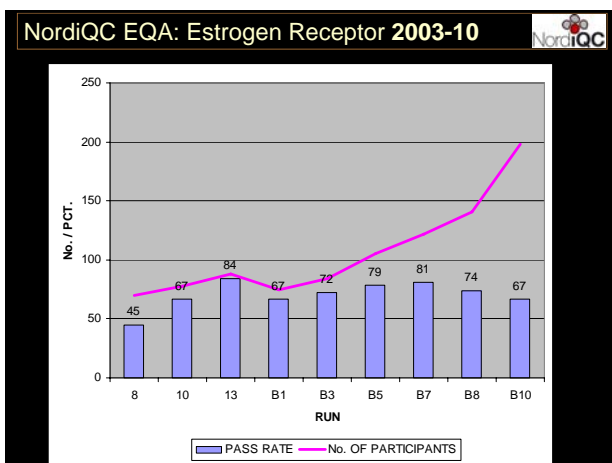
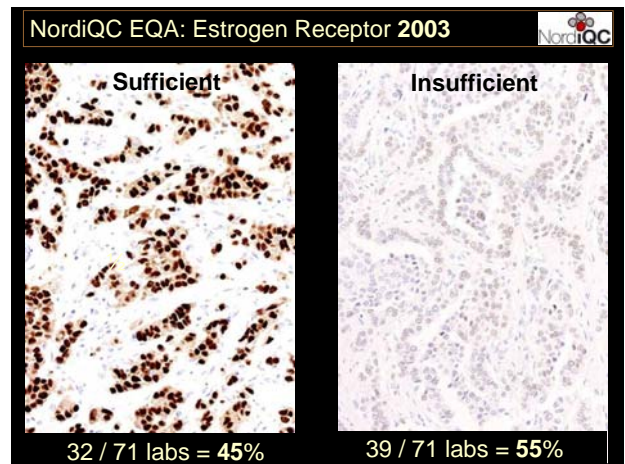
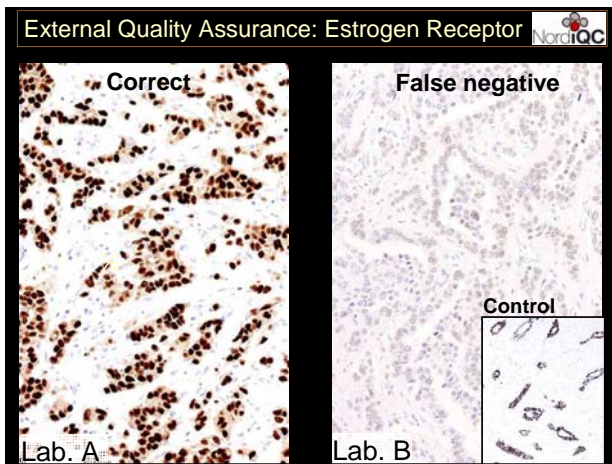
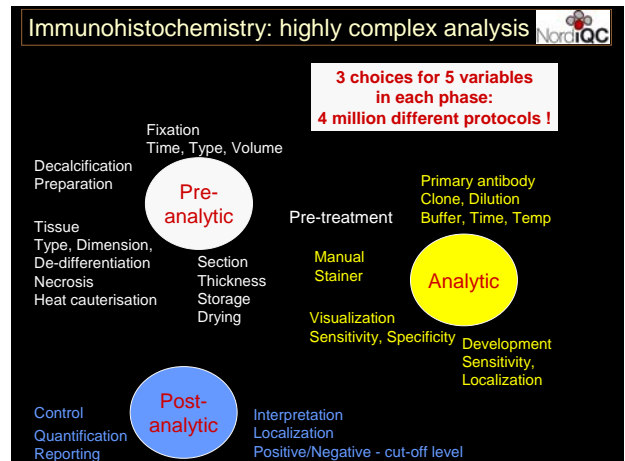




**External Quality Assessment in Diagnostic Immunohistochemistry**

**Regional HER-2 sessions for Belgian Pathologists June 2011**

Mogens Vyberg  
Assoc professor  
Scheme director, NordiQC  
Inst. of Pathology, Aalborg Hospital  
Aarhus University Hospital  
Denmark





