82 FFPE HNSCC samples stained for p16 (of 107 retrieved)

- p16 positive (n=28)
- p16 negative (n=46)

DNA extraction

- DNA extraction of LCM samples yielded insufficient amounts or insufficient quality of DNA (n=8)
- Inconsistent or borderline results in repeat measurements (n=2)
- False positive (n=6) and false negative (n=2) cases; further samples excluded acc. to DNA quality and gender and age-matching requirements

DNA

E6 qPCR

- E6 qPCR positive (n=22)
- E6 qPCR negative (n=34)

Selection of validated samples

- p16 positive E6 qPCR positive (n=6)
- p16 negative E6 qPCR negative (n=34)

Gender and age-matching

- p16 positive E6 qPCR negative (n=2)
- p16 negative E6 qPCR positive (n=2)

Histology

- p16 positive HNSCC sample
- p16 negative HNSCC sample

Analysis

- HPV+ HNSCC samples (n=20)
- HPV- HNSCC samples (n=20)

NG sequencing

- Assuming E6 qPCR to be the gold standard:
  - True positives: 22 (34.4 %)
  - True negatives: 34 (53.1 %)
  - False positives: 6 (9.4 %)
  - False negatives: 2 (3.1 %)

- 2x2 table

<table>
<thead>
<tr>
<th></th>
<th>E6 qPCR positive</th>
<th>E6 qPCR negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 positive</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>p16 negative</td>
<td>2</td>
<td>34</td>
</tr>
</tbody>
</table>

p16 staining: Sensitivity: 91.6%; Specificity: 85%