

# Proficiency Testing and Tumour Markers

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Laboratory Quality Management Conference 2015

Pinnacle Harbourfront Hotel

Vancouver BC

October 28-30, 2015

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# What is the Canadian Immunohistochemistry Quality Control (cIQc)?

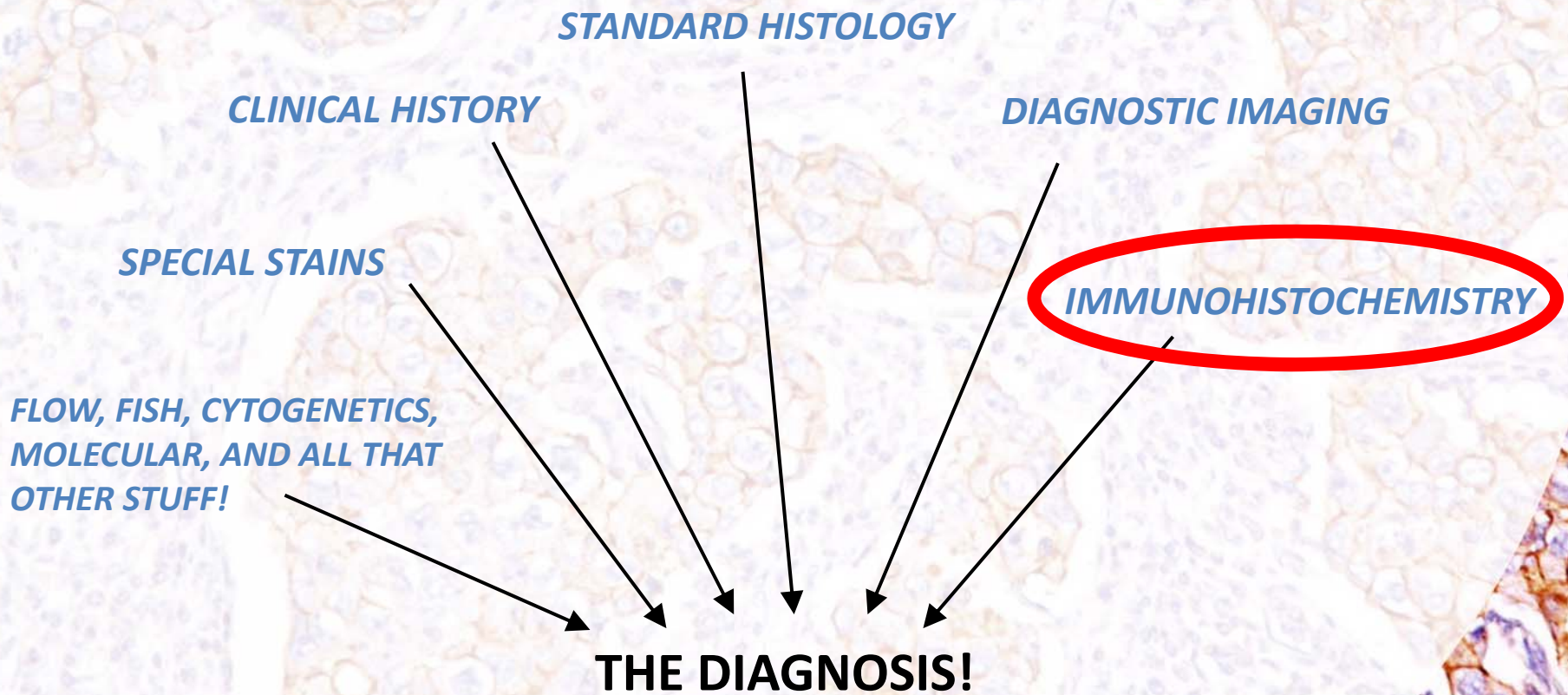


Map courtesy of Wikipedia



# Complexity of “the diagnosis”

- Diagnostic anatomic pathology is a complex, multifactorial process.
- Each piece of data has a sensitivity and specificity for “the diagnosis”.

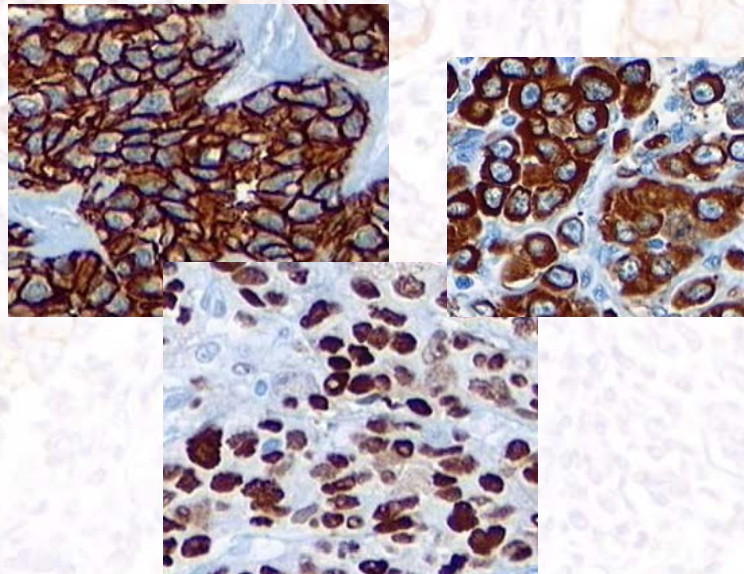




# Immunohistochemistry: A cornerstone in anatomic pathology practice

Immunohistochemistry (IHC): A method for localizing specific antigens in tissues or cells based on antigen-antibody recognition; provides valuable information used for cancer patient management.

- Diagnostics
- Prognostication
- Prediction

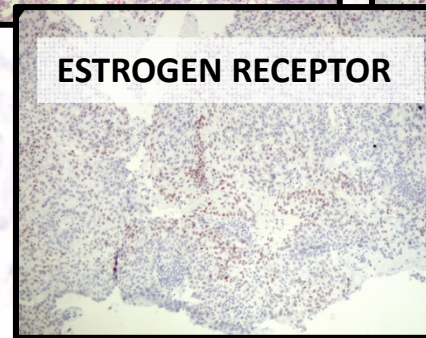
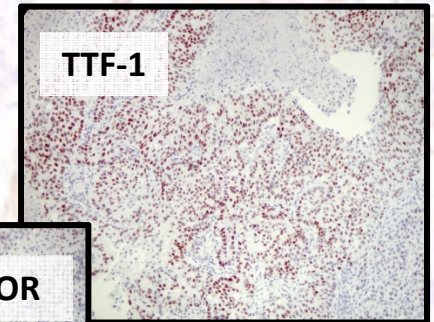
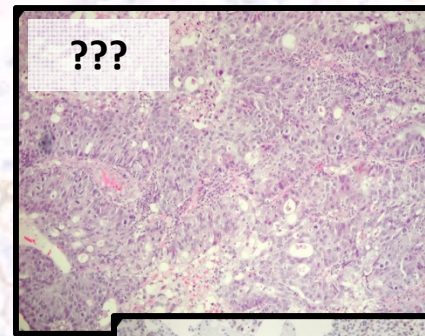
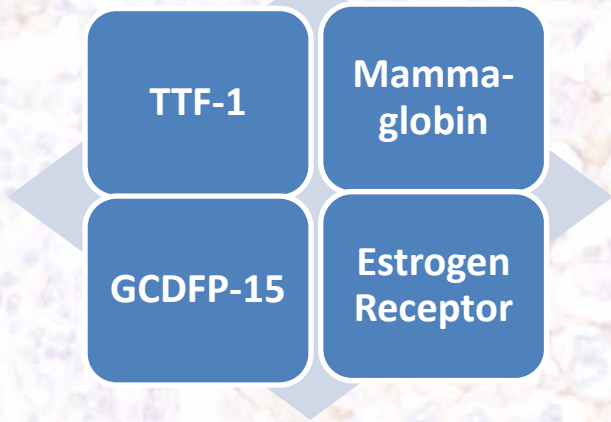




# Why do we do immunohistochemistry?

To help tell us what we are looking at!

