

# Identification of BRAF Variant V595E in urine, smears and paraffin-embedded tissue: a new diagnostic tool for canine transitional cell carcinoma

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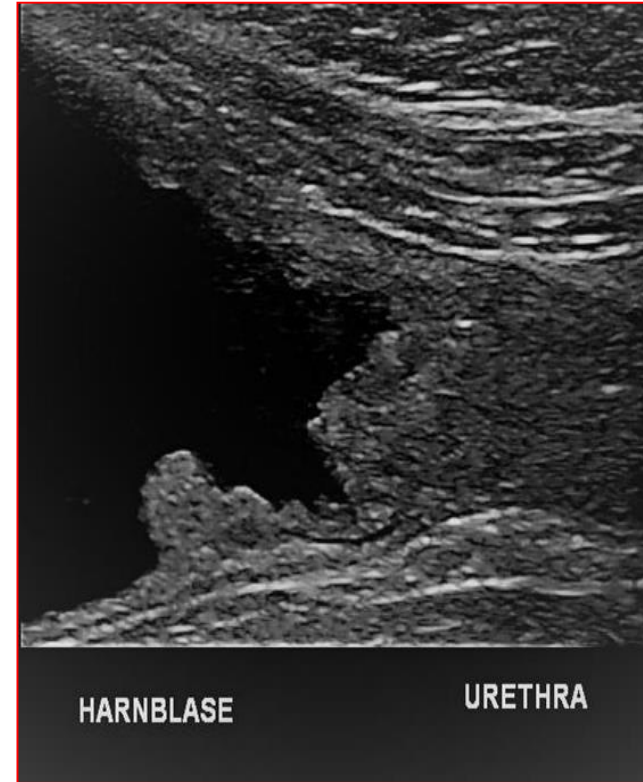
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## Introduction

### Mutation of the BRAF gene

- Common cause of tumor formation in humans  
→ Cellular proliferation and dedifferentiation
- Study of Mochizuki et al. (2015a+b) in the USA: BRAF variant V595E (syn. V450E) in ca. 85% of the canine transitional cell carcinomas (TCC)  
(PLoS ONE 2015a, 10(6):e0129534; PLoS ONE 2015b, 10(12):e0144170)



## Dog population and Methods

- **66 Dogs:** 0.5-17 years of age (median 10 years)  
15x f, 21x fs, 18x m, 12x mc
- **Material:** 45 biopsies  
48 urine samples  
31 cytological smears
- **Diagnoses:** 33x transitional cell carcinoma  
23x cystitis  
7x urinary bladder polyp  
3x various other lesions
- **Routine cytology and histology:**  
for example: cell count of the smear, TCC grading
- **Molecular genetics:**  
DNA-Isolation with commercially available test kits  
Sanger Sequencing of BRAF Variante c.1784T>A



## Study Goals

- Examination of the **prevalence** of the BRAF Variante V595E in our routinely submitted samples
- Establishment of a diagnostic method for urine, cytological smears and tissue biopsies → Improvement of a non-invasive diagnostic method for TCC

## Results

- **Successful DNA-isolation from:**  
39/45 Paraffin-embedded tissue  
38/48 Urine samples  
16/18 „Cell-rich“ cytological smears  
3/13 „Cell-poor“ cytological smears

