

Op weg naar optimale HER2 testing in Nederland!!!

Resultaten Externe Kwaliteitstoetsing NordiQC-2009

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Patholoog, LUMC

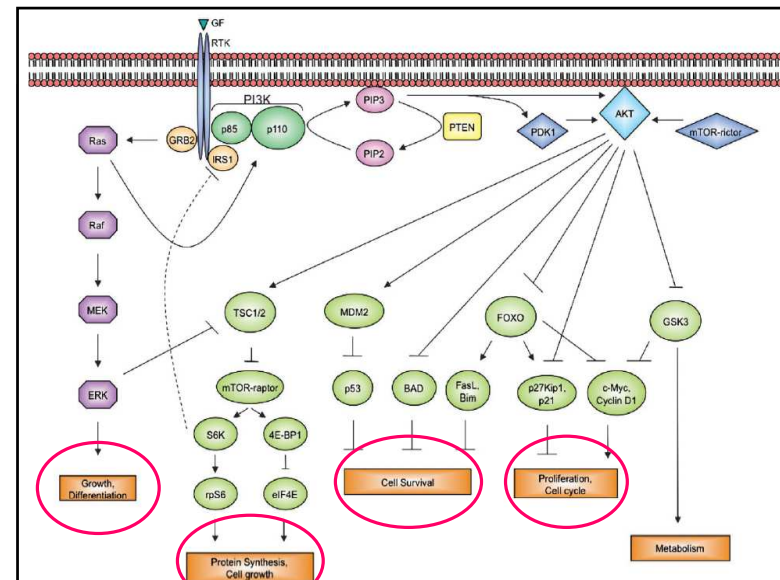
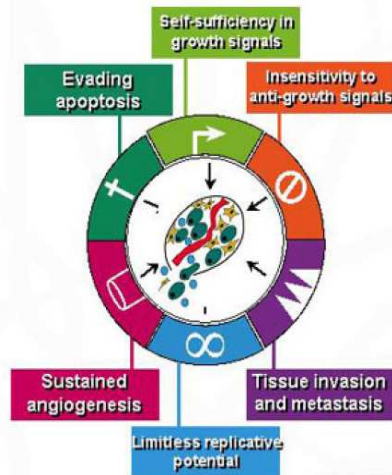


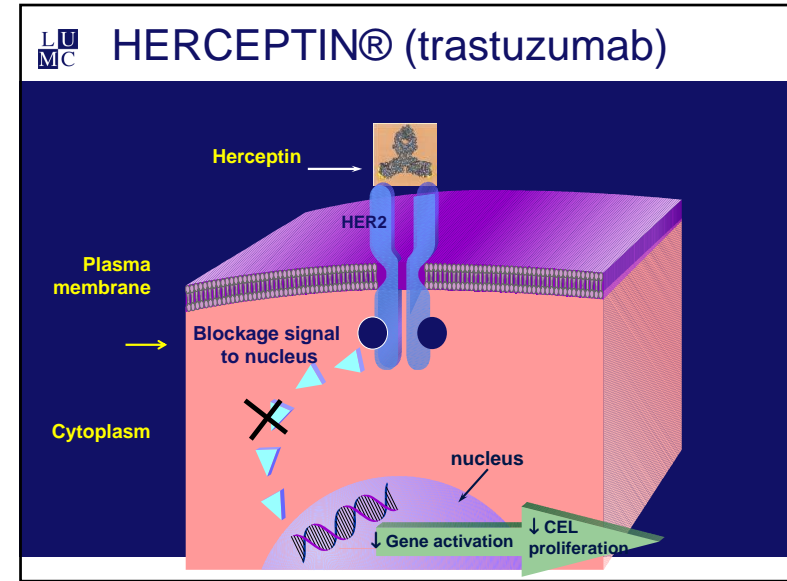
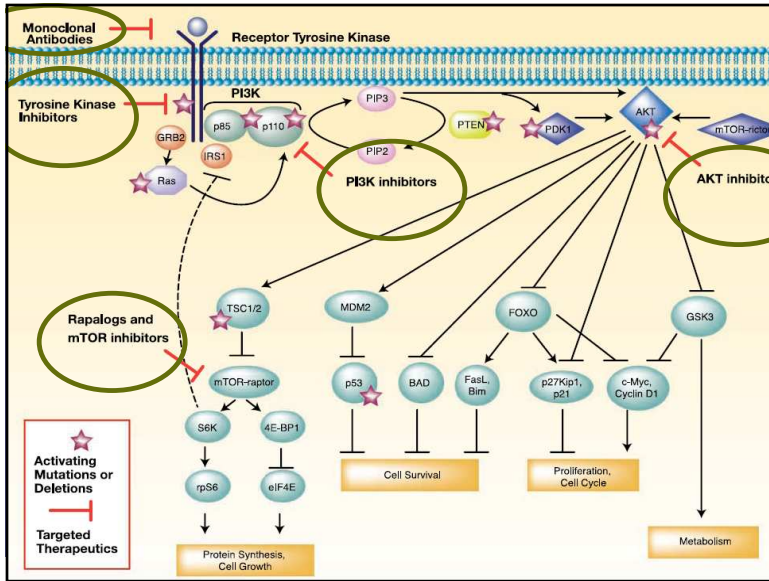
LUMC Farmacodiagnostiek in de Pathologie

