



building towards

CIQC

canadian Immunohistochemistry Quality control

Assessors' report for CIQC Run 103: Class I (CDX2, CK7, CD45)

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Assessment performed November 18, 2019 at Lions Gate Hospital, North Vancouver, BC.

OVERVIEW

The CIQC continues to support and encourage the use of iCAPCs (immunohistochemistry Critical Assay Performance Controls) to ensure quality of IHC staining. For this evaluation four tissues that make a good basic control for the majority of IHC stains were included: liver, pancreas, appendix and tonsil. Each participant was asked to determine appropriate targets of each marker for each tissue type and provide a readout for each cell and tissue type as follows:

- **Optimal (O)** – All expected targets are identified appropriately
- **Adequate (A)** – All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation
- **Poor (P)** – None or only strong targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation
- **Unsatisfactory (U)** – Technical problem e.g. loss of tissue

Pass/Fail Criteria:

All four tissue types should demonstrate optimal or adequate staining. A “fail” is considered if two (or more) of four tissue types are scored as “Poor” during CIQC assessment. In such an instance, the laboratory is advised to investigate and perform remediation on standard control tissue at the facility with an improved protocol if necessary.

RESULTS

CK7 is one of the workhorses of IHC and is primarily used in the identification of the primary site of origin of metastatic tumours. CK7 can be used as a prognostic marker in, for example, squamous esophageal cancer¹. It is found in normal non-keratinizing epithelium like ductal epithelium and mesothelium. In normal liver there should be moderate to strong staining cytoplasmic staining of almost all epithelial cells in the bile ducts. We expect to see virtually no staining in the appendix except for endothelial cells or occasional columnar cell. Establishment of staining at the lower limits of detection (LLOD) in the pancreas where epithelial cells of intercalated ducts must show weak to moderate staining of the cytoplasm while the epithelium of the large pancreatic ducts should show strong staining is a critical indicator². Hepatocytes should be negative, though the occasionally reactive hepatocytes may stain positive for CK7. Overall excellent staining was observed in the majority of laboratories. Most participants satisfactorily stained the intercalated ducts of the pancreas, which confirmed their ability to identify CK7 at its LLOD.

[1] Fei, F, et al. "CK7 expression associates with the location, differentiation, lymph node metastasis, and the Dukes' stage of primary colorectal cancers." *Journal of Cancer* 10.11 (2019): 2510-2519. [2] Torlakovic, Emina E., et al. "Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee." *Applied immunohistochemistry & molecular morphology* 23.1 (2015): 1-18.

Garratogram after CIQC assessment of CK7:

Lab ID	Pancreas	Liver	Tonsil	Appendix	Comments
101	O	A	O	O	Slight background
102	O	O	O	O	
107	O	O	O	O	
112	O	O	O	O	
114	O	O	O	O	
120	O	O	O	O	
126	A	O	O	A	Slightly weak compared to other labs
128	O	O	O	O	Nice staining
132	O	O	O	A	Slightly weak compared to other labs
138	O	O	O	O	
147	O	O	O	O	Nice staining
148	O	O	O	O	
149	O	A	O	A	Slight background
175	P	P	A	P	Very weak staining; no LLOD in pancreas
176	O	O	O	O	
190	O	O	O	O	
202	O	O	O	O	
230	O	O	O	O	
231	O	O	O	O	