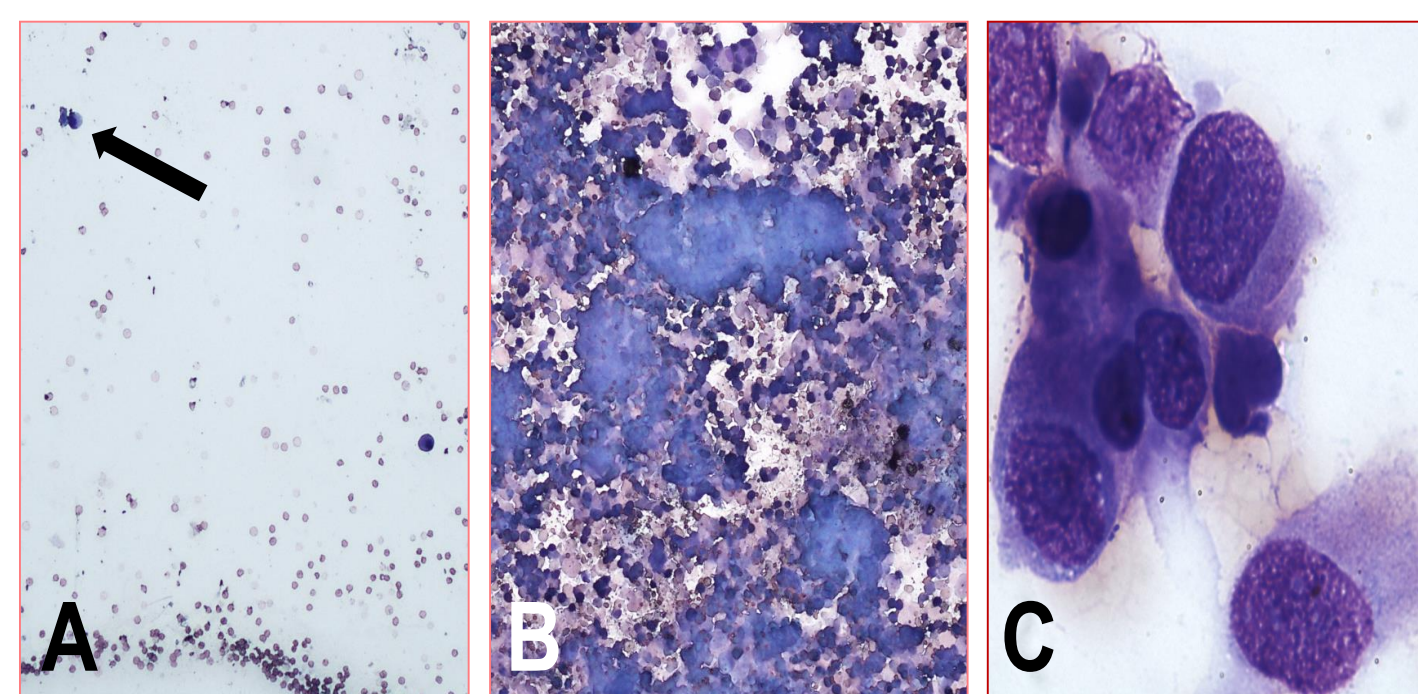
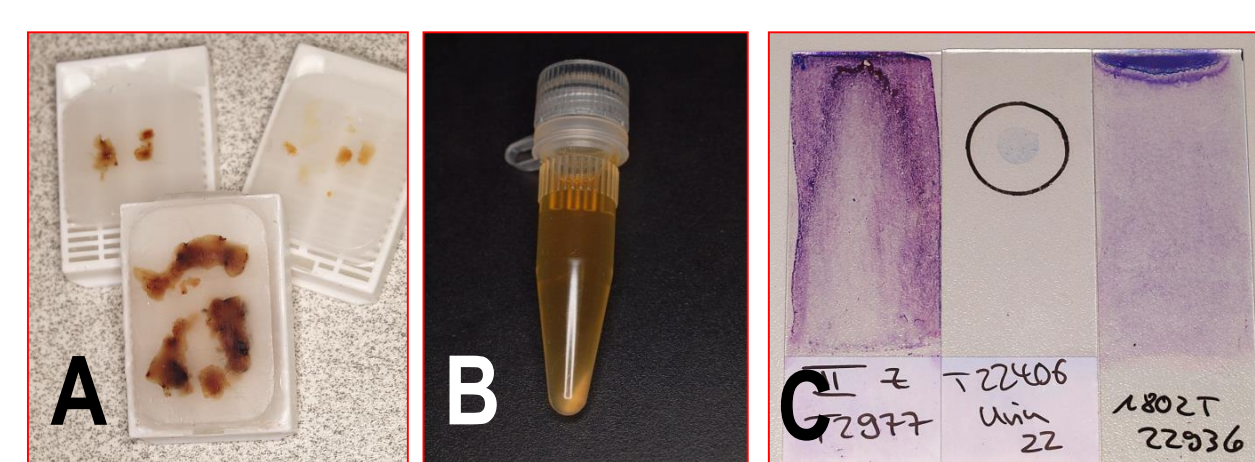


Figure 1:

- A:** „Cell-poor“ cytological smear with solitary epithelial cells (arrow)  
→ the number of cells is *too low* for BRAF-Mutation analysis
- B:** „Cell-rich“, **thick**, cytological smear: epithelial cells can not be evaluated  
→ typical indication for BRAF-Mutation analysis
- C:** „Cell-rich“, **easily evaluable** smear with transitional epithelial cells showing multiple criteria of malignancy