- HER2 testen bij mammacarcinomen
- EGFR/K-RAS mutatie analyse bij NSCLC
- K-RAS/BRAF mutatie bij coloncarcinomen
- C-Kit mutaties bij GIST tumoren/melanomen
-???????

LUMC Kanker-ontwikkeling (Weinberg)

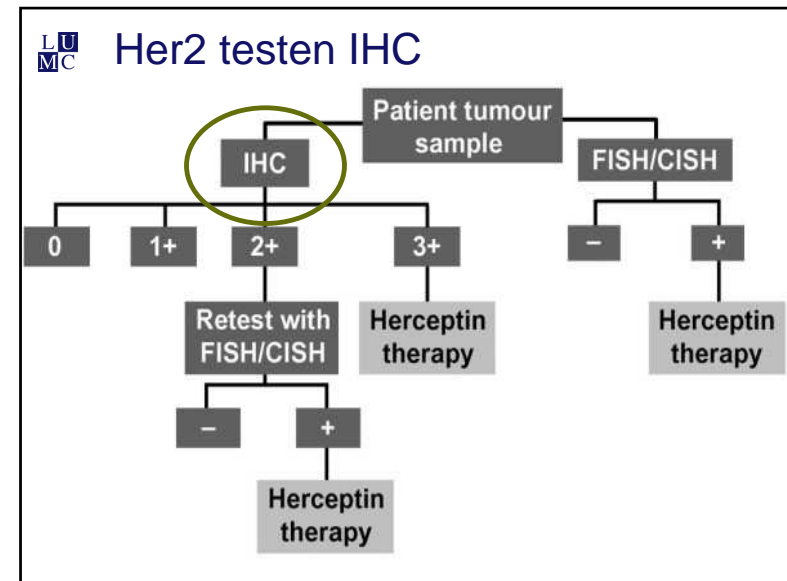
- Groei-stimulerende factoren
- Ongevoeligheid groeiremmende factoren
- Ontsnappen aan apoptose
- 'Continue' angiogenese
- Verlengde replicatie vermogen
- Weefselinvasie
- Metastasering





LU MC Her2-positive breast cancer

The diagram shows **trastuzumab** binding to **HER2** receptors on a **borstkankercel** (cancer cell). **groefactor** (growth factor) is also shown. The text **colkem** is present in the diagram.