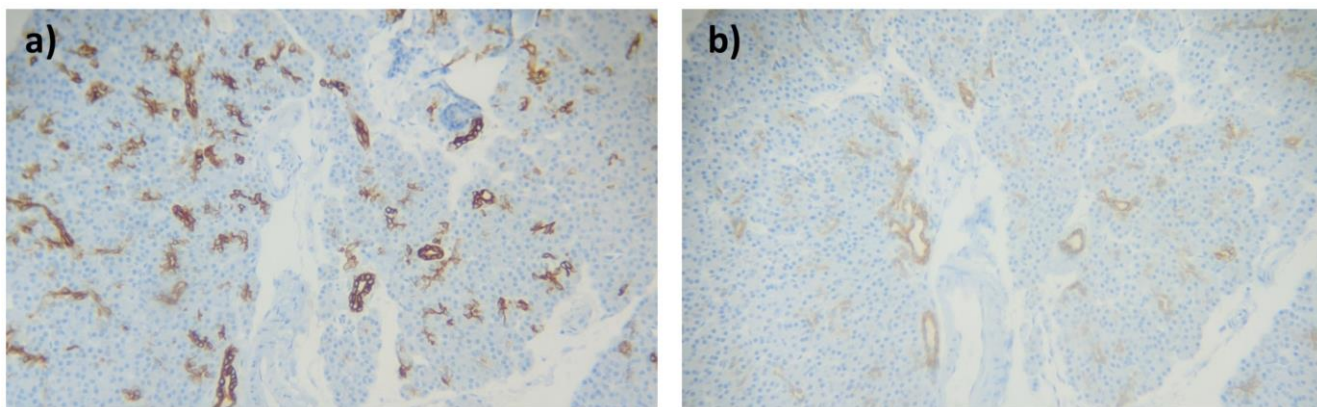


Figure 1. Representative images of CK7 staining in pancreas. **a)** Optimal staining. **b)** Very weak staining.

CDX2 is primarily used to differentiate adenocarcinomas with it being fairly specific for gastrointestinal adenocarcinomas. Normal tissues used in the CIQC assessment contain ideal material for determining the performance of CDX2 staining. It is expected that almost all the epithelial cells in the appendix have strong nuclear staining with weak cytoplasmic staining. The lower limit of detection (LLOD) is found in the pancreas where the epithelial cells of the intercalated ducts show weak to moderate staining. While no staining is expected in the tonsil there is the potential for the occasional lymphocyte to stain weakly positive. The endothelium and smooth muscle in the tonsil should be negative. Staining of the epithelial cells of the appendix was generally optimal². Most labs were able to stain the nuclei of the intercalated ducts of the pancreas which are considered to be the LLOD of CDX2.

[1] Fei, F, et al. "CK7 expression associates with the location, differentiation, lymph node metastasis, and the Dukes' stage of primary colorectal cancers." *Journal of Cancer* 10.11 (2019): 2510-2519. [2] Torlakovic, Emina E., et al. "Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee." *Applied immunohistochemistry & molecular morphology* 23.1 (2015): 1-18.

Garratogram after CIQC assessment of CDX2:

Lab ID	Pancreas	Liver	Tonsil	Appendix	Comments
101	O	O	O	O	
102	O	O	O	O	
107	O	O	O	O	
112	O	O	O	O	
114	A	O	O	O	Slightly weak compared to other labs
120	A	O	O	O	Slightly weak compared to other labs
126	P	O	O	A	Very weak staining; no LLOD in pancreas
128	O	O	O	O	
132	O	O	O	O	
138	P	O	O	A	Very weak staining; no LLOD in pancreas
147	O	O	O	O	Nice staining
148	O	O	O	O	Sligh background
149	O	O	O	O	
175	O	O	O	O	
190	O	O	O	O	
202	A	O	O	O	Slightly weak compared to other labs
230	O	O	O	O	
231	O	O	O	O	

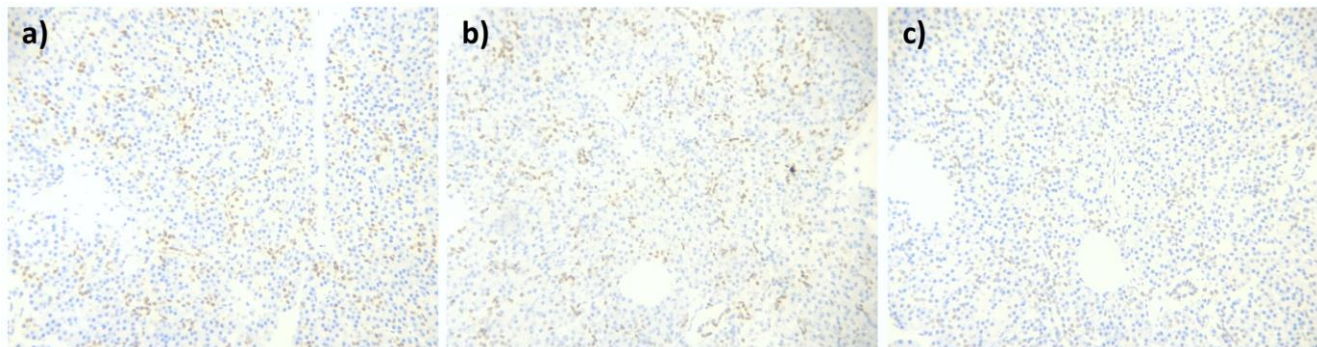


Figure 2. Representative images of CDX2 staining in pancreas. a) Optimal staining. b) Adequate staining that is slightly weak. c) Very weak staining.

