intensity of IHC staining represents a particular inherent weakness of this testing method [20]. Studies examining the issue of intra-observer variability of HER2 IHC interpretation have found a high level of agreement with regard to the presence or absence of membrane staining, but a low level of agreement with intensity and extent

of tumour membrane staining. Intra-observer reproducibility was generally not satisfactory [68]. IHC-based tests are more susceptible to intra-observer variation than FISH [44], [24]. Studies found FISH testing by two independent observers highly reproducible in determining HER2 DNA amplification, independent of intratumour heterogeneity [48]. The concordance was 99%. Even at stratification in low-level and high-level amplification, concordance was achieved in 98%.

In the study of McCormick et al. [12], pathologist concordance was 78% overall and 95%, 62%, 75% and 83% for the 3+, 2+, 1+ and 0 IHC group, respectively. When samples were grouped as IHC positive (2+, 3+) or IHC negative (0, 1+), the concordance was 92% and 96%, respectively; FISH slides were scored by one observer. Another study [54] found FISH testing by two independent observers to be highly reproducible in determining HER2 DNA amplification, independent of intratumour heterogeneity (there was a concordance of 100% between the invasive and intraductal components in all fourteen carcinomas) [54]. FISH was found to be correlated with the status of the DNA amplification detected by Southern blot hybridization.

In another study [69] a significant number of laboratories changed their interpretation from one year to the next for the same case with low-level amplification, indicating potentially poor reproducibility within individual laboratories. The study examined the results of the eighty-three laboratories that submitted interpretation for both 2002 and 2003.

**poor reproducibility
within laboratories**

Table 5-3: Intra-laboratory Interpretation of Repeated Equivocal/Borderline Cases, taken from [69]*

	Amplification 2003	No Amplification 2003	Equivocal/ Borderline 2003
Amplification 2002	44	3	2
No Amplification 2002	23	6	5

* Total number of laboratories = 83

An inter-laboratory study [27] discussed the reproducibility of HER2 testing. HER2 was evaluated by IHC on archival primary breast cancer samples. Both laboratories used the primary antibody NCL-CB 11. The study found that HER2 evaluation by IHC was not reproducible when there is no standardisation of the procedure. In an Italian Quality Assurance Initiative [70], twenty immunostained slides were distributed to twelve pathologists. Inter- and intra-observer reproducibility was good. The authors concluded that One of the reasons why a high percentage of tumours classified as positive for HER2 do not respond to anti-HER2 therapy may be in the lowest class for both the percentage of immunostained cells and membrane intensity. In the Italian Assurance Initiative from 2005 [68], the intra-observer reproducibility was generally satisfactory, but the inter-observer reproducibility was not. Seventeen slides were scored by twenty-three observers – the HercepTest did not allow to discriminate optimally between scoring classes 2+ and 3+.

Largely due to inadequate resources, probes sent to different pathological laboratories are not estimated by pathologists twice. According to the literature, eighteen percent [71] and twenty-six percent [49] of patients who entered into clinical trials as HER2 positive on the basis of local testing, were negative when their probes were tested centrally. Scientific literature has discussed the issue of poor concordance between local and central testing [49].