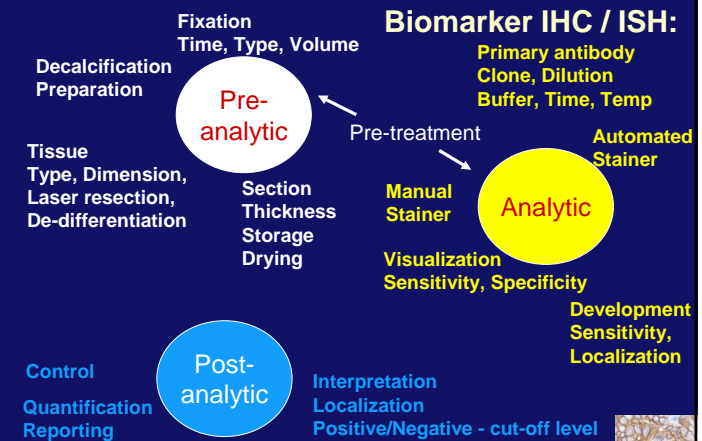


Betrouwbaarheid HER2-testing?

- HERA trial (centrale/perifere testing)
- 10-15% fout positieve testuitslagen (score 3+)
- 12.000 vrouwen (15% HER2 positief) = 1800
 - 10-15% fout positief 180-270
- Adjuvant HERCEPTIN (€40.000 per jaar)

€7.200.000-€10.800.000

Betrouwbaarheid HER2-testing



Her2 testen-IHC

- Antilichamen
- Scoringsystemen
- Interobserver variabiliteit
- Kwaliteitstoetsing/programma's

Keuze van antilichamen?

Thermo DATA SHEET (Analyte Specific Reagent)

PATHWAY™ HER2

Scoring Guide for the Interpretation of Ventana PATHWAY® HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody Staining of Breast Carcinoma

Innovations in Science and Medicine

VENTANA®

Cat. #RM-9103-R7 (7.0ml) (Ready-to-Use for Immunohistochemical Staining)
 Cat. #RM-9103-PCS (5 Slides) (Positive Control for Histology)

A histological image showing HER2 staining in breast carcinoma tissue is visible on the right side of the data sheet.

Sensitivity/specificity antibodies

Table 4 Sensitivity and specificity of antibodies compared to CISH

Antibodies	Sensitivity	95% CI	Specificity	95% CI
SP3	100.0	88.6 to 100.0	88.5	68.7 to 97.0
A0485	100.0	90.2 to 100.0	69.2	48.1 to 84.9
HercepTest	100.0	89.6 to 100.0	97.0	82.0 to 99.8
NCL-CB11	92.1	77.5 to 97.9	100.0	87.7 to 100.0
CM-CB11	93.9	78.4 to 98.9	97.4	83.8 to 99.9
4D5	97.2	83.8 to 99.9	94.3	84.2 to 99.9

CISH, chromogenic in situ hybridisation; NCL, Novocastra; CM, Cell Marque.

J Clin Pathol 2008;**61**:934–938. doi:10.1136/jcp.2007.053892

Type antilichaam?

TABLE 3. Comparison Between the IHC HER2 Overexpression Using Five Different Antibodies

IHC Score	HER-2/neu Antibodies				
	Polyclonal		Monoclonal		
	Hercep Test	A0485	4D5	NCL-CB11	Biog-CB11
0	51 (59.3%)	25 (37.9%)	69 (80.2%)	59 (68.6%)	58 (67.4%)
1+	4 (4.6%)	16 (24.2%)	2 (2.3%)	2 (2.3%)	3 (3.5%)
2+	9 (10.5%)	14 (21.2%)	2 (2.3%)	6 (7.0%)	6 (7.0%)
3+	22 (25.6%)	11 (16.7%)	13 (15.1%)	19 (22.1%)	19 (22.1%)
Total	86 (100.0%)	66 (100.0%)	86 (100.0%)	86 (100.0%)	86 (100.0%)

