Report on
IMMUNOPEROXIDIASE TRAINING
JEWISH GENERAL HOSPITAL
MONTREAL, QUEBEC
JANUARY 16 – 27, 2006

Prepared For:

Immuno-Pathology Department
Eastern Health Corporation
Health Sciences Centre
St. John's, NL

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REPORT

Immunoperoxidiase Training
Jewish General Hospital, Montreal, Quebec
January 16 –27, 2006

Prepared for:
Immuno-Pathology Department
Health Sciences Centre

Arrived at the Jewish General Hospital, Montreal on Monday, January 16th, 2006 and met with the Pathology Supervisor. I was then taken on a tour of the hospital and orientated in the Pathology Lab.

In our initial discussion Edward and I felt that even though my prime focus would be immunoperoxidiase some time devoted to routine pathology would be beneficial. I could observe the overall lab operation from tissue receiving to slide distribution to the Pathologist.

My first day was spent in tissue receiving. Surgical specimens were delivered to The lab, sorted, numbered and assigned to a pathologist using the following processing criteria:

Biopsy
Dermatology
Gynecology
Placenta
Surgical

Notable differences from our laboratory:

Gross attendants gross using the above classification and will call the pathologist only for clarification purposes. The JGH tend to use a specialty system for specimen division. JGH sort and fill their processing baskets by color code and classification and are embedded and cut, stained and sorted accordingly. They generate a master gross list to check off blocks and to help with embedding. There is no scraping of blocks as the embedders do not use excess wax. They add 5 ml of alcoholic eosin to the 2nd alcohol on the tissue processor to facilitate easier embedding and orientation. (white tissue/ white wax/ white lens paper).

They use Histogell (Richard Allen) for processing fine needle aspirations, minute

Specimens and bone marrows – less messy and easier to embed.

The gross attendant and pathologist specializing in breast pathology spend considerable time explaining the breast dissection and the blocking sequence in particular areas where the blocks would be used for ER/PR requests to follow.

Day Two was spent in the routine pathology lab where I observed the typical day. Cutting was very similar to our method. Staining on automated stainer, coverslipping and sorted using the previous classification method. Special stains are done manually.

The next eight working days were spent in the immunoperoxidiase lab.

The immuno lab is a separate lab on its own. They use the Ventana system, utilizing two Benchmarks and two Nexes IHC stainers.

The first couple of days I observed the work flow in the immuno lab. They typically have four immuno runs per day with any excess being processed on overnight runs.

The antibodies are divided into two groups, one incubated at 37 degrees C the others at 56 degrees C. I have included a list for future reference.

I brought a list of our antibodies and protocols to compare with theirs. Most of our protocols were very similar. The most notable difference being antibody supplier and dilution on some antibodies. They used very few protocols with block as the claim background staining (blush) is easily different from positive staining. After each run their technologist would check the slide and show me the controls, both internal and external.

Controls are not run with every run but the pathologist will sign off on a new control. The following are always run with controls and always signed by a pathologist.

H pylori
ER/PR
Her 2 neu
CD117
EBV
Hepatitis B core

The above tests will determine if a patient will or will not be treated and will be run with controls and signed off by a pathologist.

The technologist will read the controls to validate the system but under no circumstance report on the patient tissue. This is the responsibility of the pathologist.

I was given full access to the JGH lab protocol and protocol manuals with permission to copy any documents that I needed.

I was given full access to the immumo protocols and was at liberty to copy any or all protocols. Considerable time was spent on review of the Benchmark system, maintenance, protocols and configuration.

We reviewed ER/PR and Her 2 neu protocols, results and controls,

We ran a panel of H.C.C.S.J. slides for ER/PR using J.G.H. protocols to compare to the same panel already ran at H.C.C.S.J.

Discussed the merits of Antibodies, titrations, pitfalls and troubleshooting as well as recommeded protocols to follow when introducing new antibodies into the immuno lab.