**problem of local and
central laboratories**

few diagnostic laboratories have expertise in FISH	<p>In general, studies of reliability between/within observers/laboratories have concluded that the reliability of FISH is better than of IHC.</p> <p>However, currently relatively few diagnostic laboratories have expertise in FISH analysis. Therefore, poor agreement often occurs between results from local and central laboratories. It seems that although intra-observer reproducibility is generally satisfactory; inter-observer reproducibility is not [68].</p>
less experience means less concordance	<p>In regards to inter-/intra-laboratory reproducibility, studies have found a better agreement between outside and central tests on the FISH than on the HercepTest [50]. IHC 2+ category is most likely to be discordant with FISH. False-positive and false-negative HER2 results can have different sources of error, most likely resulting from methodological problems (i.e., lack of quality-control procedures in local laboratories). For example, among patients found to be strongly HER2 positive by local laboratories, 18.4% from IHC (HercepTest) and 11.9% from FISH (PathVysion) were unconfirmed when re-tested at a central laboratory [72]. The inter-observer variability is likely to be higher than in studies in which consensus IHC scores are obtained before performing FISH [51]. In the reliability study of Dowsett et al. [16], the discrepancies concerned primarily the 2+ category. The proportion – 13% of tumours IHC 2+ and overall 48% of these FISH positive – varied markedly between the centres. Sixty IHC-stained slides selected to be enriched with 2+ cases were circulated among the three laboratories and scored (twenty cases with discordance in scoring, the consideration with FISH → concordance in nineteen cases). The proportion of 2+ varied from seven percent in centre 2 to nineteen percent in centre 1.</p> <p>Overall, approximately fifty percent (30%/40%/82% in the three centres) of the IHC 2+ tumours were found to be FISH positive. The relatively subjective nature of the interpretation of the IHC data may mean that less experienced centres are less likely to reach the same level of concordance.</p>
potential for over-diagnosis	<p>Every pathology laboratory involved in IHC testing should validate its IHC test and interpretation by comparing IHC test and FISH results on a sizeable set of tumours (e.g. 100 cases) and using that set as a guide for IHC scoring [73].</p> <p>In another study [63], the concordance rate between two laboratories was 92%, indicating that FISH results were reproducible in archived specimens, even when they had been previously processed using IHC staining. The results suggest that FISH would exclude a significant number of women from clinical trials in both the IHC 2+ and IHC 3+ categories whose breast cancers do not have HER2 gene amplification. The study discussed possible over-diagnosis of HER2+ status in small-volume laboratories.</p>
problem of hormone status	<p>Because strongly positive (3+) cases (IHC) represent only 15–20% of newly diagnosed breast cancer cases, pathologists in small-volume laboratories may over-anticipate positive cases, leading to interpretation bias. Such bias would be likely to occur less in a large-volume setting [74].</p> <p>HER2 status at enrolment was determined in one of 65 local laboratories (IHC: 50% by HercepTest and 42% by other methods, and FISH: 7% by PathVision and 1% by Ventana). A poor concordance (74%) between local and central testing for HER2 status was found [49].</p>
	<p>An inverse relationship exists between oestrogen receptor (ER) positivity and HER2 positivity. One study [13] correlated HER2 amplification with lower ER and HER2 protein over-expression. The data showed that the ER levels</p>

are one-half to one-third lower in HER2 negative/ER positive cancers than in HER2 negative/ER positive tumours. The same study found FISH/IHC concordance in 84.6%. Taucher et al. [75] showed in their study (923 consecutive patients) that HER2 over-expression is correlated significantly with negative estrogen (ER) and progesterone receptor (PR) status, Grade 3 lesions and young age. Gene expression data (profiled on Affymetrix U133A Gene Chips) of 495 breast cancer samples (FNA samples and tissue samples) were used [9] to assess the correlation between mRNA concentrations and the routinely established clinical receptor status of oestrogen receptor and HER2. More than twenty percent of HER2 positive tumours showed moderate or strong staining for ER [76]. But the oestrogen status could not be used to select tumours for evaluation of HER2 status, and ER and PR positivity does not preclude a positive HER2 status according to these results.

Elkin et al. presented a cost-effectiveness analysis [77]. A state-transition model was created to simulate clinical practice in a hypothetical cohort of 65-year-old metastatic breast cancer patients and cost-effectiveness of alternative HER2 testing was estimated. Compared with IHC, FISH is more expensive, time consuming, and labor-intensive. But FISH has a more objective and quantitative scoring system and is also more predictive than IHC of response to trastuzumab. The additional costs must be weighed against the savings that result from avoiding treatment of women with false positive result. Base-case analysis suggests that no alternative exists to a policy of IHC with FISH conformation of all positive results.

In spite of quality control, sometimes the FISH assay needs to be repeated. This can occur, for example, when two appropriate areas of invasive tumour cannot be identified in the tissue section, controls fall outside the expected values, hybridization signals are not uniform, background is too high or obscures the signals, or enzymatic digestion is not optimized to produce scorable signals. The additional time and the direct laboratory expense involved with FISH testing need to be carefully weighed against the cost of inappropriate classifications, and thus, inappropriate treatments of patients. The cost of treating a patient with trastuzumab vastly exceeds the direct laboratory expenses of a FISH test [78].

One of the cost-effectiveness analyses indicates that it is more cost-effective to use FISH alone or as a confirmation of all positive (scores 2+ and 3+) results than using FISH to confirm only weakly positive results (2+) or using IHC alone [54].

