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 – Draft Compliance Policy Guidance Issued: “Commercialization of IVD’s for Research or Investigational Use Only
- 1998 – Final IHC and ASR Regulations and IHC Guidance Document Published

History

- 1993 – First issue of Compliance Policy Guideline for “Commercialization of Unapproved IVD Services Labeled for Research and Investigation
- 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
• **cIQc** (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

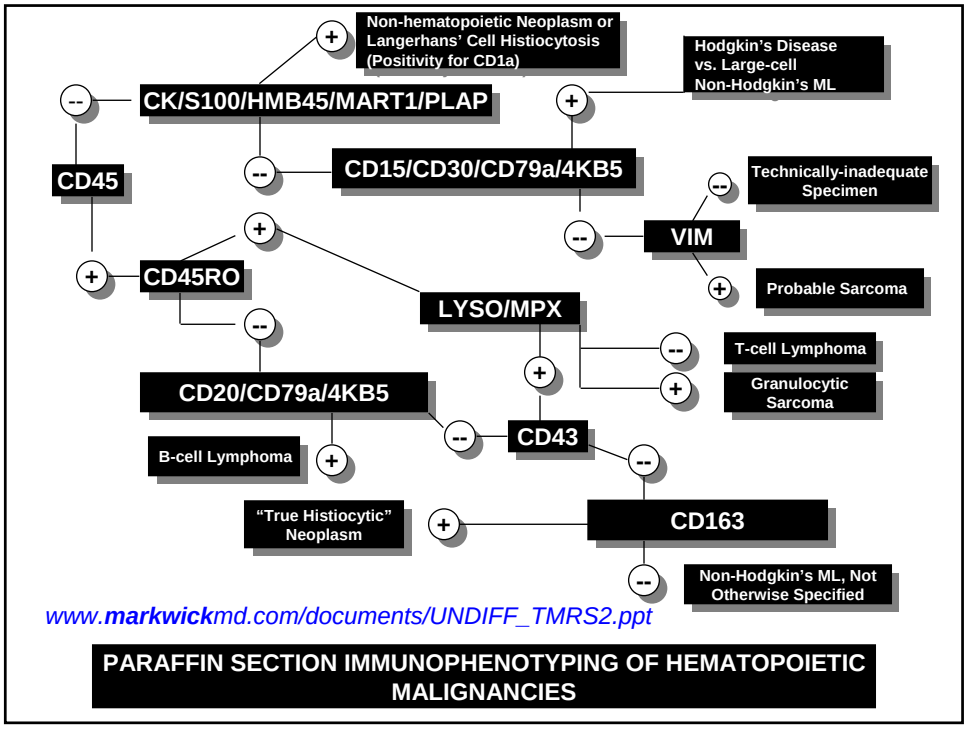
Class II

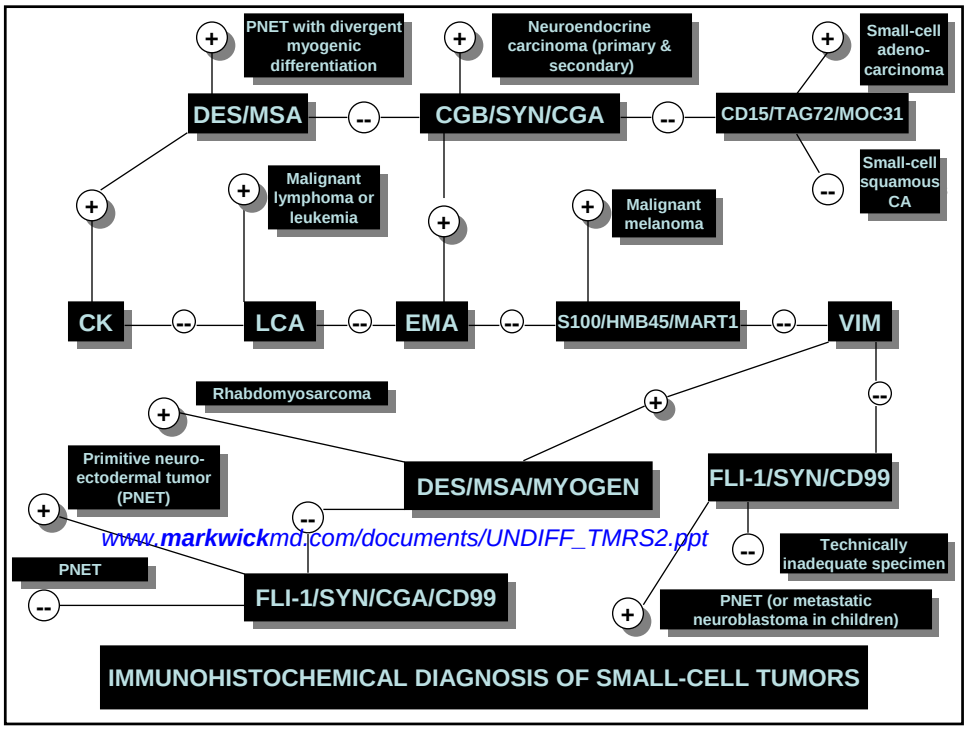
- So-called “stand alone” test that are reported independently of other clinical or laboratory information.
- The results of these tests are used as either predictive or prognostic markers and are often critically relied upon to stratify patients for appropriate therapies.
- The tests are accepted as 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 (undifferentiatiated 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)


