

True Quantitative Calibrators/Reference Materials for IHC Testing;  
Novel Tools That Are Re-defining IHC Methodology

clQC 2018 Symposium

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## Presentation Summary

- IHC Errors: Examples and Causes
- IHC Calibrators

## Disclosures

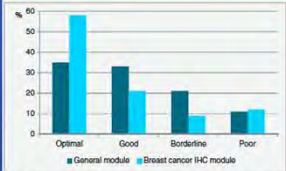
- Funded by National Cancer Institute, NIH
  - R44CA183203
  - R44CA213476
  - R44CA106847
  - R44CA094557
- Patent/ownership interest in the technology (MDP)

## IHC Error Rate

External proficiency testing (PT) programmes . . . have demonstrated a general truth that, year after year, there are about one-third of laboratory participants that achieve optimal results, while one-third are 'good' and one-third fail.

Torlakovic E, Nielsen S, Vyberg M, Taylor CR. Getting controls under control: the time is now for immunohistochemistry. *J Clin Pathol*. 2015;68(11):879-82.

## IHC Error Rate



Assessment Score	General module (%)	Breast cancer IHC module (%)
Optimal	~35	~55
Good	~30	~20
Borderline	~20	~10
Poor	~10	~10

- Cumulative experience 2003 – 2015
- 700 laboratories, 80 countries

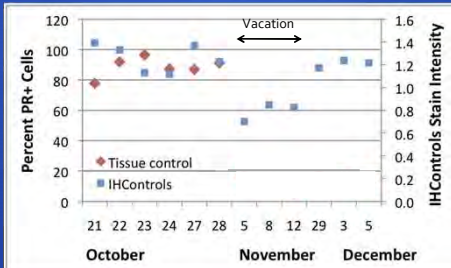
Fig 1 Proportion of assessment scores applied to more than 20,000 assays in the general module and more than 9000 assays in the breast cancer IHC module

M Vyberg & S Nielsen. Proficiency testing in immunohistochemistry—experiences from Nordic Immunohistochemical Quality Control (NordIQC). *Virchows Arch* (2016) 468:19–29

## IHC Errors

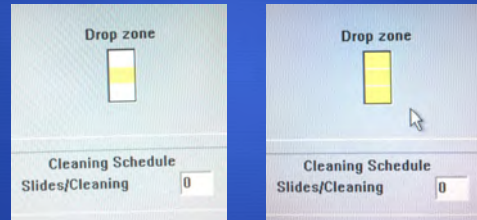
- IHC Error Rate compared to other clinical laboratory disciplines
- IHC errors can sometimes be difficult to anticipate and prevent

## Case #1



KVani, et. al., *Appl Immunohistochem Mol Morphol* 2016 24(10):688-694.

## Instrument Has User-Specified Drop Zones



## Case #2

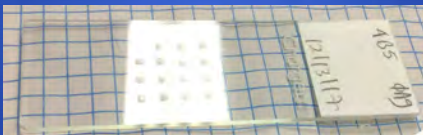
- Boston-area hospital with weaker than expected HER-2 immunostain.
- Below peer group for that primary antibody
- Insufficient reagent volume dispense

## Case #3

- Failed HER2 PT challenge on only one core
- Controls stained appropriately

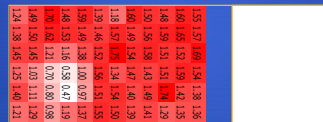
## Re-creating the Problem With An IHControl Array

IHControl spot placed in each square on the slide: 12 x 6 array.



Reflected light from overhead fluorescent fixture, highlighting IHControl spots.

## IHControl Stain Intensity Array Heat Map



Immunostain intensity

## Manufacturer: Label Placement Is Important



## Root Cause Analysis

- Pre-analytic process control
- cGMP reagents
- Automation (analytic process control)
- Continuous accurate feedback

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## Root Cause Analysis

- Pre-analytic process control
- cGMP reagents
- Automation (analytic process control)
- Continuous accurate feedback
  - Reference standards
  - Calibrators
  - Standardized controls
  - Traceable Units of Measure

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## Clinical Chemistry Reference Standards