Lung versus  
Metastatic Breast Carcinoma





# Why else do we do immunohistochemistry?

## TREATMENT!

~10% of all surgical pathology cases have IHC performed

~1/3 of those cases are invasive breast carcinomas

All invasive breast carcinomas require IHC testing for ER, PR and HER2

ER+ or PR+ *premenopausal* = TAMOXIFEN

ER+ or PR+ *postmenopausal* = AROMATASE INHIBITOR

HER2+ = HERCEPTIN



# The many variables of IHC (at least some of them!)

## Pre-analytical

- Acquisition (delay in putting samples into fixative)
- Fixation type and time
- Decalcification type and time
- Tissue processing
- Slide-drying time and temperature

## Analytical

- Antibody selection (different clones, polyclonal, vendor/supplier)
- Antibody optimization (antigen retrieval, antibody dilution, incubation time)
- Antibody validation
- Instrumentation (different automated platforms, manual stains)
- Qualification of IHC assay personnel
- Laboratory certification/accreditation

## Post-analytical

- Positive and negative tissue controls
- Interpretation
- Results reporting
- Pathologist performance
- Digital pathology with imaging analysis

**Quality assurance and quality control are a necessity for immunohistochemistry!**



# In anatomic pathology, where does quality assurance come in?

- Unlike the clinical laboratory where “QA rules”, quality depends on
  - education
  - skill
  - experience
- Since diagnosis is complex and multifactorial in anatomic pathology, proficiency testing is underutilized and of questionable value.
- Quality activities have mainly involved just *quality control*
  - positive controls within the lab
  - negative controls within the lab



B.C. EDITION / SATURDAY, MARCH 15, 2008

VANCOUVER, VARIABLY CLOUDY, HIGH 9 LOW 1

# THE GLOBE AND MAIL

CANADA'S NATIONAL NEWSPAPER



SUBPRIME CRISIS

WEEKEND SPECIAL // PAINFUL MISTAKES

## Bankers step in, bail out Bear Stearns

Famed institution hit by run on liquidity

BY SARVE MEENNA WASHINGTON

U.S. banking authorities have tapped a Depression-era financial tool to save Bear Stearns & Co., Wall Street's storied fifth-largest investment bank, from succumbing to a wave of contagion sweeping through the lending industry.

The loan to the 87-year-old Bear Stearns, which survived the Great Depression and a world war, marks the first time the U.S. Federal Reserve Board has stepped in to bail out an investment bank.

After repeatedly and vehemently denying rumours of liquidity woes, Bear Stearns said it sought help from the Fed late Thursday after what analysts described as a classic run on the bank. The Fed is providing the infusion through JPMorgan Chase, Bear Stearns's main banker.

And so it goes with the U.S. credit crunch, which erupted last summer in the evanescent market for high-risk home mortgages.

70¢ "SUBPRIME" PAGE 6



## Botched tests cast doubts on cancer screening

Thousands at risk across Canada as Newfoundland errors highlight deficiencies, pathologists caution

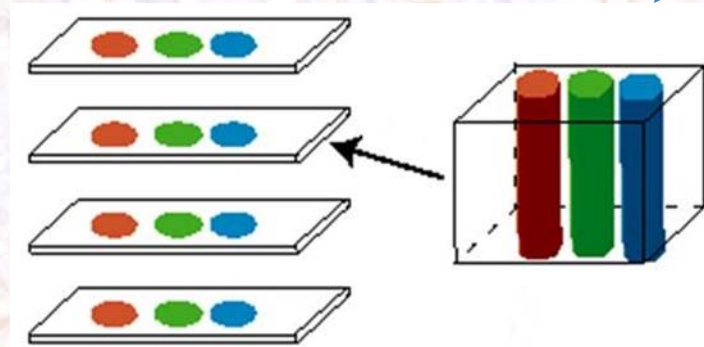
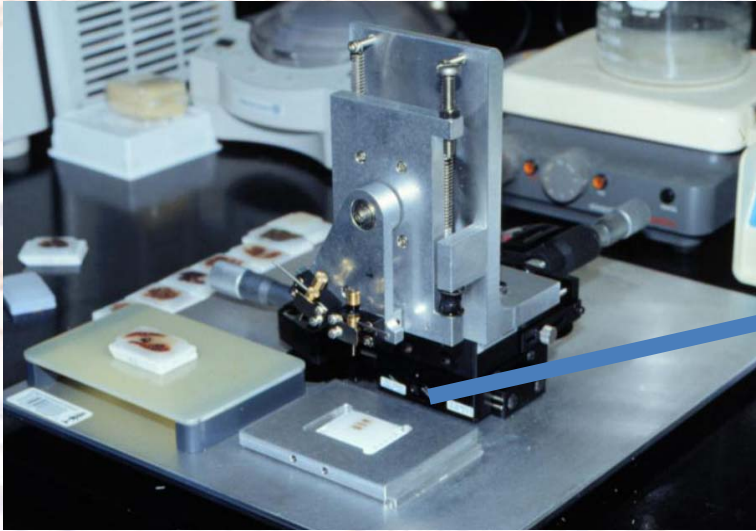
BY JESSICA LEINER ST. JOHN'S

The Newfoundland laboratory that botched hundreds of breast cancer tests has not investigated whether results of other specialized lab work done during the same period were correct, leading experts to question whether the province's testing tragedy is more extensive than has been revealed.

Beverley Green, left, found a lump in her breast in 2006, but tested negative for a hormonal treatment that can drastically reduce chances of the cancer's



# The cIQc Approach





# A Typical cIQc Challenge



cIQc sends unstained slides to participating laboratories, where slides are then stained according to each lab's usual protocol.



Laboratories evaluate each core and submit their results online through the TMA Scorer website.