Non-hematopoietic Neoplasm or Langerhans' Cell Histiocytosis (Positivity for CD1a)
Hodgkin's Disease vs. Large-cell Non-Hodgkin's ML
CK/S100/HMB45/MART1/PLAP
CD45
CD15/CD30/CD79a/4KB5
Technically-inadequate Specimen
VIM
Probable Sarcoma
CD45RO
LYSO/MPX
T-cell Lymphoma
Granulocytic Sarcoma
CD20/CD79a/4KB5
CD43
B-cell Lymphoma
"True Histiocytic" Neoplasm
CD163
Non-Hodgkin's ML, Not Otherwise Specified
www.markwickmd.com/documents/UNDIFF_TMRS2.ppt
PARAFFIN SECTION IMMUNOPHENOTYPING OF HEMATOPOIETIC MALIGNANCIES


PNET with divergent myogenic differentiation
Neuroendocrine carcinoma (primary & secondary)
Small-cell adeno-carcinoma
DES/MSA
CGB/SYN/CGA
CD15/TAG72/MOC31
Small-cell squamous CA
Malignant lymphoma or leukemia
Malignant melanoma
CK
LCA
EMA
S100/HMB45/MART1
VIM
Rhabdomyosarcoma
Primitive neuro-ectodermal tumor (PNET)
DES/MSA/MYOGEN
FLI-1/SYN/CD99
www.markwickmd.com/documents/UNDIFF_TMRS2.ppt
Technically inadequate specimen
PNET
FLI-1/SYN/CGA/CD99
PNET (or metastatic neuroblastoma in children)
IMMUNOHISTOCHEMICAL DIAGNOSIS OF SMALL-CELL TUMORS

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:

  Spec = True negatives/True negatives + 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

	Tissues as Pos. Controls			
Cell Lines	**Optimal**	**Good**	**Borderline**	**Poor**
Optimal	37	1	0	12
Good	4	1	0	1
Borderline	3	0	1	8
Poor	0	1	0	11

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

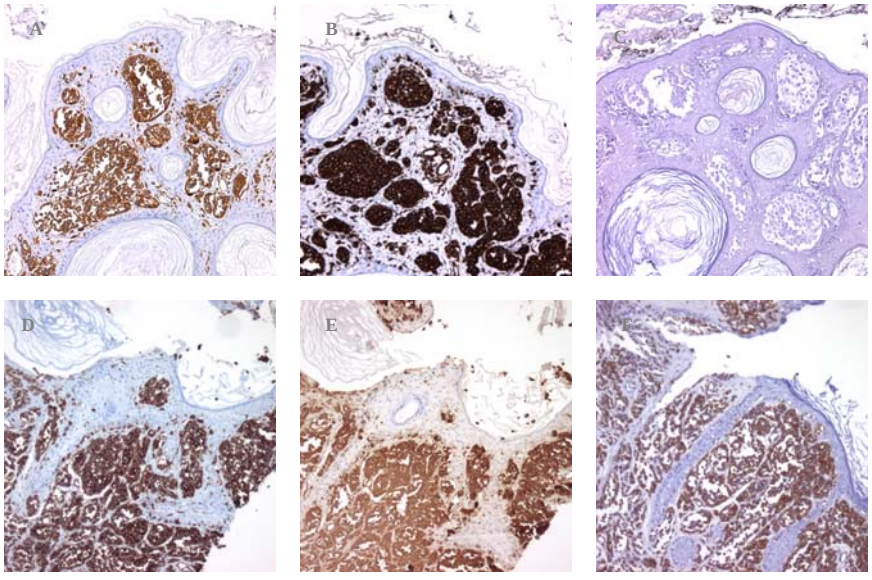
- No standardized positive controls – No standardization.
- Standardization of protocols is meaningless without control standardization.
- Standardization of positive controls also includes agreement or standardization of expected results in control tissues.
- “Standardization” is greatly misused term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

ER NordiQC Pass Rates

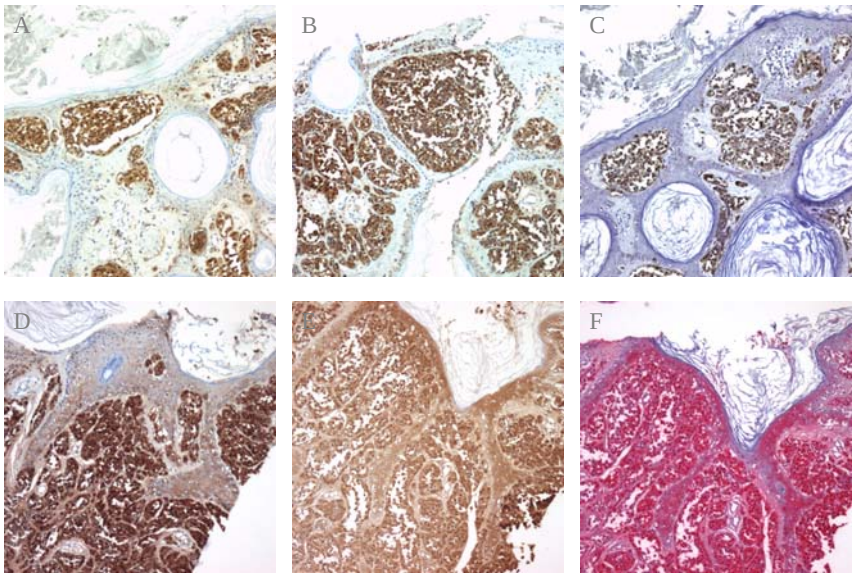
http://www.nordiqc.org

NordiQC	Participants (N)	Sufficient Results (%)
Run 8 2003	71	45
Run 10 2004	77	67
Run 13 2005	89	84
Run B1 2006	68	75
Run B3 2007	73	84
Run B5 2008	107	79