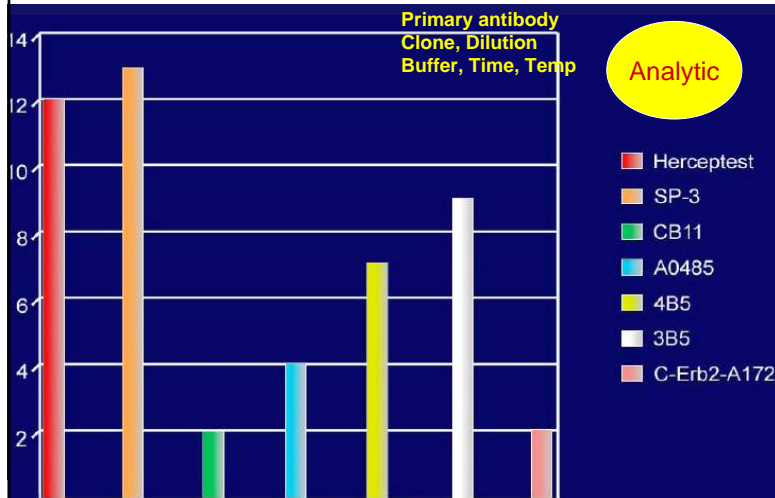
IHC Score 2-3+

36%-38%

IHC Score 2-3+

17%-29%

PA-labs. in Nederland (N=48),2008



Her2 testen-IHC

- Antilichamen
- **Scoringsystemen**
- Interobserver variabiliteit
- Kwaliteitstoetsing

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MC **Scoringsysteem**

Staining pattern	score	Her-2/neu protein overexpression assessment
No staining is observed or membrane staining is observed in less than 10% of the tumor cells	0	negative
A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells . the cells are only stained in part of their membrane	1+	negative
A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells	2+	weakly positive
A strong complete membrane staining is observed in more than 10% of the tumor cells	3+	strongly positive

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MC **Uniform scoringsysteem (in NL)**

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JOURNAL OF CLINICAL ONCOLOGY ASCO SPECIAL ARTICLE

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. Hegerl, 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, and Daniel F. Hayes

JCO 25;118-145, 2007

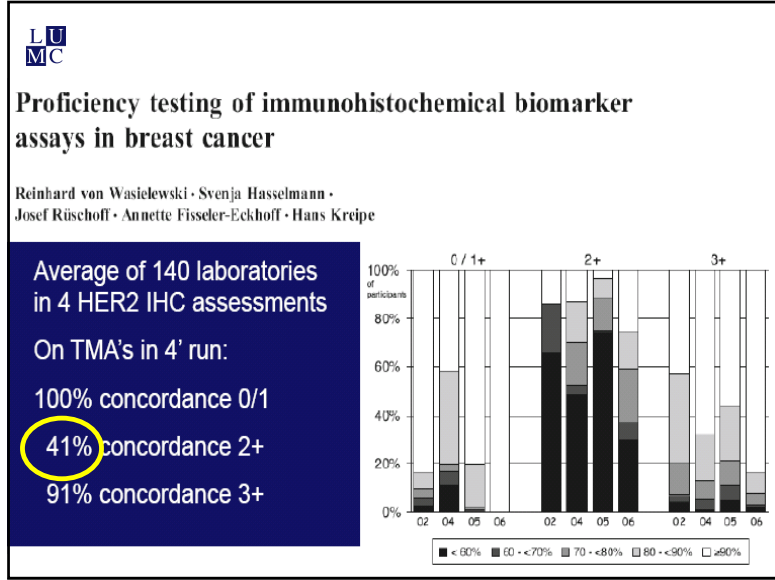
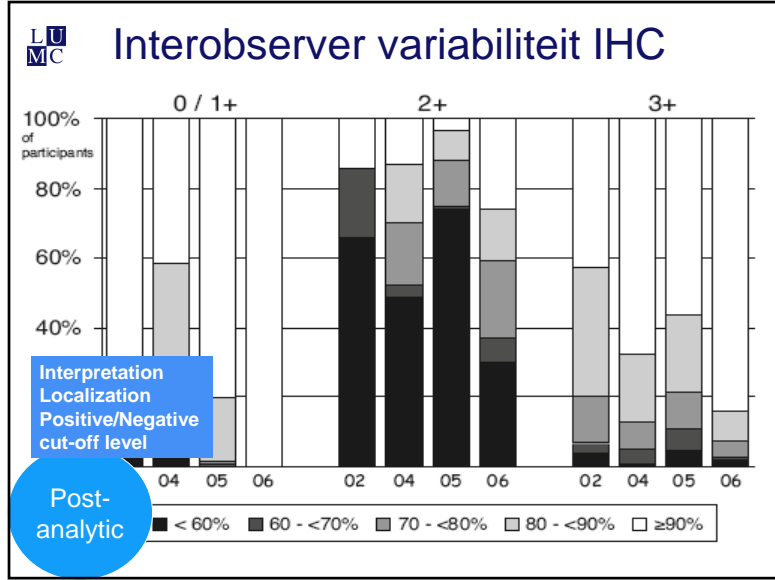
Table 7. IHC Interpretation Criteria