Many studies comparing the HER2 status between primary and metastatic sites reported concordance rates as 87.6%–100%; the HER2 status of 37.5% of patients with HER2 negative primary breast cancer became positive when relapse occurred; among distant metastatic foci, there was heterogeneity in the HER2 status [54]. For the evaluation of HER2 status in the primary site, the invasive component should be determined, and the non-invasive component should not be determined because only the invasive component is believed to metastasize.

A study conducted by Di Leo et al. found that the evaluation of HER2 status on primary tumour appears to reflect the HER2 status of corresponding metastatic sites from the same patient [79]. The authors refer to studies where the level of concordance ranged from approximately 80%–100%. In an other study [59] Rossi et al. found that there was a good concordance between HercepTest negativity and FISH non-amplification. Eighty percent of 25 cases (2+) turned out to be non amplified in FISH and only twenty percent were

problem of costs

avoidance of inappropriate treatment of false positive

problem of primary/metastatic site

amplified. As a consequence of this study the authors recommend that all 2+ and 3+ IHC scored cases should be tested by FISH.

Volpi et al. [80] tested the pure prognostic value of HER2 over-expression, according to internationally proposed guidelines and failed to find a relevance of any cut-off value of HER2 over-expression on RFS/ recurrence-free survival.

6 Summary & Conclusion

This assessment summarizes results from studies concerning routine use of state of the art testing for HER2 status. Determination of HER2 status is now an integral part of clinical-pathological exams for breast cancer patients. The development of novel methods and new markers has also led to new insights in the physiology status of HER2 and has gained increasing significance in helping at interpretation of results during the past years.

Discussions involving published HER2 testing results have to take into consideration the number of included specimen, the art of tissue extraction, histology of the specimen, and methods used (particularly studies involving large multi-local trials may utilize different methods). Data should include exact information on which tests were used because of small variations in commercial tests and the influence of different (even approved) tests on the interpretation of results.

IHC results show much greater variability than FISH – test results, particularly for the FISH negative cases [81]. The results of most presented studies indicate that high-level HER2 amplification and an IHC score of 3+ well identify breast carcinoma; the low-level amplification and/or IHC score of 2+ should be carefully interpreted.

Furthermore, an uncertainty exists on the clinical significance of low level gene amplification concerning response to trastuzumab. In addition, the target for trastuzumab is the protein and the gene is only the surrogate marker of the true target [82]. There are patients who respond to trastuzumab, if the tumour is positive for HER2 by IHC, irrespective of whether or not gene amplification is present. Using current methods, the ability of positive predictive values (PPV) of the receptor status to predict benefits from trastuzumab treatment is modest (25%–40%) [9]. There might be alternative methods of protein over-expression to gene amplification.

IHC errors in interpretation potentially could represent an overestimate of “minimal” (0, 1+) staining or an underestimate of “strong” (3+) staining. A dual probe FISH assay should be used because of its ability to distinguish HER2 gene amplification from chromosome 17 polysomy. As breast cancer research continues to identify additional factors associated with HER2 amplification, data models try to include those factors but provide only guidelines to clinical expectations. New findings on the molecular or genetic level can influence the interpretation of results. Results from large trials are interesting for clinical practice: a multivariable analysis [83] of prospectively collected data (1083 patients – 872 HER2 non-amplified, 211 HER2 amplified) showed that correlates of HER2 amplification are higher SBR grade, younger age at diagnosis and a comedo ductal carcinoma in situ component.

Many problems and uncertainties still exist. Only a few studies refer to moderate inter-observer reliability. Discussing the testing results of one probe tested in the same location but at different times is rarely possible. The findings concerning different results from local/central laboratories are important.

assessment on “state of the art” of HER2 testing

many studies are not comparable:

differences of tissue extraction, variations in tests used etc.

IHC less reliable than FISH, low level amplification/ 2+ borderline: careful interpretation needed

clinical significance: target for trastuzumab is protein, gene amplification only surrogate!