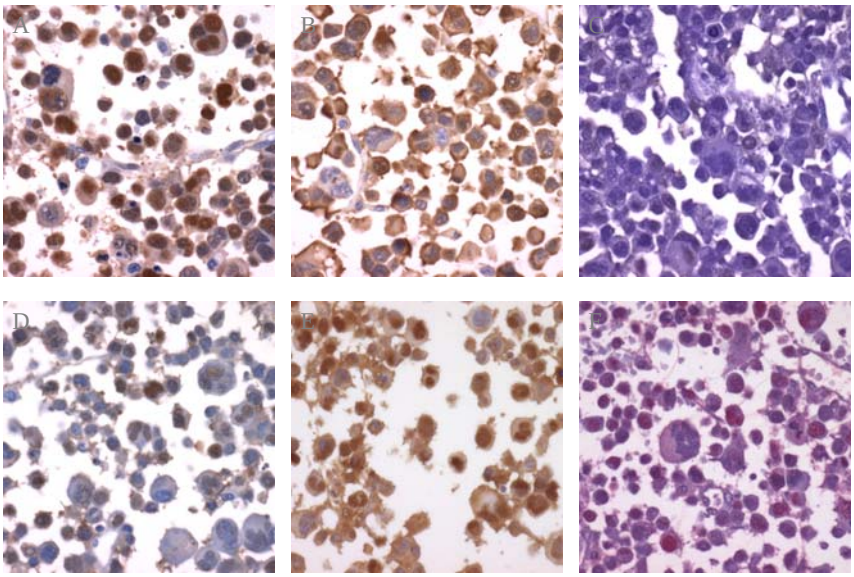
VIMENTIN



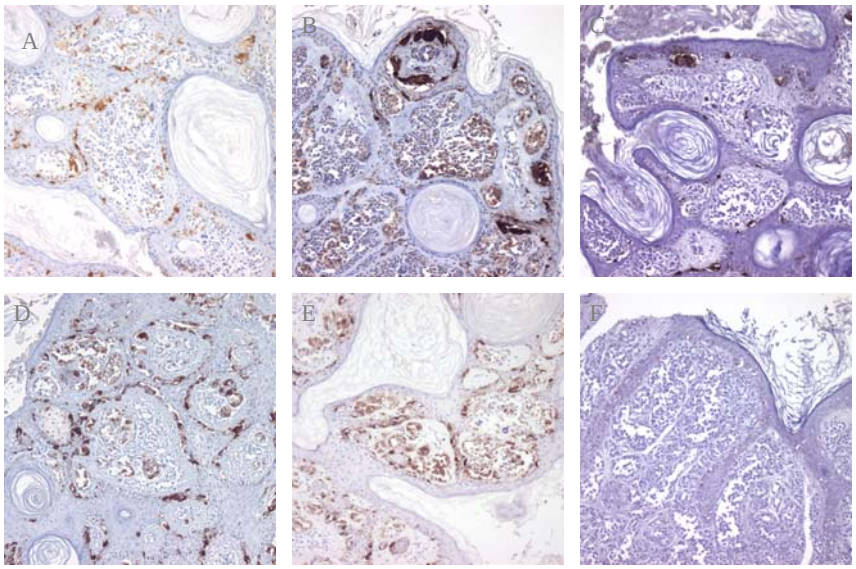
S-100



S-100



HMB-45



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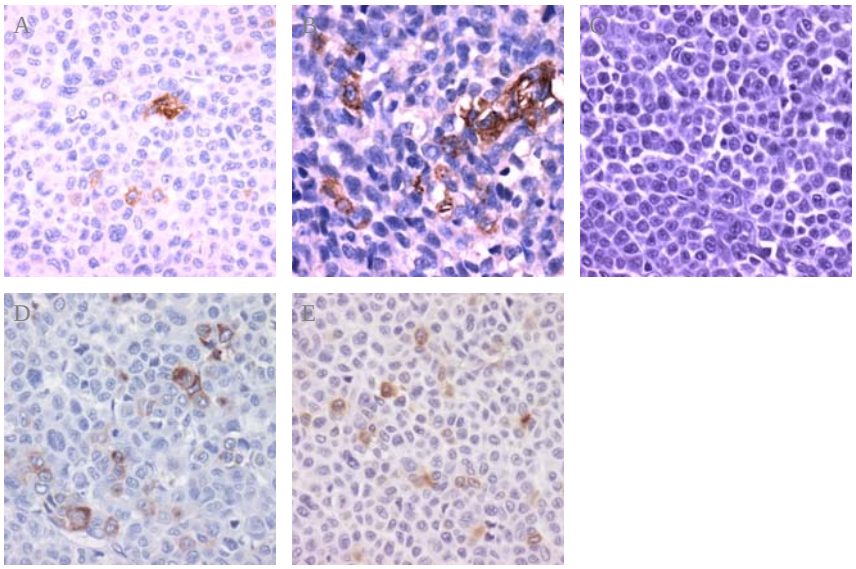
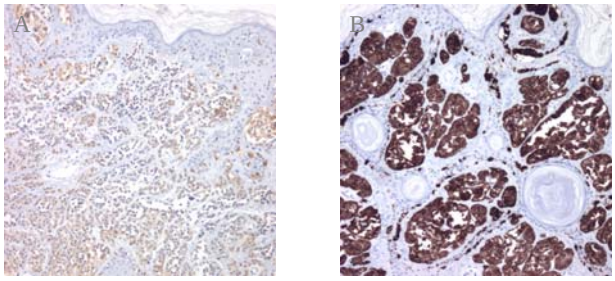


