

Calculations from a High Risk Population study

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Purpose

HPV mRNA testing may be a more suitable screening method than cytology in low resource settings in Africa with few pathologists and experienced gynecologists. The aim of the study was to compare for the first time colposcopy, cytology, histology, E6/E7 HPV mRNA/DNA detection in 100% of a selected outpatient population in Congo.

Material and methods

Colposcopy and samples for conventional PAP smear, Liquid-Based Cytology (ThinPrep, Cytec cooperation, Boston, US), histology and HPV-testing were collected in 343 women (median age 37) from DR Congo. Colposcopy was performed by several medical doctors with limited experience with the technique. The cytology and histology was performed by laboratories in Norway and Sweden. The PreTect HPV-Proofer assay (NorChip AS, Klokkestua, Norway), based on Real-Time NASBA, was used to identify mRNA from HPV 16, 18, 31, 33 and 45. HPV was also detected by Consensus PCR (GP5+/6+) followed by EIA (Jacobs MV et al JCM 1997) and typed by Reverse Line Blot (RLB) (A van den Brule et al JCM 2002). In this study histology was selected as the "gold standard". One colposcopically directed biopsy was taken from all women due to an expected high frequency of cervical lesions in this area. If the transformation zone was not seen endocervical curettage (ECC) was performed.

Results

Cervical smears, biopsies and samples with high quality mRNA were successfully collected and stored in Preserv Cyt medium, despite low resource setting. Qualitative human mRNA and DNA was detected in 100% of the samples. The overall prevalence of positive colposcopy, high-grade histology (CIN 2+), Pap smear, ThinPrep, PreTect HPV-Proofer and Consensus PCR are shown in table 1. Only four (33%) and five (42%) of the 12 histological CIN 2+ cases revealed positive cytology (\geq HSIL). The clinical sensitivity was almost twice as high for the PreTect HPV Proofer test (compared to PAP smear and ThinPrep (Fig 1A)). Since this study is without verification bias it is possible to claim that the clinical specificity of the PreTect HPV-Proofer test was almost as high as both cytological methods. PreTect HPV-Proofer and Consensus PCR-testing showed 83% concordance when HPV types from both tests were compared.

Conclusions

1. PreTect HPV Proofer test are well suited for use in low resource settings. PreTect HPV-Proofer testing revealed higher prognostic value compared to cytology and HPV Consensus PCR.
2. Follow-up is necessary in the cases with positive HPV mRNA test and negative cytology and histology to exclude underlying or progressive high-grade cervical neoplasia.
3. PreTect HPV-Proofer may provide a significant improvement in diagnosing cervical neoplasia in developing countries.

Acknowledgements

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Table 1: Frequency of positive results detected with colposcopy, HPV Proofer /Consensus PCR, cytology related to histological CIN 2+

Method	Positive samples N=343*	Prevalence CIN2+ ** N=343	Kappa values (N=297)	
			Histology	HPV-Proofer
Colposcopy	32%	8%	0.102	0.062
PAP Smear	6.7%	30%	0.369	0.314
LBC	6.4%	45.5%	0.585	0.436
HPV-Proofer	7.6%	38%	0.521	1
Consensus PCR	30%	12.5%	0.211	0.352
Consensus PCR (carcinogenic types)***	20%	19%	0.319	0.480
Histology	3.8%	100%	1	0.521

Footnote*: Unsatisfactory samples within each method:

Colposcopy 21%; PAP Smear 2.6%; LBC 4.1%; Histology 8.7%; HPV Proofer and Consensus PCR 0 %

Footnote:** Prevalence of CIN2+ in positive cases of each relevant method.

Footnote*:** See Table 2 (to the right) defining carcinogenic HPV types.