- External Quality Assurance**
- Staining quality varies greatly between different laboratories depending on the individual selection of methods and the technical expertise
  - Internal quality control will often not identify a poorly calibrated IHC system giving insufficient staining results
  - Standardization of staining methods is not possible but standardization of staining results is mandatory
  - External quality assurance of staining results through laboratory proficiency testing is mandatory
  - External quality assurance of antibodies and other reagents through producer proficiency testing is mandatory

### Establishment of a Nordic EQA program for IHC

Nordic Immunohistochemical Quality Control  
**NordIQ** founded 2003 by Nordic pathologists

- Independent, scientific, not-for-profit organisation
- Institute of pathology  
Aalborg Hospital
- General module:  
3 annual runs  
~16 different markers/tests
- Breast cancer module:  
2 annual runs  
HER-2 IHC & BRISH, ER/PR



### Establishment of a Nordic EQA program for IHC

- Central assessment with consensus between experienced pathologists
- Correlate stains with central protocol parameters
  - Identify less successful Abs
  - Identify inappropriate protocol settings
- Publish general results and recommended protocols on an open website
- E-mail individual results to the participants
  - Give specific explanations for insufficient results
  - Give tailored recommendations for improvement



**Nordic Immunohistochemical Quality Control**

Home | Participation | Assessments | Epitopes | Protocols | Techniques | Links

NordIQ is an independent scientific organisation, promoting the quality of immunohistochemistry by arranging schemes for pathology laboratories, assessing tissue stains, giving recommendations for improvement and providing good protocols.

Last update: 19-04-2011

General results from **Run 31** (General module) and **G1** (Breast cancer pilot module) are available by 7th April, see [Newsletter](#). Individual results are e-mailed.

Run 32 and B11 are closed for protocol submission (Deadline was 18th April). See the tests on [Participation](#). Unstained slides are circulated about 18th April.

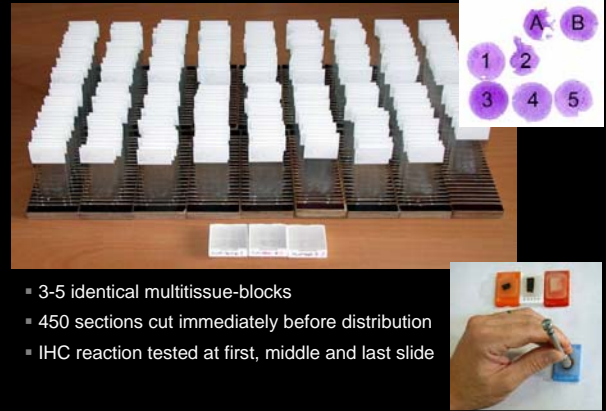
NordIQ Seminar in cell and molecular pathology - 28th October and 29th November 2011. [Website: www.nordiq.org](#)

There are still seats available at the NordIQ Workshop September 2012, click on [Workshop 2012](#)

Fig. CD56 staining of nerves in appendix (A,B) and a pancreatic neuroendocrine carcinoma (C,D). A. Optimal staining of appendix. B. Staining of appendix using clone 123C3 on a Benchmark platform, the staining is only slightly weaker than in (A). C. Optimal staining of neuroendocrine carcinoma. D. Staining of endocrine carcinoma using clone 123C3 on a Benchmark platform, the staining is false negative. Clone 123C3 is platform dependent!


Logos: BIOCARE MEDICAL, CELL MARQUE, Dako, EPITOMICS, Leica, mtm, NordIQ Workshops and Seminars, Roche, Thermo Scientific, visiopharm.

