

# Difference in coverage rate between DNA- and mRNA-based methods for HPV positive invasive cervical carcinoma samples - A summary of studies

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## Background

A number of different HPV DNA-based methods have been used to study the prevalence of HPV DNA in invasive cervical carcinoma samples. Recently, studies have also been done evaluating the prevalence of HPV mRNA in these cancers. In malignant tumours, HPV DNA is usually integrated in the human genome. One result of integration is disruption of viral genes and/or deletion of viral DNA segments. However, for successful carcinogenesis, expression of E6 and E7 genes is imperative and is consistently observed in carcinomas. More than 30 HPV-types have been identified in invasive cervical carcinoma cases. However, if oncogene expression of E6/E7 proteins is absent, these HPVs may not have contributed to the carcinogenic process.

## Objectives

- Identify coverage rate of different HPV-types in invasive cervical carcinoma samples from Europe, North-America and Australia documented in international peer-reviewed publications.
- Compare the coverage rate between DNA- and mRNA-based methods for invasive cervical carcinoma samples.
- Discuss the possible biological aspects behind different findings between DNA and RNA based HPV detection methods and the implications for choice of method in cervical cancer detection.

## Results

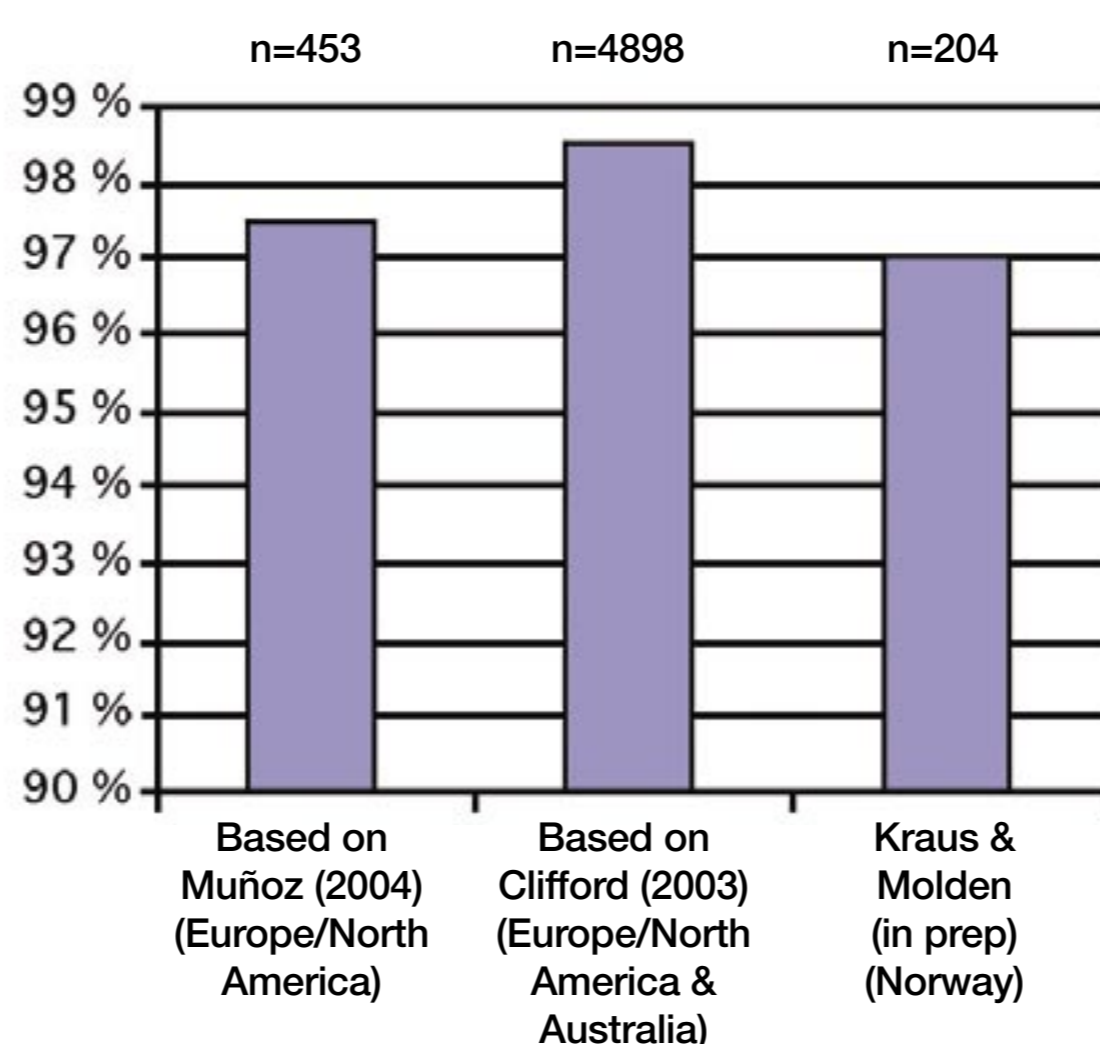
Over the last two years, some important publications regarding the relationship between different HPV-types and invasive cervical carcinoma have been published. These publications include over 90 studies and over 10 000 cervical carcinoma samples. In Europe, North-America and Australia the equivalent numbers are over 40 studies and over 5000 invasive cervical carcinomas. To relate different HPV-types to the risk of developing invasive cervical carcinoma, it is important to know the differences in coverage rate between HPV-types in invasive cervical carcinomas.