### Different samples:

- Biopsy / paraffin-embedded tissue (A)
- Urine (sediment) (B)
- Cytology smears (C)

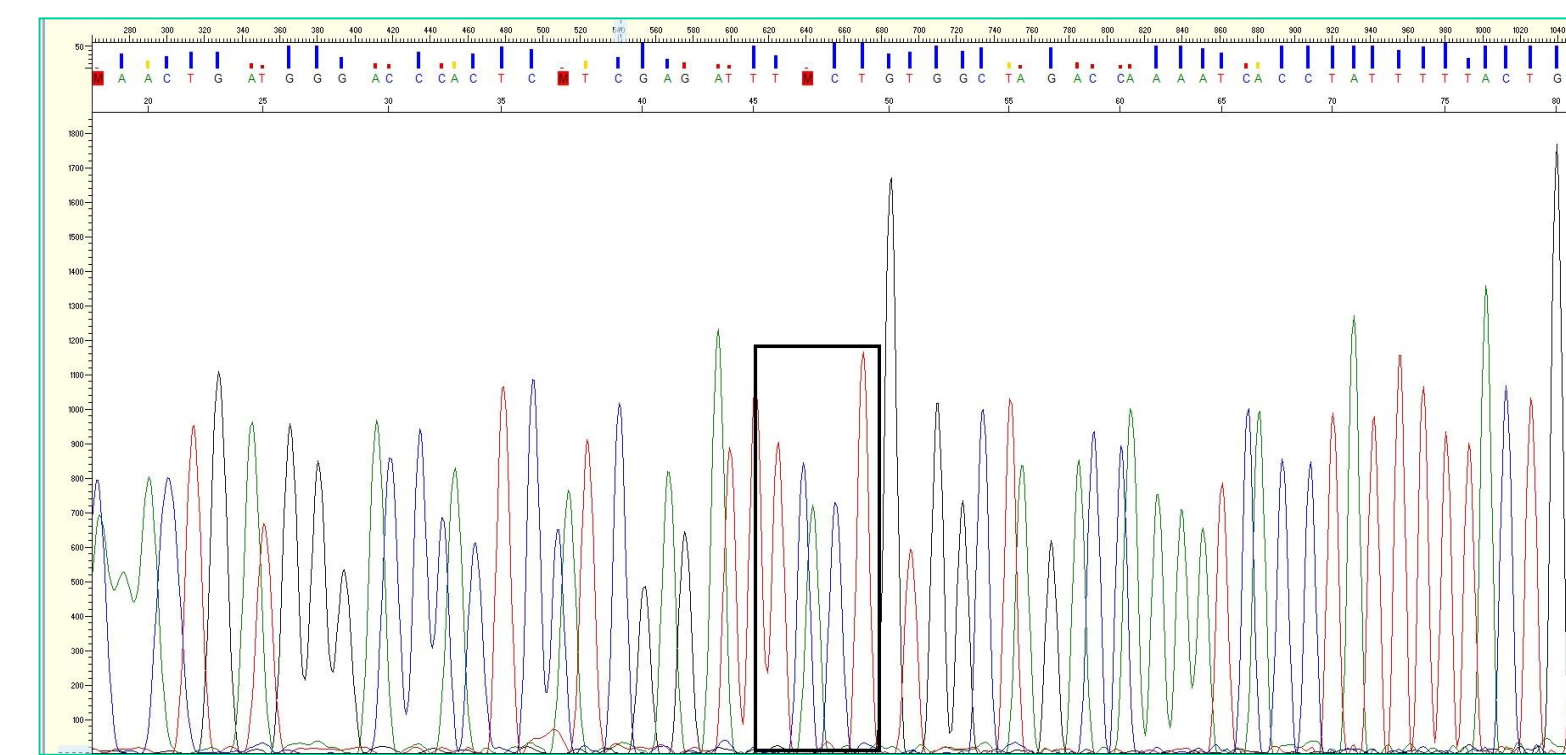


→ After successful DNA-isolation, BRAF-analysis results were similar in different types of material originating from one dog

