

# E6 and E7 mRNA expression from carcinogenic Human papillomavirus (HPV) in 4136 cervical samples collected from an outpatient population

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## Objective:

High-risk human papillomavirus (HPV) infection is the main cause of cervical carcinoma and HPV DNA has been detected in more than 90% of cervical carcinoma samples, with the most common HPV types identified as HPV 16, 18, 31, 33, and 45. Naturally, it is tempting to include HPV detection and typing in screening programs in order to increase sensitivity. However, due to the high prevalence of HPV infections in normal samples the number of HPV DNA positive results may be too high for screening purposes.

Assays currently in use for HPV detection are based on L1 or E1 DNA amplification or hybridisation. However, the actual oncogenic effect is dependent on continuous expression from the E6 and E7 genes for successful transformation and maintenance of a neoplastic phenotype of the cervical carcinoma cells. Therefore, monitoring HPV mRNA expression in cervical smears may provide an accurate and informative diagnostic approach in cervical screening in addition to cytology.

The aim of this study was to identify the presence of E6/E7 mRNA and DNA in cytological HGSIL/CIN3 samples confirmed by histology.

## Material and Methods:

The samples were collected from a well-screened outpatient population, including women older than 30 years of age (n=4136). E6/E7 transcripts from each of the high-risk HPV types 16, 18, 31, 33, and 45 were detected by the PreTect HPV-Proofer assay (NorChip AS, Klokkestua, Norway), based on real-time multiplex NASBA. The presence of HPV DNA was investigated by Gp5+/6+ consensus PCR, and HPV DNA positive samples were then subjected to type specific PCR for HPV types 16, 18, 31, 33 and 45. Women with a cytological HGSIL diagnosis were referred to biopsy and histology. Histologically confirmed cases were registered at the Norwegian Cancer Registry. In Norway, cytological HGSIL can be divided into HGSIL/AGUS, HGSIL/ASC-H, HGSIL/CIN2, and HGSIL/CIN3.

## Results:

**Table 1:** Presence of HPV and E6/E7 mRNA in cytological HGSIL and histological CIN2+ samples

HPV positive by	Cytological HGSIL (n=25)	Histological CIN2+ (n=14)	Cytological HGSIL not verified by Histology (n=11)
PreTect HPV-Proofer	13 (52%)	12 (86%)	1 (9%)
Gp5+/6+ PCR	16 (64%)	13 (93%)	3 (27%)

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Results from HPV testing are summarized in **Table 1**. Of 25 cytological HGSIL cases, 14 were by histology confirmed as CIN2+. Two histological CIN2+ cases were by cytology diagnosed as HGSIL/ASC-H and HGSIL/CIN2. PreTect HPV-Proofer detected 52% (13/25) of the cytological HGSIL cases, 86% (12/14) of the histological CIN2+ cases, and 9% (1/11) of the cytological HGSIL cases not verified by histology. The numbers for Gp5+/6+ PCR are 64% (16/25), 93% (13/14), and 27% (3/11), respectively. The one histological CIN2+ sample positive by consensus PCR, yet negative by PreTect HPV-Proofer, was identified as HPV 35. The prevalence of HGSIL/CIN3 was 0.29% (12/4136) and the prevalence of histological CIN2+ was 56% (14/25).

