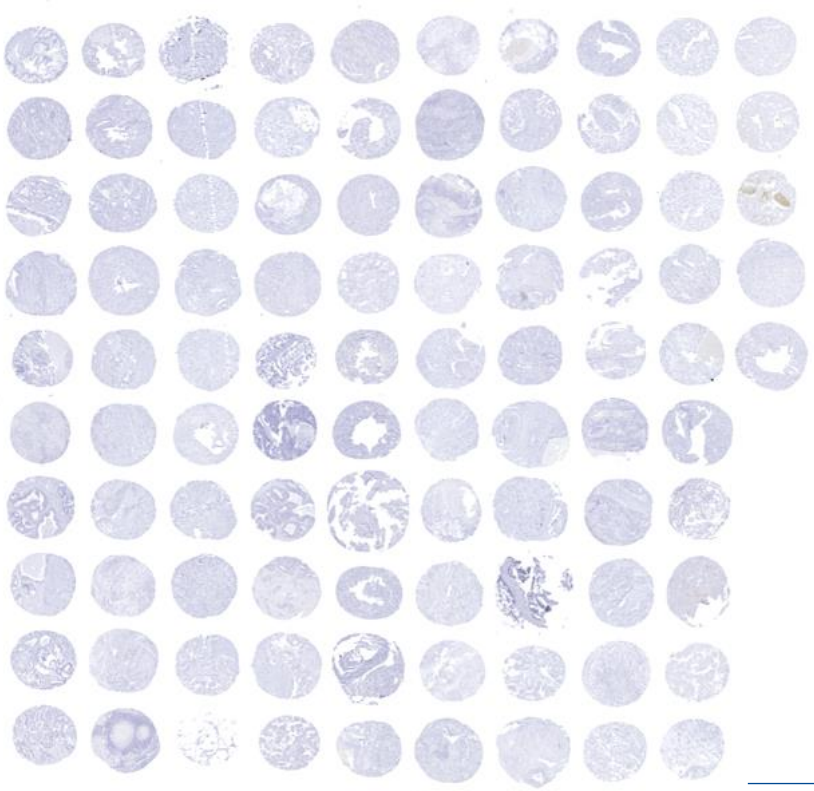
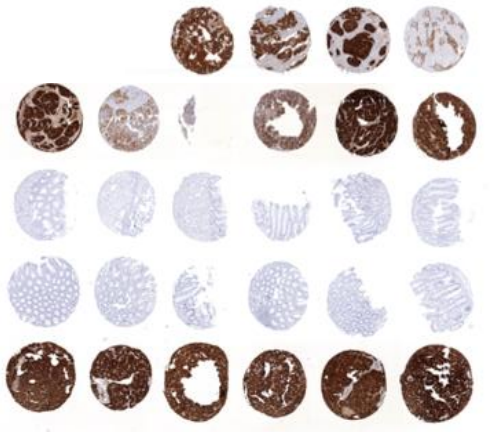
# LDT Manual – Caboxypeptidase A1 (CPA1): MSVA-601M



## Part 1 – Science

## What is CPA1

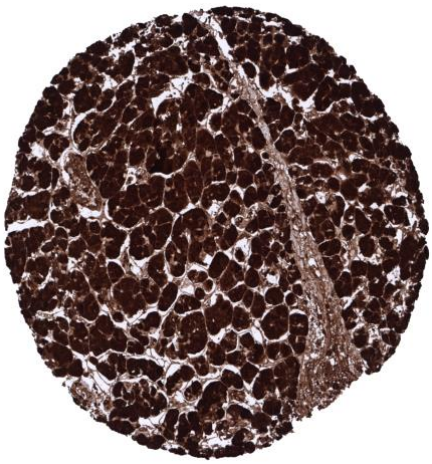
### Biology and staining pattern in normal tissues

Carboxypeptidase A1 (CPA1) is a zinc metalloprotease which is solely produced in acinar cells of the pancreas. CPA1 is released to the intestine and involved in zymogen inhibition and cleavage of aromatic amino acids from dietary proteins. RNA analyses of different kinds of normal tissues have confirmed exclusive expression of CPA1 in the pancreas (HPA).