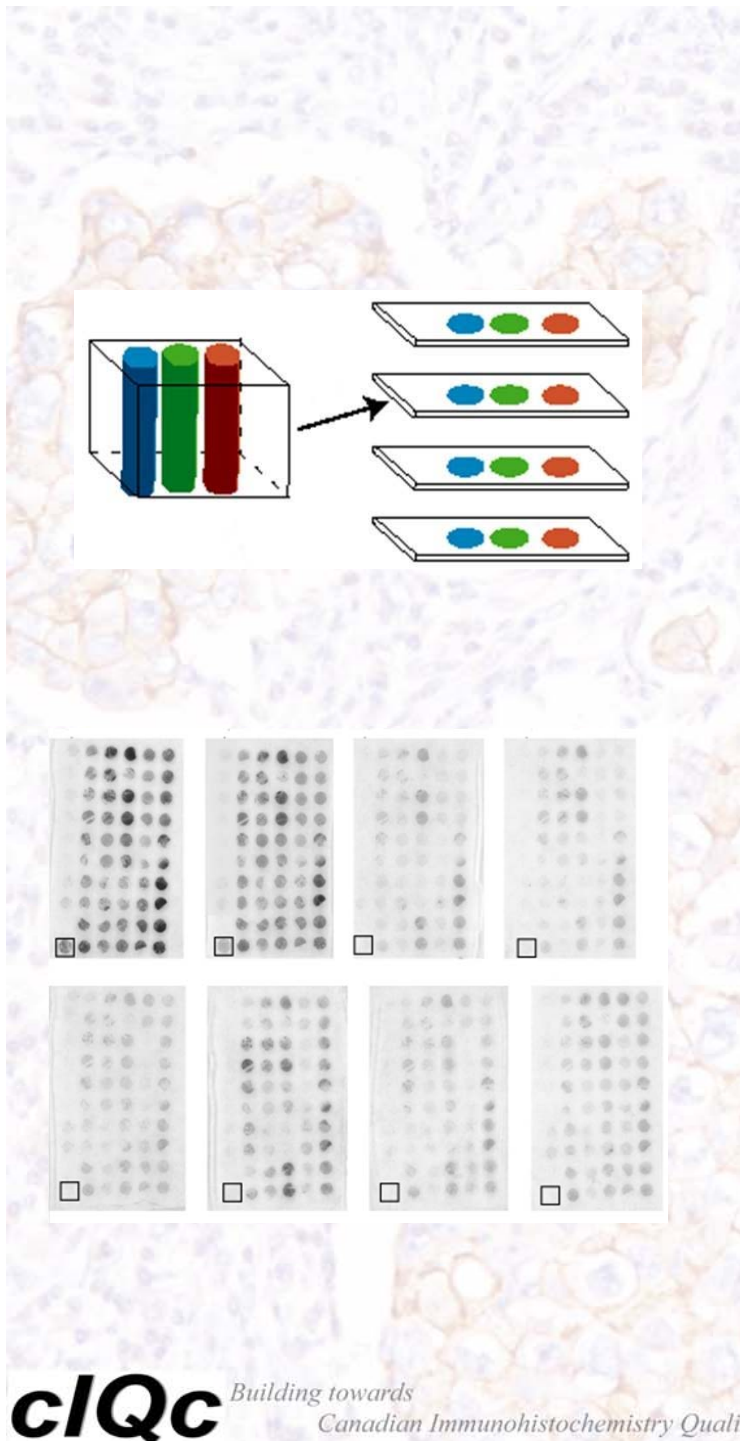
Lab/ Core	101	102	103	107	110	111	112	114	123	125	126	149	162	175	191	202	R1
1	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
3	N	E	E	P	N	N	N	E	E	P	P	N	N	N	P	P	P
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P
6	N	N	N	N	P	N	N	N	N	N	N	N	N	N	N	N	N
7	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P	P
9	N	N	N	N	U	N	E	N	U	N	N	N	N	N	N	N	N
11	N	P	P	P	P	N	E	P	P	P	P	E	P	P	P	P	P
12	N	N	N	N	N	E	N	N	E	N	N	N	N	N	N	P	N
13	E	E	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P
14	P	P	P	P	P	E	P	P	P	P	P	P	P	P	P	P	P
16	N	N	N	E	E	N	N	N	N	N	N	N	N	U	E	P	N
17	U	N	U	U	U	U	N	N	U	U	U	U	N	U	U	N	N
18	P	P	P	P	N	P	P	P	P	P	P	P	P	P	P	P	P
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	N
20	P	P	P	P	E	E	P	P	P	P	P	P	P	P	P	P	P
21	N	N	N	N	N	E	N	N	E	N	N	N	N	N	E	E	N
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
24	P	U	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
25	N	U	P	P	N	N	P	N	P	P	P	N	P	E	P	P	P
26	N	N	E	N	N	N	P	N	N	E	N	N	N	N	N	N	N
27	N	N	N	N	N	N	E	N	N	N	N	N	N	N	N	N	N
28	E	E	P	P	N	E	P	P	P	P	P	P	P	P	P	P	P



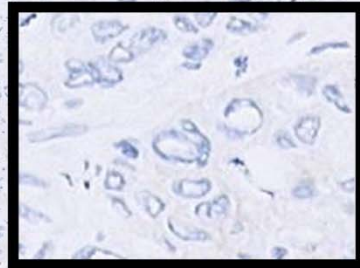
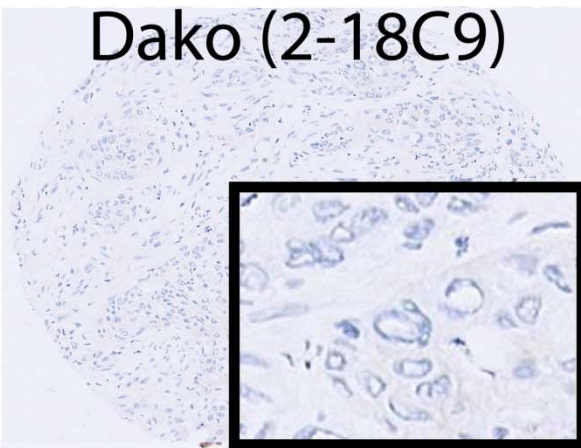
Labs return stained slides to cIQc for expert assessment by cIQc personnel and invited pathologists.



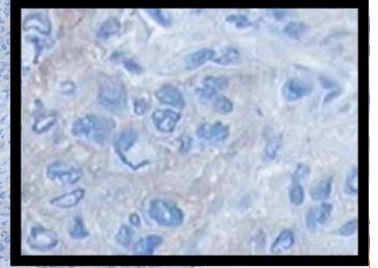
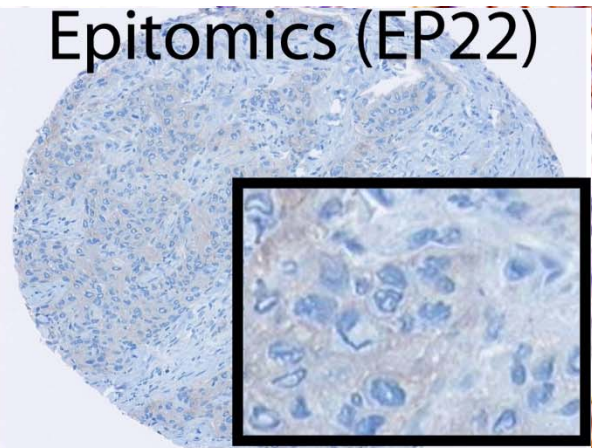




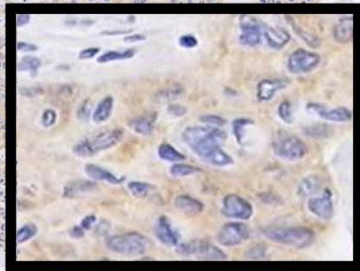
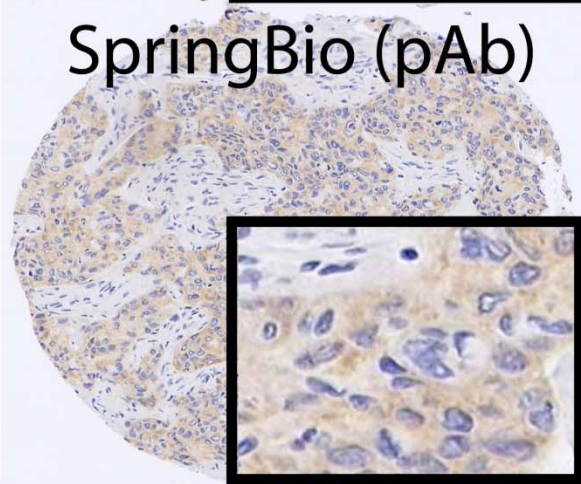
Dako (2-18C9)