role of retesting with FISH

HER2 characteristics for clinical practice: higher SBR grade, younger age, comedo DCIS

problems: moderate inter-observer reliability

<p>volume/experience of laboratories: high volume >100 HER2 probes, advantage; low volume <100 HER2 probes, potentially problematic</p>	<p>A high number of probes in a reference or central laboratory seems to be an advantage. Quality assurance at small-volume laboratories (those processing fewer than approximately one hundred HER2 tests a month) appears to be a problem due to less practice in processing methods. Furthermore, the algorithms of testing differ in various countries: the results often do not represent the same relevant outcome because of a different procedure implementation.</p>
<p>standardization; adherence to strict instructions</p> <p>external QA</p>	<p>A problem is the difference in the interpretation of probes. Tests should be performed according to strict guidelines. Quality control is very important when performing HER2 tests, both internal and external, for routine diagnosis and in clinical protocol studies [54]. All clinical laboratories using assays for HER2 diagnostics (as predictive or prognostic tests) should participate in an appropriate external quality assurance (EQA) program.</p>
<p>performance evaluation: rules for handling discordant results and validation with clinical results</p>	<p>Standards based on criteria that has been clinically validated have to be set for assay performance and interpretation of assay results. In spite of prevalent agreement between HER2 results obtained by IHC and FISH, there are cases in which results are discordant, particularly among tumours with intermediate results. One way to make results more accurate is to implement higher standards and the attachment to interlaboratory comparisons, for example, organized by Networks for quality assessment of tumour biomarkers [68]. The implementation of more accurate tests can influence the analogy of the results of other studies.</p>
<p>cost-effective testing strategy:</p> <p>all patients with IHC, followed by FISH for IHC of 2+ (or of 2+ and 3+)</p>	<p>Cost-effectiveness is also important. How necessary and essential is more accurate testing from the very beginning? Authors from various studies seem to concur that the most cost-effective strategy is to screen all patients with IHC, and follow up with confirmatory FISH testing in those patients with an IHC of 2+ (or of 2+ and 3+). As pathological departments make more of their results available on national and international levels, their results will gain greater acceptance. All laboratories performing HER2 testing for clinical purposes should use methods that have been proven to have results with high levels of concordance with another validated test.</p>
<p>identification of patients that benefit from trastuzumab should be prioritized for clinical and cost reasons</p> <p>many unsolved questions</p>	<p>Based on the results of this assessment, there seem to be less HER2 positive women than generally reported in clinical treatment studies: not 20-30%, but rather 15-20% are amplifying HER2 positive in “real life” settings. From a societal perspective, the diagnostic performance of the test used to identify trastuzumab candidates has considerable influence on cost-effectiveness – independent of test cost – due to the high cost of treating patients with false-positive test results and the inability of patients with false-negative results to benefit from trastuzumab [77]. Increased efforts in accurate testing results might lead to less trastuzumab treatments associated with clinical important adverse events: less might be more. True evidence-based clinical validation of the prognostic and predictive utilities of biomarker tests is performed only through prospective clinical studies in which rigorous quality control measures are systematically implemented and reported [84]. Although more than 23,000 patients (specimen) have been reviewed in the seventy-five studies published in recent years, many unsolved questions remain.</p>

7 Excursus: Interpretation for Austria

About 4,000 breast carcinomas are newly diagnosed in Austria each year. HER2 status testing is performed as part of a routine clinical exam for any patient newly diagnosed with invasive breast cancer.

**HER2 testing
part of routine**

Immunohistochemical determination of HER2 status represents an easy and standardized method that can be performed in any of the thirty-six pathology laboratories that have an IHC department. The mostly used test in Austria is the HercepTest by DAKO. Standardization of fixation protocols, automation of the immunohistochemical procedure, and pathologist training in scoring need to be priorities in assuring the reliability of results.

**IHC testing in
36 laboratories**

Attendance at national inter-laboratory tests for IHC is organized.

Cases with equivocal IHC results are analysed by FISH (that can be performed in 22 pathology departments). The most commonly used test is PathVision by Vysis, distributed by Abbott.

**FISH testing in 22
pathologies: highly
decentralised**

Table 7-1: FISH diagnostic in Austria (Jan 2007)

Vienna	General Hospital, Hietzing Hospital, SMZO Hospital, KFJ, Hanusch, Baumgartner Höhe, Rudolfstiftung
Lower Austria	Mistelbach, Wiener Neustadt, Horn
Upper Austria	Linz General Hospital, Linz Barmherzige Schwestern, Wels Kreuzschwestern, Vöcklabruck
Salzburg	State Hospital
Tirol	State Hospital (only surgery, Gyn à Graz)
Vorarlberg	State Hospital Feldkirch
Carinthia	State Hospital Klagenfurt, State Hospital Villach
Styria	Graz State Hospital, State Hospital West
Burgenland	Oberwart

National and international inter-laboratory tests for FISH do exist, but attendance is optional.

