



HER-2 GUIDELINES

GBECAM
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Consultoria em Patologia





Cancer Fight: Unclear Tests for New Drug

By GINA KOLATA

Published: April 10, 2010

The New York Times

Dr. Linda Griffith was at a conference in Singapore in early January when she felt a lump in her breast. She assumed it was nothing — a cyst. And anyway, she had no time for it. She was returning on a Sunday night and the next Tuesday morning was leaving for a conference in Florida.

The test on Dr. Griffith's tumor was negative. Or was it? One small area of her tumor stained chocolate brown, indicating lots of HER2. The rest was a cream color, indicating no extra HER2 protein.

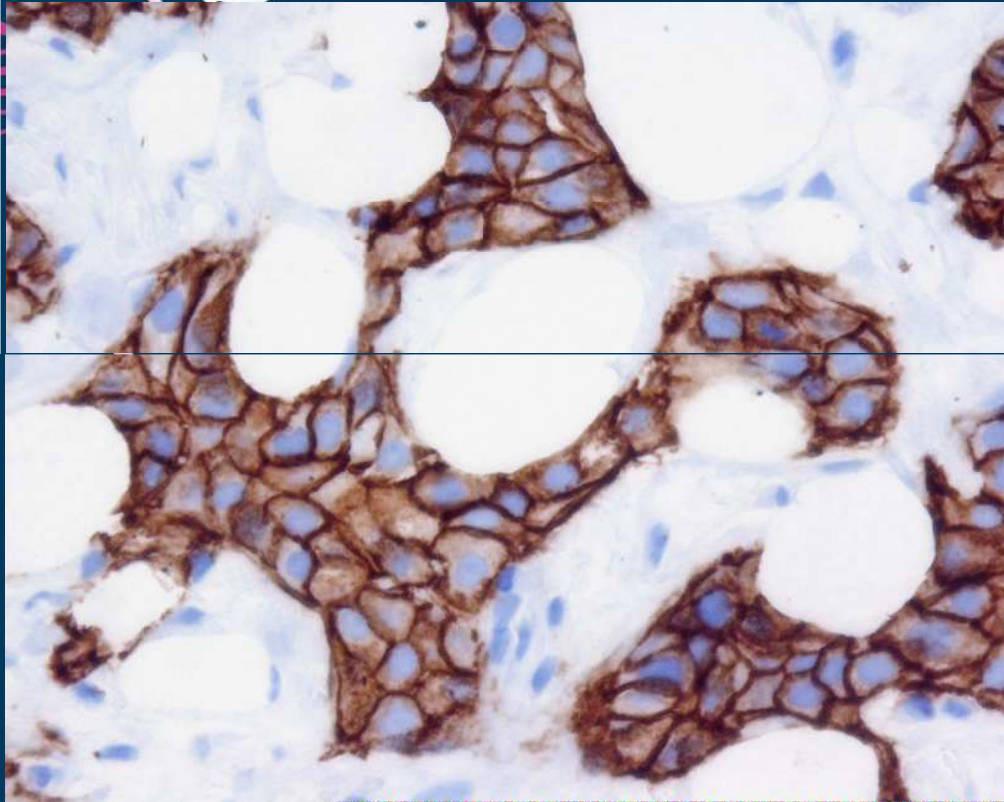
HER2 tests, for instance, can give false-positives up to 20 percent of the time, wrongly telling women they need the drug when they do not. Five percent to 10 percent of the time the tests can falsely tell a woman that she should not take the drug, when she should. And Herceptin testing for breast cancer is easy compared with what is coming next.