### NordIQ serial sections



- 3-5 identical multitissue-blocks
- 450 sections cut immediately before distribution
- IHC reaction tested at first, middle and last slide

### NordIQ assessment



40 runs =  
 > 25,000 slides =  
 > 125,000 tissue sections assessed

### Assessment of immunostains

- **Optimal**  
Perfect or close to perfect in all of the included tissues.
- **Good**  
Fully acceptable in all of the included tissues. However, the protocol may be optimized to ensure the best staining intensity and signal-to-noise ratio.
- **Borderline**  
Insufficient, e.g., because of a generally too weak staining or a false negative staining of one of the included tissues, or a false positive staining reaction.
- **Poor**  
Very insufficient e.g., because of false negative staining of several of the included tissues, or a marked false positive staining reaction, or one false neg./pos. class II staining.

### Over-all assessment results

NordiQC consensus marks (average 2003-08)

- Optimal: **36 %**
- Good: **33 %**
- Borderline: } **31 %** { too weak / false neg.: ~ 90 %
- Poor: } { over-stained / false pos.: ~ 10 %

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### NordiQC over-all assessment results

Major causes of insufficient stains:

- Less successful antibodies **18 %**
- Inappropriate antibody dilution **39 %**
- Inappropriate epitope retrieval **31 %**
- Other inappropriate lab. performance **12 %**
  - Inappropriate calibration / home brews
  - Endogenous biotin reaction (EBR)
  - Section drying-out after HIER
  - Technical stainer errors
  - .....
  - Unexplained

14

### Results of NordiQC tailored recommendations

Lab response to 419 advices (11 markers)

	No.	Improved	%
Positive	268	195	73
Negative	151	21	14

15

### External IHC Quality Assurance

- Almost 1/3 of all IHC stains produced by NordiQC participants are still insufficient !
  - New labs
  - New antibodies, techniques, platforms
  - New challenges
- How many IHC stains produced by labs not participating in an EQA scheme are insufficient ?
- How many scientific publications are based on insufficient IHC stains ?
- What are the consequences for the patients ?

### External Quality Assurance – ER

JNCI Journal of the National Cancer Institute Advance Access published June 10, 2008

NEWS |

#### Breast Cancer Testing Scandal Shines Spotlight on Black Box of Clinical Laboratory Testing

By Karyn Hede

“Through the inquiry, the public learned that between 1997 and 2005 nearly 400 of about 1,000 breast cancer patients received incorrect test results of the ER status of their breast tumors.”

“There are no good data on the quality of ER testing in the United States. The scary thing about the debacle in Canada is that we would never have known about this if results hadn't been checked in a central lab.”

Craig Allred

### IHC – Recommendations

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. Higgins, D. Craig Allred, Richard J. Coo, Mitchell Dowsett, Patrick L. Fitzgibbon, Wendal M. Hanna, Amy Langer, Liu M. McShane, Sooramsung Park, Mark D. Pignatelli, Edith A. Perez, Michael F. Press, Anthony Rhodes, Catherine Steeghs, Sheila E. Tarpe, Raymond Tubbs, Gail H. Vance, Marc van de Vijver, Thomas M. Wheeler, Daniel F. Hayes

Appl Immunohistochem Mol Morphol • Volume 16, Number 6, December 2008

#### Consensus Recommendations on Estrogen Receptor Testing in Breast Cancer By Immunohistochemistry

Hadi Yaziji, MD,\* Clive R. Taylor, MA, MD, D.Phil,† Neal S. Goldstein, MD,‡ David J. Dabbs, MD,§ Elizabeth H. Hammond, MD,|| Bryan Hewlett, ART (CSMLS), MLT,¶ CMLT,¶\* Alton D. Floyd, PhD,¶ Todd S. Barry, MD,|| Alvin W. Martin, MD,\*\* Stuart Radvai, MD,†† Frederick Buchner, MD,†† Richard W. Carraway, MD,‡‡ Richard N. Eisen, MD,§§ Paul E. Swanson, MD,||| Stephen M. Hewitt, MD, PhD,\*\* Mogen Vyberg, MD,||| and David G. Hicks, MD\*\*\* and Members of the Standardization Ad-Hoc Consensus Committee

FIX:  
6 - 48h  
8 - 72h

### IHC – Effect of fixation time

(Am J Surg Pathol 2011;35:545-552)

**The Effect of Prolonged Fixation on the Immunohistochemical Evaluation of Estrogen Receptor, Progesterone Receptor, and HER2 Expression in Invasive Breast Cancer: A Prospective Study**

Leung Chu Tong, BA, MD,\* Nahid Nelson, BSc, PhD,† Jim Tsourigiamis, BSc, MLT,† and Anna Marie Mulligan, MB, MSc, FRCPath\*†

13 hours versus 79 hours in 10% NBF (the week-end dilemma.....)

