

## Experiences from NEQAS

*“Speak softly and carry a big stick”*


Dr John. M.S. Bartlett,  
Director of Transformative Pathology OICR

**The UK National External Quality  
Assessment Service for HER2-ISH tests**



### QUALITY ASSURANCE


- **Internal Quality Control (IQC)**
  - monitors within- & between-analytical run variability
  - Internal standards - plot performance over time.
    - Ideally included in *every* analytical run.
- **External Quality Assessment (EQA)**
  - participating clinical laboratories are sent samples on a regular basis which they test as if they had come from patients.
  - Results are returned to EQA centres which provide a report that compares the participant's performance with that of all laboratories and/or groups of laboratories using the same test method(s).
  - Ensures high quality of testing – may affect ability to deliver tests.



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### EXTERNAL QUALITY ASSURANCE

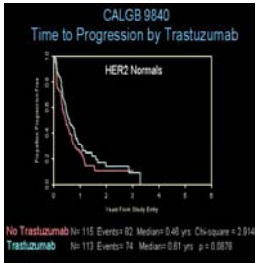
1. Why perform EQA?
2. NEQAS EQA for ISH
  - a. Scope
  - b. Assessment (reference laboratories)
  - c. Participants
  - d. Training and trouble shooting.
3. What have we learnt?
4. Is it just EQA?



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### Why perform QA?

- Impact on patient selection
  - Inappropriate treatment.
  - Exposure to unwanted side effects
  - Failure to control disease.
- Impact on health care costs:
  - Wasted financial resources.
- **Patients are the imperative for accuracy, reproducibility and quality in diagnostic pathology.**
- “Approximately 20% of current HER2 testing may be inaccurate”
- “Such a disorganised practice and high rate of inaccuracy – is not acceptable” Wolff AC et al. Journal of Clinical Oncology 25:118-145 2007



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### Both QA & Methods matter:

Local Test	N	Sens	Spec	Accuracy
All IHC	1457	0.91	0.75	0.79 (0.77-0.81)
Herceptest	862	0.92	0.73	0.79 (0.76-0.81)
Ventana	153	0.94	0.74	0.78 (0.71-0.85)
FISH	131	0.98	0.90	0.92 (0.86-0.96)


Local Test	N	Central
Herceptest	1063	81.6% (79.1-83.9)
IHC	636	75.0% (71.4-78.3)
FISH	813	88.1% (85.6-90.2)

(Fisher's exact test P < .001, Herceptest vs FISH, local vs central).

Perez et al, JCO 24:3032-8 2006

Press M.F. et al, CCR 11:6598-6607, 2005

Choice of method explains 6.5-13% of “errors”  
Size of lab may play a role –  
Choice of method has a critical impact on accuracy – high levels of inaccuracy may persist.



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### ASCO Guidelines


2. Requirement for Quality assurance

*“testing inaccuracy remains a major issue with both IHC and FISH”*

*“A precise definition of accuracy is how close the measure values are to a supposed true value”.*

*“Participation in external proficiency testing”*

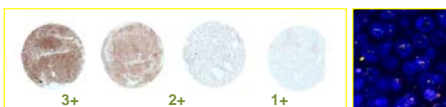
*“Unsatisfactory performance results in suspension of laboratory”*



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## UK NEQAS ISH

- Ensure quality of diagnostic performance: -
- Provide objective information
- Support & advise laboratories to improve quality
- HER2: Both IHC and FISH EQA modules



	HER2 Chr 17 Ratio			HER2 copy number		
	Average	Range	Borderlin e	Average	Range	Borderlin e
MDA-MB-231						
Run 1	1.09±0.05	0.87-1.43	0.78-1.57	2.41±0.12	1.55-2.85	1.40-3.14
Run 2	1.16±0.08	0.88-1.44	0.79-1.58	2.25±0.25	1.53-2.80	1.38-3.08
Run 3	1.04±0.05	0.71-1.19	0.64-1.31	2.62±0.22	1.45-3.65	1.31-4.02

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9

## NEQAS ISH EQA Scheme

- Goals:
  - To assess the quality of HER2 diagnostic procedures
  - To provide support and training to improve poor performance where identified.
  - To disseminate information on best practice
  - To prevent poor practice via reference to NQAAP and CPA.