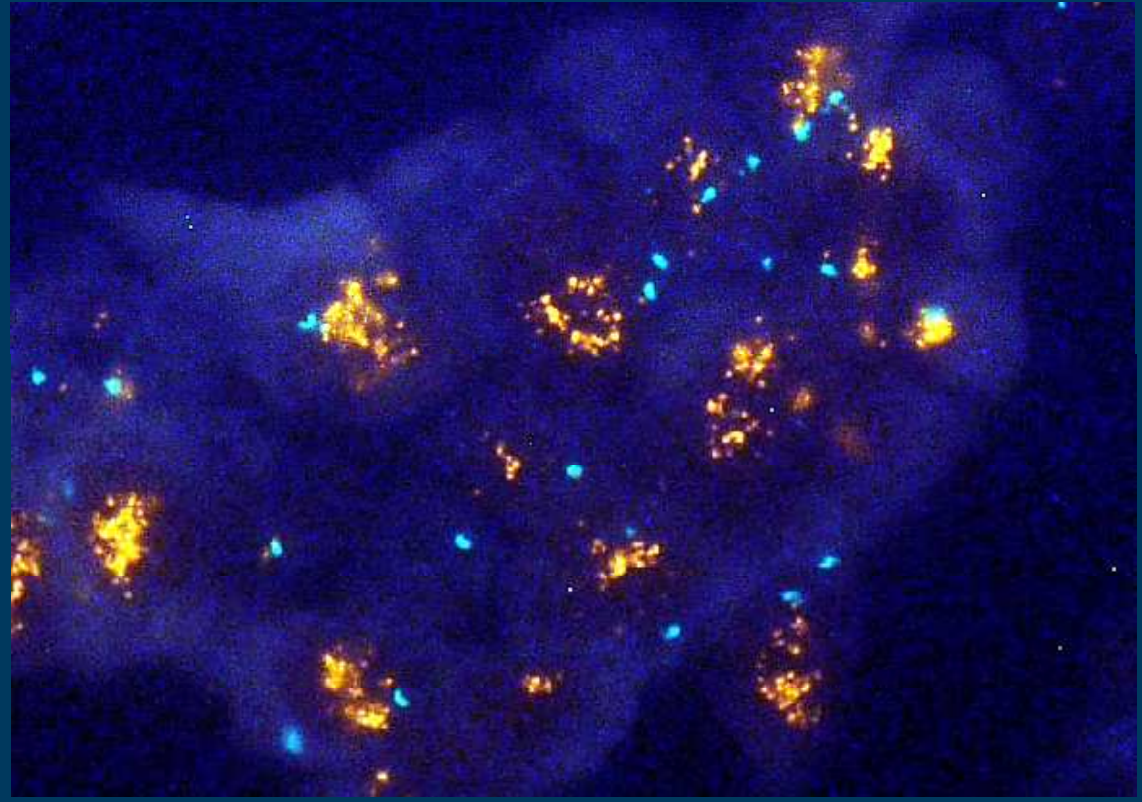
HER-2 Testing

- Crucial tool in the management of breast cancer: prognostic and predictive factor
- Every case diagnosed as invasive breast cancer should be tested for HER-2 expression (ASCO, 2001)
- Incorrect result is devastating: lack of benefit (false-negative) and high-cost treatment and potential toxicity (false-positive)

HER-2 Assays




IHC: HER-2
Protein



FISH: HER-2
Gene



What is the real situation of HER-2 testing?



| Authors, Countries, Year | Number of Cases | Type of HER-2 Results | Concordance Rate (%) |
|--|-----------------|-----------------------|----------------------|
| Paik et al, USA, 2002 | 104 | HER-2 positive | 79% |
| Roche et al, USA, 2002 | 119 | HER-2 positive | 74% |
| Press et al, USA and Switzerland, 2002 | 1291 | Score 0 and 1+ | 96% |
| | | Score 2+ and 3+ | 51% |
| Perez et al, USA, 2006 | 1699 | HER-2 positive | 75-81% |
| Reddy et al, USA, 2006 | 973 | Score 0 | 34% |
| | | Score 1+ | 54% |
| | | Score 2+ | 26% |
| | | Score 3+ | 77% |
| Papadopoulos et al, Greece, 2007 | 369 | Score 0 and 1+ | 85% |
| | | Score 2+ | 80% |
| | | Score 3+ | 63% |
| O'Malley et al, Canada, 2008 | 505 | HER-2 positive | 79-90% |
| | 205 | HER-2 negative | 95-100% |



**What is the real
situation of HER-2
testing in Brazil?**



HER2 Testing in Breast Carcinoma

Very Low Concordance Rate Between Reference and Local Laboratories in Brazil

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Tácio R. Berto e Silva, MD,* Filomena M. Carvalho, MD, PhD,† Lawrence M. Weiss, MD,‡
and Carlos E. Bacchi, MD, PhD*†*