101 breast carcinomas:

- 99 % Concordance between short and long fixation for ER (SP1)
- 95 % Concordance between short and long fixation for PR (1E2)
- 98 % Concordance between short and long fixation for HER2 (A0485)

### IHC – Effect of fixation time

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	1+	1+	1+	1+
Tumour 2	3+	3+	3+	3+
Tumour 3	0	0	0	0
Tumour 4	1+	1+	1+	1+
Tumour 5	0	0	0	0
Tumour 6	3+	3+	3+	3+
Tumour 7	0	0	0	0
Tumour 8	0	0	0	0
Tumour 9	0	0	0	0

Breast carcinomas, HER-2 PATHWAY, rmAb 4B5  
(CC1 Mild, Ab inc. 20 min. 36°C, UltraView DAB)

### IHC – Effect of fixation time

Breast carcinoma 3+, HER-2 PATHWAY, rmAb 4B5

### IHC – Effect of fixation time

Breast carcinoma 1+, HER-2 PATHWAY, rmAb 4B5

### IHC – Effect of fixation time

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	1+	1+	1+	1+
Tumour 2 3+	3+	3+	3+	3+
Tumour 3	0	0	0	0
Tumour 4	1+	1+	1+	1+
Tumour 5	0	0	0	0
Tumour 6 3+	3+	3+	3+	3+
Tumour 7	0	0	0	0
Tumour 8	0	0	0	0
Tumour 9	0	0	0	0

Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	+	+	+	+
Tumour 2	-	-	-	-
Tumour 3	+	+	+	+
Tumour 4	+	+	+	+
Tumour 5	+	+	+	+
Tumour 6	+	+	+	+
Tumour 7	-	-	-	-
Tumour 8	+	+	+	+
Tumour 9	+	+	+	+

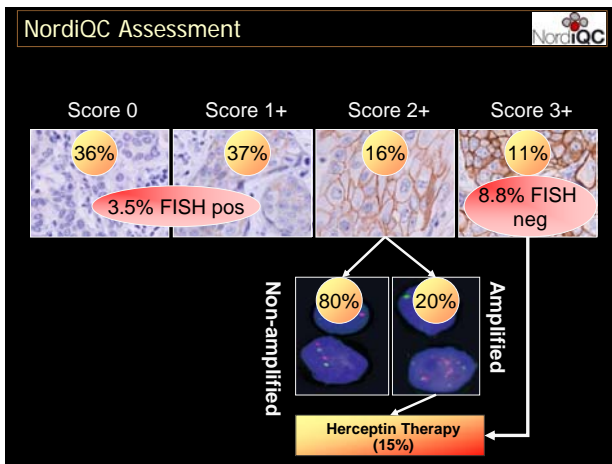
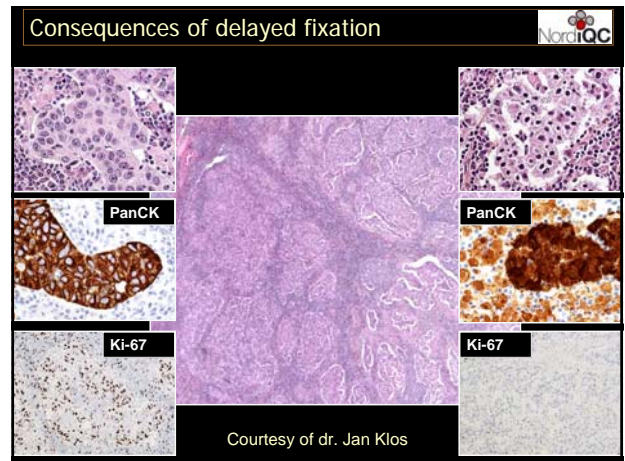
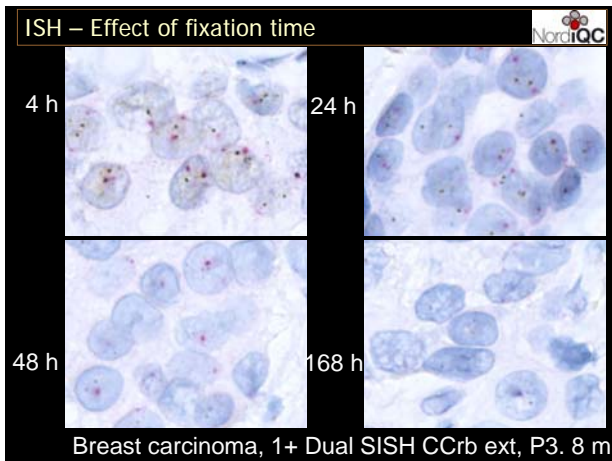
Internal IHC validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	+	+	+	+
Tumour 2	-	-	-	-
Tumour 3	+	+	+	+
Tumour 4	+	+	+	+
Tumour 5	+	+	+	+
Tumour 6	+	+	+	+
Tumour 7	-	-	-	-
Tumour 8	+	+	+	+
Tumour 9	+	+	+	+

