Canadian Pathology Quality Assurance
CPQA AQCP
Assurance qualité canadienne en pathologie

# Summary Report – Run 112 p63/AMACR/HMWCK

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## Overview

Expression of AMACR combined with loss of expression of basal cell markers, HMWCK and p63, enhance diagnostic accuracy of prostate cancer. The survey consisted of eight (8) 2mm tissue cores: five (5) prostate adenocarcinomas and three (3) benign prostate cores. Overall assessment of immunohistochemical staining in all eight cores combined was used to evaluate staining of each marker. The adenocarcinoma in Core 7 was cut through for some participants. It was also noted that Cores 2 and 7 may not have been as well-fixed as other cores in the tissue microarray, leading to generally weaker staining for p63 in particular. The expected staining criteria are described below:

**p63**

- Nuclear staining in benign basal cell nuclei of moderate intensity
- No nuclear staining in carcinoma
- Sufficient staining intensity
- Minimal to no non-specific staining

**AMACR (α-methylacyl-coenzyme A racemase)**

- Granular cytoplasmic staining of tumour with luminal accentuation in glands of adenocarcinoma that lack p63
- At most, only weak focal staining in benign
- Sufficient staining intensity

**Prostate basal epithelial keratin**

- Nuclear staining in benign basal cell nuclei of moderate intensity
- No nuclear staining in carcinoma
- Sufficient staining intensity
- Minimal to no non-specific staining

## Results

The technical quality of staining for participants was excellent. Participant-specific feedback is below:

| Lab ID | p63 | | AMACR | | HMWCK | |
|---|---|---|---|---|---|---|
| | IHC Status* | Comment | IHC Status* | Comment | IHC Status* | Comment |
| 101 | Optimal | | Optimal | | Optimal | |
| 102 | Optimal | | Optimal | | Optimal | |
| 103 | Optimal | | Optimal | | Optimal | |
| 111 | Optimal | Slightly week | Optimal | | Optimal | |
| 112 | Optimal | | Optimal | Slightly more staining of benign prostate than other labs | Optimal | |
| 113 | Optimal | | Optimal | Stromal background | Optimal | |
| 120 | Optimal | | Optimal | | Optimal | |
| 132 | Optimal | | Optimal | | Optimal | |
| 147 | Optimal | | Optimal | Stromal background | Optimal | |
| 149 | Optimal | | Optimal | | Optimal | |
| 151 | Adequate | Cores 2 and 7 poor staining due to weak staining | Optimal | | Optimal | |
| 183 | Optimal | | Optimal | | Optimal | |
| 198 | Optimal | | Optimal | Slight stromal background | Optimal | |
| 207 | Optimal | Slightly weak | Optimal | | Optimal | |
| 230 | Optimal | | Optimal | Nice staining | Optimal | |
| 231 | Optimal | | Optimal | | Optimal | |
| 234 | Optimal | | Optimal | Slightly weak | Optimal | |

*based on CPQA assessor consensus

Garrattogram after CPQA assessment (provided as an overall assessment of all tissue microarray cores):

| Lab ID | 102 | 103 | 111 | 112 | 113 | 120 | 132 | 147 | 149 | 151 | 183 | 198 | 207 | 230 | 231 | 234 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| p63 | O | O | O | O | O | O | O | O | O | A | O | O | O | O | O | O |
| AMACR | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |
| HMW CK | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O | O |