(Appl Immunohistochem Mol Morphol 2011;19:112–118)




Local Labs, Central Labs and Reference Lab

- Local Pathology Lab: low-volume load (ex: <250 cases of HER-2 tests per year/pathologist), local community
- Central Pathology Lab: high-volume load (ex: >250 HER-2 tests), regional activity
- Reference Pathology Laboratory: very high-volume load, nationwide activity, CAP certified
- Comparison in this study:
 - Consultoria em Patologia (Reference Path Lab) vs Local Path Labs
 - Consultoria em Patologia >6,000 HER-2 tests per year; CAP certified



HER-2 Testing Results in Brazil: Local Labs vs Reference Lab

- 500 consecutive cases of invasive breast cancer (2008/2009)
- Previously tested by IHC in 149 different LOCAL Path Labs (original path report available) (70% Southeast Region)
- Retested by IHC in REFERENCE Path Lab (same paraffin block)
- Validation of IHC by FISH of Reference Lab before comparison (694 cases)
- Approved by “Comissão de Ética, HC-FMUSP (#1238/09)”



Validation of IHC (SP3) by Correlation with FISH (Reference Lab)

| SP3 Scores | FISH Not Amplified (%) | FISH Amplified (%) | Total |
|------------|------------------------|--------------------|-------|
| 0 | 207 (99.0) | 2 (1.0) | 209 |
| 1 + | 144 (97.3) | 4 (2.7) | 148 |
| 3 + | 5 (1.5) | 332 (98.5) | 337 |
| Total | 356 | 338 | 694 |

(Appl Immunohistochem Mol Morphol 2011;19:112–118)

Distribution of HER-2 Results Between Reference Lab and Local Labs

| Local Laboratory <i>HER2</i> Score | | Reference Laboratory <i>HER2</i> Score | | | | Total n = 500 |
|------------------------------------|---|--|--------------|--------------|---------------|------------------|
| | | 0 n = 161 | 1+ n = 86 | 2+ n = 93 | 3+ n = 160 | |
| 0 | n | 10 | 4 | 1 | 1 | 16 |
| | % | 6.2 | 4.7 | 1.1 | 0.6 | 3.2 |
| 1+ | n | 21 | 12 | 2 | 3 | 38 |
| | % | 13.0 | 14.0 | 2.2 | 1.9 | 7.6 |
| 2+ | n | 110 | 63 | 79 | 86 | 338 |
| | % | 68.3 | 73.3 | 84.9 | 53.8 | 67.6 |
| 3+ | n | 20 | 7 | 11 | 70 | 108 |
| | % | 12.4 | 8.1 | 11.8 | 43.8 | 21.6 |

(*Appl Immunohistochem Mol Morphol* 2011;19:112–118)

HER-2 Testing Results in Brazil: Local Labs vs Reference Lab

| Authors and Country | No. Cases | Concordance (%) | Type of Cases |
|---|-----------|-----------------|---------------|
| Wludarski et al, ⁵⁰ Brazil (this study) | 500 | 6.2 | Score 0 |
| | | 14.0 | Score 1+ |
| | | 84.9 | Score 2+ |
| | | 43.8 | Score 3+ |

56% discordance

(*Appl Immunohistochem Mol Morphol* 2011;19:112–118)



HER-2 Testing Results in Brazil: Local Labs vs Reference Lab

- False-positive results: 36% of the patients positive in the Local Lab with HER-2-NEGATIVE in the Reference Lab
- False-negative results: 5% of the patients negative in the Local Lab with HER-2-POSITIVE in the Reference Lab
- Inconclusive results (2+):
 - Local Lab: 67%
 - Reference Lab: 18%



Why These Results?