CD45 (LCA) is one of the antibodies that were in the original panel in the early days of IHC in the clinical laboratory. Its primary use is in the identification of hematopoietic cells (macrophages, lymphocytes, granulocytes, plasma cells, mast cells) either benign or malignant in tissues. In the CIQC test slides it is expected that there will be moderate to strong staining of lymphocytes in all tissues. The Kupffer cells of the liver are an indicator that the LLOD of detection have been established and the staining is satisfactory². Generally, there was excellent staining for CD45 with the Kupffer cells of the liver showing moderate to strong staining in most cases thus showing that the CD45 stain is calibrated appropriately for the LLOD. However, it was noted that a few labs did not have a sensitive enough method to stain all the lymphoid cells in the tonsil control.

[1] Fei, F, et al. "CK7 expression associates with the location, differentiation, lymph node metastasis, and the Dukes' stage of primary colorectal cancers." *Journal of Cancer* 10.11 (2019): 2510-2519. [2] Torlakovic, Emina E., et al. "Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee." *Applied immunohistochemistry & molecular morphology* 23.1 (2015): 1-18.

Garrattogram after CIQC assessment of CD45:

Lab ID	Pancreas	Liver	Tonsil	Appendix	Comments
101	O	A	A	O	Slightly weak compared to other labs
102	O	O	O	O	
107	O	O	O	O	
112	O	O	O	O	
114	O	O	O	O	
120	O	O	O	O	
126	O	O	U	O	Staining artifact for tonsil
128	O	O	O	O	
132	O	O	O	O	
138	O	O	O	O	
147	O	O	O	O	
148	O	A	P	A	Very weak staining compared to other labs
149	O	O	O	O	Nice staining
175	O	O	A	O	Slightly weak compared to other labs
176	O	O	O	O	
190	O	O	O	O	
202	O	O	O	O	
230	O	O	O	O	
231	O	O	O	O	