Review controls; if not as expected, test should be repeated
More than 30% of tumor must show circumferential membrane staining for positive result*
Membrane staining must be intense and uniform
A homogeneous, dark circumferential (chicken wire) staining pattern should be seen
Ignore incomplete or pale membrane staining
Quantitative image analysis is encouraged for cases with weak membrane staining (1-2+) to improve consistency of interpretation
If cytoplasmic staining obscures membrane staining, repeat assay or do FISH
Reject sample if normal ducts and lobules show obvious staining
Reject sample if there are obscuring artifacts
Avoid scoring DCIS; score only infiltrating ductal carcinoma

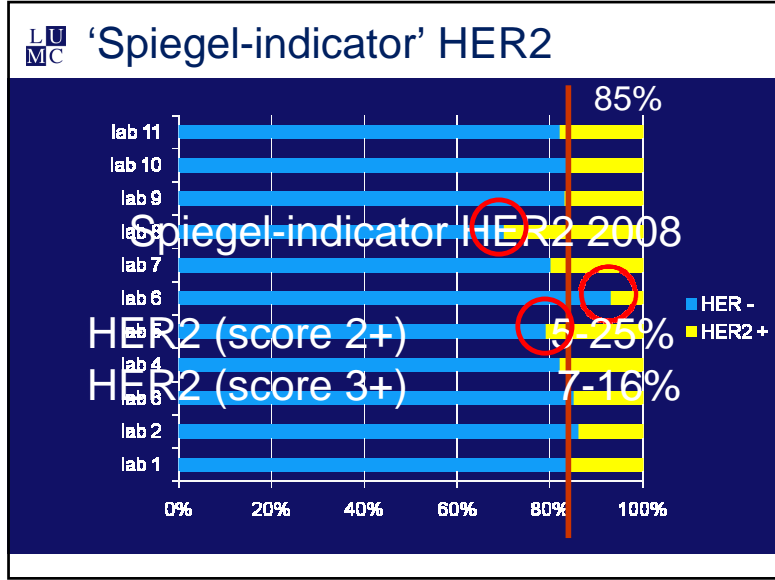
Abbreviations: IHC, immunohistochemistry; FISH, fluorescent in situ hybridization; DCIS, ductal carcinoma in situ.
*This percentage is greater than that specified in US Food and Drug Administration-approved tests but value was based on expert panel consensus that this level is a more realistic threshold.

LU
MC **Her2 testen-IHC**

- Antilichamen
- Scoringsystemen
- Interobserver variabiliteit
- Kwaliteitstoetsing



- LU MC Her2 testen-IHC**
- Antilichamen
 - Scoringsystemen
 - Interobserver variabiliteit
 - Kwaliteitstoetsing/programma's
 - Spiegelindicatoren (CBU)
 - EQA



LU
MC Spiegelindicator HER2-IHC 2009 (n=10)

	HER2 (score 2+)	HER2 (score 3+)
1	6%	
2	20%	
3	34%	
4	17%	
5		14%
6		14%
7		7.5%
8		8%
9		14%
10	8%	12%

HER2 2+ 6-34%

HER2 3+ 3-33%

LU
MC Kwaliteitsprogramma's (EQA)

- Eigen controlemateriaal lab (intern/externe)
- Regionale rondzendingen (IK's)
- SKML/SKCP 2x per jaar
 - eigen beroepsgroep, NVVP
- Externe EQA, 2x per jaar
 - NordiQC (2009/2010)
 - UKNEQAS