- Local Labs are probably not following any guidelines
 - Lack of pertinent information in the reports (specifications of reagents, interpretation of the results, etc)
- Low-volume load of HER-2 assay
 - Recommended: >250 HER-2 test/pathologist/year
 - Vast majority of the Local Labs <<<250 cases/year
- Not applied for Central Pathology Laboratories



**What should be done
in order to change this
scenario?**

Arch Pathol Lab Med—Vol 131, January 2007

American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

Antonio C. Wolff, M. Elizabeth H. Hammond, Jared N. Schwartz, Karen L. Hagerty, D. Craig Allred, Richard J. Cote, Mitchell Dowsett, Patrick L. Fitzgibbons, Wedad M. Hanna, Amy Langer, Lisa M. McShane, Soonmyung Paik, Mark D. Pegram, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catharine Sturgeon, Sheila E. Taube, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, Daniel F. Hayes

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ASCO SPECIAL ARTICLE

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ASCO/CAP Guidelines: Sources of HER-2 Testing Variation

SURGEON

PATHOLOGIST

Training and competency assessment of staff
Type of antigen retrieval
Test reagents
Use of standardized control materials
Use of automated laboratory methods

PATHOLOGIST

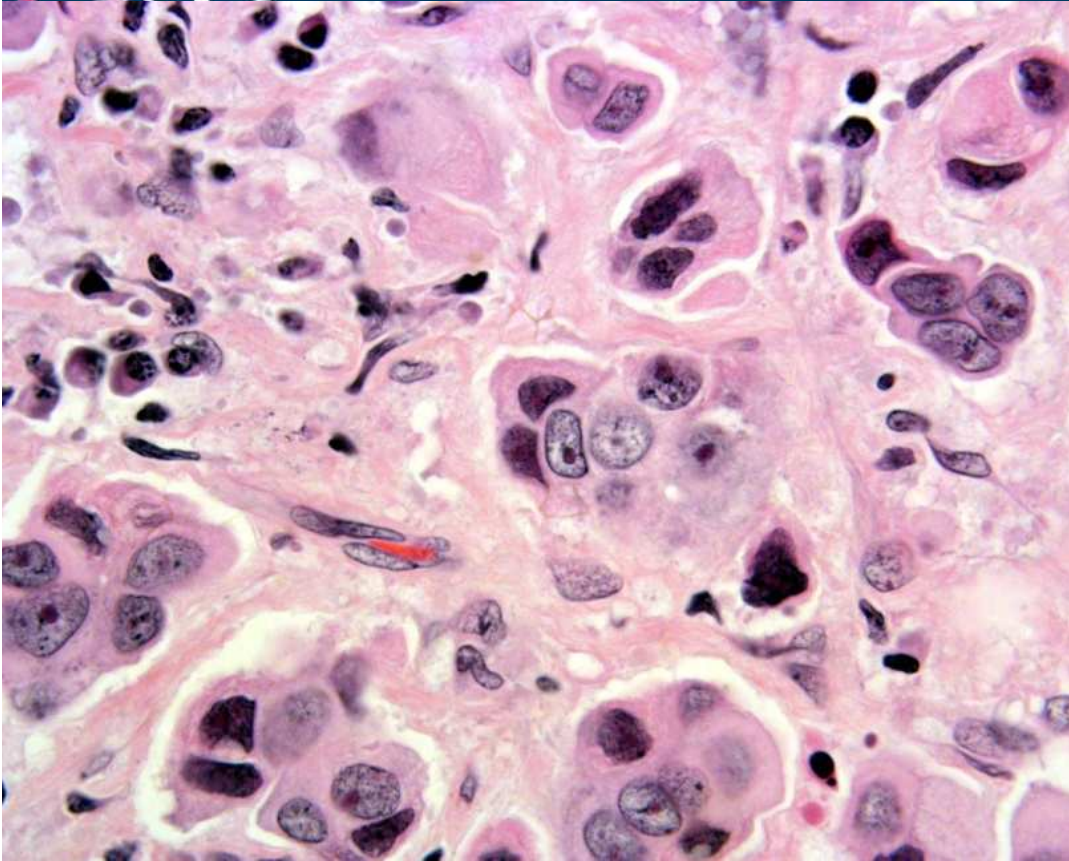
Postanalytic
Interpretation criteria
Use of image analysis
Reporting elements
Quality assurance procedures
Laboratory accreditation
Proficiency testing
Pathologist competency assessment