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8

## NEQAS ISH EQA Scheme

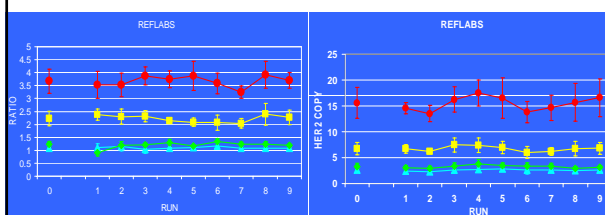
- Close relationship/parallels to IHC scheme
  - 3-4 assessments per annum
- Designed to assess scoring/technical performance.
  - Not to recapitulate pathology EQA
- Launched 2005 – pilot phase 2005-2006.
  - 30 assessments completed to date.
  - Now 266 participants world wide (*ISH only*).
- Initially multiple cell lines
  - Reference laboratories/steering group to assess performance
- Now using TMAs



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9

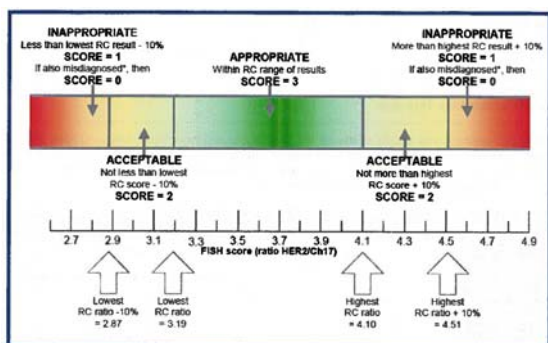
## Reference results over time



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10

## NEQAS assessment:



11

## HER-2 FISH EQA: Appropriate Result

Scheme:	FISH	Assessment code:	3	Module:	HER-2 FISH
<b>TECHNICAL EVALUATION:</b> Cell Lines displayed as distributed on Slides					
Cell Line	Participant Diagnosis	Participant HER2:trstom: 17 ratio or (Copy no.)	Mark		
A SK-BR-3 (3+)	Amplified	4.34	3		
B MDA-MB-453 (2+)	Amplified	2.21	3		
C MDA-MB-175 (1+)	Non-amplified	1.29	3		
D MDA-MB-231 (0)	Non-amplified	1.17	3		
Method Used by Participant: ratio		Total Mark: 12			
<b>OVERALL RESULT: Appropriate</b>					
<small>RANGE HER2:trstom: 17 ratio and Copy no. OF SCORES ACHIEVED BY THE REFERENCE CENTRES USED TO EVALUATE THE CELL LINES</small>					
SK-BR-3 (3+) Amplified: 3.57 - 4.41		MDA-MB-453 (2+) Borderline / Amplified: 2.10 - 2.70			
11.70 - 20.7		4.27 - 10.00			
MDA-MB-175 (1+) Non-amplified: 1.13 - 1.29		MDA-MB-231 (0) Non-amplified: 0.71 - 1.19			
2.10 - 4.17		1.40 - 3.65			

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### HER-2 FISH EQA: Inappropriate Result

Scheme:	FISH	Assessment code:	3	Module:	HER-2 FISH
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**TECHNICAL EVALUATION:** Cell Lines displayed as distributed on Slides

Cell Line	Participant Diagnosis	Participant HER2/chr. 17 ratio or (Score/eq.)	Mark
A SK-BR-3 (3+)	Amplified	3.5	1
B MDA-MB-453 (2+)	Amplified	2.6	3
C MDA-MB-175 (1+)	Non-amplified	1.5	1
D MDA-MB-231 (0)	Non-amplified	1.3	2
Total Mark:			7