Discussed and learned the Automated Fluorescence techniques for the Benchmark For kidney and skin bx's. Developed protocols and studied the kidney bx's techniques

and procedures.

ISH (In-situ Hybridization)

IHC uses antibody/antigen

ISH nuclei acid)probe) to look for a nucleir acid (tanget)

Iber PROBE

These probes are grouped and done every couple of weeks as the probes are expensive and it takes seven hours on the machine. (usually done on overnight runs).

SUMMARY:

It is my opinion that this trip has been a worthwhile venture. It has been a rewarding educational experience.

It is an opportunity to bring back to our immuno lab some valuable practical and theoretical knowledge and skills.

It also provides us with a valuable contact should we need help or advise in our future immuno protocols.

Most importantly it reinforces our belief that we are performing the protocols and procedures of the Ventena system using the same checks and balances used anywhere in North America.

Appendix

Hôpital Général Juif - Sir Mortimer B. Davis - Jewish General Hospital
Département de Pathologie - Pathology Department

TRAINING CHECKLIST FOR PATHOLOGY'S EMPLOYEES

DATE: JAN. 24 /06 to JAN. 27/06

TRAINED BY: MARTINE BOURDEAU **EMPLOYEE'S SIGNATURE:** Martine Bourdeau

ITEMS	TRAINEE'S INITIALS	TRAINER'S INITIALS	DURATION	COMMENTS
BENCHMARK	KG	MB		
MAINTENANCE DAILY MONTHLY QUARTERLY	KG	MB		
PROTOCOLS CREATE	KG	MB		
PRINTING RUN REPORTS PROTOCOLS	KG	MB		
BAR CODE CREATE TEMPLATE	KG	MB		
" CREATE PANEL	KG	MB		
" CONFIGURATION	KG	MB		

Hôpital Général Juif - Sir Mortimer B. Davis - Jewish General Hospital
Département de Pathologie - Pathology Department

TRAINING CHECKLIST FOR PATHOLOGY'S EMPLOYEES

DATE: JAN 24 to JAN 27/06

TRAINED BY: MARTINE BOURDEAU **EMPLOYEE'S SIGNATURE:** Martine Bourdeau

ITEMS	TRAINEE'S INITIALS	TRAINER'S INITIALS	DURATION	COMMENTS
ISH TECHNIC EBER	KG	MB		
PRIMARY ANTIBODY (CLONE DILUTION)	KG	MB		
TITRATION (New Ab)	KG	MB		
READING INTERNAL CONTROL	KG	MB		
" TROUBLESHOOTING	KG	MB		
REQUISITION FOR IMMUNO FLUORESCENCE	KG	MB		
" IMMUNO PEROXIDASE	KG	MB		
ER/PR Protocol, Control, Result	KG	MB		
Her2New Protocol, control Result	KG	MB		

Hôpital Général Juif - Sir Mortimer B. Davis - Jewish General Hospital
Département de Pathologie - Pathology Department

TRAINING CHECKLIST FOR PATHOLOGY'S EMPLOYEES

DATE: JAN. 23 / 06

TRAINED BY: MARTINE BOURDEAU **EMPLOYEE'S SIGNATURE:** Martine Bourdeau

ITEMS	TRAINEE'S INITIALS	TRAINER'S INITIALS	DURATION	COMMENTS
FLUORESCENCE TECHNIC (KIDNEY BX)	KG	MB		
FREEZING PROCEDURE	KG	MB		
CUTTING	KG	MB		
STAINING	KG	MB		
PROTOCOLS	KG	MB		
SOLUTION	KG	MB		

Procedure: Fluorescence (Protocol Summary)