### NIST Standard Reference Materials (SRM):

- |                                      |                                       |
|--------------------------------------|---------------------------------------|
| 1. SRM 911c Cholesterol              | 18. SRM 965b Glucose in serum         |
| 2. SRM 912a Urea                     | 19. SRM 967a Creatinine in serum      |
| 3. SRM 913b Uric acid                | 20. SRM 968f Fat-soluble vitamins     |
| 4. SRM 914a Creatinine               | 21. SRM 971 Hormones in serum         |
| 5. SRM 915b Calcium                  | 22. SRM 972a Vitamin D                |
| 6. SRM 917c D-glucose                | 23. SRM 998 Angiotensin               |
| 7. SRM 918a Hemoglobin               |                                       |
| 8. SRM 919a Hematocrit               |                                       |
| 9. SRM 920a Hematocrit               |                                       |
| 10. SRM 921a Hematocrit              |                                       |
| 11. SRM 922a Hematocrit              |                                       |
| 12. SRM 923a Hematocrit              |                                       |
| 13. SRM 929a Magnesium gluconate     | 27. SRM 2389 Amino acids              |
| 14. SRM 937 Iron metal               | 28. SRM 2389 Amino acids              |
| 15. SRM 955c Toxic elements in blood | 29. SRM 2670a Toxic elements in urine |
|                                      | 30. SRM 2921 Cardiac troponin         |



### WHO INTERNATIONAL REFERENCE MATERIALS

- 1. SRM 918a Hemoglobin
- 2. SRM 919a Hematocrit
- 3. SRM 920a Hematocrit
- 4. SRM 921a Hematocrit
- 5. SRM 922a Hematocrit
- 6. SRM 923a Hematocrit
- 7. SRM 924a Hematocrit
- 8. SRM 925a Hematocrit
- 9. SRM 926a Hematocrit
- 10. SRM 927a Hematocrit
- 11. SRM 928a Hematocrit
- 12. SRM 929a Magnesium gluconate
- 13. SRM 930a Magnesium gluconate
- 14. SRM 931a Magnesium gluconate
- 15. SRM 932a Magnesium gluconate
- 16. SRM 933a Magnesium gluconate
- 17. SRM 934a Magnesium gluconate
- 18. SRM 935a Magnesium gluconate
- 19. SRM 936a Magnesium gluconate
- 20. SRM 937 Iron metal
- 21. SRM 938 Iron metal
- 22. SRM 939 Iron metal
- 23. SRM 940 Iron metal
- 24. SRM 941 Iron metal
- 25. SRM 942 Iron metal
- 26. SRM 943 Iron metal
- 27. SRM 944 Iron metal
- 28. SRM 2389 Amino acids
- 29. SRM 2670a Toxic elements in urine
- 30. SRM 2921 Cardiac troponin



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## Importance Of Reference Standards

"The reliability of analytical results from clinical laboratories has been questioned by many individuals, and several independent surveys have more than justified the suspicion."

Journal of  
Clinical Pathology

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## Importance Of Reference Standards

"In these surveys, blame has been placed on poor supervision of personnel, poorly trained and insufficient personnel, poor equipment, poor choice of methods available, and so on.

It is the purpose of this paper to discuss the importance of running routine standards . . . ."

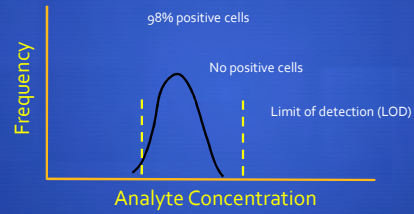
RJ Henry and M Segalove. Running of standards in clinical chemistry and the use of the control chart. *J Clin. Path.* 1952; 5:305-311.

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## Goals

- Standards
- Calibrators
- Standardized controls
- Traceable units of measure

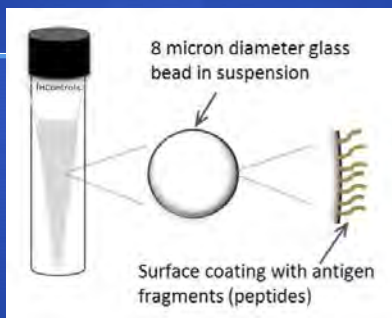
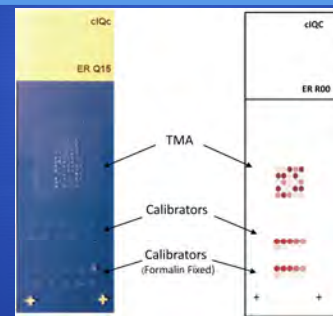
## Importance of Immunostain Calibration



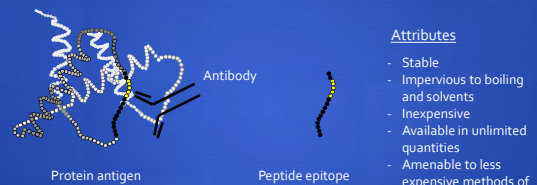
## Presentation Summary

- IHC Errors: Examples and Causes
- • IHC Calibrators

## IHC ER Calibrators



## Peptide Epitopes In Lieu of Proteins



Sompuram, SR, et al., Antibodies immunoreactive with formalin-fixed tissue antigens recognize linear protein epitopes. *Am J Clin Pathol* 2006 125(3):82-90.  
 Sompuram, SR, et al., A molecular model of antigen retrieval using a peptide array. *Am J Clin Pathol* 2006 125(3):91-98.