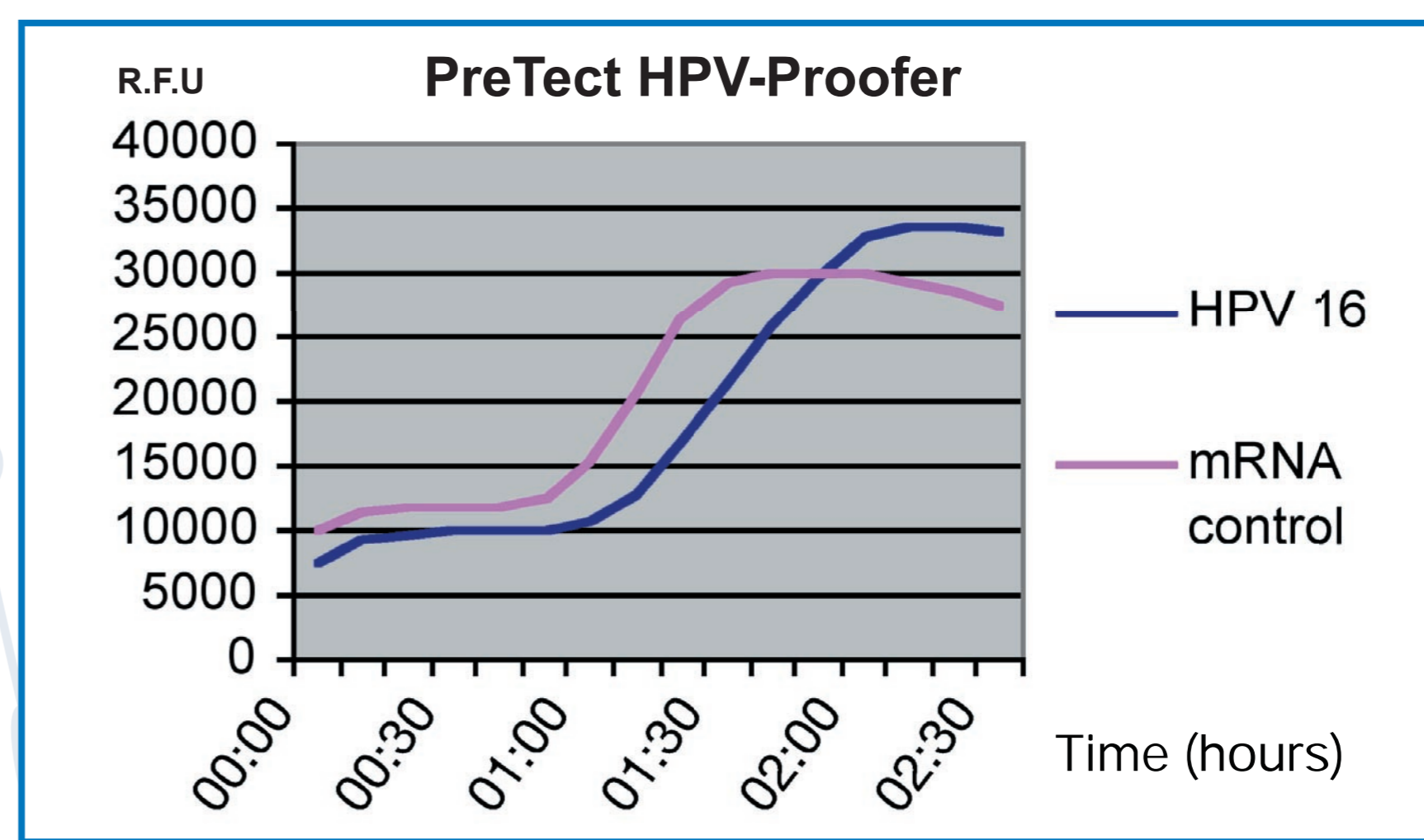
Sensitivity, specificity, and positive and negative predictive values for PreTect HPV-Proofer and consensus PCR, are given in **Table 2**.

**Table 2:** Sensitivity, specificity, PPV and NPV for PreTect HPV-Proofer and Consensus PCR

	Total (n=4136) Endpoint cytological HGSIL/CIN3		Cytological HGSIL (n=25)* Endpoint histological CIN2+	
	PreTect HPV-Proofer	Consensus PCR	PreTect HPV-Proofer	Consensus PCR
Sensitivity	75,0%	83,3%	85,7%	92,9%
Specificity	97,2%	89,9%	90,9%	72,7%
PPV	7,3%	2,3%	92,3%	81,3%
NPV	97,2%	99,9%	83,3%	88,9%

\* Histology has only been performed on cytological HGSIL cases. PPV=Positive Predictive Value. NPV=Negative Predictive Value.