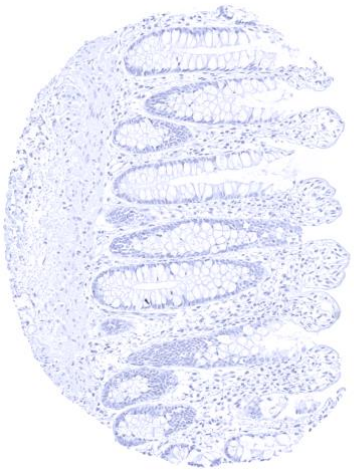
### Published data on tumors

A study by Uhlig et al. has described 100% sensitivity and 99.5% specificity of CPA1 expression analysis by using MSVA-601M for the identification of pancreatic acinar cell carcinomas in 12,274 tumors from 132 different tumor entities.[1] These data are very promising but given the still small number of analyzed pancreatic acinar cell carcinomas – a very rare tumor entity - it cannot be excluded that the sensitivity may be somewhat lower if more tumors are analyzed.

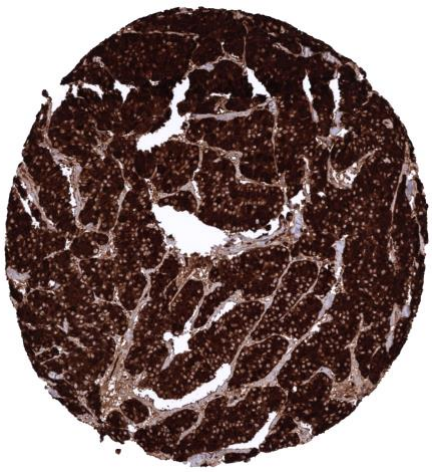
### Characteristic Images



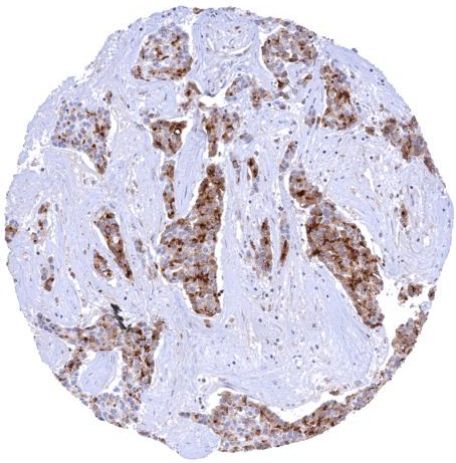
**Strong CPA1 staining of normal pancreas (Note: Stroma is also staining due to contamination artifact).**