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10/2/2008

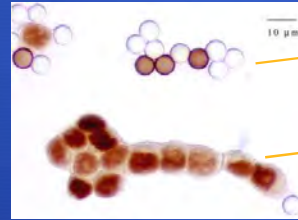
## Calibrators Pipetted Onto Slide



They stay on the slide through deparaffinization, AgR, and immunostaining.

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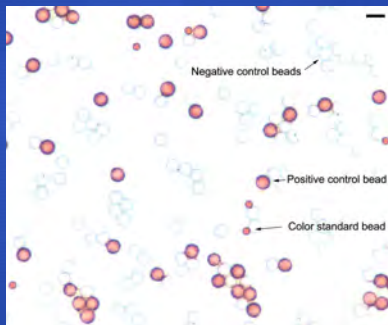
## Microbeads Size Comparison



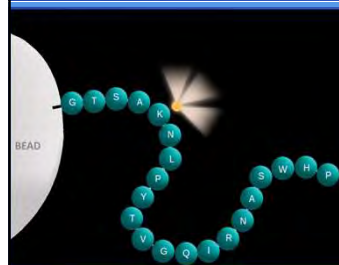
Some microbeads coated with other antigen

MCF-7 grown in culture

Built-in color comparisons



## Traceability of Analyte Concentration

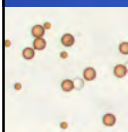


Traceability of concentration through a fluorescent tag

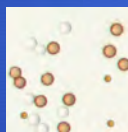
Vani, K, et al., Analytic Ranges Of Commercial Breast Cancer IHC Tests: Quantification With IHCcontrols. *J Histochem Cytochem.* 2021;65(5):273-83.

## Measuring Limit of Detection

1D5 mAb Immunostain



934,651  
MEF/microbead



77,913  
MEF/microbead

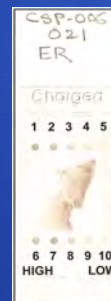


8,187  
MEF/microbead



1,331  
MEF/microbead

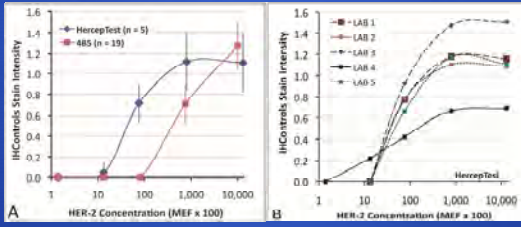
## IHC Proficiency Test Survey



- Each participating laboratory received 3 slides (HER-2, ER, & PR).
- 39 participating laboratories
- Slides evaluated by pathologist and also returned for central evaluation (image analysis).
- Data: LOD and image quantification

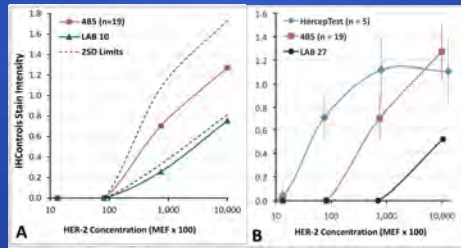
Sompuram, SR, et al., Quantitative Assessment of IHC Laboratory Performance Using IHCcontrols. *Arch Pathol Lab Med* In press.

## HER-2 Consensus Response Curves



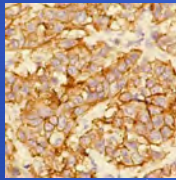
Sompuram, SR, et al., Quantitative Assessment of IHC Laboratory Performance Using *IHCControls*. *Arch Pathol Lab Med* 2018 142(7):851-862 .

## HER-2 Survey Outliers

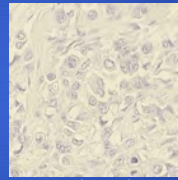


Sompuram, SR, et al., Quantitative Assessment of IHC Laboratory Performance Using *IHCControls*. *Arch Pathol Lab Med* 2018 142(7):851-862 .