MELAN-A

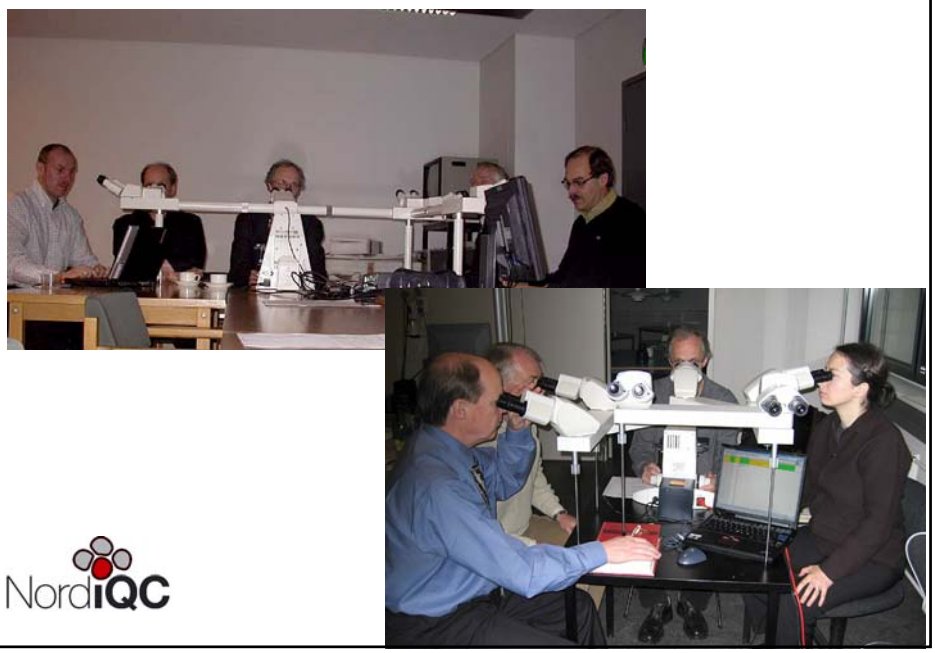


USE OF IHC FOR CLINICAL PURPOSES: Class I Tests

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

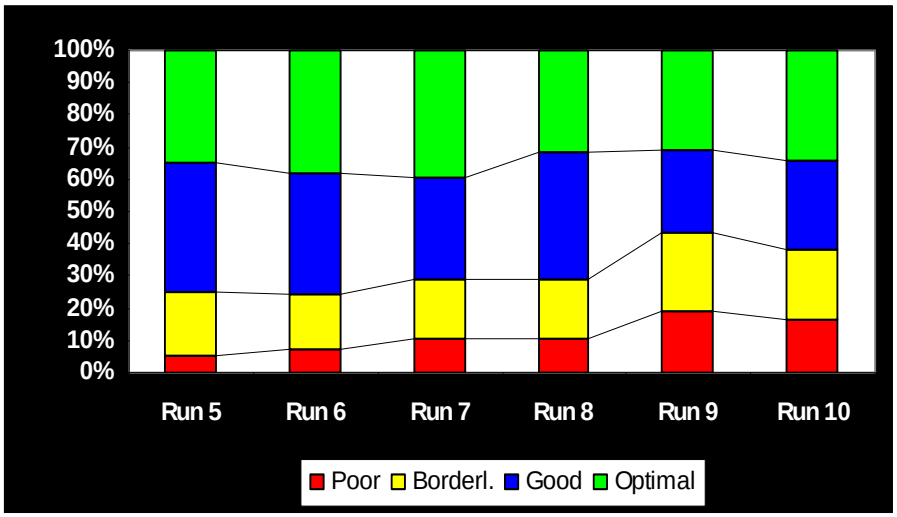
	LAB A	LAB B	LAB C	LAB D	LAB E	LAB F
VIM	++	++++	-	+++	++	+++
S-100	++++	+++	+	++	+++	++
HMB-45	+++	++++	+	++++	++++	NA
MELAN-A	+	++++	NA	NA	NA	NA
	Pi	**P**	**F**	**Pc**	**Pi**	**F**