**Fig 1:** E6/E7 mRNA amplification by real-time multiplex NASBA



R.F.U = Relative Fluorescence Units

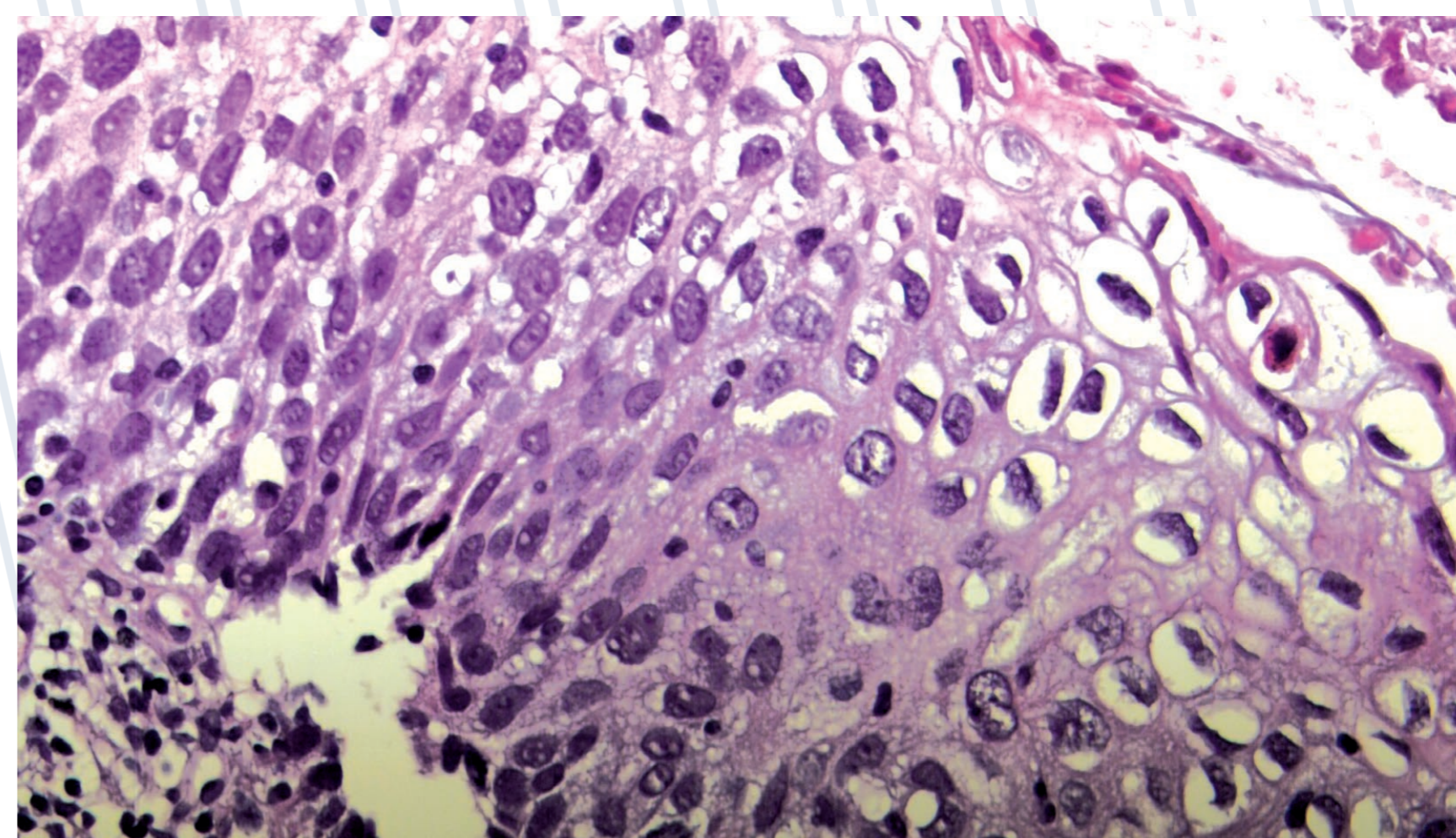
## Discussion and Conclusion:

There was a good agreement between cytological HGSIL/CIN3 and histological CIN2+ cases, with only one cytological HGSIL/CIN3 not confirmed as CIN2+ by histology. In histologically verified CIN2+ cases, the detection grade for both HPV DNA and mRNA were high, and nearly identical. In cytological HGSIL cases not verified by histology, the detection grade for PreTect HPV-Proofer was lower than for consensus PCR. Together, cytological HGSIL/CIN3 and PreTect HPV-Proofer detected all histological CIN2+.

In conclusion, HPV E6/E7 transcripts from the five most frequently found carcinogenic HPV types, HPV 16, 18, 31, 33, and 45, seem to be present in nearly all histological CIN2+ cases.

The high specificity and positive predictive value for PreTect HPV-Proofer may be an advantage in HPV diagnostic and hence mRNA detection is a suitable supplement to cytology and histology.

**Fig 2:** Histological section of a CIN3 lesion.



HPV related cell changes (koilocytosis) are evident in the upper layers.