Quality Control/Quality Assurance in Diagnostic Immunohistochemistry

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- **Academic Credentials:**
  - 1986  M.D. Zagreb Medical School, Zagreb, Croatia
  - 1989  Epidemiology Degree, School of Public Health, Zagreb Medical School, Zagreb, Croatia
  - 2005  Ph.D. University of Oslo, Medical Faculty, Oslo, Norway

- **Training in Pathology:**
  - 1989-1991  Anatomic/Clinical Pathology Resident, St. Luke’s/Roosevelt Hospital, Columbia University, NYC, NY, USA.
  - 1991-1993  Anatomic/Clinical Pathology Resident, University of Minnesota Hospital, Minneapolis, MN, USA.
  - 1993-1994  Hematopathology Fellow, Department of Laboratory Medicine and Pathology, Division of Special Hematology, University of Minnesota Hospital, Minneapolis, MN, USA.
  - 1994-1996  Surgical Pathology Fellow, Division of Surgical Pathology, University of Minnesota Hospital, Minneapolis, MN, USA.

- **Other Credentials:**
  - Special Licentiate  Medical Council of Canada, January 2004 – present
  - Diplomate  American Board of Pathology, Hematology, 2000 – present
  - Special Licentiate  Medical Council of Norway, January 1998 – present
  - Diplomate  American Board of Pathology, Anatomic and Clinical Pathology, 1996 – present
  - Licentiate  Minnesota Board of Medical Practice, MN, USA, 1994 – present
  - FLEX  USA, 1991
  - ECFMG  USA, 1988
  - Licentiate  Medical Council of Croatia, 1988 - present
Immunohistochemistry: Personal Background

- Director of Immunohistochemistry
  - 1997-2003 Department of Pathology, The Norwegian Radium Hospital, Oslo, Norway
  - 2004-Present Department of Pathology and Laboratory Medicine, College of Medicine, University of Saskatchewan
- NordiQC Core Group
  - 1999-2004 Norwegian representative
  - 2004-Present External Contributor (review articles)
- Canadian Immunohistochemistry Quality Control (cIQC)
  - 2005-Present
- Chair, CAP National Standards Committee/Immunohistochemistry
  - 2007-Present
- Leader, European Bone Marrow Working Group Immunohistochemistry Committee (introducing standardization for bone marrow IHC for all European countries)
- Member, ASCO/CAP ER/PR Expert Panel,
- Publications:
  - 34/64 of my articles in PubMed are searchable under "torlakovic"+"immunohistochemistry"
  - Bone Marrow Immunohistochemistry, book published by ASCP Press 2008
- Lectures:
  - Many invited lectures in USA, Europe, Canada

Objectives

1. History of QC in IHC (USA)
2. Terminology and definitions
3. NordiQC program
4. Elements of QC in IHC
5. Clinical significance
6. Specific challenges in IHC QC
7. Status in Canada
History

- 1989 – NIH Workshop on IHC Standardization
- 1991 – Biological Stain Commission (BSC) Established IHC Steering Committee
- 1992 – BSC and FDA Publish Proposed Format for Package Inserts of IHC Products
- 1996 – Proposed IHC and ASR Regulations Published
- 1998 – Final IHC and ASR Regulations and IHC Guidance Document Published

History

- 1994 – FDA Panel meeting to recommend classification of IHC devices
- 1995 – Draft IHC Guidance Issued
- 1996 – FDA Panel Meeting to recommend regulation of Analyte Specific Reagents (ASRs)
Extralaboratory Quality Assurance (EQA)

- **UKNEQAS** (1968, 1990) UK
- **CAP** (1949, 1961, 2003, 2006) USA
- **NordiQC** (1999/2003) Scandinavia
- **cIQCc** (2006) Canada
- Other regional/provincial programs (Finland, Ontario, BC)

The Role of Medical Laboratories in Patients’ Care

- Dr. J. Butany:
  “Canada’s medical laboratory system is the foundation upon which good patient care, diagnosis and treatment rest.”
What is Immunohistochemistry?

- Application of immunoassay in tissue sections.
- Immunological localization of the protein of interest in its natural environment.
- Simultaneous evaluation of morphology and staining of the localized protein provide very complex information.
- The intensity of signal may or may not represent the real quantity of the protein in tissue.

Class I

- Class I IHC tests provide adjunctive diagnostic information not independently reported to clinical physicians.
- They are used after the tumor is diagnosed by other methods and are used only by pathologists.
- E.g. cytokeratin, vimentin, CD45, and other differentiation markers
Class II

- So-called “stand alone” test that are reported independently of other clinical or laboratory information.
- The results of these tests are used as either predictive or prognostic markers and are often critically relied upon to stratify patients for appropriate therapies.
- The tests are accepted as such after widely accepted valid scientific claims. National and international guidelines for these tests are usually published.
- E.g. hormone receptors in breast cancer.