Method Used by Participant: *not visible*

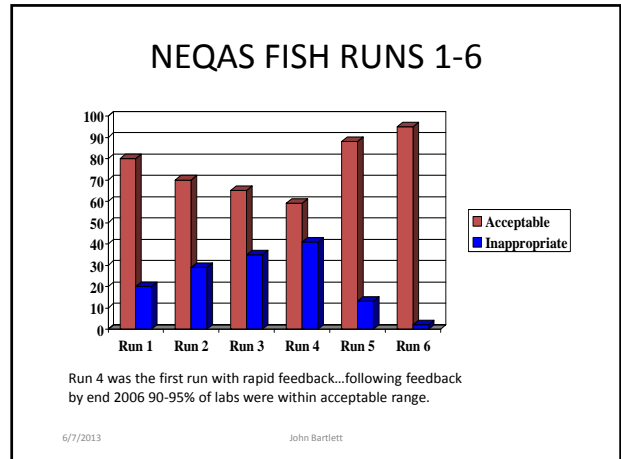
**OVERALL RESULT: Inappropriate**

RANGE (HER2/chromosome 17 ratio and Copy no. OF SCORES ACHIEVED BY THE REFERENCE CENTRES USED TO EVALUATE THE CELL LINES)

SK-BR-3 (3+) Amplified: 3.37 - 4.4	MDA-MB-453 (2+) Borderline / Amplified: 2.10 - 2.70
MDA-MB-175 (1+) Non-amplified: 1.33 - 1.29	MDA-MB-231 (0) Non-amplified: 0.71 - 1.19

Possibly due to loss of chr 17 signal/underscoring of 17?  
Risk of overdiagnosis of amplification.

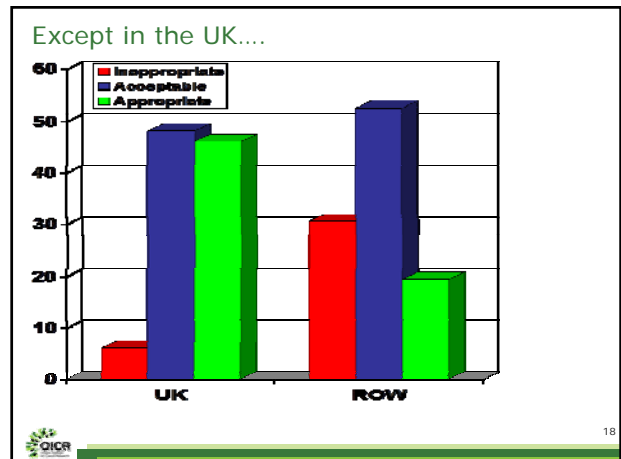
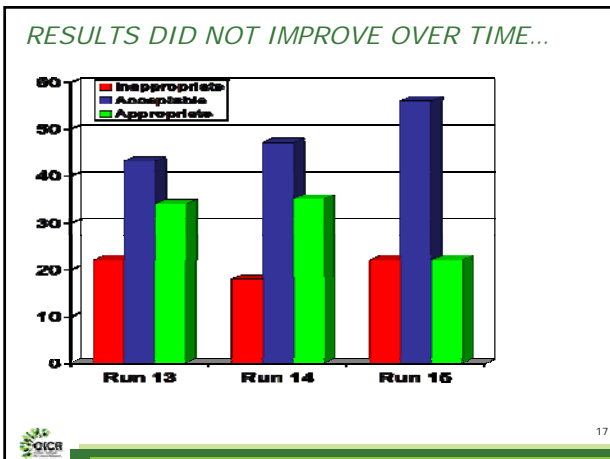
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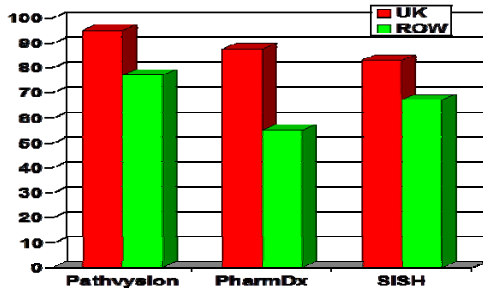
## LESSON 1 from EQA

IT WORKS!