NexES IHC Staining Module

JEWISH GENERAL HOSPITAL, 3755 Cote Ste-Catherine Montreal, Quebec H3T 1E2

Protocol No	Protocol Name	Creation Date
2	IgG	06/27/2002
1	Apply One Drop of [FITC ANTI-IgG] (Fluorescent Ab), and Incubate for [8 Minutes]	
3	IgM	06/27/2002
1	Apply One Drop of [FITC ANTI-IgM] (Fluorescent Ab), and Incubate for [8 Minutes]	
4	IgA	11/19/2003
1	Apply One Drop of [FITC ANTI-IgA] (Fluorescent Ab), and Incubate for [10 Minutes]	
5	C3	01/04/2002
1	Apply One Drop of [FITC ANTI-C3] (Fluorescent Ab), and Incubate for [8 Minutes]	
6	FIBRIN	01/04/2002
1	Apply One Drop of [FITC ANTI-FIBRIN] (Fluorescent Ab), and Incubate for [8 Minutes]	
7	KAPPA	01/04/2002
1	Apply One Drop of [FITC ANTI-KAPPA] (Fluorescent Ab), and Incubate for [8 Minutes]	
8	LAMBDA	11/28/2003
1	Apply One Drop of [FITC ANTI-LAMBDA] (Fluorescent Ab), and Incubate for [16 Minutes]	
10	C1Q	06/27/2002
1	Apply One Drop of [FITC ANTI-C1Q] (Fluorescent Ab), and Incubate for [8 Minutes]	

IMMUNOPATHOLOGY (G-151)

IMMUNO# _____ PREVIOUS IMMUNO# _____

PATHO# _____ PREVIOUS PATHO# _____

UNIT# _____ **TISSUE RECEPTION:**

ROOM# _____ FREEZE: ☐

AGE: _____ MICHELS SOLUTION: ☐

DOCTOR: _____ FIXED IN B5: ☐

NAME: _____ FORMALIN: ☐

SPECIMEN: _____ TISSUE FIX: ☐

DATE OBTAINED: _____ BRAZIL: ☐

DATE PROCESSED: _____ OTHERS: _____

TECHNICIAN: _____ DIAGNOSIS: _____

RESULTS OBTAINED WITH EACH ANTISERUM:

* FITC IgG: _____

* FITC IgM: _____

* FITC IgA: _____

* FITC C3: _____

* FITC C1q: _____

* FITC FIBRIN: _____

* FITC KAPPA: _____

* FITC LAMBDA: _____

* FITC ALBUMIN: _____

* FITC C4: _____

REMARKS: _____

Procedure: BMK iVIEW DAB Paraffin (Protocol Summary)

BenchMark IHC/ISH Staining Module

JEWISH GENERAL HOSPITAL, 3755 Cote Ste-Catherine Montreal, Quebec H3T 1E2

Protocol No	Protocol Name	Creation Date
35	ER	01/04/2005

1 Deparaffinization [Selected]
2 Cell Conditioning [Selected]
3 Conditioner #1 [Selected]
4 Mild CC1 [Selected]
5 Standard CC1 [Selected]
6 Antibody [Selected]
7 Apply One Drop of [ANTI-ER (6F11)] (Antibody), Apply Coverslip, and Incubate for [32 Minutes]
8 Counterstain [Selected]
9 Apply One Drop of [HEMATOXYLIN] (Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

Protocol No	Protocol Name	Creation Date
36	PR	06/29/2005

1 Deparaffinization [Selected]
2 Cell Conditioning [Selected]
3 Conditioner #1 [Selected]
4 Mild CC1 [Selected]
5 Antibody [Selected]
6 Apply One Drop of [PgR (Clone 16)] (Antibody), Apply Coverslip, and Incubate for [32 Minutes]
7 Counterstain [Selected]
8 Apply One Drop of [HEMATOXYLIN] (Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

Completed Staining Run

JEWISH GENERAL HOSPITAL, 3755 Cote Ste-Catherine Montreal, Quebec H3T 1E2

Run Number 6280
Instrument Name BMK 2
Instrument Type BenchMark IHC/ISH Staining Module
Run Operator LOGINS DISABLED
Run Started 01/23/2006 3:08:46 PM
Run Completed 01/24/2006 7:03:40 AM
Delay Started 13:04