Classification of IHC Tests

- Class I, class II, class III
- Qualitative, quantitative
- Test - drug combo vs. all other tests

- Panels (undifferentiated tumor panel, melanoma panel) in which non-specific tests when used together are considered highly specific vs. single specific test used in appropriate context has high specificity (ALK-1, CD117)
PARAFFIN SECTION IMMUNOPHENOTYPING OF HEMATOPOIETIC MALIGNANCIES

- Non-hematopoietic Neoplasm or Langerhans’ Cell Histiocytosis
  (Positivity for CD1a)
- Hodgkin’s Disease vs. Large-cell Non-Hodgkin’s ML
- Technically-inadequate Specimen
- Probable Sarcoma
- 1-cell Lymphoma
- Granulocytic Sarcoma
- Non-Hodgkin’s ML, Not Otherwise Specified
- “True Histiocytic” Neoplasm

IMMUNOHISTOCHEMICAL DIAGNOSIS OF SMALL-CELL TUMORS

- PNET with divergent myogenic differentiation
- Neuroendocrine carcinoma (primary & secondary)
- Small-cell adeno-carcinoma
- Small-cell squamous CA
- Malignant lymphoma or leukemia
- Malignant melanoma
- Rhabdomyosarcoma
- Primitive neuro-ectodermal tumor (PNET)
- PNET (or metastatic neuroblastoma in children)
Class II IHC tests

• Despite the need for finely tuned calibration and quantitative nature of the tests, they are usually reported simply as positive or negative.
• The simplicity of the report masks the true biological and technical complexity of the testing.

Specificity and Sensitivity of IHC tests

• Classical definition given by Galen & Gambino:
  \[ \text{Spec} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}} \]
• Specificity, sensitivity, and concordance with reference laboratory are usually not reported for IHC tests.
• “Specificity” of IHC reagents must be evaluated in well-defined contexts. Hence, “specificity” is a relative term in this applied clinical setting.
• There is no reason not to report on sensitivity, concordance, and kappa-values in relation to reference laboratory values.
Standards and Optimization

- True standardization in IHC is not possible because standard controls for daily QC programs are not available.
- Cell culture positive controls are currently the closest to what standardized controls for breast Ca markers need, but they are very expensive and cannot fully replace tissue controls at the moment. More studies are needed to truly validate this type of controls for clinical practice.

NordiQC Results with Cell Culture Positive Controls

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Optimal</th>
<th>Good</th>
<th>Borderline</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal</td>
<td>37</td>
<td>1</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Good</td>
<td>4</td>
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<td>Borderline</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

http://www.nordiqc.org/Run-23-B5/Assessment/Assessment-HER-2.htm
Use of Cell Lines as Positive Controls: Results and Conclusion

1. An insufficient (false negative) reaction in the breast ductal carcinoma no. 3 in combination with an optimal staining of the cell lines. This was seen in 13/17 cases.
2. A sufficient staining in the histological specimens in combination with an insufficient staining of the cell lines due to impaired morphology of the cell lines, probably as a results of excessive retrieval.
3. These data indicate that histological specimens should be preferred for EQA of HER-2. However, due to potential heterogeneity of tissue material, cell cultures may be valuable as a supplement.

http://www.nordiqc.org/Run-23-B5/Assessment/Assessment-HER-2.htm

Main Conclusions Regarding Standardization

- No standardized positive controls – No standardization.
- Standardization of protocols is meaningless without control standardization.
- Standardization of positive controls also includes agreement or standardization of expected results in control tissues.
- “Standardization” is greatly misused term in this context.
- Standardization is possible only if there are so-called “gold standards” for reference values.
### ER NordiQC Pass Rates

http://www.nordiqc.org

<table>
<thead>
<tr>
<th>NordiQC</th>
<th>Participants (N)</th>
<th>Sufficient Results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run 8 2003</td>
<td>71</td>
<td>45</td>
</tr>
<tr>
<td>Run 10 2004</td>
<td>77</td>
<td>67</td>
</tr>
<tr>
<td>Run 13 2005</td>
<td>89</td>
<td>84</td>
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<td>Run B1 2006</td>
<td>68</td>
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<tr>
<td>Run B3 2007</td>
<td>73</td>
<td>84</td>
</tr>
<tr>
<td>Run B5 2008</td>
<td>107</td>
<td>79</td>
</tr>
</tbody>
</table>

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### VIMENTIN

![VIMENTIN](Image)
S-100

S-100
### USE OF IHC FOR CLINICAL PURPOSES: Class I Tests

Good results with one test may cover the failure of the other tests; however, this is not possible for Class II tests.