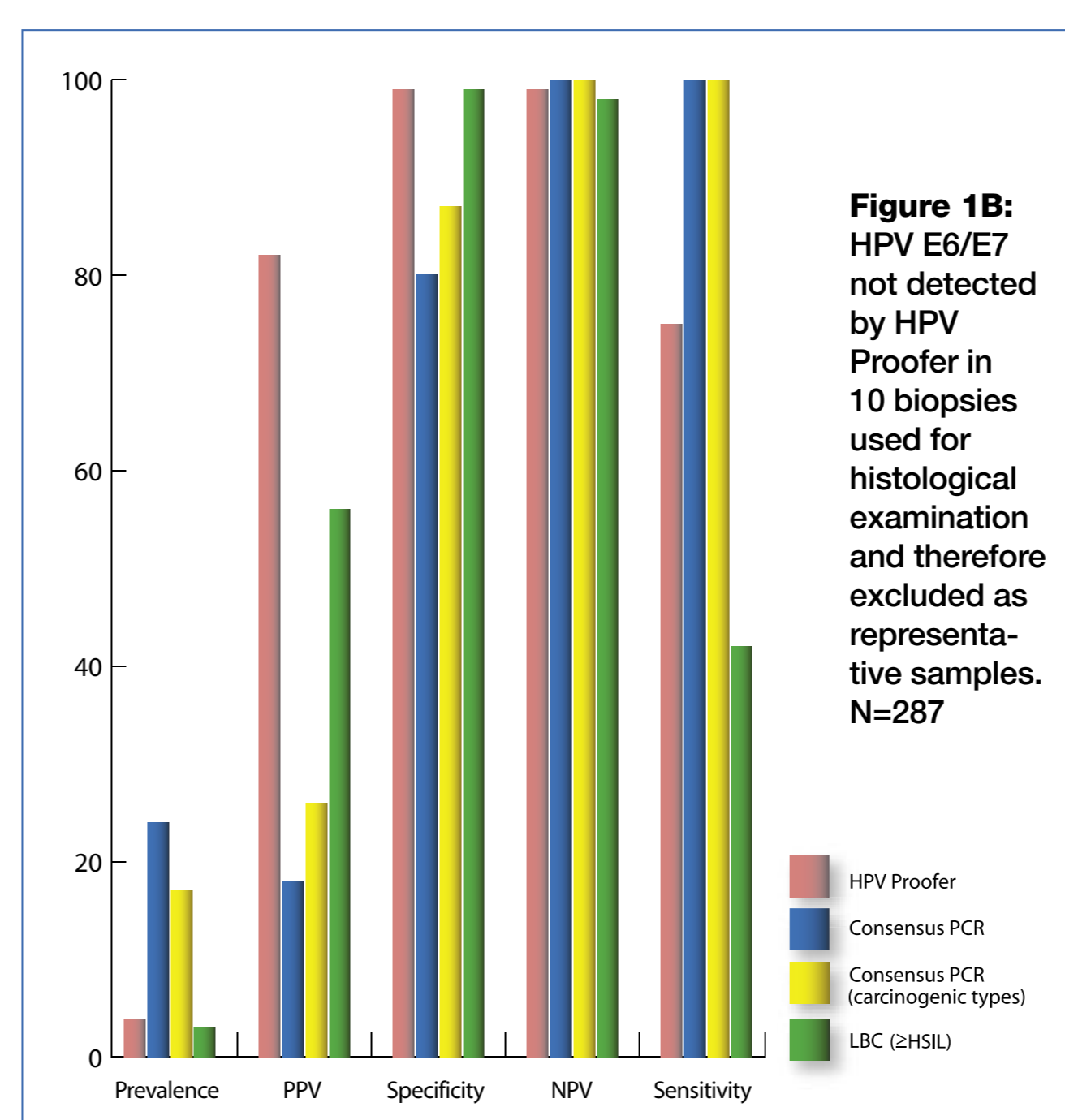
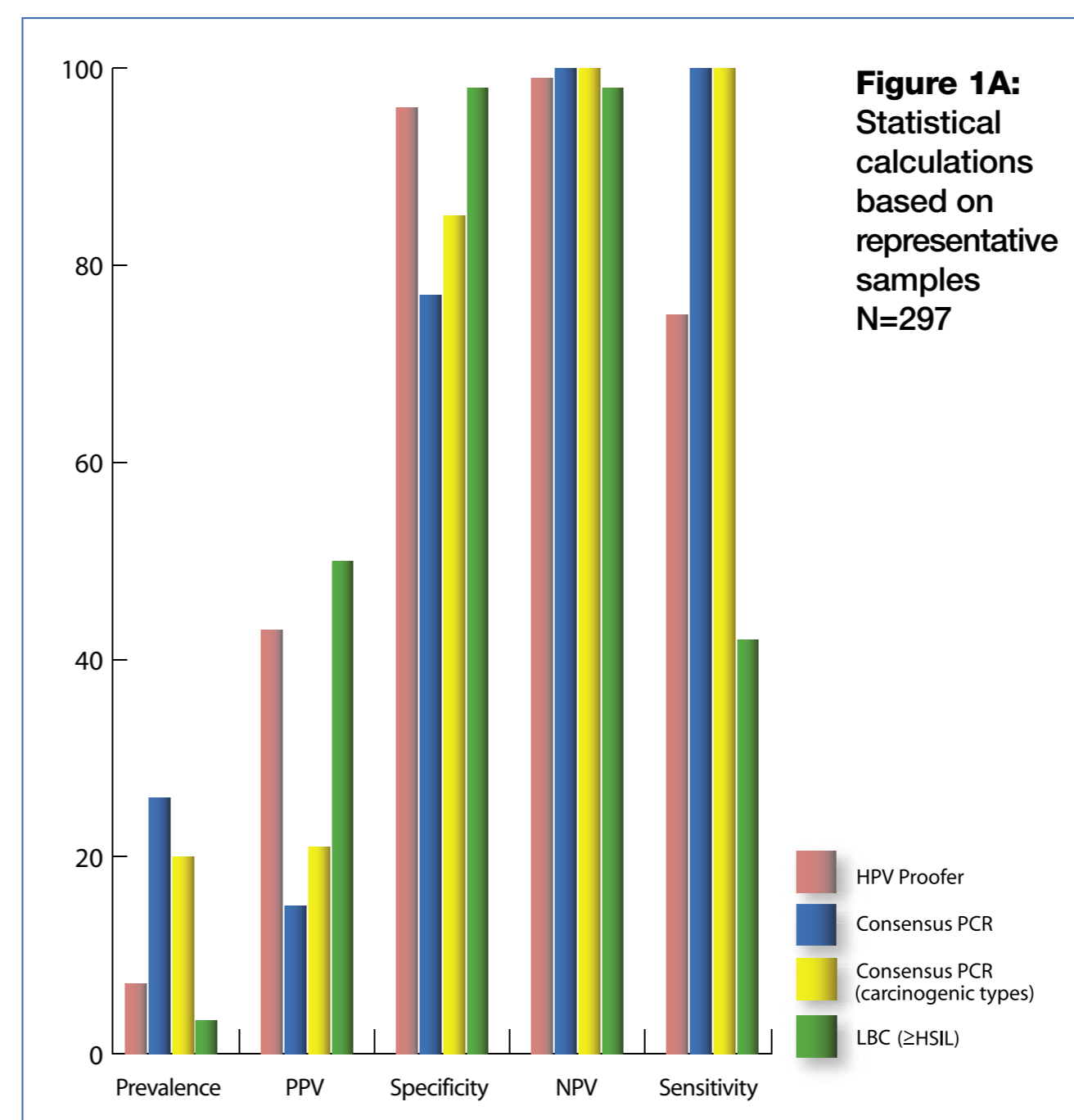


Table 2. Overview HPV Types included in the study. (van den Brule et.al.: JCM 2002)

Carcinogenic types (IARC 2005)
Covers 98% of cervical cancers in Europe and US (Clifford et.al.: 2003)

DNA* Multiple infections detected in 42%
mRNA** Multiple infections detected in 19%

HPV TYPES

DNA*	mRNA**
16 N=8	16 N=5
18 N=7	18 N=9
31 N=7	31 N=3
33 N=5	33 N=3
45 N=10	45 N=10
35 N=11	
39 N=2	
51 N=3	
52 N=7	
56 N=10	
58 N=2	
59 N=7	
66 N=7	
82 N=2	
6 N=2	
11 N=3	
26 N=0	
30 N=3	
34 N=0	
40 N=1	
42 N=7	
43 N=2	
44 N=0	
53 N=2	
54 N=1	
55 N=4	
57 N=0	
61 N=0	
64 N=0	
67 N=2	
68 N=1	
69 N=2	
70 N=3	
71 N=0	
72 N=3	
73 N=3	
81 N=2	
CP6108 N=0	
32, 83, 84, 85, 86, JC9710 COCTAIL N=19	