Conclusion: IHC biomarkers not affected by NBF fixation time and patient material and control material can be fixed from 4 - 168h in 10% NBF .... but

### ISH – Effect of fixation time

Internal SISH validation	4 h. NBF	24 h. NBF	48 h. NBF	168 h. NBF
Tumour 1	-	-	-	FN
Tumour 2 Amp	+	+	+	+
Tumour 3	(?)	-	FN	FN
Tumour 4	-	-	FN	FN
Tumour 5	-	-	-	-
Tumour 6 Amp	+	+	+	+
Tumour 7	FN	-	-	FN
Tumour 8 poly.	-	-	-	FN
Tumour 9 poly.	-	-	-	FN

HER-2 ISH: 9/36 cores could not be assessed..!  
Breast carcinomas, Dual SISH CCr6 ext, P3. 8 m



### Nordic immunohistochemical Quality Control

Assessment Run B10 2010

#### HER-2 IHC

The slide to be stained for HER-2 comprised the following 5 tissues:

	IHC HER-2 Score* (0, 1+, 2+, 3+)	FISH HER-2/chr17 ratio**
1. Breast ductal carcinoma	0	1.0 - 1.2
2. Breast ductal carcinoma	1+	1.1 - 1.3
3. Breast lobular carcinoma	2+	1.2 - 1.5
4. Breast ductal carcinoma	2+	2.8 - 2.9
5. Breast ductal carcinoma	3+	> 6.0, clusters

\* HER-2 immunohistochemical score (guidelines below) as achieved by using the two FDA approved kits and antibodies (Herceptin™, Dako & PATHWAY®; Ventana) in NordiQC reference laboratories.  
\*\* HER-2 gene/chromosome 17 (HER-2/chr17) ratio as achieved by using HER-2 FISH pharmDX™ Kit, Dako.

All carcinomas were fixed for 24 - 48 h in 10 % neutral buffered formalin.

IHC scoring system according to the guidelines given by ASCO/CAP:

Score 0 No staining is observed or cell membrane staining is observed in less than 10% of the tumour cells.  
Score 1+ A faint perceptible membrane staining can be detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane.  
Score 2+ A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells.