LU
MC Activiteiten Werkgroep Farmacodiagnostiek (HER2)

- TMA-project (6 referentielabs 2009)
 - 1000 mammatumoren op TMA's
 - IHC: 4B5 (Pathway), SP3 en A0485 (HercepTest)
 - ISH: mono-SISH en duo-color SISH, (mono-CISH)
 - Correlatie met oorspronkelijke PA-uitslagen
 - Informatie over fout-positief én fout negatief % HER2
 - In 2010 uitbreiding andere labs (op verzoek)
- HER2 testing op maagtumoren (2010)
- Externe EQA (NordiQC/Roche)

LU
MC NordiQC/Roche (2009/2010)

- Initiatief werkgroep Farmacodiagnostiek
- Taakgroep Moleculaire diagnostiek (QC)
- Vier rondzendingen in 2 jaar (2009/2011)
- Deelname gratis
- Onafhankelijk



Nordic immunohistochemical Quality Control





NordiQC/Roche (2009/2010), B8

- Deelname van 136 labs (worldwide)
- 50 laboratoria uit Nederland (NL)
 - 4 laboratoria inactief
 - 9 laboratoria geen protocol ingestuurd
 - 1 laboratorium geen 'slides' teruggestuurd
- 36 labs NL uiteindelijk beoordeeld!! (75%)

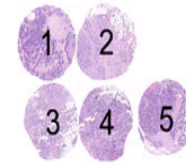


Overall results HER2 (n=136 labs)

HER-2 IHC

The slide to be stained for [HER-2](#) comprised the following 5 tissues:

	IHC	FISH
	HER-2 Score* (0, 1+, 2+,3+)	HER-2 gene/chr.17 ratio**
1. Breast ductal carcinoma	0	1.0 - 1.2
2. Breast ductal carcinoma	1+	1.1 - 1.3
3. Breast lobular carcinoma	1+/2+***	1.2 - 1,5
4. Breast ductal carcinoma	2+	2.5 - 2.9
5. Breast ductal carcinoma	3+	> 6.0, clusters



* HER-2 immunohistochemical score (see table below) as achieved by using the two FDA approved kits and antibodies (HerceptTest™, Dako, and PATHWAY®, Ventana) in NordiQC reference laboratories.

** HER-2 gene/chromosome 17 Ratio achieved by using HER-2 FISH pharmDX™ Kit, Dako

*** Staining varied through the tissue block.

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



Criteria voor beoordeling

- **Optimal**
 - 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+/2+** in the breast carcinoma **no 3.**
 - A clear and unequivocal immunohistochemical staining marked as **score 2+** 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.



Criteria voor beoordeling

- **Good**
 - Als 'optimal' behalve als no.5 was gescored als 2+
- **Borderline**
 - Signaal/Ruis ratio was te hoog (e.g. intracytoplasmatic staining)
- **Poor**
 - Fout positief of fout negatief (e.g. 3+ tumor /2+ tumor met gen amplificatie toonde een 1+ reactie)

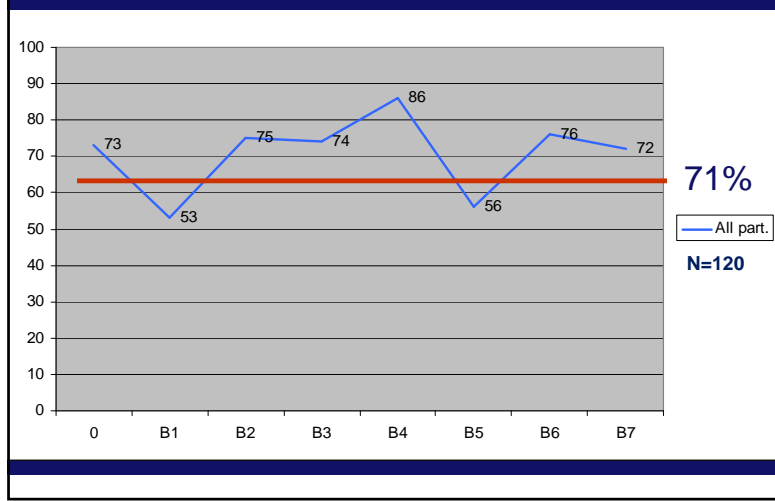
LU MC Overall results HER2 (n=136 labs)