<table>
<thead>
<tr>
<th></th>
<th>LAB A</th>
<th>LAB B</th>
<th>LAB C</th>
<th>LAB D</th>
<th>LAB E</th>
<th>LAB F</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIM</td>
<td>++</td>
<td>++++</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
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<tr>
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<td>++</td>
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<tr>
<td>HMB-45</td>
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<td>++++</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>NA</td>
</tr>
<tr>
<td>MELAN-A</td>
<td>+</td>
<td>++++</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

- Pi
- P
- F
- Pc
- Pi
- F
NordiQC Assessment: Assessments by Experts are Critical

Run 5 - 10: 23 different epitopes
Run 8, 9 & 10  n: 382 insufficient staining:

False negative:
1. Too dilute primary ab. conc.  127 (33%)
2. Inappropriate primary ab.  51 (13%)
3. Insufficient HIER  94 (25%)
4. Inappropriate epitope retrieval  54 (14%)
5. Unexplained  22 ( 6%)

False positive:
1. Too high primary ab. conc.  7 ( 2%)
2. Inappropriate primary ab.  14 ( 4%)
3. Excessive retrieval  1 (<1%)
4. Unspecific reaction of the detection system  10 ( 3%)
5. Unexplained  2 (<1%)

European Bone Marrow Working Group
EBMWG: Survey Results

- 95% believes that their quality control system is good, but only 65% achieved clinically acceptable results.

- Nevertheless, 89% believes that external quality control system is necessary.
What do we want to optimize or standardize?

- METHODS - Not necessarily!
- RESULTS - Obligatory!

How to standardize results?

- First step:
  - Standardization of what is considered "optimal result", based on current standard of practice.
  - Each laboratory should consider that standardization of tissue processing would make it easier to standardize results.
Risks to Health

- Based on the results obtained with the IHC diagnostic test to the patient may result from:
  - misdiagnosis and initiation of inappropriate therapies or
  - withholding of appropriate therapies

- The degree of risk depends on whether the product is used as an adjunct to conventional histopathological diagnostic techniques or provides information that is used independently of the usual diagnostic process.

- The highest risk products are those used as independent, stand-alone diagnostic tests that are the sole or major determinant for a medical decision and cannot be confirmed by conventional histopathologic techniques or other diagnostic tests or clinical procedures.
FDA is focused on whether this level of regulation is adequate for the protection of public health

- FDA is aware that variability in IHC results may be introduced at every step:
  - Collection and fixation of the specimen,
  - Automated processing,
  - Embedding and sectioning,
  - Staining of the final slide preparation, and
  - Microscopic interpretation by the pathologist.

FDA counts on:

Ongoing initiatives by professional organizations and manufacturers directed at ensuring that pre- and postanalytic, as well as analytic procedures, are properly performed.

That there is clear distinction in laboratory practices regarding Class I and Class II tests in regard quality control/quality assurance measures by the laboratories.
NHL

CD20 and/or Pax-5

CD34-positive mononuclear cells

4%

11%

21%
Metastatic breast carcinoma

QC/QA in IHC in Canada

• No national standards for diagnostic IHC.

• No fully established national program for extralaboratory quality assurance in diagnostic IHC.

• No national body to evaluate current practices.

• No national accreditation body to ensure compliance with national standards.
QC/QA in IHC in Canada

- No national list of diagnostic laboratories that perform the IHC testing for patients’ care.
  - Not able to contact laboratories for surveys.
  - Not able to determine the extent of problem.
  - No insight how far we are from standardization.
  - No information to plan the size or other components of the national program needed for standardization and EQA.

- Many, if not most Canadian laboratories take participation in programs provided by USA (CAP), Scandinavia (NordiQC), and UK (UKNEQAS). These programs are not the same and they do not provide the same information to the laboratories.

- Recent initiative from the Canadian Association of Pathologists:
  - National Standards Committee/Immunohistochemistry

Canadian Immunohistochemistry Quality Control

- [www.clQc.ca](http://www.clQc.ca)
- RUN1: Undifferentiated tumor panel
- RUN2: ER/PR and HER2/neu
- RUN3: ER/PR
- 12 labs in RUN1, 18 in RUN2, 23 in RUN3
- No funding so far.
- Provides extensive feedback to participating laboratories, who can use this information to improve results immediately.
- The program is adequate to fulfill the criteria for mandatory certification.
- The program provides testing material adequate for sensible statistical analyses currently recommended in new guidelines for class II tests (e.i. HER2).
The CAP Five-Point Plan

1. Mandatory certification for each prognostic and predictive test performed by a medical laboratory;
2. An external validation system where test results from one laboratory would be verified by another, independent external laboratory (external quality assurance program);
3. Dissemination and use of the Canadian National Checklists for Diagnostic IHC.
4. Creation of a national body, separate from government, to accredit all medical laboratories in Canada and ensure they need quality and critical mass standards;
5. Immediate and ongoing support from federal, provincial, and territorial governments to address the critical workforce and resource shortages undermining laboratory medicine.

In brief, the CAP is calling for an appropriately resourced national system to promote excellence in the laboratory medicine in Canada. Canadian laboratories are not unique in facing workloads, human-resource issue, or problems related to quality control. Canada is lacking a national quality assurance program to link laboratories, provide support and administer national standards.