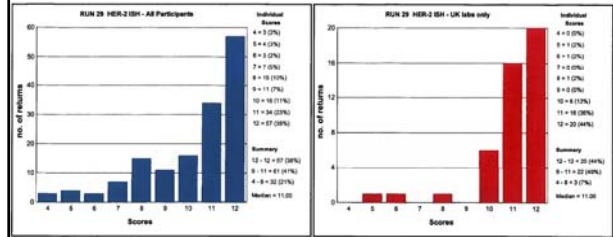
## BUT.....



Performance is independent of test format



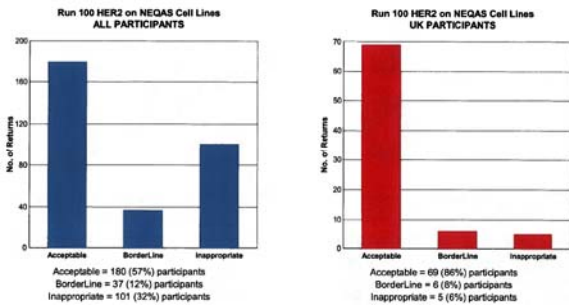
It's still happening....



- ISH Run 29 2013.



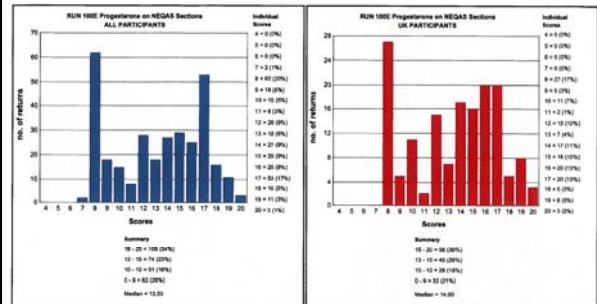
And it's not just ISH -



HER-2 IHC Assessment Pass Rates: Data From multiple countries - UK & Overseas



But it's just HER2....

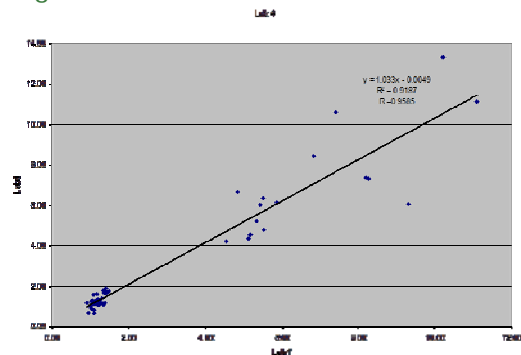


Lessons from EQA

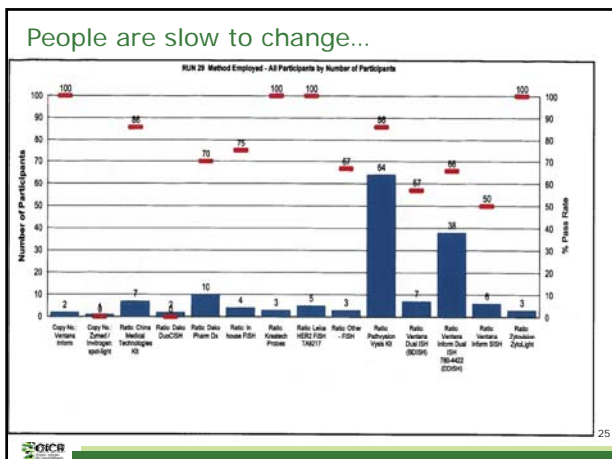
- Lesson 2:
  - *Speak softly and carry a big stick.*
    - Theodore Roosevelt 26<sup>th</sup> US President
  - EQA schemes need "teeth" - or they don't work.



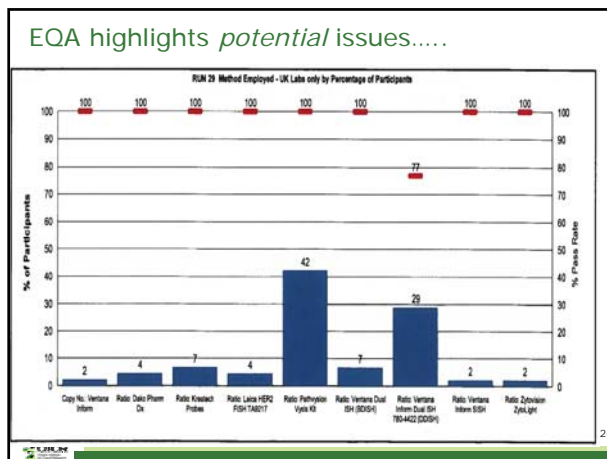
Use of alternative methods supported by "Ring" studies



People are slow to change...