**PROBLEMS
WITH LOCAL
PATH LABS**

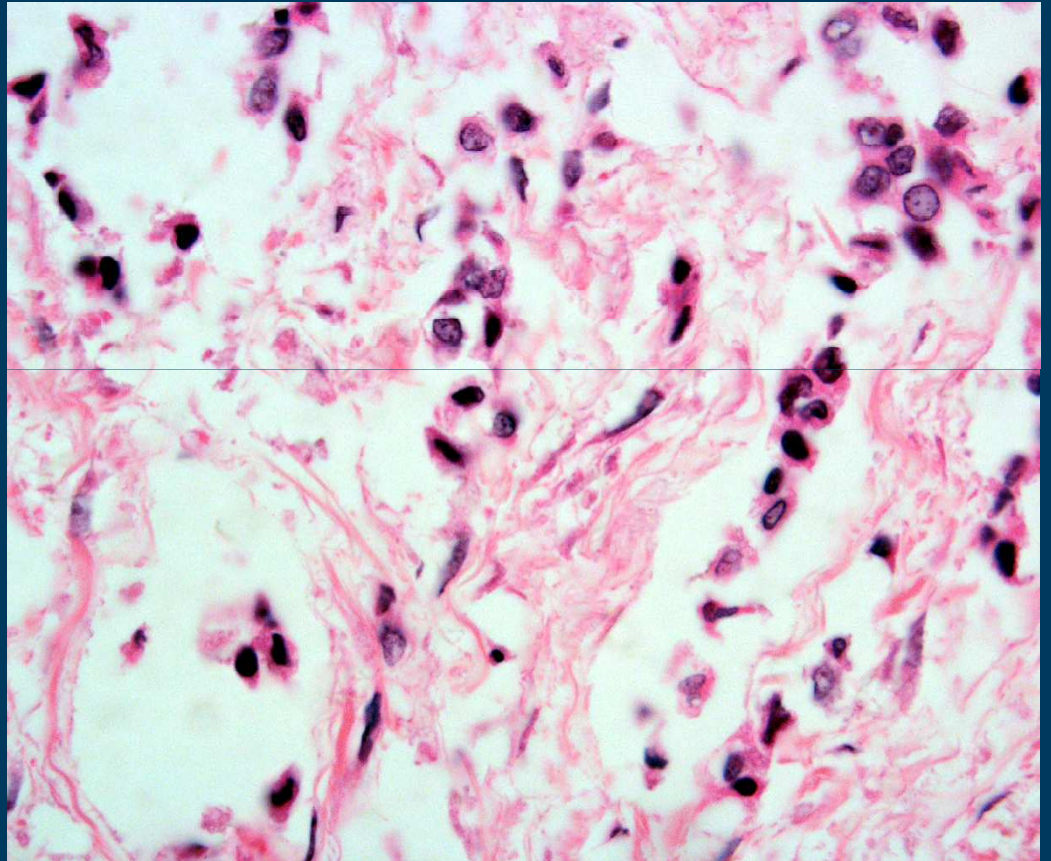


What can go wrong with HER-2 testing?