NordiQC Assessment: Assessments by Experts are Critical



NordiQC

Run 5 - 10: 23 different epitopes



NordiQC

NordiQC

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%)**

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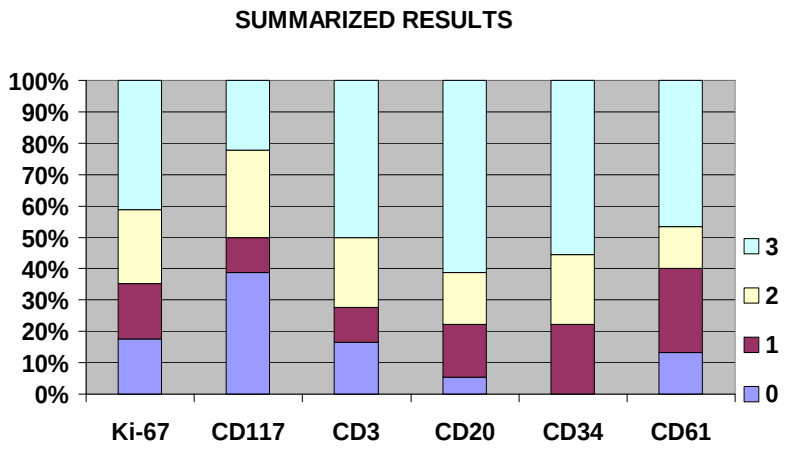
European Bone Marrow Working Group



Center	Ki-67	CD117	CD3	CD61	CD34	CD20	Suboptimal/Poor	Total
1	1	1	3	1	1	1	5	6
2	1	0	2	2	1	2	6	6
3	1	3	3	3	3	3	1	6
4	3	1	3	3	2	3	1	6
5	2	2	2	2	3	2	0	6
6	2	3	2	3	3	3	0	6
7	0	0	0	.	3	3	3	5
8	3	2	3	3	3	3	0	6
9	3	0	3	0	2	3	2	6
10	3	2	0	1	3	3	2	6
11	2	2	2	.	3	3	0	5
12	0	0	1	.	2	2	3	5
13	3	0	3	1	1	1	4	6
14	0	3	3	0	3	3	2	6
15	.	0	0	1	1	0	5	5
16	2	2	3	3	3	3	0	6
17	3	3	1	3	3	1	2	6
18	3	0	3	3	2	3	1	6
Total							35.50%	104

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.

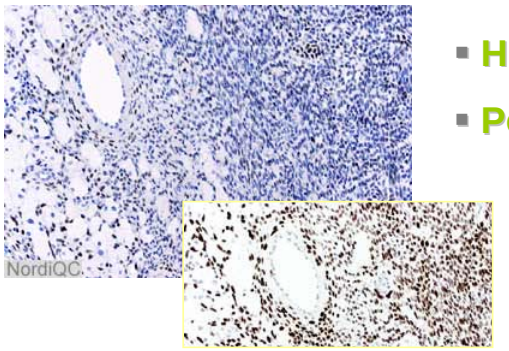
What do we want to optimize or standardize?

- METHODS - Not necessarily!
- RESULTS - Obligatory!

How to standardize results?

- Fist 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.

Marker	ASMA	CD15	EMA	ER	PR
Assessment:	Not received	Poor	Poor	Good	Poor
Comments to the protocol:	-	False neg.	False neg.	-	False neg.
Suggestions for improvement:	-	Use HIER and consider change of primary ab.	Increase primary ab. conc.	-	Optimize HIER (use TE pH 9) and/or increase primary ab. conc.



- 1A6 1:50
- HIER Ci pH 6 20 min
- Polymer based

NordiQC

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.

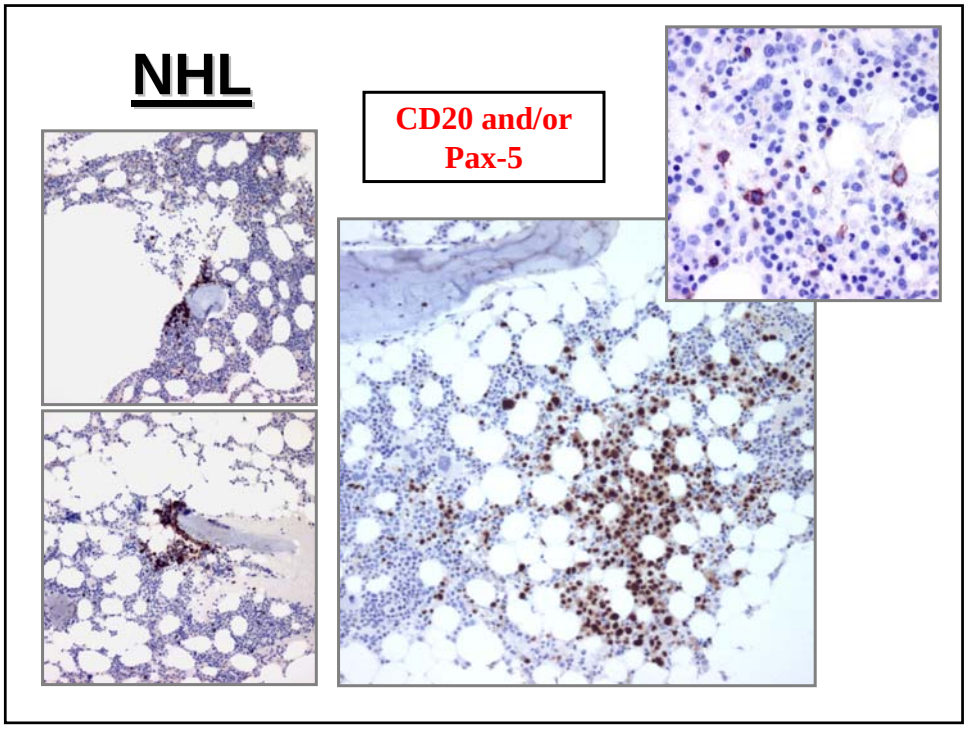
FDA is focused on whether this level of regulation is adequate for the protection of public health