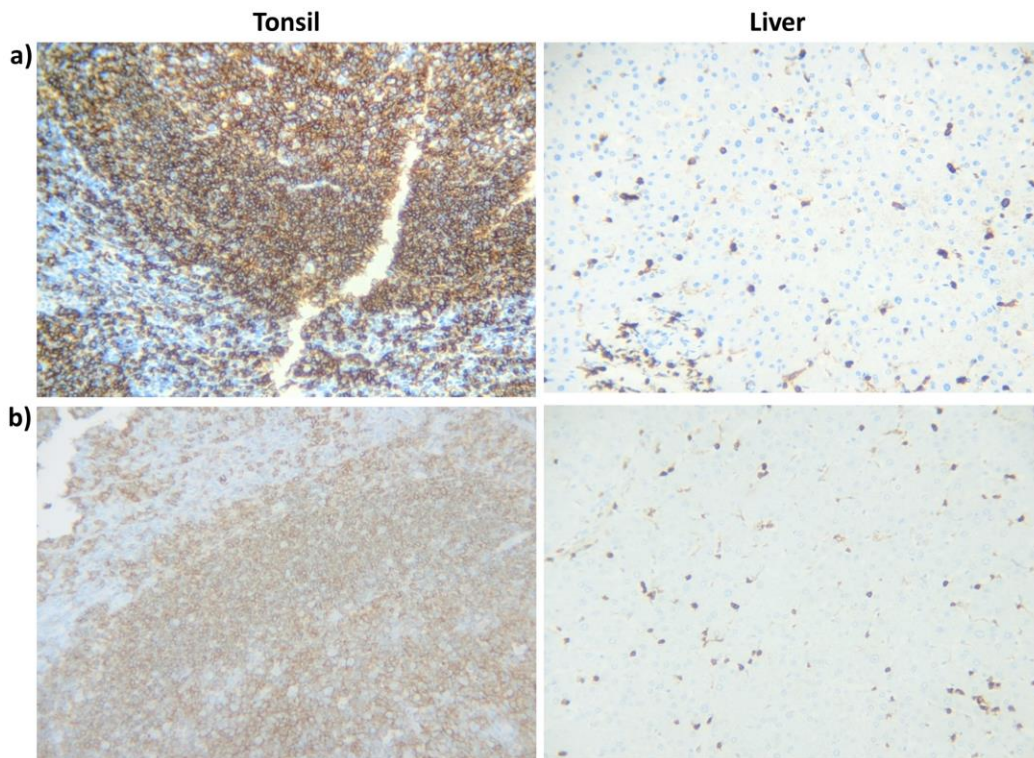


Figure 3. Representative images of CD45 staining. **a)** Optimal staining. **b)** Very weak staining.

Supplementary Tables 1-3 summarizing staining protocols can be found at the end of this document. Your participation in CIQC is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

Table S1. Reported CK7 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	DAKO PT - HIGH PH	20	OV-TL 12/30	1:70	DAKO	20058523	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
107	EnV FLEX TRS, High pH	30	OV-TL12/30	RTU	Dako	20062268	12	EnV FLEX/HRP	N	N	DAB
112	BOND epitope retrieval 1 pH 6.0	20 minutes	RN7	RTU	LEICA	63724	15 minutes	BOND polymer refine	none	none	DAB
114	Envision Flex TRS, High pH	30	OV-TL 12/30	RTU	Dako	20062268	10	Envision FLEX DAKO omnis	N	N	Envision Flex DAB
120	HIER Waterbath	20	OV-TL 12/30	RTU	Dako	20051777	20	Envision Flex+	N	N	DAB
128	Ultra CC1	64 Minutes	SP52	Ready-To-Use	Ventana/Roche	E30305	16 Minutes	UltraView Universal Dab Detection Kit	No	No	DAB
132	High pH	20	OV-TL 12/30	RTU	Dako	20056101	10	Envision Flex	N	N	DAB
138	EDTA HIER	20	OV-TL-12-30	RTU	Dako	20056101	20	Polymer/Dako	N	N	DAB
147	ER 2	20	OV-TL12/30	500	DAKO	92976	15	LEICA REFINE	N	N	DAB
148	CC1	36 min	SP52	RTU	Ventana	F03788	16 min	Ultraview	NO	NO	DAB
149	high pH OMNIS	20 min at 97 C	OVTL12/30	RTU	Dako Agilent	20062268	20	EnVision Flex OMNIS	yes	no	DAB
175	HIER	36	OV-TL	1 in 400	Cell Marque	1414806M	16	Ultra-DAB	N	Y (copper)	DAB
176	CC1	32	SP52	Predilute	Ventana	F03788	32	Optiview	n	n	DAB
190	Protease 2	4	OV-TL 12/30	1:100	Dako	20034208	24	Ventana Optiview	N	N	DAB
202	Epitope retrieval 1	20 min	RN7	RTU	Leica	64134	15 min	Polymer Refine detection Kit	n	n	DAB
230	HIER	40	SP52	predilute	Roche	E30305	32	Optiview	N	N	DAB
231	HIER	36 mins	SP52	RTU	ROCHE/VENTANA	E30305	8 MINS	ULTRAVIEW (VENTANA)	N	Y	DAB