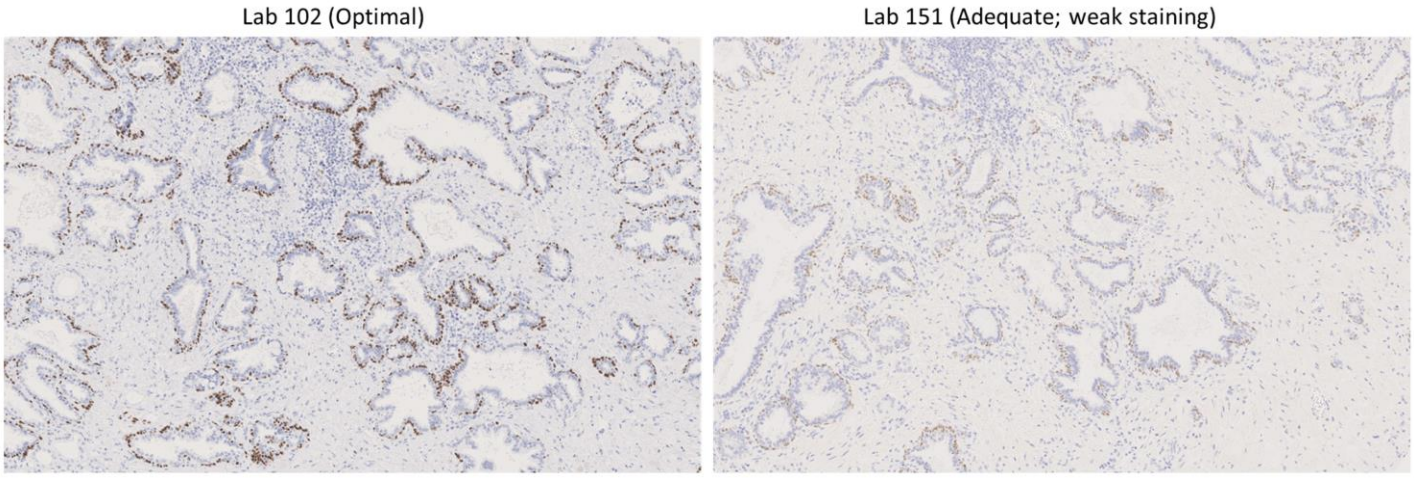


**Figure 1. Representative images of the qualitative variability of p63 staining.**

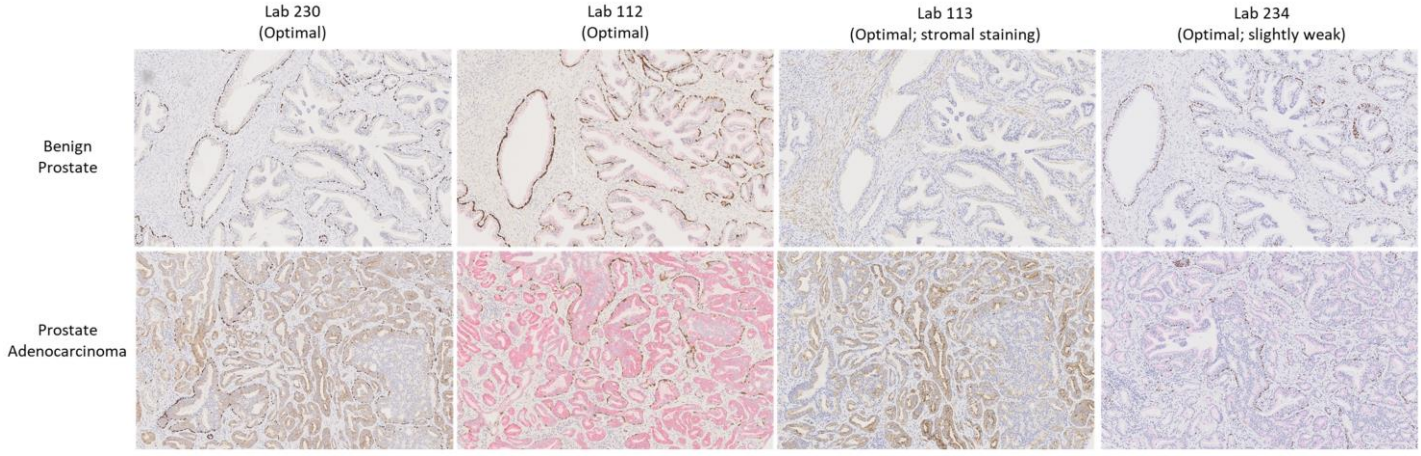


**Figure 2. Representative images of the qualitative variability of AMACR staining in benign prostate and prostate adenocarcinoma.**

Supplementary Tables 1-3 summarize the reported staining protocols for p63, AMACR and prostate basal epithelial keratins, respectively, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 4 provides the definitions of IHC Status and recommended participant action. Your regular participation in CPQA is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association Canadienne des Pathologistes.