**Complete lack of CPA1 staining in the normal colon mucosa.**

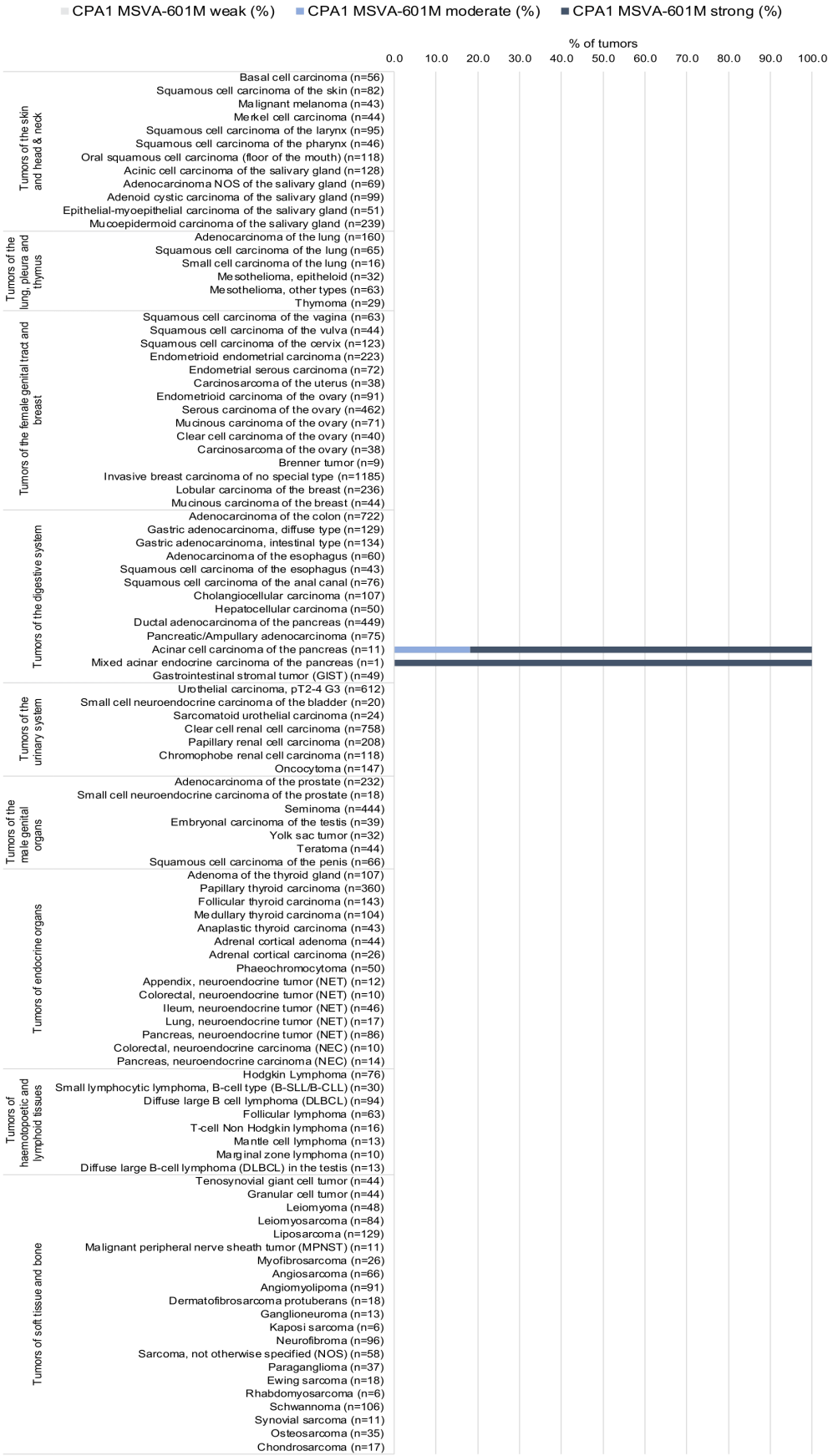


**Pancreatic acinar cell carcinoma showing strong positivity for CPA1 in all tumor cells.**



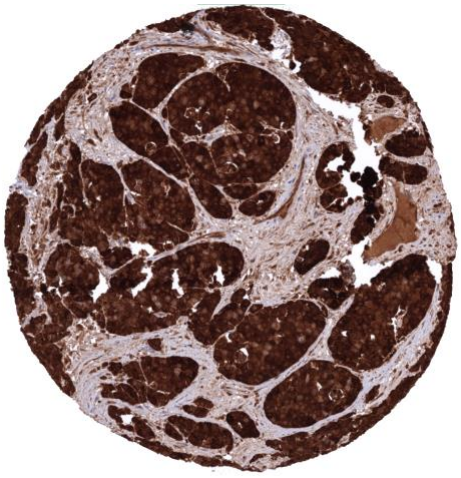
**Pancreatic acinar cell carcinoma showing moderate staining for CPA1 in most tumor cells.**

**The complete results of Uhlig et al. are shown below**: Data of Uhlig et al. from MSVA Homepage.[1,2]



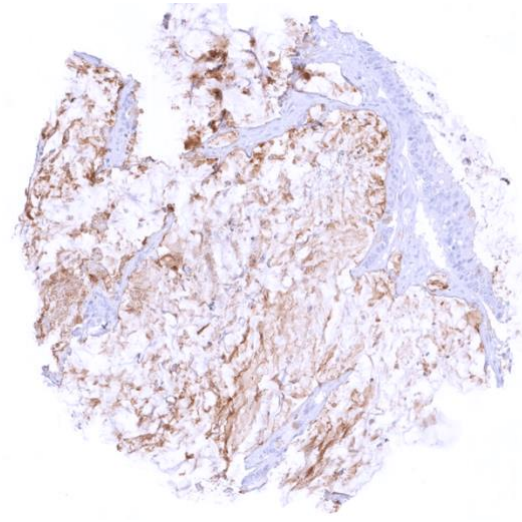
**Potential pitfalls**

**-contamination artifacts:** The CPA1 expression level in normal acinar cells is very high. Therefore, acinar-cell adjacent structures can show some staining ("pseudo-positivity") due to a diffusion of CPA1 protein from neighboring acinar cells. The spread of CPA1 protein may be facilitated by a mild tissue damage caused by early autolysis during ischemia time under surgery.