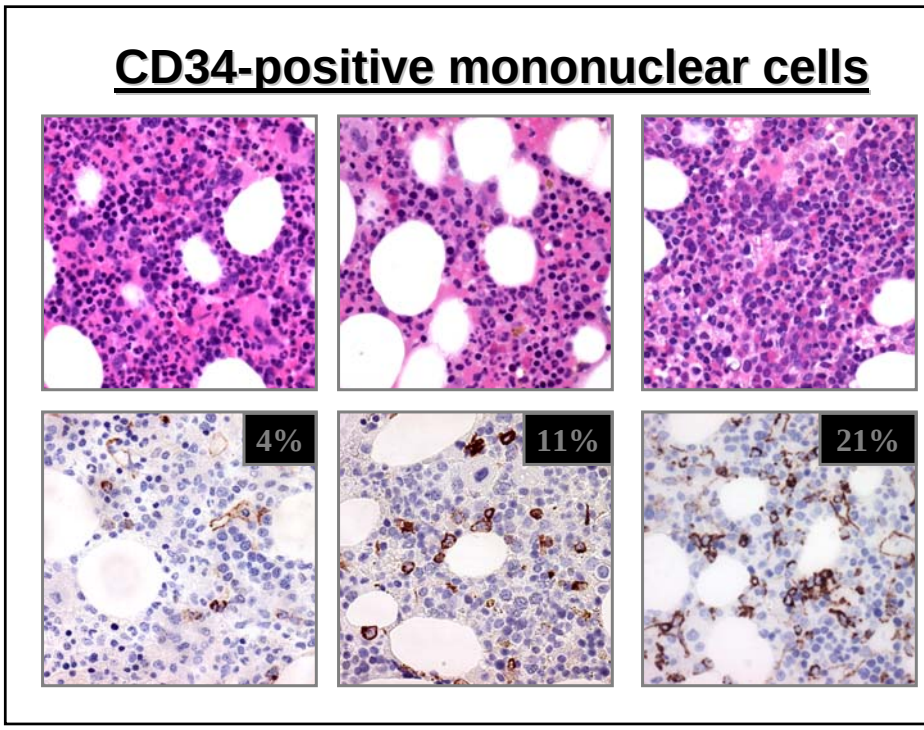
- **FDA** is aware that variability in IHC results may be introduced at every step:
  - Collection and fixation of the specimen,
  - Automated processing,
  - Embedding and sectioning,
  - Staining of the final slide preparation, and
  - Microscopic interpretation by the pathologist.

FDA counts on:

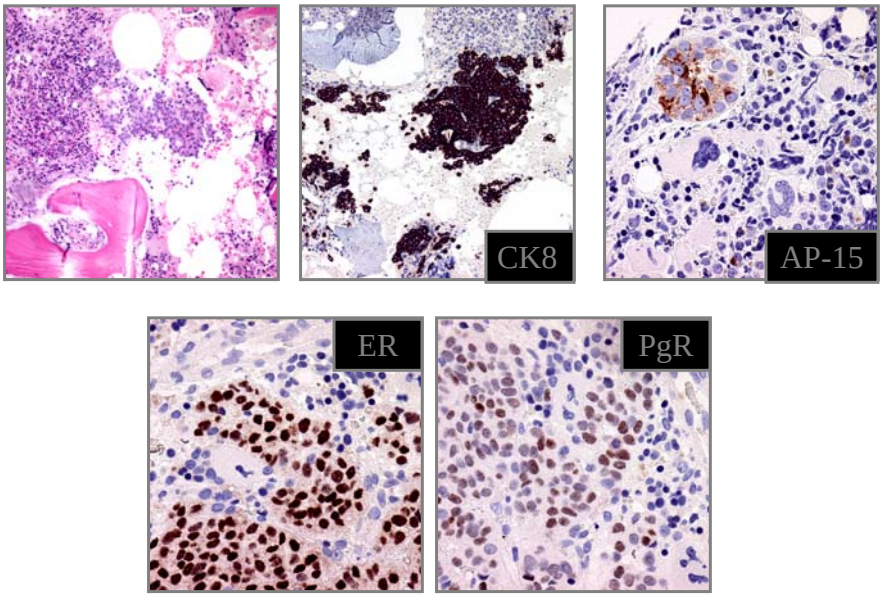
Ongoing initiatives by professional organizations and manufacturers directed at ensuring that pre- and postanalytic, as well as analytic procedures, are properly performed.

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.