**Table 1: Prevalence** of the BRAF-gene mutation (BRAF Variante c.1784T>A) in the urinary bladder. Samples from 66 dogs (2/66: DNA-isolation was not sufficient)

|                                | TCC (n=31) | Polyp (n=7) | Cystitis (n=23) | Other (n=3) |
|--------------------------------|------------|-------------|-----------------|-------------|
| <b>Homozygote Normal TT</b>    | 9          | 7           | 23              | 3           |
| <b>Heterozygote Variant TA</b> | 22         | 0           | 0               | 0           |

- **Cystitis, polyps or other diseases** → BRAF-Mutation is absent (100%)



A

Figure 2:

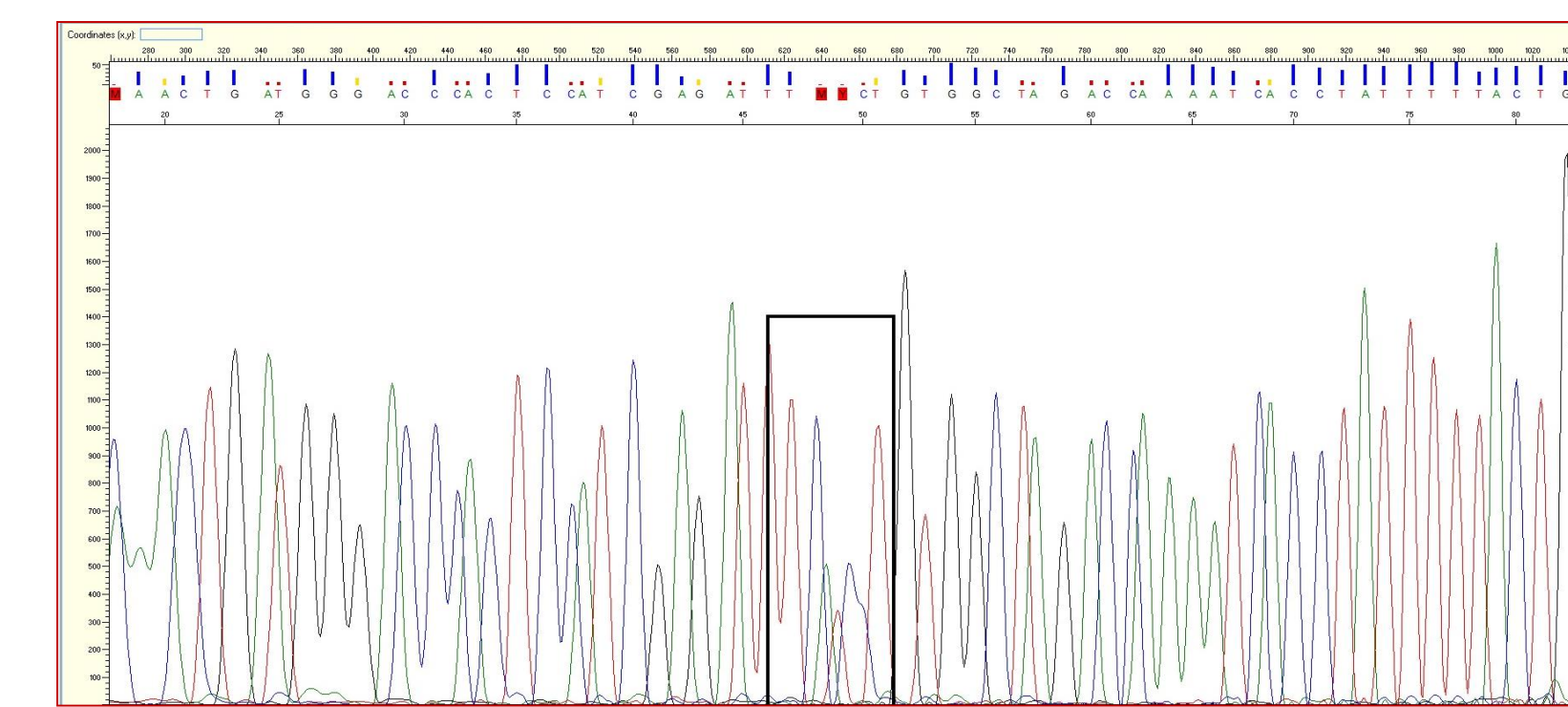
- A: Normal Sanger Sequencing result** of the BRAF gene:  
Electropherogram of a dog: All dogs without TCC are **homozygote TT** (black rectangle)  
→ BRAF-gene mutation is absent