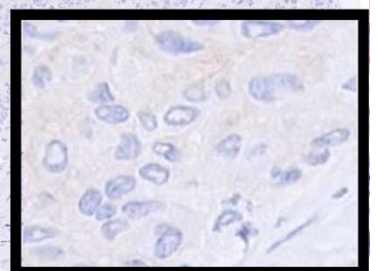
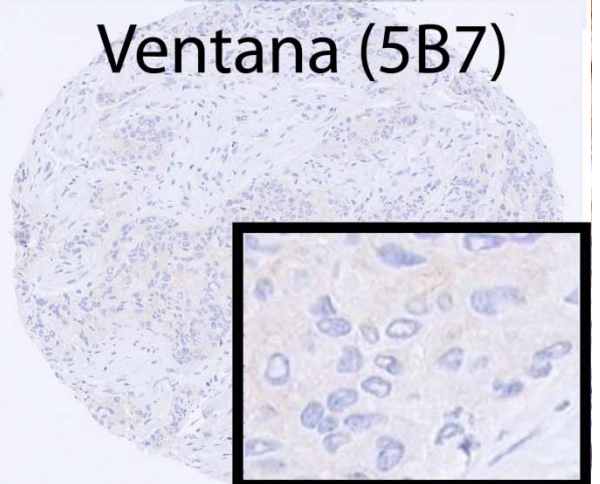
Epitomics (EP22)



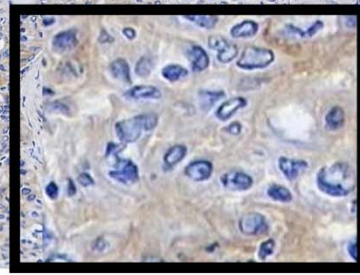
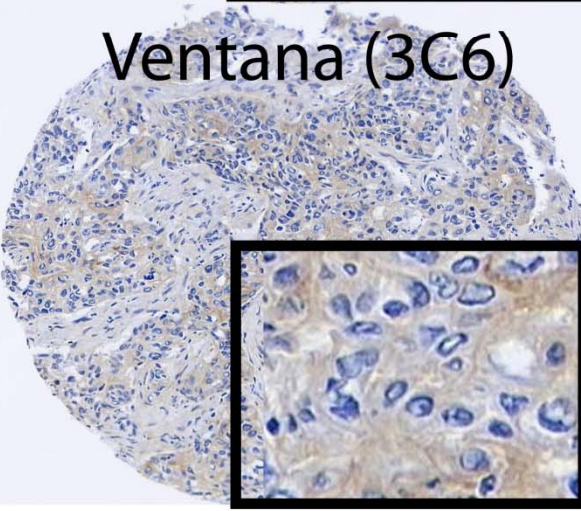
SpringBio (pAb)



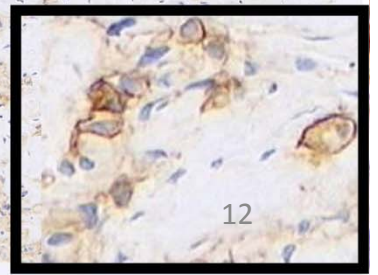
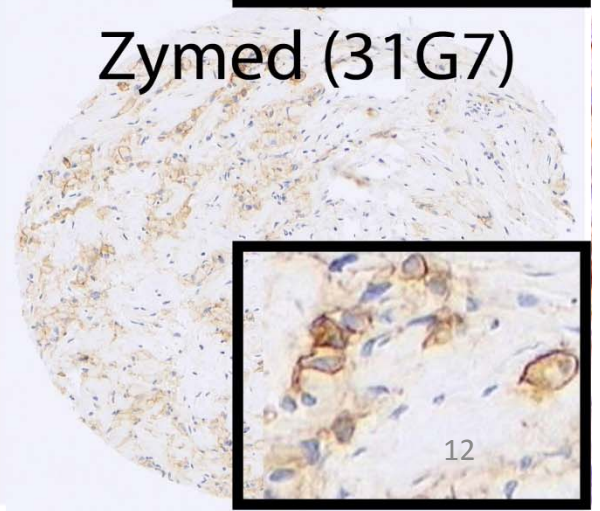
Ventana (5B7)



Ventana (3C6)



Zymed (31G7)





# The cIQc schedule

	2014	2015	2016	2017
<b>January</b>	Run 35 IDH1	*Run 44 IDH1	*Run 56 IDH1	*Run 72 ATRX
			*Run 57 ALK	*Run 73 ALK
<b>February</b>		*Run 45 ALK ISH	*Run 58 p53	*Run 74 p53
		*Run 46 MMR		
<b>March</b>	Run 36 Breast	*Run 47 Breast	*Run 59 Breast	*Run 75 Breast
<b>April</b>	*Run 37 BRAFV600E	*Run 48 BRAFV600E	*Run 60 BRAFV600E	Run 76 BRAFV600E
			*Run 61 MMR	Run 77 MMR
<b>May</b>	*Run 38 MMR		*Run 62 ALK	Run 78 ALK
			*Run 63 ATRX	Run 79 IDH1
<b>June</b>		*Run 49 ATRX		Run 80 p53
<b>July</b>	*Run 39 Breast	*Run 50 Breast	*Run 64 Breast	Run 81 Breast
<b>August</b>	Run 40 HER2 ISH	Run 51 HER2 ISH	Run 65 HER2 ISH	Run 82 HER2 ISH
		*Run 52 MMR	*Run 66 MMR	Run 83 MMR
<b>September</b>	*Run 41 ALK	*Run 53 ALK IHC	*Run 67 ALK	Run 84 ALK
			*Run 68 IDH1	Run 85 IDH1
<b>October</b>	*Run 42 p53/WT1/Napsin	*Run 54 p53	*Run 69 p53	Run 86 p53
<b>November</b>	*Run 43 Breast	*Run 55 Breast	*Run 70 Breast	Run 87 Breast
<b>December</b>			*Run 71 MMR	Run 88 MMR



# The importance of accurate IHC: Breast cancer as a illustrative model

- Breast cancer is the most common cancer among Canadian women.
  - Incident rate: 99 per 100,000
  - 2<sup>nd</sup> leading cause of death from cancer
- Molecular insights now make it possible to classify breast cancer according to intrinsic subtype.
  - Luminal A
  - Luminal B
  - HER2-enriched
  - Basal-like