Comparative studies have shown high concordance rates between IHC analyses and FISH in cases with IHC scores of 0 or 1+ (negative) and 3+ (strongly positive) and low concordance rates among cases with IHC scores of 2+. Since the meta-analysis [41] and further studies discussed in this assessment include a great variety of different HER2 testing methods, different problems concerning quality, validity, etc. have become apparent.

In Austria for IHC the HercepTest by DAKO is used most often, for FISH the PathVision by Vysis, both approved by FDA. Studies included in this review include results of more than 23,000 specimens of patients. Only a part of these specimens were available for both IHC and FISH performed by FDA-approved tests, and among them only partially by HercepTest and by PathVision. The presented results allow an interpretation for/comparison with Austrian data because of the implementation of the same tests in most pathology departments.

Table 7-2: IHC/FISH sensitivity
 ([18], [16], [32], [50], [49], [51], [12], [28], [64], [85], [47])

		FISH	
		Pos	neg
IHC	3+	1028	142
IHC	0.1+	124	4409

**sensitivity of IHC/
 FISH diagnostic:
 2.7% false negative,
 12.1% false positive**

The results of eleven studies ([18], [16], [32], [50], [49], [51], [12], [28], [64], [85], [47]), in which *only IHC by HercepTest and FISH by PathVision were used*, do not include IHC 2+ scored results because of diversified interpretations of the diagnosis of borderline findings and the general acceptance of ambiguity of the findings. Of the patients with 0.1+ results (IHC) 2.7% (124) were false negative, if retested with FISH, of the patients with 3+ results 12.1% (142) were false positive.

8 Recommendations

One has to stay aware, even if FISH is the more reliable and reproducible test compared to IHC, nevertheless the therapeutic antibody trastuzumab targets the protein. Amplification is merely a surrogate for protein-expression. HER2 diagnostic is broadly used and decisive for the clinical management of HER2 positive breast cancer, but it is still a field “under way”. Internationally the “state of the art” is to screen all patients with IHC, and follow up with confirmatory FISH testing in those patients with an IHC of 2+ (or of 2+ and 3+).

The following recommendations are extrapolated from the results of this review:

- ❖ Due to the high variability between the different IHC-tests, we recommend using only standardized and approved tests.
- ❖ Due to the consequential costs (non-monetary costs/side effects of therapy and monetary costs), we recommend to establish SOPs/standard operating procedures.
- ❖ Due to high inter-laboratory variabilities, we recommend using a small number of reference centers rather than decentralised testing locations with lack of routine.
- ❖ Due to high inter-laboratory variabilities, we recommend national and international inter-laboratory exchange on results of diagnostic outcome.

**standardization,
performance control,
reference centers**

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Appendix

Search in Databases

Search strategy for Cochrane CENTRAL

Date: 2007-01-25

Database: EBM Reviews – Cochrane Central Register of Controlled Trials
<4th Quarter 2006>

Provider: Ovid

- 1 exp breast neoplasms/
- 2 (breast adj4 (cancer\$ or carcinoma\$ or tum?r\$ or malignant\$ or oncolog\$)).ti,ab.
- 3 or/1-2
- 4 immunohistochemistry/
- 5 IHC.ti,ab.
- 6 immunohistochemi\$.ti,ab.
- 7 in situ hybridization, fluorescence/
- 8 FISH.ti,ab.
- 9 or/4-8
- 10 receptor, erbB-2/
- 11 Her2.ti,ab.
- 12 HER2.ti,ab.
- 13 or/10-12
- 14 (assay adj method\$).ti,ab.
- 15 algorithms/
- 16 “quality assurance (health care)”/
- 17 quality control/
- 18 (quality adj control).ti,ab.
- 19 reproducibility of results/
- 20 validity.ti,ab.
- 21 gene amplification/
- 22 concordan\$.ti,ab.
- 23 correspond\$.ti,ab.
- 24 discordan\$.ti,ab.
- 25 or/14-24
- 26 9 and 13
- 27 3 and 26
- 28 25 and 27

Search strategy for Medline*Date:* 2007-01-26*Database:* Medline 1950 to Present with Daily Update*Provider:* Ovid