EQA highlights potential issues....

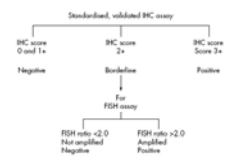


Lessons from EQA

- Lesson 2:
  - Speak softly and carry a big stick.
    - Theodore Roosevelt 26<sup>th</sup> US President
  - EQA schemes need "teeth" – or they don't work.
- Lesson 3
  - ISH, CISH, FISH, DDISH, SISH, phish..
    - If done according to correct standards, with EQA all ISH tests are of similar quality.
    - But QC can highlight "issues"
- Lesson 4:
  - We need to do more
    - Assessment of in house sections – technical assessment scheme
    - Assessment of multiple scorers – "designated hitters"?
    - Change is slow..

NEQAS: more than just EQA

- Practice Guidelines:
  - 2000, 2004, 2008, Bartlett JMS, Starczynski J, Atkey N et al. HER2 testing in the UK: recommendations for breast and gastric in-situ hybridisation methods. J Clin Path 2011; 64(8): 649-653.



NEQAS: more than just EQA

- Practice Guidelines:
- New methods
  - FISH and CISH
- Key papers:
  - A UK NEQAS ISH multicentre ring study using the Ventana HER2 dual-color ISH assay: *AJCP* 2011 135: 157-162
  - A multicenter study comparing silver in situ hybridisation with FISH *AJCP* 2009 132: 514-520.
  - Determination of HER2 amplification in primary breast cancer using dual-colour chromogenic in situ hybridisation is comparable to fluorescence in situ hybridisation: a European multicentre study involving 168 specimens. *Histopathology* 2010 56: 472-480.

NEQAS: more than just EQA

- Practice Guidelines:
- New methods
- Challenging cases
  - Heterogeneity & Rogues
- Key papers:
  - Starczynski J, Atkey N, Connelly Y et al. HER2 Gene Amplification in Breast Cancer: A Rogues Gallery of Challenging Diagnostic Cases: UKNEQAS Interpretation Guidelines and Research Recommendations. *American Journal of Clinical Pathology* 2012; 137(4): 595-605.
  - Bartlett AJ, Starczynski J, Robson T et al. Heterogeneous HER2 Gene Amplification. *American Journal of Clinical Pathology* 2011; 136(2): 266-274.

### HER2 Gene Amplification in Breast Cancer

A Rogues' Gallery of Challenging Diagnostic Cases: UKNEQAS Interpretation Guidelines and Research Recommendations

- Guiding principles:
  - For unusual cases there is no likelihood of a **robust** evidence base that will determine treatment efficacy with HER2-directed therapies.
  - Report what you see: Provide a detailed, adequate description of the ISH/IHC staining pattern.
  - Reach a conclusion: Is there sufficient evidence of amplification/overexpression of HER2?
    - Existing treatments are all validated for HER2 overexpressing/amplified cases
  - Recognise HER2 status is not the final arbiter of clinical decisions
    - Age, ER, PgR, Grade, Nodal status, size, Ki67, comorbidity etc etc all play a part.
  - Identify ways to improve "certainty" – research recommendations

31

### HER2/CEP17 "co-amplification"

- Accounts for ca 1-2% of all ISH cases.
- High copy number of *both* HER2 and CEP17
- Reduced HER2/CEP17 ratio to around 1
- Signals may co-localise

32

### HER2 CEP17 "co-amplification"

- Signals "co-localise": HER2 copy 24.3, CEP17 23.4 **RATIO HER2/CEP 1.04!**

33

- Only HER2 amplified
- HER2 + one or more contiguous targets amplified, followed by telomeric non-amplified or deleted targets
- All targets amplified
- Complex patterns