# The importance of accurate IHC: Breast cancer as a illustrative model

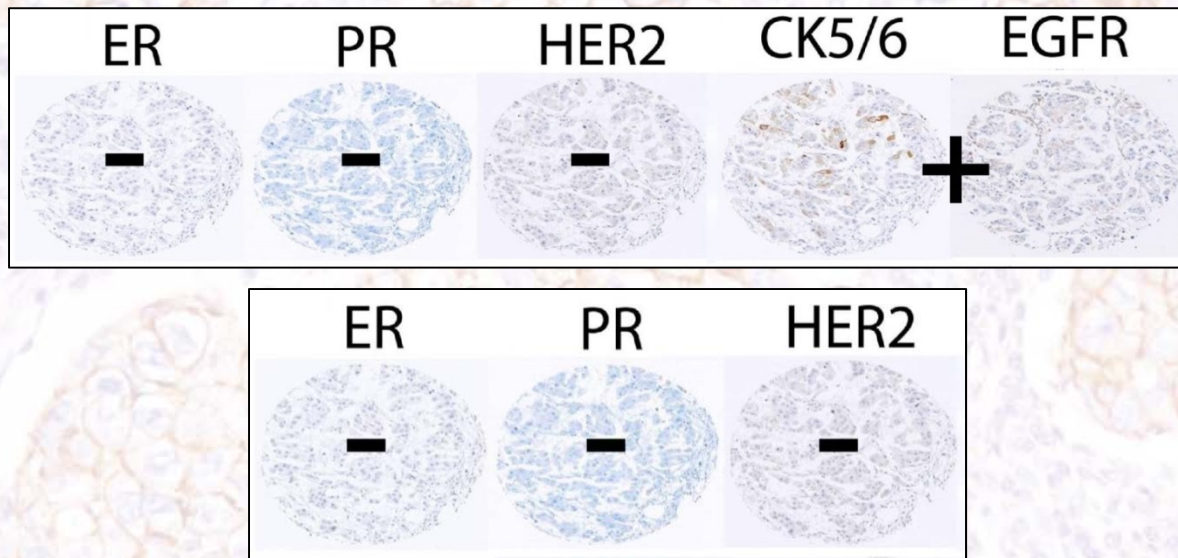
- Breast cancer classification
  - *Molecular assays*: PAM50, BluePrint
  - IHC panel

ER	PR	HER2	Ki67	CK5/6	EGFR	
						Lum A
						Lum B
						HER2E
						Basal-like



# The importance of accurate IHC: Breast cancer as an illustrative model

- Basal-like breast cancer defined by IHC:



- Basal-like breast cancer
  - Increased risk of early relapse
  - Aggressive pathologic features
  - Afflicts younger women
  - **No targeted therapies**
  - Poor prognosis



# clQc at the forefront

- The concept of molecular breast cancer subtypes in a clinical setting is becoming of great interest.
- IHC surrogate definitions are practical and cost effective alternatives to gene expression-based assays for hospital diagnostic laboratories.

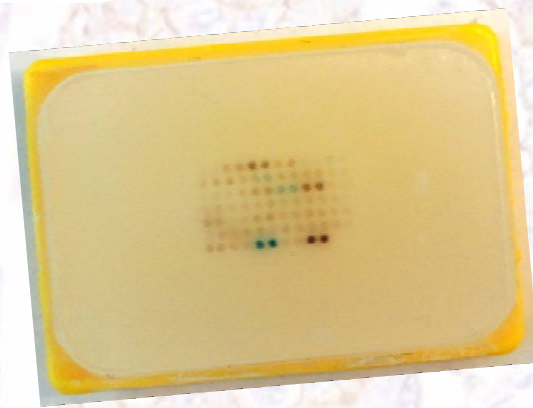
*Are diagnostic laboratories able to correctly identify cases of basal-like breast carcinoma using established biomarkers and surrogate immunopanel?*



# clQc Run 27

## Methods

- A 40-case breast cancer tissue microarray enriched for basal-like breast carcinomas was constructed (aka triple negative array).
- Sections were distributed to 50 laboratories for staining with ER, PR, HER2, CK5 and an optional additional basal-like biomarker.





# clQc Run 27 Garrattogram

Based on “triple negative”  
(ER/PR/HER2-negative) definition

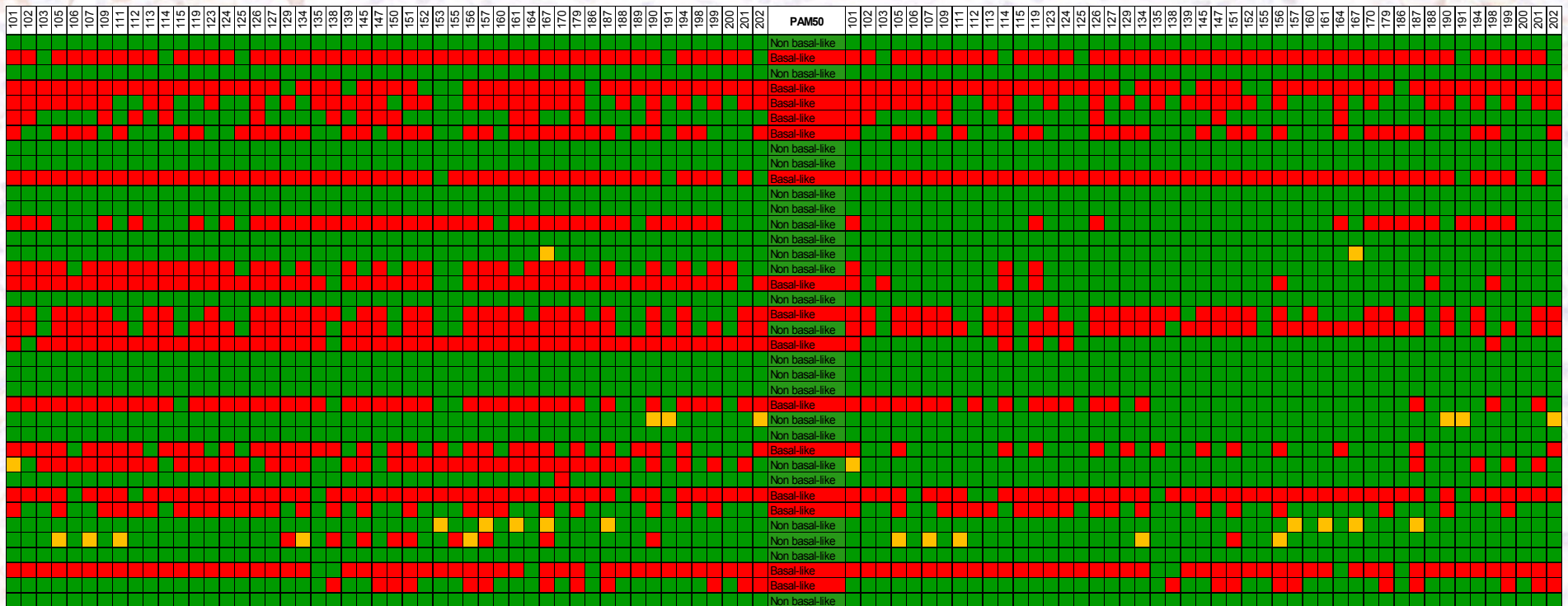
*SENSITIVITY: 27% to 100%*

*SPECIFICITY: 76% to 100%*

Based on surrogate  
basal-like immunopanel

*SENSITIVITY: 13% to 93%*

*SPECIFICITY: 86% to 100%*



■ Case defined as basal-like

■ Case defined as non basal-like

■ Case Missing



# Run 27 Independent Review

## GPEC Review

- Stained slides were independently evaluated to assess interobserver variability and its influence on surrogate immunopanel interpretation.