### NordiQC Assessment

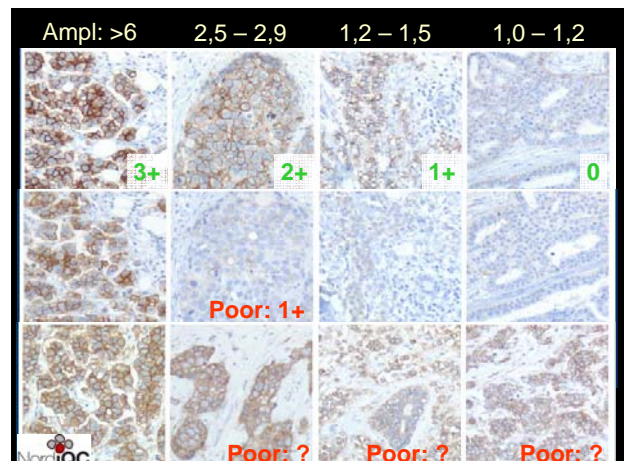
Criteria for assessing a HER-2 staining as optimal included:

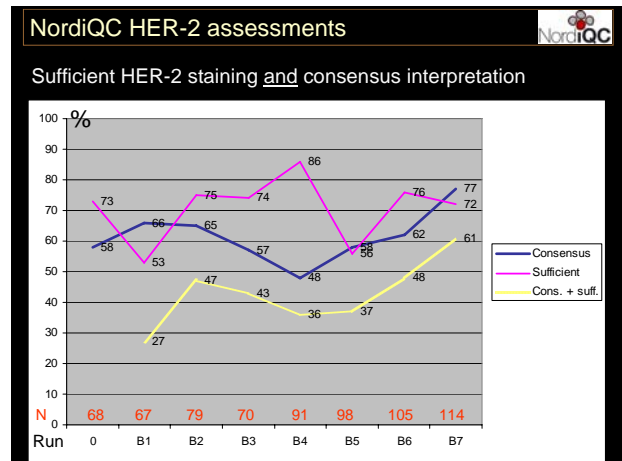
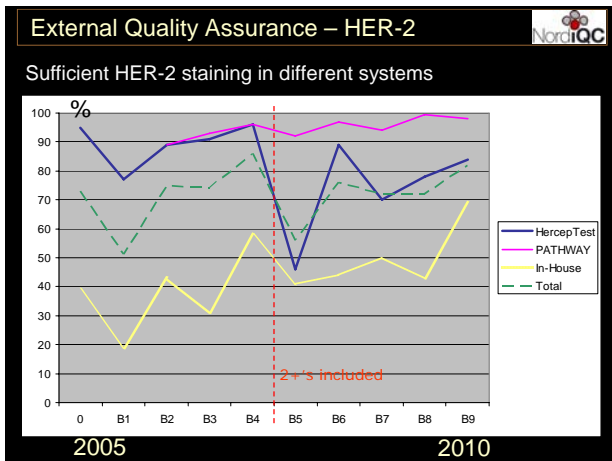
- A clear and unequivocal immunohistochemical staining marked as score 0 or 1+ in the breast ductal carcinomas no. 1 & 2.
- A clear and unequivocal immunohistochemical staining marked as score 1+ or 2+ in the breast carcinoma no. 3.
- A clear and unequivocal immunohistochemical staining marked as score 2+ or 3+ in the breast ductal carcinoma no. 4.
- A clear and unequivocal immunohistochemical staining marked as score 3+ in the breast ductal carcinoma no. 5.
- No or only a weak cytoplasmic reaction that did not affect the interpretation of the true membranous HER-2 reaction.

A staining was assessed as good, if the HER-2 gene amplified tumour no. 5 showed a 2+ reaction (an equivocal 2+ IHC staining should always be analyzed by FISH/BRISH according to the ASCO/CAP guidelines and the national guidelines in Scandinavia) and the other breast carcinomas showed a reaction pattern as described above.

A staining was assessed as borderline if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive counterstaining or excessive retrieval hampering the interpretation.

A staining was assessed as poor in case of false negativity (e.g. the 3+ tumour and the 2+ tumour with gene amplification showing a 1+ reaction) or false positivity (e.g. the 0, 1+ and 2+ tumours without gene amplification showing a 3+ reaction).





### External Quality Assurance – HER-2

Reference: 2+ ampl.

### External Quality Assurance – HER-2

Reference: 2+ ampl.