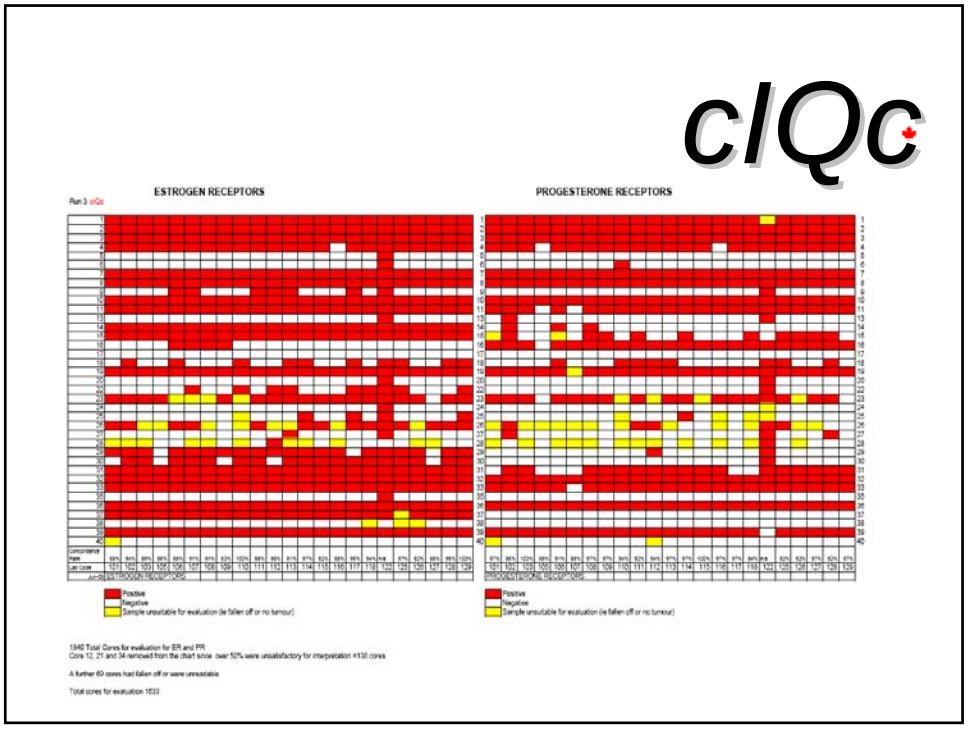
QC/QA in IHC in Canada

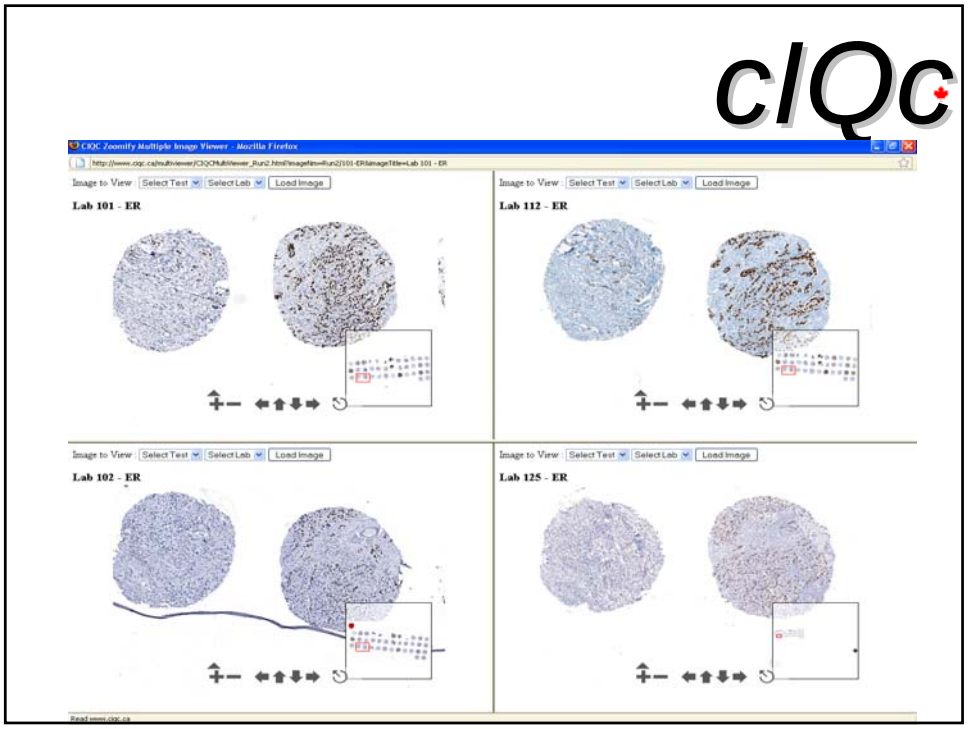
- No national list of diagnostic laboratories that preform the IHC testing for patients' care.
  - Not able to contact laboratories for surveys.
  - Not able to determine the extent of problem.
  - No insight how far we are from standardization.
  - No information to plan the size or other components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

clQc

- 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 fulfil 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).