## HER2 Tissue Staining



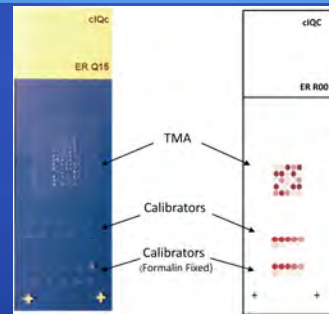
Comparison Lab



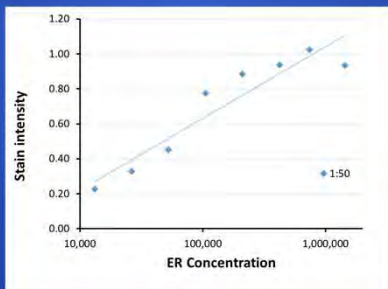
Outlier Lab

SR Sompuram et al., Quantitative Assessment of Immunohistochemistry Laboratory Performances by Measuring Analytic Response Curves and Limits of Detection. *Arch. Pathol. Lab. Med.* 2018 142(7):851-862 .

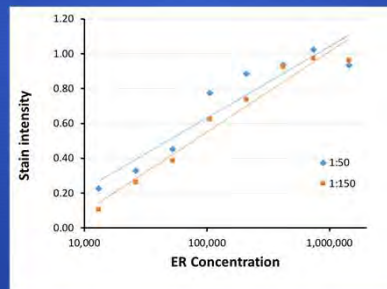
## IHC ER Calibrators



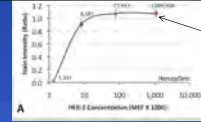
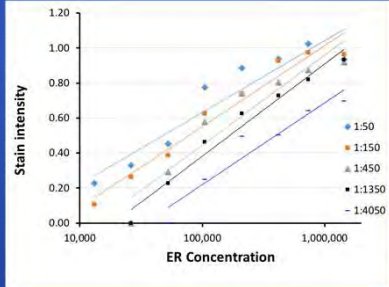
## ER SP1 Analytic Response Curve



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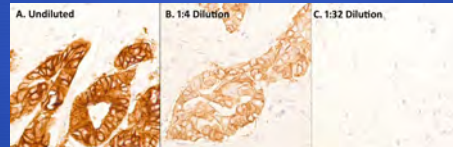
## HercepTest Analytic Response Curve

Vani, K, et. al., The importance of epitope density in selecting a sensitive positive IHC control. *J Histochem Cytochem* 2017 65(8):463-477.

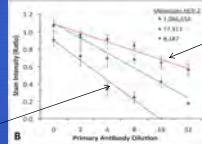
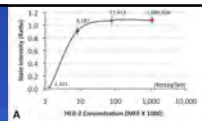
## Immunostain Failure Simulation



## Immunostain Failure Simulation



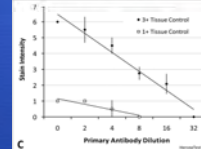
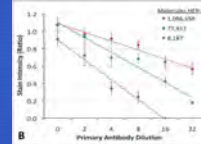
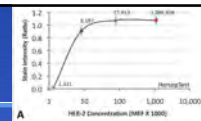
3+



Most sensitive in revealing 1° antibody degradation

Worsening primary antibody failure →

Vani, K, et. al., The importance of epitope density in selecting a sensitive positive IHC control. *J Histochem Cytochem* 2017 65(8):463-477.



Vani, K, et. al., The importance of epitope density in selecting a sensitive positive IHC control. *J Histochem Cytochem* 2017 65(8):463-477.

## Guidelines: Use Low/Interm Concentration

- CAP ANP22550: QC – Antibodies  
“... Ideally, positive control tissues possess **low levels of antigen expression**, ... use of ... tissues that have high levels of antigen expression may result in ... false-negative results.”
- CLSI I/LA28-A2  
“Optimal tissue controls should contain an **intermediate level of the analyte** in reference to the dynamic range of the assay. Early reagent degradation may occur without detection if only strongly positive controls are used.” p.73

## Conclusions

- Sources of error can be hard to predict.
- Importance of **continuous accurate feedback**:
  - Reference standards, calibrators, standardized controls and traceable units of measure
- New IHC controls and calibrators
- For controls, “accurate” feedback requires using the lowest concentration that yields easily visible staining.

## Closing Remarks

- Use of calibrators for calibration verification during immunostain validation
- Composition of controls & calibrators
- Rapidly expanding menu, funded by National Cancer Institute

## Acknowledgments



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Dr. Monika Pilichowska  
Dr. Jeffrey D Goldsmith  
Dr. Kueikwun G Chang

Prof. Brian Tracey [Image analysis](#)

Ms. Drorit Bogen [Admin.](#)