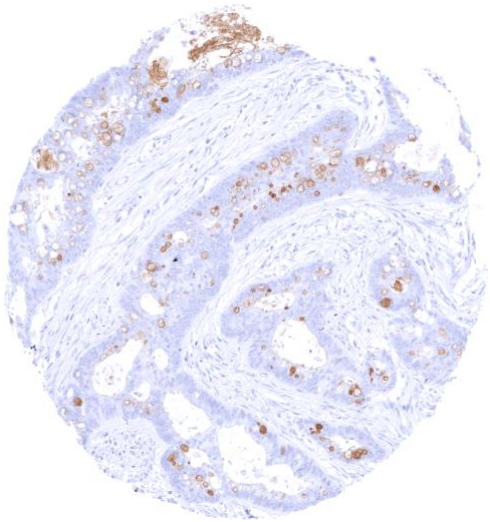


**Pancreatic acinar cell carcinoma showing strong positivity for CPA1 in all tumor cells and also significant stroma staining (contamination artifact).**

**-mucus staining:** Because CPA1 is released to the intestine some CPA1 staining can occasionally be found in mucus of the lower gastrointestinal tract. Very rarely, CPA1 staining can even be seen in goblet cells of colorectal carcinomas. Pancreatic origin of these mucins is supported by identical staining patters for Chymotrypsin (data not shown).



**CPA1 staining of mucins in a colorectal adenocarcinoma.**



**CPA1 staining of mucins in goblet cells of a colorectal adenocarcinoma.**

## Part 2 – Regulatory

### A) Justification of selection of MSVA-601M for CPA1 detection

MSVA-601 is the most extensively validated CPA1 antibody on the market and the validation data of this antibody are much broader than what is available for any other CPA1 antibody, irrespective of IVD label.

The antibody has been validated by Uhlig et al. according to the guidelines of the International working group of antibody validation (IWGAV) which requests orthogonal validation and/or a comparison with a different independent antibody for antibodies to be used on formalin fixed tissues.[1] The results of this validation are extensively documented and also explained on the homepage of the vendor under “evidence for antibody specificity”.[2] No other vendor provides a comparable explanation of the validation process for a CPA1 antibody.

The antibody has been evaluated for cross-reactivity in >50 different normal tissues and the results have been described by Uhlig et al..[1] In addition, further images of these experiments are documented on the homepage of the vendor.[3] No other CPA1 antibody has been evaluated that extensively for possible cross-reactivities and a comparable image documentation of the staining patterns in normal tissues is not available for other CPA1 antibodies.

The performance of the antibody in tumors has been comprehensively evaluated in a study by Uhlig et al. on 12,274 cancers from 132 different tumor entities.[1] No other CPA1 antibody has been evaluated for real life performance in a comparable number of tumors.

The vendor has described a protocol for DAKO autostainer Link48 that results in comparable staining results as obtained by the manual protocol of Uhlig et al..[1] The availability of dozens of images (tumors and normal tissues) obtained by the original protocol of the Uhlig study can serve as a reference for adjusting the own protocol. No other antibody comes with this level of documentation.

### B) Relevant statements

A commercial CE-IVD marked anti CPA1 antibody with a comparable level of documentation of its performance characteristics is not available for the intended use.

The risk class of the antibody is class C according to rules 3f, 3h, and 3k of Appendix VIII EU regulation 2017/746.

Antibody MSVA-601M will be continuously supervised during usage so that corrective measures can be taken if necessary”.

The antibody MSVA-601M meets the basic safety and performance requirements in accordance with Annex 1 of the IVDR.

This declaration is publicly available at:

## C) Intended use of the antibody MSVA-601M

The antibody ABC is used for detection of CPA1 protein in formalin-fixed human tissue samples.

## D) Protocol

### Autostainer: Agilent / Dako – Autostainer Link 48

**Procedure:** Pretreatment in PT-Link for 30 minutes at 95°C (pH high); FLEX peroxidase blocking for 5 minutes (room temperature), MSVA-601M **1:150** for 20 minutes (room temperature), FLEX+ mouse/rabbit (LINKER) for 15 minutes (room temperature), horseradish peroxidase (HRP) for 20 minutes (room temperature), FLEX DAB+Sub-Chromo for 10 minutes (room temperature), FLEX hematoxylin for 5 minutes (room temperature).