clQc
ESTROGEN RECEPTORS
PROGESTERONE RECEPTORS
Positive
Negative
Sample unsuitable for evaluation (ie fallen off or no tumour)
1840 Total Cores for evaluation for ER and PR
A further 60 cores had fallen off or were unreadable
Total cores for evaluation 1033


clQc
CIQC Zoomify Multiple Image Viewer - Mozilla Firefox
Image to View :
Select Test
Select Lab
Load Image
Lab 101 - ER
Lab 112 - ER
Lab 102 - ER
Lab 125 - ER

cIQc

Lab Code	Ag Retrieval	Instrument	Temp	Time	Buffer	Prim Ab Clone	Supplier	Dilution	Diluent Type	Incubation Time	Detection System	Detection System Name	Supplier	Incubation Time	Enhancement	Type of Enhancement
101	Yes	Ventana XT	90	30 min	CC1	SP1	Labvision	1:50	Ventana	32 min	iView DAB		Ventana			
102	Yes	Dako	100	13 min + 20 min cool down	1mM EDTA pH 9	SP1	Labvision	1:30	Dako Ab Dil	30 min RT		Envision +	Dako	30 min RT	Yes	$CuSO_4$
103	Yes	Ventana Benchmark	42	60 min	CC1	SP1	Ventana	Predilluted		8 min	iView	LSAB	Ventana	N/A	No	
105	Yes	Ventana Discovery XT	100	Standard	CC1 (Cat# 950-124)	SP1	Labvision (Cat# RM 9101-S0)	1:50	Labvision (Cat# TA 125-UC)	60 min	LSAB	DAB MAP	Ventana	Standard Ventana Protocol	No	
106	Yes	MicroMed T/Tmega	115	3 min	0.1M TRIS HCl pH9	GFII	Vector	1:75	Dako Flex	60 min RT	Vectastain Elite ABC	Vectastain	Vector	30 min		
108	Yes	Biocare Medical	98	8 min	Dako Target Retrieval System	SP1	Labvision/Neo Markers	1:200	Dako Ab diluent	30 min	HRP-DAB	Envision + Rabbit polymeric HRP system	Dako	30 min	No	
109	Yes	Biocare Decloaker	125	1 min	low pH Reveal-Biocare	GFII	Vector	1:80	Zymed	1 hour		Histo +	Zymed	link= 10 min, label = 10 min	No	
110	Yes	Ventana ES	Unknown	19:45 min	Citrate Buffer	SP1	Ventana	Predilluted		28 min		AEC detection	Ventana	Unknown	No	
111	Yes	Benchmark XT	95	30 min	CC1 (mild)	SP1	Ventana	Predilluted	N/A	32 min		Ultraview	Ventana		Yes	Copper Sulfate
112	Yes	Benchmark from Ventana	95-100	30 min	CC1 TRIS buffer pH 8 Ventana	SP1	Ventana (Cat#760-4325)	Predilluted		20 min	Multimer based detection system	Ultraview universal DAB Detection kit	Ventana (Cat# 760-500)			
113	Yes	Panasonic Microwave	100	20 min + 20 min cool down	Citrate pH 6	GFII	Ventana	N/A	N/A	32 min		iView	Ventana	As per Ventana protocols	Yes	Copper Sulfate
114	Yes	Ventana Benchmark XT	90	4 min	2x SSC	SP1	Labvision	1:50	Ventana	32 min		XT, iView, DAB	Ventana		Yes	Copper
115	Yes	Ventana Benchmark XT	95	30 min	CC1 Solution	SP1	Ventana	Predilluted		32 min		iView DAB Detection kit	Ventana		Yes	Copper
116	Yes	Benchmark XT or LT Ventana	95-100	30 min	CC1 (EDTA)	SP1	Neomarkers for Labvision Corp	1:100	Antibody Diluent (Ventana)	48 min 37° C	Phosphatase Red	Ultraview Universal Alkaline	Ventana	32 min (total)	No	
117	Yes	Benchmark	According to Protocol	According to Protocol CC1 long	CC1 Ventana	SP1	Ventana	No	No	32 min	iView DAB	iView DAB detection kit	Ventana	According to Protocol		
118	Yes	Discovery XT Ventana	Max 100	Standard	Cell Conditioning 1	SP1	Ventana medical Systems	Ready-to-use		32 min, 37° C		DAB-MAP	Ventana Medical Systems		Yes	DAB MAP Copper Sulfate Solution
122	Yes	Biocare Decloaking Chamber	120	30 sec	Dako Target Retrieval Sol/A pH 9	GFII	Biocare	Predilute		30 min RT	Polymer	MAch4	Biocare	10/10/5 (Mouse probe/HRP polymer/DAB)		
125	Yes	Ventana XT	95-100	30 min	CC1	SP1	Ventana	Predilute		16 min	DAB	Ultraview	Ventana		No	
127	Yes	Panasonic Microwave 1000 Watts	60 % Power	7 min/ 7 min/ 6 min, 20 min cool down	EDTA pH 8 ± 0.05	SP1	Ventana	Predilute		32 min	Biotin/Streptavidin iView Det Kit	iView Det	Ventana	As per detection kit (approx 1 1/2 hours) total	No	
128	Yes	Ventana Benchmark XT	95	30 min	CC1	SP1	Ventana	Predilute	Ready-to-use	32 min @ 37° C	DAB	iView	Ventana	As per Ventana protocols	Yes	Copper
129	Yes	Vision Biosystems Bond Max	100	20 min	Bond Max ER2	GFII	LEICA									

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.