Reagent Usage Detail

Reagent Position	Reagent	Serial #	Tests Dispensed	Dispenses Remaining*	Dispenser Life*	Lot # / MasterLot # **	Expiration Date
12	I-VIEW INHIBITOR	131114	20	105	105	502848 **	05/14/2007
13	I-VIEW BIOTIN Ig	117586	20	105	105	502848 **	05/14/2007
14	I-VIEW SA-HRP	123019	20	105	105	502848 **	05/14/2007
15	I-VIEW DAB	131567	20	105	105	502848 **	05/14/2007
16	I-VIEW H2O2	136141	20	105	105	502848 **	05/14/2007
17	I-VIEW COPPER	118658	20	105	105	502848 **	05/14/2007
18	HEMATOXYLIN	159218	20	105	105	503634A	07/18/2007
19	ANTI-ER (6F11)	27713	10	15	15	497497	10/31/2007
20	PgR (Clone 16)	15419	10	6	6	501273	08/31/2006

Protocol Detail

Slide Position	Protocol	Protocol #	Case ID	Staining	Background	Comments	Sign Off
1	KEN ER	208	SS5914 052A	+ / -			
2	KEN ER	208	SS5914 052B	+ / -			
3	KEN ER	208	SS6024 052A	+ / -			
4	KEN ER	208	SU 2026 03Q	+ / -			
5	KEN ER	208	SU 11542 05 5G	+ / -			
6	KEN ER	208	SS1587 02K	+ / -			
7	KEN ER	208	RI 490 05	+ / -			
8	KEN ER	208		+ / -			
9	KEN ER	208	SS 7545 05 2D	+ / -			
10	KEN ER	208	SU 13552 05C	+ / -			
11	KEN PR	207	SS 7545 05 2D	+ / -			
12	KEN PR	207	SU 13552 05C	+ / -			
13	KEN PR	207	RI 490 05	+ / -			
14	KEN PR	207		+ / -			
15	KEN PR	207	SU 11542 05 5G	+ / -			
16	KEN PR	207	SS1587 02K	+ / -			
17	KEN PR	207	SS6024 052A	+ / -			
18	KEN PR	207	SS5914 052B	+ / -			
19	KEN PR	207	SU 2026 03Q	+ / -			
20	KEN PR	207	SS5914 052A	+ / -			

Bulk Usage Detail

Bulk Name	Application Slide Count	Lot # / MasterLot #	Expiration Date
CC1	20	500288	04/20/2006
EZ Prep	20	500282	10/19/2006
LCS	20	503631	06/14/2007
Reaction Buffer	20	504655	12/20/2007

** Remaining dispenses are as of time of this report* *** Indicates master lots*

Procedure: BMK iVIEW DAB Paraffin (Protocol Summary)

BenchMark IHC/ISH Staining Module

JEWISH GENERAL HOSPITAL, 3755 Cote Ste-Catherine Montreal, Quebec H3T 1E2

Protocol No	Protocol Name	Creation Date
94	CB11	01/03/2006

1 Deparaffinization [Selected]
2 Cell Conditioning [Selected]
3 Conditioner #1 [Selected]
4 Mild CC1 [Selected]
5 Antibody [Selected]
6 Apply One Drop of [PREP KIT 41] (Antibody), Apply Coverslip, and Incubate for [32 Minutes]
7 Counterstain [Selected]
8 Apply One Drop of [HEMATOXYLIN] (Counterstain), Apply Coverslip, and Incubate for [4 Minutes]