Bad fixation effects

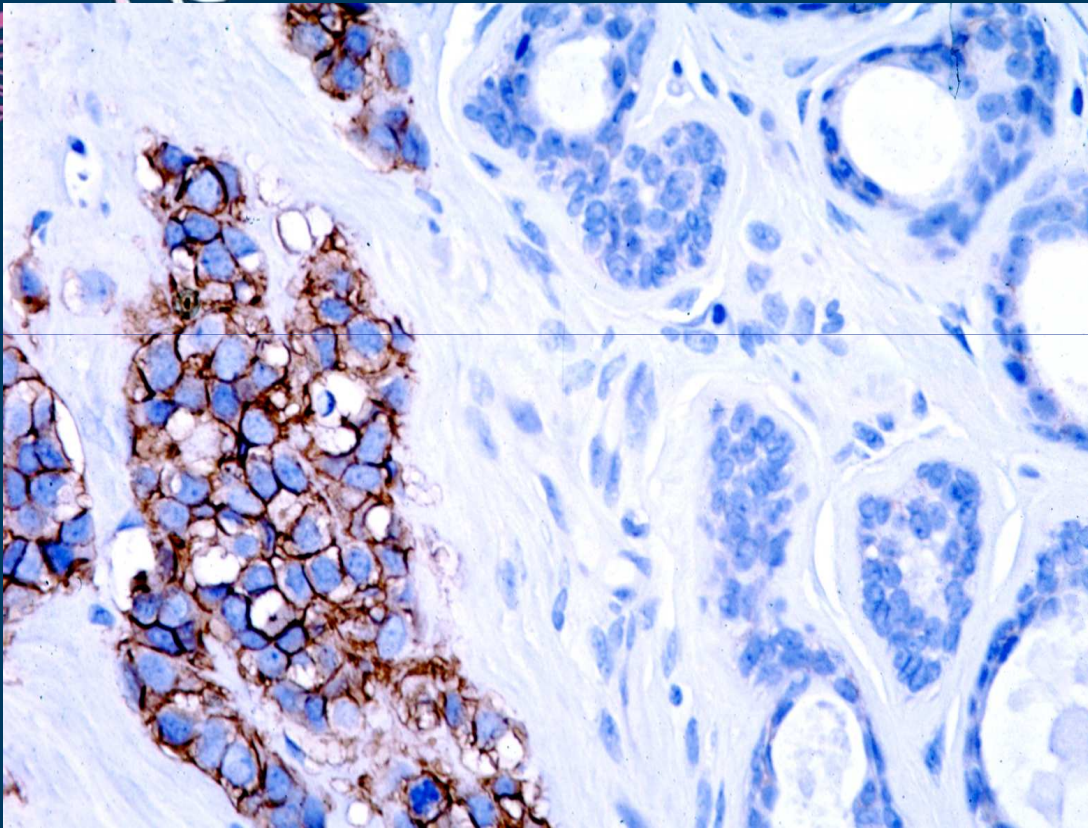


GOOD FIXATION

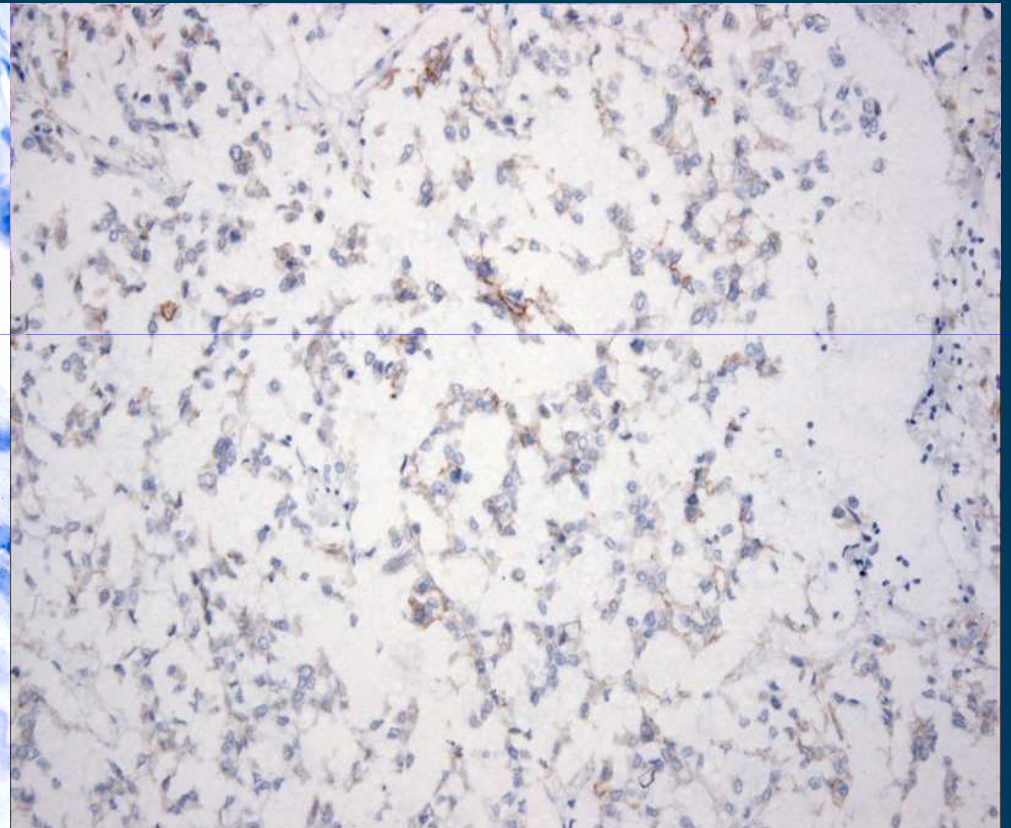


BAD FIXATION

Effects of Fixation (IHC HER-2)