## E) Positive and negative control tissues

**Positive tissue control**: Pancreas: A strong staining of acinar cells should be seen. Adjacent structures can also be stained due to contamination artifacts.

**Negative tissue control:** Colon: CPA1 immunostaining must be absent in all cell types.

## F) Assessment of precision

Use one block of normal pancreas and one block of normal colon

-day 1: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of intra-run consistency)

-day 2: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of inter-run consistency)

-day 3: stain 3 sections each of both blocks for CPA1 by MSVA-601M by using the predefined staining protocol (analysis of inter-run consistency)

All results are documented on the form (CPA1-staining: Precision assessment; page 8)

**Expected result:**

-Strong CPA1 positivity in all 9 sections from normal pancreas,

-Absence of CPA1 staining in all 9 sections from normal colon.

## G) Assessment of sensitivity and specificity

Analyze 10 samples of normal pancreas (expected to be positive) and 10 samples of other normal tissues (colon, kidney, placenta, brain, skin, smooth muscle, liver, gallbladder, adrenal gland) which are all expected to be negative.

All results are documented on the form (CPA1-staining: Specificity/sensitivity assessment) which describes the necessary calculations for determining the specificity and sensitivity (page 9).

**Expected result:**

Sensitivity = 100%

Specificity = 100%

## References:


1. Uhlig R, Contreras H, Weidemann S, et al. Carboxypeptidase A1 (CPA1) Immunohistochemistry Is Highly Sensitive and Specific for Acinar Cell Carcinoma (ACC) of the Pancreas. *Am J Surg Pathol*. 2022;46(1):97-104. doi:10.1097/PAS.0000000000001817

2. https://ms-validatedantibodies.com/product/cpa1-msva-601m/

3. https://ms-validatedantibodies.com/product-gallery/normal-tissue-gallery-cpa1/

**Form: CPA1 staining: Precision assessment**

**DAY 1**

| | **normal tissue Pancreas** | | **normal tissue Colon** | | Date of experiment |
|---|---|---|---|---|---|
| | NEG | POS | NEG | POS | |
| slide 1 | | | | | |
| slide 2 | | | | | Pathologist |
| slide 3 | | | | | |

**DAY 2**

| | **normal tissue Pancreas** | | **normal tissue Colon** | | Date of experiment |
|---|---|---|---|---|---|
| | NEG | POS | NEG | POS | |
| slide 1 | | | | | |
| slide 2 | | | | | Pathologist |
| slide 3 | | | | | |

**DAY 3**

| | **normal tissue Pancreas** | | **normal tissue Colon** | | Date of experiment |
|---|---|---|---|---|---|
| | NEG | POS | NEG | POS | |
| slide 1 | | | | | |
| slide 2 | | | | | Pathologist |
| slide 3 | | | | | |

## Form: CPA1 staining: Specificity/sensitivity assessment

| Expected positive tissue type | IHC result NEG | IHC result POS |
|---|---|---|
| Tissue Pancreas #1 | | |
| Tissue Pancreas #2 | | |
| Tissue Pancreas #3 | | |
| Tissue Pancreas #4 | | |
| Tissue Pancreas #5 | | |
| Tissue Pancreas #6 | | |
| Tissue Pancreas #7 | | |
| Tissue Pancreas #8 | | |
| Tissue Pancreas #9 | | |
| Tissue Pancreas #10 | | |

| Expected negative tissue type | IHC result NEG | IHC result POS |
|---|---|---|
| Assumed neg tissue Colon | | |
| Assumed neg tissue Kidney | | |
| Assumed neg tissue Placenta | | |
| Assumed neg tissue Brain | | |
| Assumed neg tissue Skin | | |
| Assumed neg tissue Smooth muscle | | |
| Assumed neg tissue Liver | | |
| Assumed neg tissue Gallbladder | | |
| Assumed neg tissue Adrenal gland | | |
| Assumed neg tissue Tonsil | | |

# of false negative _____

# of true negative _____

# of false positive _____

**Sensitivity*** _____

**Specificity**** _____

Date of experiment _____

Pathologist _____

* Sensitivty = number of true positive / (number of true positive + number of false negative)

**Specificity = number of true negative / (number of true negative + number of false positive)