IMMUNOPATHOLOGY REQUISITION

JGH – PATHOLOGY DEPARTMENT

PATHOLOGY # :_____ BLOCK # :_____ HOSPITAL # :_____ CUT BY:_____

PATIENT: _____ CLINICIAN: _____ PATHOLOGIST:_____

SITE:_____ DATE REQUESTED: ___/___/___ DATE PROCESSED: ___/___/___ TECHNOLOGIST: _____

- ☐ CD 1a
- ☐ CD 10
- ☐ CD 15 (Leu M1)
- ☐ CD 20 (L26)
- ☐ CD 21
- ☐ CD 23
- ☐ CD 3
- ☐ CD 30 (BerH2)
- ☐ CD 4
- ☐ CD 40
- ○ CD 43 (MT1) (56BM)
- ○ CD 45 (LCA) (56BM)
- ☐ CD 45RO (UCHL1)
- ☐ CD 5
- ☐ CD 56
- ☐ CD 57(Leu7)
- ○ CD 68 (KP1)
- ☐ CD 79a
- ☐ CD 8
- ☐ CD 138
- ☐ KAPPA
- ☐ LAMBDA
- ☐ BCL-1 (CYCLIND1)
- ☐ BCL-2
- ☐ BCL-6 (F orm only)
- ☐ ALK-1
- ☐ FASCIN
- ○ LYSOZYME
- ☐ TDT
- ○ DBA44
- ☐ MYELO
- ☐ TIA-1
- ☐ PAX-5 (test)

CYTOKERATINS:

- ☐ CAM 5.2
- ☐ AE1/AE3
- ☐ Lu-5 (test)
- ☐ 34βE12
- ☐ CK-5/6
- ☐ CK-7
- ☐ CK-19
- ☐ CK-20
- ○ EMA (Epith. mem. antigen)
- ○ CEA (poly)
- ○ PSA
- ☐ TTF-1
- ☐ C-KIT (CD-117)
- ☐ SYNAPTOPHYSIN
- ○ CHROMOGRANIN
- ☐ TAG 72(B72.3 + CC49)
- ☐ β -HCG

- ☐ PLAP (Plac. alka. phosph.)

- ○ GLYCOPHORIN A
- ☐ IgG
- ☐ IgM
- ☐ IgA
- ☐ VIMENTIN
- ○ ACTIN
- ○ DESMIN
- ☐ MYOGENIN

- ○ MYOGLOBIN
- ☐ CD 34
- ☐ CD 31
- ☐ FACTOR VIII
- ☐ F XIIIa
- ☐ S-100 protein
- ○ HMB-45
- ☐ MART-1
- ☐ INHIBIN
- ☐ ER (Estrogen Receptor)
- ☐ PR (Progesterone Receptor)
- ☐ ANDROGEN R

HER-2/neu

- ○ CB-11
- ☐ TAB-250
- ☐ E-CADHERIN
- ○ BRST-2 (GCDFP-15)
- ☐ P 16
- ☐ P 53
- ☐ P 63
- ☐ P 63 / 34βE12
- ☐ AMACR (P504S)
- ☐ CA 125
- ☐ CD 99 (MIC2)
- ○ α - FP
- ○ α 1-ANTITRYPSIN
- ☐ CALRETININ
- ○ THYROGLOBULIN
- ☐ HBME-1
- ☐ CALCITONIN
- ☐ PTH
- ☐ CALDESMON

- ○ FSH
- ○ LH
- ○ PRL
- ○ TSH
- ○ ACTH
- ○ HGH
- ☐ Neu N
- ☐ CASPASE 6

- ☐ α-SYNUCLEIN
- ○ α B - CRYSTALLIN
- ☐ NEUROFILAMENTS
- ☐ TAU
- ○ UBIQUITIN
- ○ GFAP
- ☐ PG-M1
- ☐ TOXOPLASMA
- ☐ KI 67
- ☐ PARVOVIRUS B19
- ☐ AMYLOID-A
- ☐ MSH-2
- ☐ MLH-1 (test)
- ☐ SV40
- ☐ EBER (PROBE)
- ☐ HSV1-2
- ☐ HBsAg
- ○ HB CORE Ag
- ☐ HEPATOCYTE
- ☐ H PYLORI
- ☐ CMV (early)
- ☐ WT1 (Wilm's Tumor)

○ - 37°C