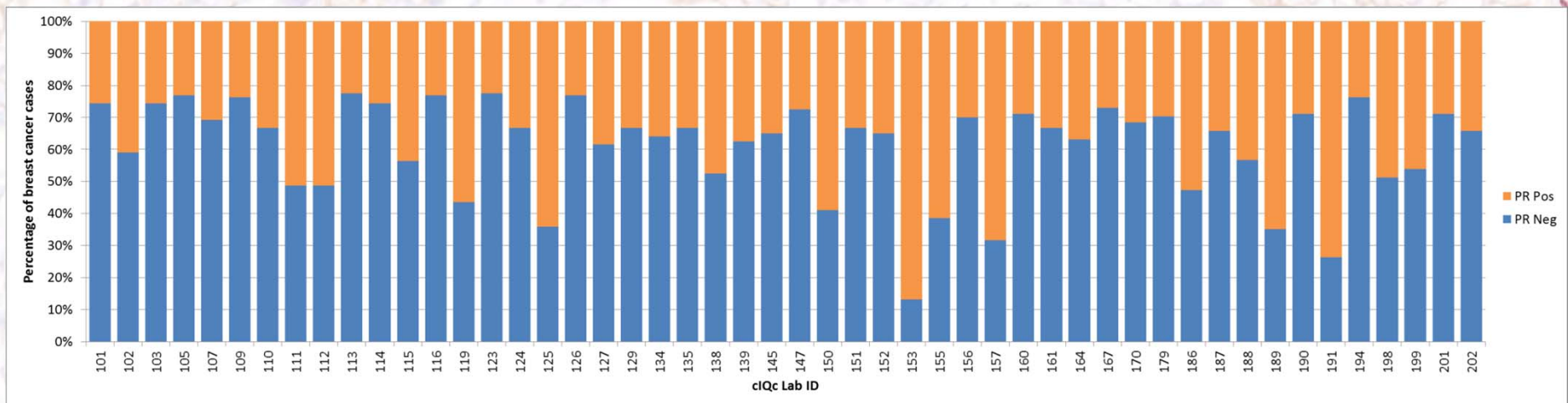
## Results of Review

- Sensitivities and specificities were similar to self-assessments.
- Discordance in staining interpretation of individual positive basal-like biomarkers (i.e. EGFR, CK5, etc.) was evident.
- PR staining had surprisingly high interlaboratory variability.



# Interlaboratory variability of PR immunohistochemistry

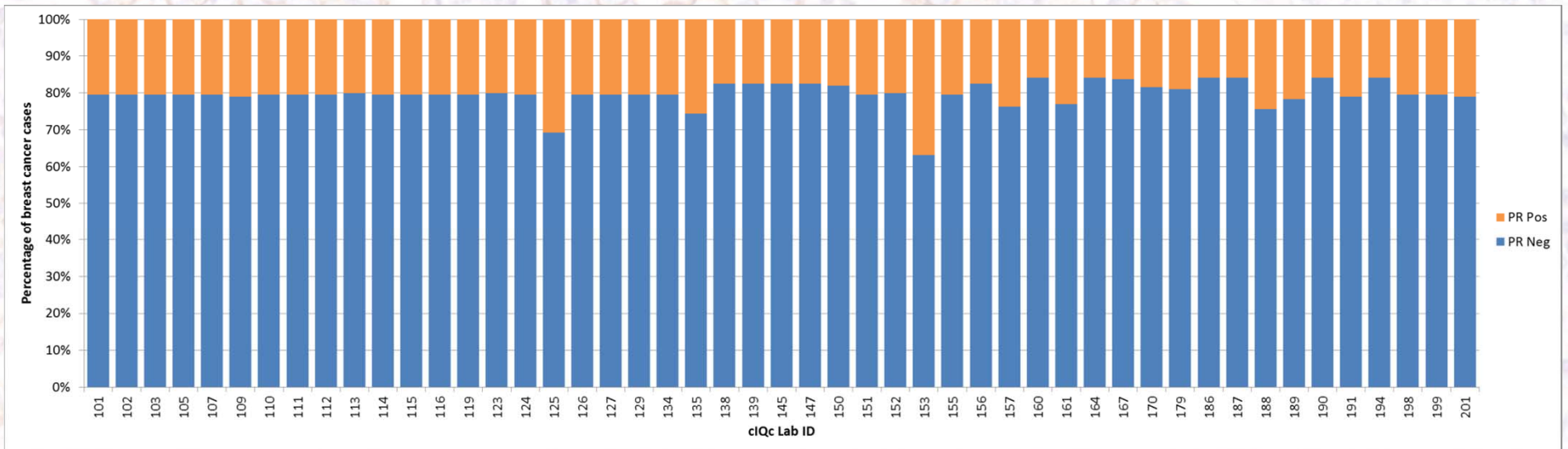
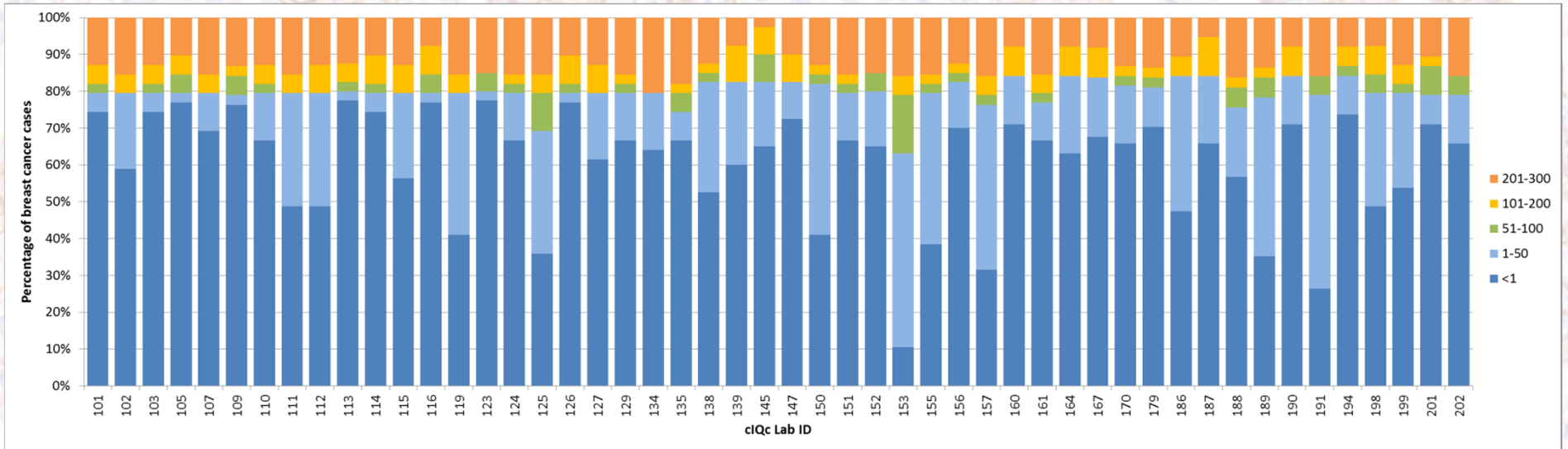
- Stacked column chart of negative vs positive PR at a cutpoint of 1%:



- Sensitivities ranged from **88-100%** (mean: 98%)
- Specificities ranged from **14-97%** (mean: 76%)