### Interpretation and reporting

- Interpretation:** HER2/CEP17 ratio = 1: high HER2/CEP17.
  - Opinion is that these rare cases should be interpreted as amplified.
  - Preliminary evidence suggests patients with extended amplicons may exhibit reduced response (again metastatic disease)
- Scoring:** Score HER2 and CEP17 signals under single-color filters, taking care to match cells within fields to ensure accurate counting of red and green signals.
  - Counting additional cells is not likely to alter the diagnosis.
- Reporting:** Amplified with centromere co-amplification
  - Based on HER2 copy and associated *balanced* CEP17 copy number, plus note co-localization of HER2/CEP17 signals in the report.

35

### NEQAS: more than just EQA

- Practice Guidelines:
- New methods
- Challenging cases
- Importance of CEP17
  - 1 in 16.5 cases Misdiagnosed

36

## NEQAS: more than just EQA

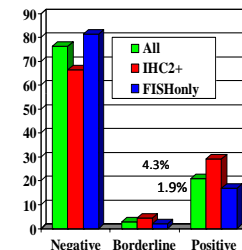
1. Practice Guidelines:
2. New methods
3. Challenging cases
4. Importance of CEP17
5. Frontline ISH testing



37

## FISH reduces equivocal results

- Approx 12% of IHC results require FISH confirmation.
  - Up to 25% in some labs.
  - 4.3% remain "borderline"
  - 0.5% of all cases
- For FISH first approx. 1.9% of "borderline" cases.
  - Improved criteria could reduce this (e.g. score more cells)
  - Borderline cases score 40-60 cells
  - ?? Reflux IHC??



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## NEQAS: more than just EQA

1. Practice Guidelines:
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Sauter G, Lee J, Bartlett JM, Slamon DJ, Press MF. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. J Clin Oncol 2009; 27(8): 1323-1333.



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39

## NEQAS: more than just EQA

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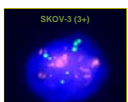
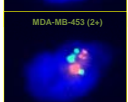
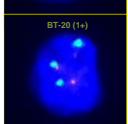
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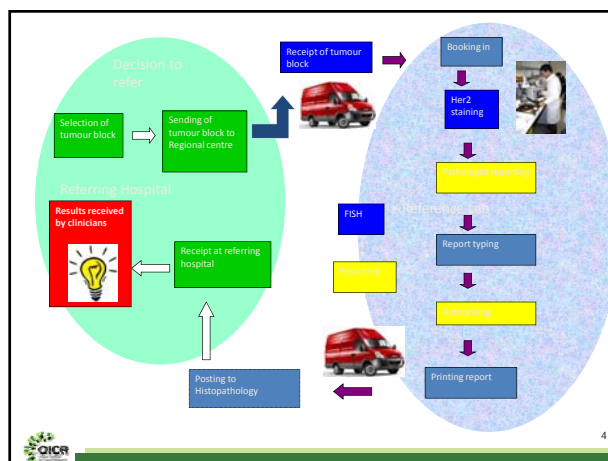
40

### Blind Study: Human V Automation

Sarah Barnett (UCL AD)

Automation

<p>No. cells counted: 20</p> <p>HER2/ chrom. 17 ratio: 4.98</p> <p>AMPLIFICATION</p>	 <p>SKOV-3 (3+)</p>	<p>No. cells counted: 35</p> <p>HER2/ chrom. 17 ratio: 4.85</p> <p>AMPLIFICATION</p>
<p>No. cells counted: 20</p> <p>HER2/ chrom. 17 ratio: 2.40</p> <p>AMPLIFICATION</p>	 <p>MDA-MB-453 (2+)</p>	<p>No. cells counted: 37</p> <p>HER2/ chrom. 17 ratio: 3.22</p> <p>AMPLIFICATION</p>
<p>No. cells counted: 20</p> <p>HER2/ chrom. 17 ratio: 1.00</p> <p>NO AMPLIFICATION</p>	 <p>BT-20 (1+)</p>	<p>No. cells counted: 25</p> <p>HER2/ chrom. 17 ratio: 1.02</p> <p>NO AMPLIFICATION</p>



42

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