Table 1. The IHC systems/Abs used and the assessment marks given

FDA approved HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. OPS ²
PATHWAY [®] rmAb clone 4B5, 790-2991, CONFIRM [™] , rmAb clone 4B5, 800-2996	40	Ventana	38	2	0	0	100 %	100 %
HercepTest [™] K5204, K5206, K5207, SK001	49	Dako	34	4	1	10	78 %	81 %
CE IVD approved HER-2 systems								
Oracle [™] mAb clone CB11, TA9145	3	Leica	1	0	0	2	-	-
Abs for in-house HER-2 systems								
pAb clone A0485	19	Dako	7	1	2	9	42 %	64 %
mAb clone mAb clone CB11	1	Monosan 1 Novocastra 1 NeoMarkers	0	2	0	1	-	-
mAb clone 3B5	4	NeoMarkers	0	0	0	4	-	-
mAb clone e2-4001+3B5	2	NeoMarkers	0	0	0	2	-	-
rmAb clone SP3	13	NeoMarkers 1 Epitomics 1 Zytomed	7	2	4	2	60 %	62 %
rmAb clone EP1045Y	1	Biocare	0	0	0	1	-	-
Total	136		87	11	7	31		
Proportion			64 %	8 %	5 %	23 %	72 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only. See below.

LU MC Pass-rate in 8 NordiQC HER2 runs



LU MC Overall results HER2 (n=136 labs)

Table 1. The IHC systems/Abs used and the assessment marks given

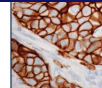


FDA approved HER-2 systems	N	Vendor	Optimal	Good	Borderl.	Poor	Suff. ¹	Suff. OPS ²
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mAb clone mAb clone CB11	1	Monosan 1 Novocastra 1 NeoMarkers	0	2	0	1	-	-
mAb clone 3B5	4	NeoMarkers	0	0	0	4	-	-
mAb clone e2-4001+3B5	2	NeoMarkers	0	0	0	2	-	-
rmAb clone SP3	13	NeoMarkers 1 Epitomics 1 Zytomed	7	2	4	2	60 %	62 %
rmAb clone EP1045Y	1	Biocare	0	0	0	1	-	-
Total	136		87	11	7	31		
Proportion			64 %	8 %	5 %	23 %	72 %	-

1) Proportion of sufficient stains (optimal or good), 2) Proportion of sufficient stains with optimal protocol settings only. See below.

LU MC Overall results HER2 (n=136 labs)

- FDA approved systems
 - Ventana (4B5) 100%
 - HercepTest (A0485) 78%
- CE IVD approved system
 - Leica (CB11) 33%
- 'In-house' systems 43%

Scoring Guide for the Interpretation of Ventana PATHWAY[®] HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody Staining of Breast Carcinoma

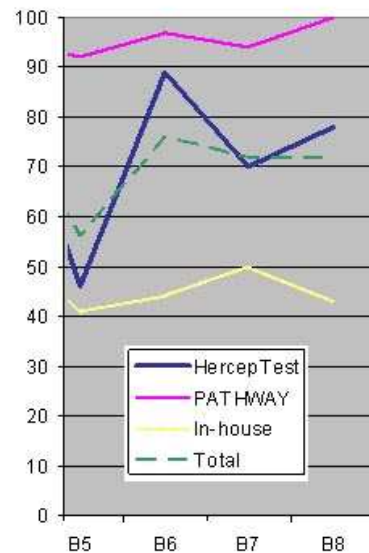
LU MC 'Pass-rate' (n=9)

Approved kits

94% Pathway (Ventana)

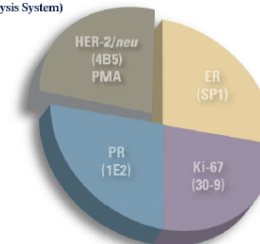
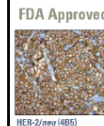
86% HercepTest (DAKO)

41% in-house systems



LU MC FDA-approved kits? Prijs per test?