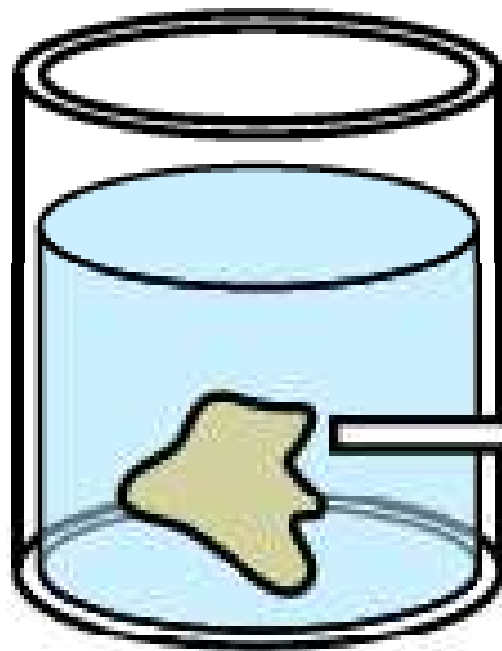


GOOD FIXATION



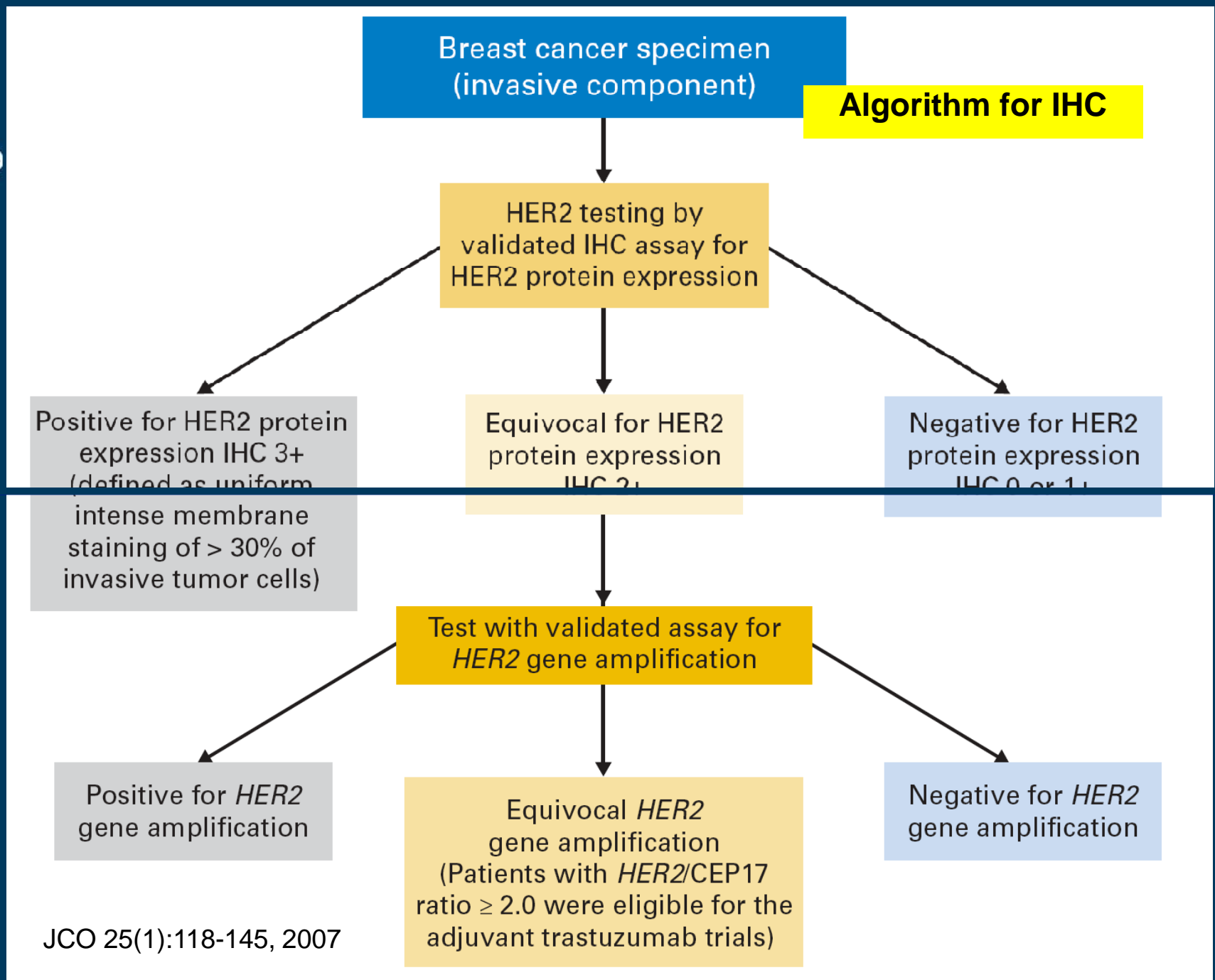
BAD FIXATION

Appropriate Fixation



10% formalin
volume 20x size of
specimen

- Any doubt? Send the specimen to the Path Lab





Breast cancer specimen
(invasive component)

Algorithm for FISH

HER2 testing by
validated FISH assay for
HER2 gene amplification

Positive for *HER2*
gene amplification
(FISH ratio > 2.2 or
HER2 gene copy > 6.0)


Equivocal for *HER2*
gene amplification
(FISH ratio 1.8-2.2 or
HER2 gene copy
4.0-6.0*)

Negative for *HER2*
gene amplification
(FISH ratio < 1.8 or
HER2 gene copy < 4.0)

Count additional cells for FISH
or retest, or test with *HER2* IHC

Equivocal *HER2* gene
amplification result
(Patients with *HER2/CEP17*
ratio ≥ 2.0 were eligible for the
adjuvant trastuzumab trials)

JCO 25(1):118-145, 2007



ASCO/CAP Guidelines: Optimal Performance (HER-2)

- Evaluated only in invasive breast cancer or invasive component
- Report type of fixative and time of fixation (difficult in Brazil)
- Utilize exclusion criteria (crush artefact; bad fixation)
- Assay procedures should be validated before offering the test (95% concordance)
- Use standardized control materials

ASCO/CAP Guidelines: Reporting Elements IHC HER-2



| |
|---|
| Patient identification information |
| Physician identification |
| Date of service |
| Specimen identification (case and block number) |
| Specimen site and type |