*This report has been updated with scanned images that were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.*

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## Table S1. Reported p63 staining protocols.

| Lab ID | Ag Retrieval Method | Time for Ag Retrieval (min) | Ab Clone | Ab Dilution | Ab Supplier/ Vendor | Ab Lot No. | Time for Ab Incubation (min) | Detection System | Amplification (Y/N) | Enhancement (Y/N) | Chromogen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 102 | DAKO PT - HIGH PH | 20" | 4A4 | 1:50 | BIOCARE | 52219 | 30" RT | DAKO ENVISION FLEX | NO | YES CUSO4 | DAB+ |
| 103 | CC1 | 36 | 4A4 | Pre | Ventana | F22650 | 32 | Ultra view | Y | Y copper | DAB |
| 112 | Bond ER2 pH 9.0 | 30 minutes | 4A4 | RTU | BioCare Medical | 80819 | 60 minutes | ChromoPlex 1 Dual Detection | no | no | DAB |
| 113 | High pH | 30min | DAK-p63 | 1/100 | Dako | 2007039 | 27.5min | Dako Envision Flex | 10min MOuse Linker | N | DAB |
| 120 | HIER Waterbath | 20 | DAK-p63 | RTU | Dako | 20066310 | 20 | Envision Flex+ | N | N | DAB |
| 132 | High pH | 20 | DAK-P63 | RTU | Dako | 20066311 | 20 | Envision flex | N | N | DAB |
| 147 | ER PH 9 | 20 | 13H4 | 50 | ZETA CORPORATION | 1708B/1902C-1 | 15 | LEICA REFINE KIT | N | N | DAB |
| 149 | high pH OMNIS | 20 min at 97 C | DAK-p63 | RTU | Dako Agilent | 20066314 | 20 | EnVision Flex OMNIS | No | No | DAB |
| 151 | BUFFER 9.0 | 20 MIN | 4A4 | 1:200 | ZETA | 181221 | 15 MIN | BOND REFINE | N | N | DAB |
| 183 | ER2 | 30 | CK5/14+ P63+P504S | RTU | BIOCARE | 21519 | 15 | CHROMOPLEX | N | N | DAB |
| 198 | HIER high pH | 30 min @ 97deg | DAK-p63 | RTU | Agilent | 20073135 | 15 min | Envision Flex | N | N | DAB |
| 207 | on line | 64 | 4A4 | prediluted | Ventana | F22650 | 32 | ULTRAVIEW | N | Y | DAB |
| 230 | HIER | 40 | A4A | predilute | Roche | F18327 | 32 | Optiview | N | N | DAB |
| 231 | HIER | 56 mins | 4A4 | RTU | ROCHE/VENTANA | F00224 | 24 mins | OPTIVIEW (VENTANA) | N | Y | DAB |
| 234 | Omnis on board | 30 | DAK-p63 | RTU | Agilent | 20073135 | 10 | Envision | Rabbitlinker | n | DAB |

## Table S2. Reported AMACR staining protocols.

| Lab ID | Ag Retrieval Method | Time for Ag Retrieval (min) | Ab Clone | Ab Dilution | Ab Supplier/ Vendor | Ab Lot No. | Time for Ab Incubation (min) | Detection System | Amplification (Y/N) | Enhancement (Y/N) | Chromogen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 102 | DAKO PT - HIGH PH | 20" | 13H2 | 1:80 | DAKO | 10148602 | 30" RT | DAKO ENVISION FLEX | NO | YES CUSO4 | DAB+ |
| 103 | CC1 | 36 | 13H4 | 1/100 | Dako | 10143476 | 24 | Ultra View | No | Y copper | Dab |
| 112 | Bond ER 2 pH 9.0 | 30 minutes | N/A (polyclonal) | RTU | BioCare Medical | 80819 | 60 minutes | ChromoPlex 1 Dual Detection | no | no | Fast Red |
| 113 | High pH | 30min | 13H4 | Pre-dilute | Dako | 11102752 | 20min | Dako Envison Flex | 10min Rabbit Linker | N | DAB |
| 120 | HIER Waterbath | 20 | 13H4 | RTU | Dako | 10147735 | 20 | Envision Flex+ | N | N | DAB |
| 132 | High pH | 20 | 13H4 | 1:50 | Dako | 10136945 | 30 | Envision flex | N | N | DAB |
| 147 | ER PH 9 | 20 | 13H4 | 50 | ZETA CORPORATION | 1708B/1902C-1 | 15 | LEICA REFINE KIT | N | N | DAB |
| 149 | high pH OMNIS | 20 min at 97 C | 13H4 | RTU | Dako Agilent | 10146449 | 15 | EnVision Flex OMNIS | Yes | No | DAB |
| 151 | BUFFER 9.0 | 20min | 13H4 +4A4 | 1:150 | ZETA | 1703A/1072A-1 | 15 MIN | BOND REFINE | N | N | DAB |
| 183 | ER2 | 30 | CK5/14+ P63+P504S | RTU | BIOCARE | 21519 | 15 | CHROMOPLEX | N | N | REFINE RED |
| 198 | HIER High pH | 30 min 97deg | 13H4 | RTU | Agilent | 11102752 | 20 | Envision Flex | Y | N | Magenta Envision |
| 207 | ON LINE | 64 | P504S | 1/200 | BIOCARE | 72318 | 44 | ULTRAVIEW | N | Y | DAB |
| 230 | HIER | 40 | P504S +4A4 | predilute | Biocare | VP201G | 32 | Optiview | N | N | DAB |
| 231 | HIER | 52 mins | polyclonal | 1/100 | intermedico (biocare) | 70319 | 32 mins | ULTRAVIEW (VENTANA) | N | Y | DAB |
| 234 | Omnis on board | 30 | 13H4 | 5000 | Agilent | 10136945 | 15 | Envision | Rabbitlinker | n | DAB |