## Definition of coverage rate:

Coverage rate of different HPV-types is the proportion of invasive cervical carcinomas harboring one or more of the HPV-types in question divided by the total number of invasive cervical carcinomas that has been found to be positive with any kind of HPV.

Muñoz et al. (2004) gives a good overview of the prevalence of different HPV-types in invasive cervical carcinomas. Clifford et al. (2003) also addresses this important question. In Norway, Kraus and Molden et al. (in prep.) has also looked at this issue. Figure 1 shows the coverage rate of HPV-types 16, 18, 31, 33, and 45 in invasive cervical carcinomas in Europe/North America/Australia.

**Figur 1.** Coverage rate of HPV-types 16, 18, 31, 33, and 45 in HPV positive invasive cervical carcinomas in Europe, North America and Australia.



## Discussion

During HPV integration the viral L1 region may be disrupted. If the L1 target sequence has been lost, HPV DNA cannot be detected by PCR methods. The same may be true in regard to *in situ* or hybridization techniques, but these methods normally use probes covering the whole HPV genome and are therefore not so vulnerable. These cancers will in be detected by PreTect HPV-Proofer if E6/E7 genes are expressed.

An obvious challenge is the presence of possible HPV "passengers" or nontransforming HPV infections that have not been involved in the carcinogenic process (Fig. 2). These HPV passengers may also be present together with disrupted HPV DNA from "carcinogenic" HPV types in tumours. This may cause a target competition that reduces the specificity and the overall sensitivity of the detection method, in particular related to L1, E2 or E1 based consensus and multiplex detection systems. Therefore, when the consensus PCR products are analysed by typing methods, the HPV passengers may be the ones that are detected instead of the cancer causing HPV-type that has produced E6 and E7 proteins. Despite the high coverage rate of the "high-risk" HPV DNA types in studies of HPV positive cervical cancer samples (Clifford et al., 2003, Muñoz et al., 2004, Kraus and Molden et al., in prep., Karlsen et al., 1996) it may be discussed whether all HPV types detected by DNA based methods have participated in the development of the cancer. Schiffman et al. (2005) documents that for both screening and triage, testing for more than about 10 HPV types decreased specificity more than it increased sensitivity. HPV 35 is very rare as a stand-alone HPV-type detected in invasive cervical carcinomas and HPV 52 and 58 may be important in Asia. Due to the necessary presence and abnormal steady state activity of the E6 and E7 genes, E6/E7 HPV mRNA detection methods are independent of a low or high copy number, or of disrupted DNA in invasive cervical carcinoma samples. Actually, more than 50% of the cytologi-

cally negative samples, yet being HPV 16, 18 or 33 DNA positive do not express E6 or E7 transcripts as analysed by the PreTect HPV-Proofer assay (Cuchierie et al., JMV, 2004, Molden et al., CEBP, 2005). It has been demonstrated that the transcription of full-length or spliced E6 and E7 transcripts from cell lines or clinical samples with a low copy number or integrated HPV DNA, is at the same high level as is found in samples from invasive cervical cancer or CIN III with high amount of HPV DNA (Dürst et al., Eurogin 2004, Sotlar et al., 2004, BJC). Carcinoma samples with disrupted DNA from the carcinogenic HPV types not detected by HPV DNA methods, may still have a high expression of oncogenic E6 and E7 proteins. The detection rate of the carcinogenic HPV 16, 18 and 45 transcripts were demonstrated to be 100% in cytological smears collected from women with invasive cervical carcinoma (Lie et al., Gynaecological Oncology, 2005, Moeckel et al., oral presentation 22th IPC, 2005).

## Conclusion

- It is very likely that a limited number of HPV-types (16, 18, 31, 33, 45 and in Asia 52 and 58) covers close to 100% of all cervical smears collected from HPV-induced invasive cervical carcinomas.
- Based on Evidence-based Medicine, diagnostics to identify women at risk for developing invasive cervical carcinomas should therefore focus on the carcinogenic HPV-types and not include the vast number of HPV-types not related to development of the disease invasive cervical carcinoma.

**Figure 2.** Presence of possible HPV "passengers" or non-transforming infections.