| Specimen fixative type |
| Time to fixation (if available) |
| Duration of fixation (if available) |
| Antibody clone/vendor |
| Method used (test/vendor and if FDA approved) |
| Image analysis method (if used) |
| Controls (high protein expression, low-level protein expression, negative protein expression, internal) |
| Adequacy of sample for evaluation |
| Results |
| Percentage of invasive tumor cells exhibiting complete membrane staining |
| Uniformity of staining: present/absent |
| Homogeneous, dark circumferential pattern: present/absent |
| Interpretation |
| Positive (for HER2 protein expression); equivocal (FISH will be done and reported); negative (for HER2 protein expression); not interpretable |



Quality Assurance Programs

- Internal quality assurance
- External quality assurance:
every lab performing HER-2 testing
should participating in proficiency
testing
- CAP-USA (x2 per year)

HER-2 Testing in Brazil

- Challenge issue for all us (breast surgeons, oncologists and pathologists) due to the numbers of variables
- Justified efforts by the benefit of patients (correct results) and devastated consequences (incorrect results)
- Enforce the use of formalin (10%, ideally buffered)
- Pathologists should use standardized approved methods after laboratory validation and engage in regular proficiency test (CAP)
- Pathologists: strictly follow the *ASCO/CAP guidelines*
- Oncologists: keep eyes open for unexpected results