- 1 exp breast neoplasms/
- 2 (breast adj4 (cancer\$ or carcinoma\$ or tum?r\$ or malignant\$ or oncolog\$)).ti,ab.
- 3 or/1-2
- 4 immunohistochemistry/
- 5 IHC.ti,ab.
- 6 immunohistochemi\$.ti,ab.
- 7 in situ hybridization, fluorescence/
- 8 FISH.ti,ab.
- 9 or/4-8
- 10 receptor, erbB-2/
- 11 Her2.ti,ab.
- 12 HER2.ti,ab.
- 13 or/10-12
- 14 "sensitivity and specificity"/
- 15 sensitivity.ti,ab.
- 16 specificity.ti,ab.
- 17 (assess\$ adj5 status).ti,ab.
- 18 (determin\$ adj5 overexpression).ti,ab.
- 19 (determin\$ adj5 status).ti,ab.
- 20 (detect\$ adj5 status).ti,ab.
- 21 (detect\$ adj5 amplification).ti,ab.
- 22 (evaluation adj5 status).ti,ab.
- 23 (evaluation adj5 method\$).ti,ab.
- 24 (assay adj method\$).ti,ab.
- 25 algorithms/
- 26 exp diagnostic errors/
- 27 "quality assurance (health care)"/
- 28 quality control/
- 29 (quality adj control).ti,ab.
- 30 reproducibility of results/
- 31 validity.ti,ab.
- 32 concordan\$.ti,ab.
- 33 discordan\$.ti,ab.
- 34 correspond\$.ti,ab.
- 35 or/14-34
- 36 9 and 13
- 37 3 and 36
- 38 35 and 37
- 39 (letter or editorial or case reports).pt.
- 40 38 not 39
- 41 *prostatic neoplasms/
- 42 *urinary bladder neoplasms/
- 43 *stomach neoplasms/
- 44 or/41-43
- 45 40 not 44

Search strategy for Embase

Date: 2007-01-26

Database: EMBASE <1988 to 2007 Week 03>

Provider: Ovid

- 1 exp breast tumor/
- 2 (breast adj4 (cancer\$ or carcinoma\$ or tum?r\$ or malignant\$ or oncolog\$ or neoplasm\$)).ti,ab.
- 3 or/1-2
- 4 immunohistochemistry/
- 5 IHC.ti,ab.
- 6 immunohistochemi\$.ti,ab.
- 7 fluorescence in situ hybridization/
- 8 FISH.ti,ab.
- 9 or/4-8
- 10 epidermal growth factor receptor 2/
- 11 Her2.ti,ab.
- 12 HER2.ti,ab.
- 13 or/10-12
- 14 "sensitivity and specificity"/
- 15 sensitivity.ti,ab.
- 16 specificity.ti,ab.
- 17 (assess\$ adj5 status).ti,ab.
- 18 (determin\$ adj5 overexpression).ti,ab.
- 19 (determin\$ adj5 status).ti,ab.
- 20 (detect\$ adj5 status).ti,ab.
- 21 (detect\$ adj5 amplification).ti,ab.
- 22 (evaluation adj5 status).ti,ab.
- 23 (evaluation adj5 method\$.ti,ab.
- 24 (assay adj method\$.ti,ab.
- 25 algorithm/
- 26 exp diagnostic error/
- 27 diagnostic accuracy/
- 28 (quality adj control).ti,ab.
- 29 reproducibility/
- 30 reliability/
- 31 validity.ti,ab.
- 32 concordan\$.ti,ab.
- 33 discordan\$.ti,ab.
- 34 intermethod comparison/
- 35 or/14-34
- 36 9 and 13
- 37 3 and 36
- 38 35 and 37
- 39 (conference paper or editorial or letter or proceeding).pt.
- 40 38 not 39
- 41 *prostate carcinoma/
- 42 *bladder carcinoma/
- 43 *stomach carcinoma/
- 44 or/41-43
- 45 40 not 44

Search strategy for Biosis Previews*Date:* 2007-01-26*Database:* BIOSIS Previews <1990 to 2006>*Provider:* Ovid