**Table 2.** Correlation of overall results of assessment of cell lines and tissues.

Cell lines	Tissues			
	Optimal	Good	Borderline	Poor
Optimal	37	1	0	12
Good	4	1	0	1
Borderline	3	0	1	8
Poor	0	1	0	11

Discrepancy: 21% Run B5 2008, 80 labs

### EQA – HER-2 Run B11 2011

B11	Slides	Optimal	Good	Borderl.	Poor
All countr.	208	75%	7%	3%	14%
Belgium	48	51%	18%	4%	27%

### EQA – HER-2 Run B11 2011

B11	N	Sufficient	Insuff.
All countr. Pathway	87 (42%)	98%	2%
Herceptest	47 (23%)	85%	15%
Homebrew/ Oracle	74 (36%)	64%	36%
All	208	83%	17%
All except Belgium	160	87%	13%

EQA – HER-2 Run B11 2011

B11		N	Sufficient	Insuff.
All countr.	Pathway	87 (42%)	98%	2%
	Herceptest	47 (23%)	85%	15%
	Homebrew/ Oracle	74 (36%)	64%	36%
	All	208	83%	17%
All except Belgium		160	87%	13%
Belgium	Pathway	15 (31%)	93%	7%
	Herceptest	6 (13%)	67%	33%
	Homebrew	27 (56%)	59%	41%
	All	48	71%	29%

EQA – HER-2 Run B11 2011

B11		N	Sufficient	Insuff.
All countr.	Pathway	87 (42%)	98%	2%
	Herceptest	47 (23%)	85%	15%
	Homebrew/ Oracle	74 (36%)	64%	36%
	All	208	83%	17%
All except Belgium		160	87%	13%
Belgium	Pathway	15 (31%)	93%	7%
	Herceptest	6 (13%)	67%	33%
	Homebrew	27 (56%)	59%	41%
	All	48	71%	29%

EQA – HER-2 Run B11 2011

B11			Sufficient	Insuff.
Belgium		Consens.	75%	38%
		Not cons.	25%	62%

Breast Cancer Res Treat  
DOI 10.1007/s10549-011-1514-2

PRECLINICAL STUDY

Digital image analysis of membrane connectivity is a robust measure of HER2 immunostains

Anja Brüggemann · Mikkel Eld · Giedrius Leikaitis · Søren Nielsen · Michael Grunkin · Johan D. Hansen · Nick T. Foged · Mogens Vyberg

Received: 18 February 2011 / Accepted: 8 April 2011  
© Springer Science+Business Media, LLC, 2011

HER2-CONNECT(TM)  
Visiopharm, Denmark

- Overall agreement between the IA software and digital scorings of 5 assessors 92%
- IA sensitivity 99% and specificity 100% when correlated to FISH

EQA – HER-2 Amplification

UK NEQAS HER-2 FISH Testing in 62 labs

Run	Inappropriate (%)	Acceptable (%)	Appropriate (%)
1	20	10	70
2	15	15	70
3	10	15	75
4	10	15	75
5	10	15	75
6	10	15	75
7	10	15	75
8	10	15	75
9	10	15	75

- Inappropriate results 5% - 29%.
- Cell lines with unchanged amplification
- No external assessment of slides !

Bartlett et al. AJCP 2009

**NordiQC Nordic immunohistochemical Quality Control**

Home ■ Participation ■ Assessments ■ Epitopes ■ Protocols ■ Techniques ■ Links

**Assessment Run B10 2010**

**HER-2 BRISH**

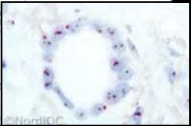
The material circulated for HER-2 BRISH (Brightfield In Situ Hybridization) assessment run 10 was identical to the material used in run B9, 2010 and comprised normal breast tissue & 3 breast ductal carcinomas showing HER-2 gene/chromosome 17 (HER-2/chr17) ratios as follows:

	HER-2/chr17 ratio		
	Duo - CISH*	Dual - SISH**	FISH***
1. Normal breast tissue <sup>1</sup>	1.1	1.0	1.1
2. Breast ductal carcinoma	> 6.0	> 6.0	> 6.0
3. Breast ductal carcinoma	1.4	1.3	1.3
4. Breast ductal carcinoma	2.8	2.6	2.3
5. Breast ductal carcinoma	2.2	2.7	2.3
6. Breast ductal carcinoma	2.4	2.8	2.5

<sup>1</sup>HER-2 DuoCISH™ kit, Dako (data from one ref. lab); <sup>2</sup>HER-2 Dual SISH kit, Ventana (data from one ref. lab); <sup>3</sup>HER-2 FISH kit, Dako (average of data from three tests performed in one ref. lab). All carcinomas were fixed for 24 h in 10 % neutral buffered formalin (NBF), except for the carcinoma fixed for 48 and 72 h, respectively.

Criteria for assessing a BRISH HER-2 analysis as optimal included:

- Staining of the normal breast tissue and the ductal carcinoma no. 3 status.
- Staining of breast ductal carcinomas no. 2, 4, 5 and 6 corresponding
- Staining with preserved morphological details and a minimal background



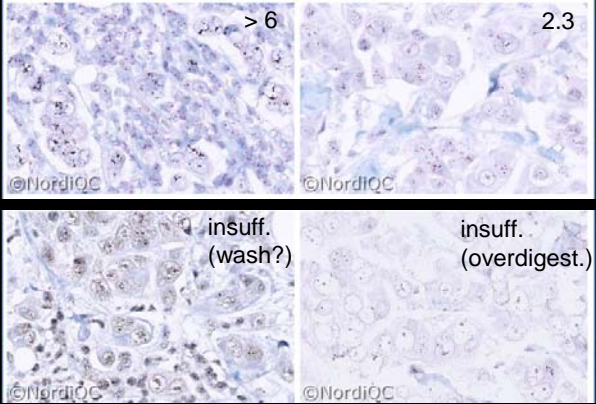
**External Quality Assurance – HER-2 ISH**

**Table 1. Abs and assessment marks for CISH/SISH HER-2, run B10**

Two colour HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. <sup>1,2</sup>
INFORM™ HER-2 Dual SISH	27	Ventana	10	11	3	3	77 %
DuoCISH™	11	Dako	3	4	3	1	64 %
ZytoDot® 2 C	2	Zytovision	0	2	0	0	-
<b>One colour HER-2 systems</b>							
SPOT-Light®	4	Invitrogen	0	3	1	0	-
ZytoDot®	7	Zytovision	0	3	1	3	43 %
INFORM™ HER-2 SISH	4	Ventana	2	1	0	1	-
"In-house"	2		1	1	0	0	-
<b>Total</b>	<b>57</b>		<b>16</b>	<b>25</b>	<b>8</b>	<b>6</b>	
<b>Proportion</b>			<b>28 %</b>	<b>44 %</b>	<b>14 %</b>	<b>14 %</b>	<b>72 %</b>

<sup>1</sup> Proportion of sufficient stains (optimal or good); <sup>2</sup> Proportion of sufficient stains (with optimal protocol settings only, see below).

**EQA – HER-2 Inform dual SISH**



**Nordic immunohistochemical Quality Control**

**Conclusion I**

- External Quality Assurance (EQA)
  - Provides objective evidence of lab proficiency
  - Identifies methodological errors
  - Provides directions for improvements
- The results of the NordiQC work indicate that
  - Improvement of IHC is strongly needed
  - EQA may have a major impact on lab proficiency
  - EQA should be implemented as a standard in all labs

**Nordic immunohistochemical Quality Control**

**Conclusion II**

- HER-2 stains
  - Pathway is very robust: ~ 95% sufficient results in 10 runs
  - Other systems mostly provide sufficient results but generally appear less robust and must be carefully calibrated
- Cell lines are not reliable for control or QA
- Fixation time is not critical for IHC but for ISH
- Training in scoring is strongly needed
- Image analysis may improve scoring reliability

**Nordic immunohistochemical Quality Control**



**Thank you for your attention !**

Aalborg Hospital