Table S2. Reported CDX2 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	DAKO PT - HIGH PH	20	DAK-CDX2	1:40	10138685	DAKO	30" RT	DAKO ENVISION FLEX+	YES	YES CUSO4	DAB+
107	EnV FLEX TRS, High pH	30	DAK-CDX2	RTU	20061987	Dako	20	EnV FLEX/HRP	Y	N	DAB
112	BOND epitope retrieval 2 pH 9.0	13 minutes	EP25	RTU	64115	LEICA	15 minutes	BOND polymer refine	none	none	DAB
114	Envision Flex TRS, High pH	30	DAK-CDX2	RTU	10142480	Dako	15	Envision FLEX DAKO omnis	N	N	Envision Flex DAB
120	HIER Waterbath	20	DAK-CDX-2	RTU	10145146	Dako	20	Envision Flex+	N	N	DAB
128	Ultra CC1	52 Minutes	EPR2764Y	Ready-To-Use	V0001407	Cell Marque	32 Minutes	UltraView Universal Dab Detection Kit	No	No	DAB
132	High pH	20	DAK-CDX2	RTU	10148019	Dako	20	Envision Flex	N	N	DAB
138	EDTA HIER	20	DAKO-CDx2	RTU	10150734	Dako	20	Polymer/Dako	N	N	DAB
147	ER 2	20	DAK-CDX2	10	10143125	DAKO	15	LEICA REFINE	N	N	DAB
148	CC1	36 min	EPR2764Y	RTU	V0001598	Ventana	24	Ultraview	NO	NO	DAB
149	high pH OMNIS	20 min at 97 C	DAK-CDX2	RTU	10145130	Dako Agilent	15	EnVision Flex OMNIS	yes	no	DAB
175	HIER	64	EPR2764Y	1 in 500	1505106g	Cell Marque	32	Opti-DAB	N	Y (copper)	DAB
190	HIER	56	DAK-CDX2	1:20	10125934	Dako	32	Ventana Optiview	Y	N	DAB
202	Epitope retrieval 1	20 min	EP 25	RTU	10387	Leica	15 min	Polymer Refine detection Kit	n	n	DAB
230	HIER	40	EPR2764Y	predilute	V0001407	Roche	32	Optiview	N	N	DAB
231	HIER	36 mins	EPR2764Y	RTU	V0001598	CELL MARQUE	48 MINS	ULTRAVIEW (VENTANA)	N	Y	DAB

Table S3. Reported CD45 staining protocols.

Lab ID	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time Ab Incubation (min)	Detection System	Amplification (Y/N)	Enhancement (Y/N)	Chromogen
102	DAKO PT - HIGH PH	20	2B11 + PD7/26	1:400	DAKO	20058725	30" RT	DAKO ENVISION FLEX	NO	YES CUSO4	DAB+
107	EnV FLEX TRS, High pH	20	2B11+PD7/26	RTU	Dako	20057868	15	EnV FLEX/HRP	N	N	DAB
112	BOND epitope retrieval 1 pH 6.0	20 minutes	X16/99	RTU	Leica	64137	15 minutes	BOND polymer refine	none	none	DAB
114	Envision Flex TRS, High pH	30	PD7/26	RTU	Dako	20053428	12	Envision FLEX DAKO omnis	N	N	Envision Flex DAB
120	HIER Waterbath	20	2B11+PD7/26	RTU	Dako	20050977	20	Envision Flex+	N	N	DAB
128	Ultra CC1	36 Minutes	RP2/18	Ready-To-Use	Ventana/Roche	E19835	16 Minutes	UltraView Universal Dab Detection Kit	No	No	DAB
132	High pH	20	2B11 + PD7/26	RTU	Dako	20055022	10	Envision Flex	N	N	DAB
138	EDTA HIER	20	2B11+PD7/26	RTU	Dako	20057867	20	Polymer/Dako	N	N	DAB
147	ER 2	20	2B11+PD7/26	1500	DAKO	20026786	15	LEICA REFINE KIT	N	N	DAB
148	CC1	8	RP2/18	RTU	Ventana	E12792	36	Ultraview	NO	NO	DAB
149	high pH OMNIS	20 min at 97 C	2B11 + PD7/26	RTU	Dako Agilent	20053428	13	EnVision Flex OMNIS	no	no	DAB
175	HIER	16	2B11+PD7/26	1 in 200	Dako	87397	16	opti-DAB	N	2B11+PD7/26	DAB
176	CC1	32	RP2/18	Predilute	Ventana	E19835	32	Optiview	n	n	DAB
190	HIER	16	2B11 + PD7/26	1:50	Dako	20026786	24	Ventana Optiview	N	N	DAB
202	Epitope retrieval 1	20 min	X16/99	RTU	Leica	64134	15 min	Polymer Refine detection Kit	n	n	DAB
230	HIER	40	RP2/18	predilute	Roche	E27360	32	Optiview	N	N	DAB
231	HIER	36 mins	2B11PD7/26	RTU	CELL MARQUE	V0001596	32 mins	ULTRAVIEW (VENTANA)	N	Y	DAB