- 1 breast neoplasms.ds.
- 2 (breast adj4 (cancer\$ or carcinoma\$ or tum?r\$ or malignant\$ or oncolog\$)).ti,ab.
- 3 or/1-2
- 4 IHC.ti,ab.
- 5 immunohistochemi\$.ti,ab.
- 6 immunohistochemistry.mq.
- 7 fluorescence in situ hybridization.mq.
- 8 FISH.ti,ab.
- 9 or/4-8
- 10 human HER2 gene.gn.
- 11 Her2.ti,ab.
- 12 HER2.ti,ab.
- 13 or/10-12
- 14 sensitivity.ti,ab.
- 15 specificity.ti,ab.
- 16 (assess\$ adj5 status).ti,ab.
- 17 (determin\$ adj5 overexpression).ti,ab.
- 18 (determin\$ adj5 status).ti,ab.
- 19 (determin\$ adj5 amplification).ti,ab.
- 20 (detect\$ adj5 status).ti,ab.
- 21 (detect\$ adj5 amplification).ti,ab.
- 22 (evaluation adj5 status).ti,ab.
- 23 (evaluation adj5 method\$.ti,ab.
- 24 (assay adj method\$.ti,ab.
- 25 (quality adj control).ti,ab.
- 26 validity.ti,ab.
- 27 (reproducibility adj5 (result\$ or test\$)).ti,ab.
- 28 concordan\$.ti,ab.
- 29 discordan\$.ti,ab.
- 30 correspond\$.ti,ab.
- 31 or/14-30
- 32 9 and 13
- 33 3 and 32
- 34 31 and 33
- 35 (letter or editorial or case reports).pt.
- 36 meeting.pt.
- 37 meeting abstract.mi,lt.
- 38 meeting poster.mi,lt.
- 39 or/35-38
- 40 34 not 39
- 41 urology.mc.
- 42 urinary system.mc.
- 43 respiratory system.mc.
- 44 gastroenterology.mc.
- 45 or/41-44
- 46 40 not 45

Search strategy for Pascal Biomed

Date: 2007-01-26

Database: Pascal Biomed <2001 to December 2006>

Provider: Ovid

- 1 breast tumor.de.
- 2 breast cancer.de.
- 3 breast carcinoma.de.
- 4 (breast adj4 (cancer\$ or carcinoma\$ or tum?r\$ or malignant\$ or neoplasm\$)).ti,ab.
- 5 or/1-4
- 6 IHC.ti,ab.
- 7 immunohistochemi\$.ti,ab.
- 8 FISH.ti,ab.
- 9 fluorescence in situ hybridization.de.
- 10 or/6-9
- 11 erbB2 gene.de.
- 12 Her2.ti,ab.
- 13 HER2.ti,ab.
- 14 or/11-13
- 15 sensitivity.ti,ab.
- 16 specificity.ti,ab.
- 17 (assess\$ adj5 status).ti,ab.
- 18 (determin\$ adj5 amplification).ti,ab.
- 19 (determin\$ adj5 overexpression).ti,ab.
- 20 (determin\$ adj5 status).ti,ab.
- 21 (detect\$ adj5 status).ti,ab.
- 22 (detect\$ adj5 amplification).ti,ab.
- 23 (evaluation adj5 status).ti,ab.
- 24 (evaluation adj5 method\$).ti,ab.
- 25 assay\$.ti,ab.
- 26 (quality adj control).ti,ab.
- 27 reproducibility.de.
- 28 validity.ti,ab.
- 29 concordance\$.ti,ab.
- 30 discordance\$.ti,ab.
- 31 correspond\$.ti,ab.
- 32 or/15-31
- 33 10 and 14
- 34 5 and 33
- 35 32 and 34
- 36 correspondence, letters.mt.
- 37 case report, clinical case.mt.
- 38 editorial.mt.
- 39 conference meeting.lt.
- 40 or/36-39
- 41 35 not 40
- 42 urology.de.
- 43 stomach.de.
- 44 lung.de.
- 45 prostate.de.
- 46 or/42-45
- 47 41 not 46

Glossary

ACIS.....	Automated Cellular Imaging System
ARR	Absolute Risk Reduction
CISH	Chromogenic In Situ Hybridization
DCIS	Ductal Carcinoma In Situ
EMA	European Agency for the Evaluation of Medicinal Products
FISH	Fluorescence In Situ Hybridization
HER2	Human Epidermal growth Factor Receptor 2
HUGO.....	Human Genome Organisation
IHC	Immuno Histo Chemistry
LCIS.....	Lobular Carcinoma In Situ
Polysomy	having one or a few chromosomes present in a greater number than is characteristic of the rest of the chromosome complement
PCR.....	Polymerase Chain Reaction
PPV	Positive Predictive Values
SBR	Scarff-Bloom-Richardson grading
TMA.....	Tissue Micro Array