## Table S3. Reported prostate basal epithelial keratins staining protocols.

| Lab ID | Ag Retrieval Method | Time for Ag Retrieval (min) | Ab Clone | Ab Dilution | Ab Supplier/ Vendor | Ab Lot No. | Time for Ab Incubation (min) | Detection System | Amplification (Y/N) | Enhancement (Y/N) | Chromogen |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 102 | DAKO PT - HIGH PH | 20" | D5/16 B4 | Predilute | DAKO | 20067284 | 30" RT | DAKO ENVISION FLEX+ | YES | YES CUSO4 | DAB+ |
| 103 | CC1 | 36 | 34BE12 | Pre | Ventana | F19791 | 16 | Ultra view | N | Y copper | DAB |
| 112 | Bond ER2 pH 9.0 | 30 minutes | XM26 & LLOO2 | RTU | BioCare Medical | 80819 | 60 minutes | ChromoPlex 1 Dual Detection | no | no | DAB |
| 113 | High pH | 30min | XM26 | 1/200 | Leica | 6065470 | 27.5min | Dako Envision Flex | 10min Mouse Linker | N | DAB |
| 120 | HIER Waterbath | 20 | 34BE12 | RTU | Dako | 10146592 | 20 | Envision Flex+ | N | N | DAB |
| 132 | High pH | 20 | 34BE12 | RTU | Dako | 10149455 | 20 | Envision flex | N | N | DAB |
| 147 | ER PH 9 | 20 | 34BE12 | 100 | DAKO | 10147751 | 15 | LEICA REFINE KIT | N | N | DAB |
| 149 | high pH OMNIS | 20 min at 97 C | 34BE12 | RTU | Dako Agilent | 10138647 | 20 | EnVision Flex OMNIS | Yes | No | DAB |
| 151 | BUFFER 9.0 | 20 MIN | 34BE12 | 1:200 | DAKO | 10147751 | 20 MIN | BOND REFINE | N | N | DAB |
| 183 | ER2 | 30 | CK5/14+ P63+P504S | RTU | BIOCARE | 21519 | 15 | CHROMOPLEX | N | N | DAB |
| 198 | HIER high pH | 30 min @97 deg | 34BE12 | RTU | Agilent | 11086028 | 25 min | Envision Flex | Y | N | DAB |
| 207 | ON LINE | 64 | 34BE12 | PREDILUTED | VENTANA | F13474 | 16 | ULTRAVIEW | N | Y | DAB |
| 230 | HIER | 40 | 34BETAE12 | predilute | Roche | F04650 | 32 | Optiview | N | N | DAB |
| 231 | HIER | 36 mins | 34BE12 | RTU | ROCHE/VENTANA | F19791 | 32 mins | ULTRAVIEW (VENTANA) | N | Y | DAB |
| 234 | Omnis on board | 30 | 34BE12 | RTU | Agilent | 10142752 | 25 | Envision | Mouselinker | n | DAB |

## Table S4. Proficiency Testing Definitions of IHC Status.

| IHC Status | Definition | Proficiency Testing Performance |
|---|---|---|
| **Optimal** | All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining). | **PASS** |
| **Adequate** | All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation. | **PASS** |
| **Sub-optimal** | None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation. | **PASS, Conditionally[1]** |
| **Failed** | The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible. | **FAIL[2]** |
| **Unsatisfactory** | Technical issue (e.g. unsuitable antibody selection, etc.) | **N/A** |

*1 – A one-time sub-optimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".*

*1,2 – Please contact the CPQA for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.*