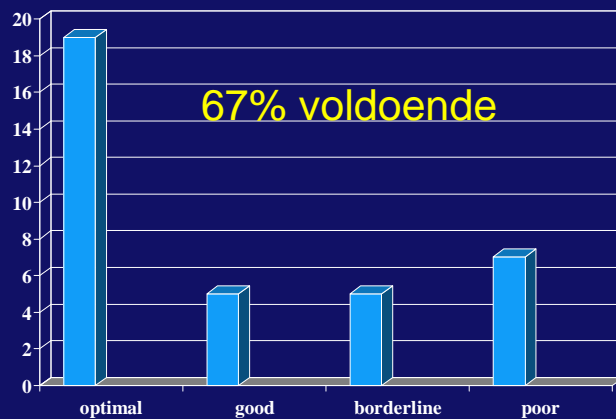
We now provide
PATHWAY® HER-2/neu
HER-2/neu (4B5)
FDA Approved for VIAS™
(Ventana Image Analysis System)



HercepTest™ for the Dako Autostainer
Code K5207



LU MC HER2 resultaten NL-laboratoria



LU MC 'Pass-rate' (O+G) NL-laboratoria (n=36)

14 A0485, (DAKO)	71%
7 4B5 (Ventana)	100%
9 SP3	55%
4 3B5	0%
1 e2-4001 en 3B5	
1 CB11	100%

Consequenties Kwaliteitstoetsing?

GEEN!!!!

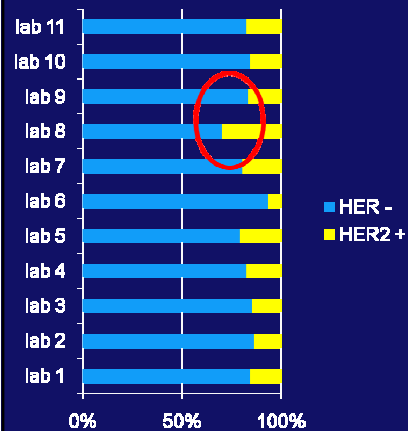
€7.200.000- €10.800.000 per jaar
HERCEPTIN

Tijdperk van vrijblijvendheid voorbij?



"I didn't actually catch anything, but I do feel I gained some valuable experience."

Spiegel-indicatoren en EQA



EQA			
Lab 1:	-	+	++
Lab 2:	+	+	+
Lab 3:	+	+/-	+
Lab 8:	-	-	-

Gewetensvraag??

Een goede vriendin van u heeft de diagnose borstkanker/mammacarcinoom en vraagt aan u in welk laboratorium ze het beste de HER2 test **NIET** kan laten uitvoeren?

- A: lab 1
- B: lab 2
- C: lab 3
- D: lab 8



Consequenties Kwaliteitstoetsing!

.....Een lab dat onvoldoende scoort zou binnen 3 maanden een plan moeten genereren waaruit **verbeterpunten** voor deze testanalyse blijkt. Bij visitaties kan dan ook uitvoering van dat herstelplan beoordeeld worden. Een lab dat **2 achtereenvolgende QC rondzendingen** onvoldoende voor dezelfde test scoort mag de analyse niet meer uitvoeren, totdat extern gevalideerde testen adequate analyse laten zien.....

Concept-voorstel QC van de WMDP



Conclusies ('take-home'-messages!)

- HER2 testing (IHC) in NL nog niet optimaal ('pass-rate' 70%)
- FDA-approved kits superieur boven 'in-house systems' (90% vs 55%)
- Stainers beter dan Manual
- Deelname aan EQA verbetert de kwaliteit
- Tijdperk van vrijblijvendheid is voorbij
 1. Netwerk van referentielabs (technische ondersteuning voor IHC)
 2. Discussie over uitbesteding van MD-onderzoek ?