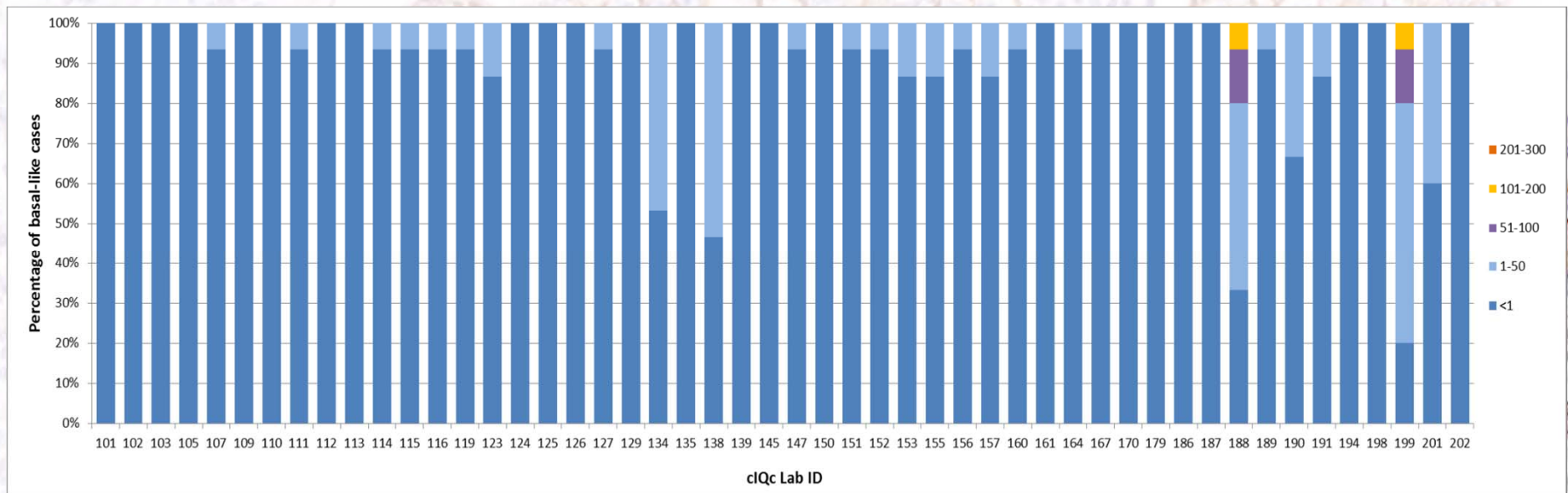
- Stacked column charts of PR staining based on H-score:





# Variable expression of ER in basal-like breast cancer

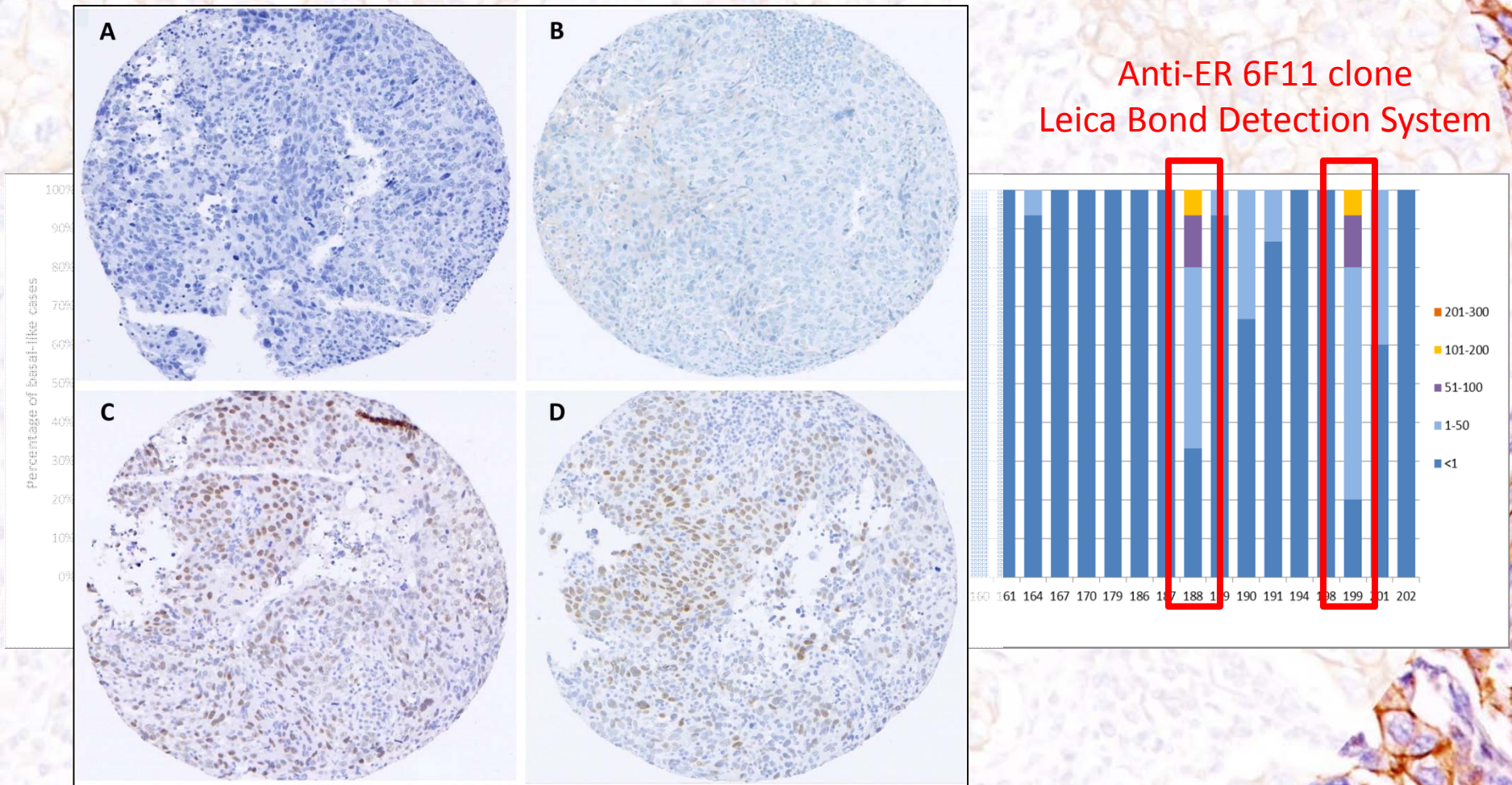
- Estrogen receptor (ER) is a standard biomarker used for breast cancer patient management.
- Most ER IHC assays in hospital diagnostic laboratories are technically optimized and validated.





# Variable expression of ER in basal-like breast cancer

Anti-ER 6F11 clone  
Leica Bond Detection System





## Run 27 Conclusions

clQc Run 27 provided the first evaluation of basal-like breast cancer diagnostics in general pathology laboratories.

Current surrogate immunopanel are specific, but lack sensitivity.

Individual IHC biomarkers and surrogate immunopanel for basal-like breast cancer have considerable staining variability in clinical laboratories.

A triple negative is **NOT** a triple negative in every anatomic pathology laboratory. Efforts to optimize staining and interpretation of basal-like immunopanel are necessary.