- B: Urinary bladder polyp** of a 12-year-old dog<sup>1</sup>



B

- **TCC: 22/31 Dogs** with two different nucleobases (TA)  
→ *Confirmation of the mutation*

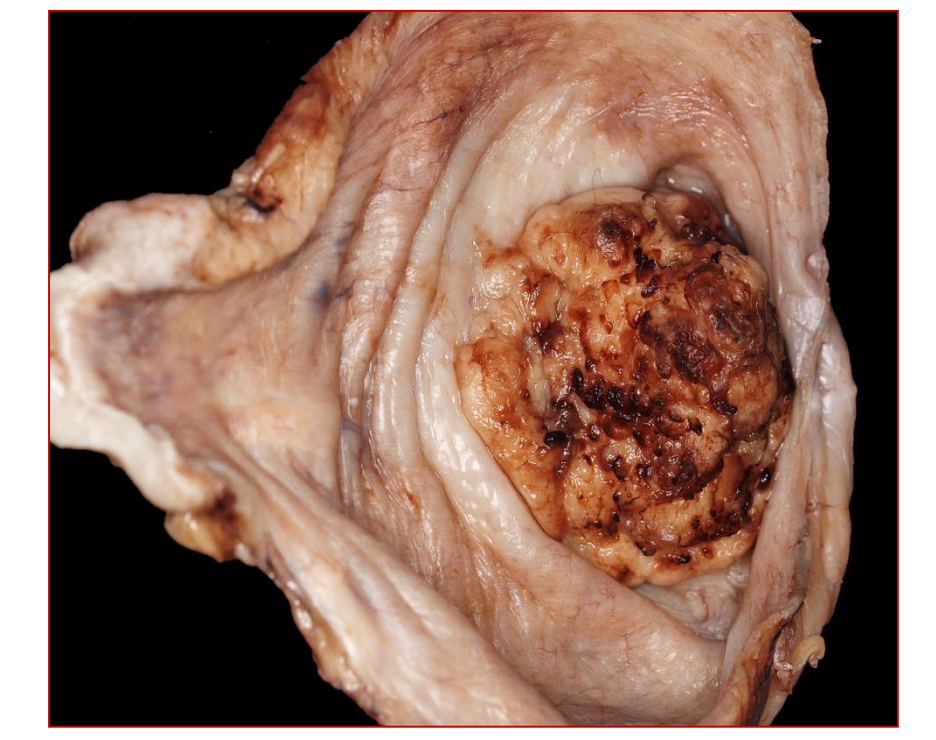


A

Figure 3:

- A: Sanger Sequencing of Exon 15** of the BRAF Gene:  
Electropherogram of a dog with TCC, with **Variant c.1784T>A**:  
Ca. 70% of evaluated TCC were **heterozygote TA** (black rectangle) → BRAF-gene mutation

- B: Transitional cell carcinoma** in the urinary bladder of a 12-year-old dog



B

## Discussion

- The BRAF-mutation analysis on routinely submitted samples (biopsies, cytological smears, urine) was established.
- **Requirement:** sufficient numbers of transitional epithelial cells in the submitted material is necessary.
- **Indications** for BRAF-mutation analysis: 1) Non-invasive diagnostic method preferred  
2) Cytologically or histologically poor quality of material or questionable dysplastic appearance of epithelial cells
- The **sensitivity of 70%** in this study is corresponding to the sensitivity described in literature (67%-87%).
- The **specificity is around 100%**, since the BRAF-mutation was absent in all samples from dogs without TCC.  
→ A **positive result** is **confirming** the diagnosis of **TCC** (or possibly prostate carcinoma, which can also show this mutation).
- **Absence of the mutation** interpretation: 1) Absence of a transitional cell carcinoma (for example: presence of a polyp or cystitis)  
2) Mutated transitional epithelial cells were absent in the submitted material (depending on sample size/representativity)  
3) The present TCC was not caused by a BRAF-gene mutation  
→ A **negative result** does not rule out the presence of a transitional cell carcinoma!
- **Prospect:** 1) Use of the test for early detection of TCC in urine  
2) Prognostic and therapeutic relevance of the BRAF-mutation (for example: MAPKinase inhibitors)