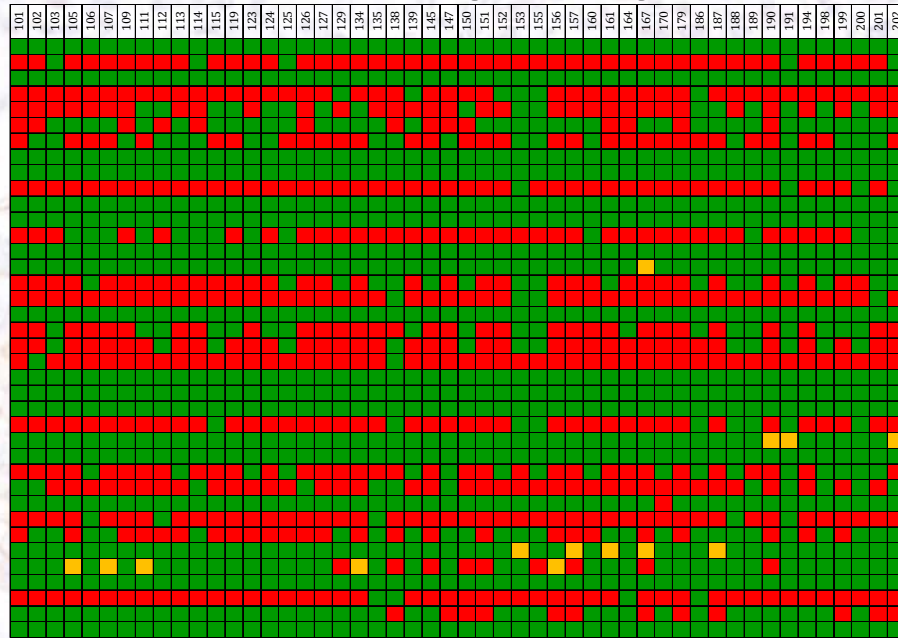
# The fruits of our labour

- Through continued participation in proficiency testing schemes, laboratories strive to achieve and maintain reproducibility with a high level of accuracy.
- For IHC, use of standardized technical protocols and platforms is an impractical and unrealistic goal.
- cIQc proficiency testing schemes are designed to facilitate optimal use of different technical protocols and platforms, but all the while yielding similar and consistent IHC results.

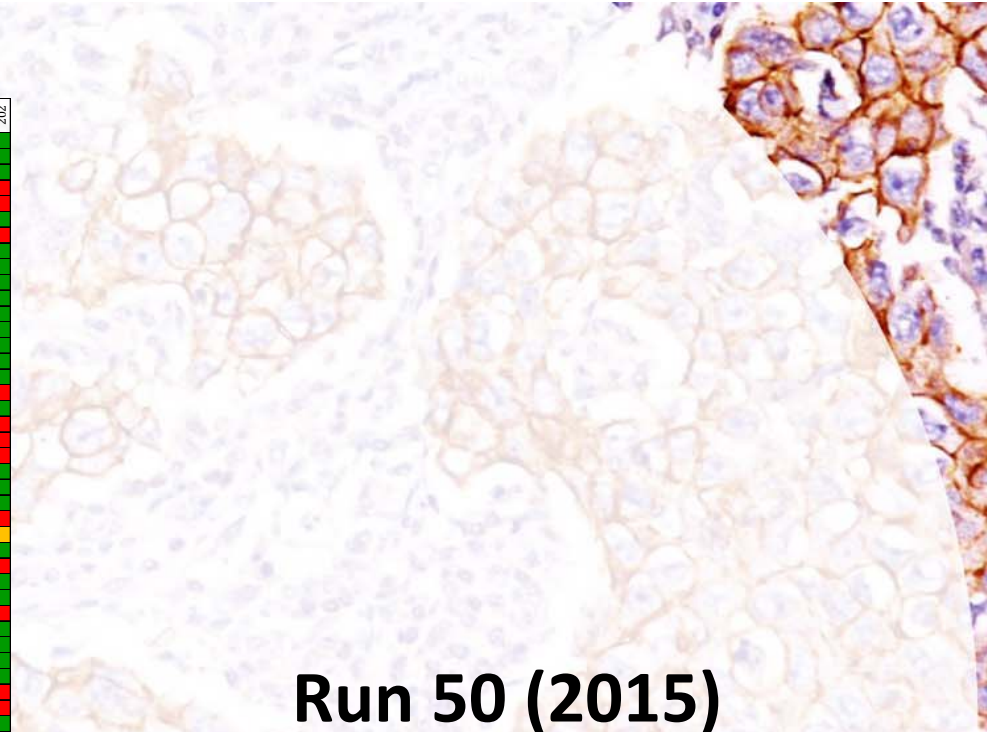
***What has continued participation in ER/PR/HER2 proficiency testing done for anatomic pathology laboratories?***



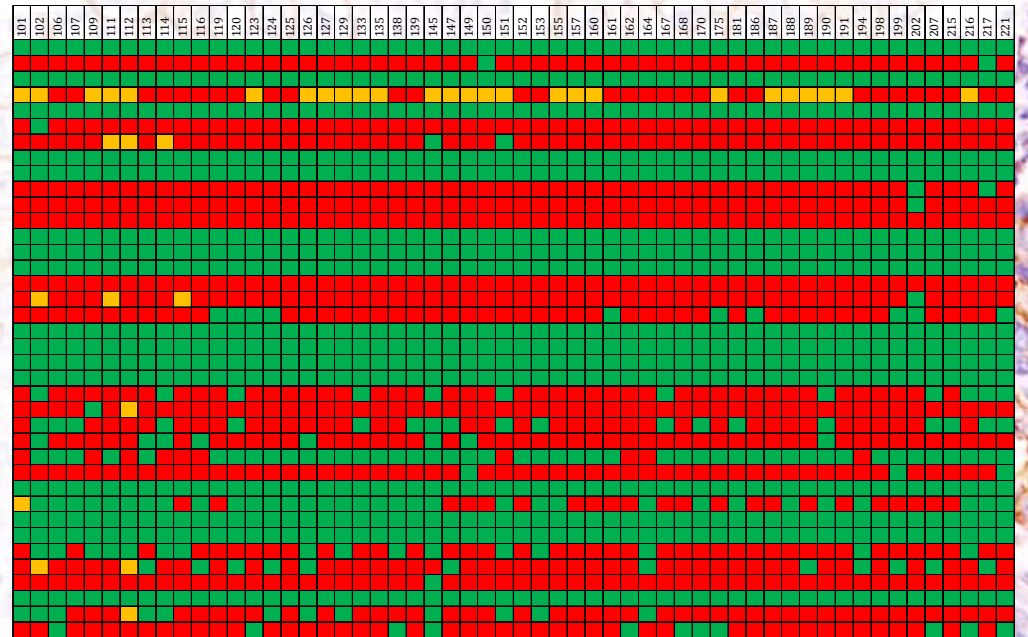
# Run 27 (2012)



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# Run 50 (2015)



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# Acknowledgements

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# Acknowledgments

## The cIQc team

- Dr. Blake Gilks, Director cIQc
- Dr. Emina Torlakovic, Director cIQc
- Mr. John Garratt, Manager cIQc

## The CMPT team



## Genetic Pathology Evaluation Centre (GPEC)

- Dr. Torsten Nielsen
- Dr. Dongxia Gao
- Dr. Zuzana Kos
- GPEC personnel