EUROGIN 2007, Monte Carlo

COMPARISON OF HPV DETECTION BY HYBRID CAPTURE II TEST, PRETECT HPV-PROOFER KIT AND FULL SPECTRUM HPV AMPLIFICATION AND DETECTION SYSTEM

Benczik M[1], Liddle S[2], Szenthe B[1], Takács T[1], Sebe A[1,3]

1. GenoID Ltd, Budapest, Hungary, 2. The Doctors Laboratory, London, England 3. Semmelweis University, Dept. of Pathophysiology, Budapest, Hungary

Abstract: Objectives: Given the complexity of multiple infections, viral persistence, vaccination and implementation of interventions, the accurate detection of Human Papillomaviruses is essential in cervical cancer patient management, screening and follow-up. We evaluated the clinical performance of three HPV detection systems: Hybrid Capture II (HCII), PreTect HPV-Proofer Kit (HPV-Proofer) and Full Spectrum HPV Amplification and Detection System (FS, published as the L1F/L1R PCR and hybridization based HPV detection and typing system).
Methods: Clinical specimens were collected at outpatient clinics in London, England. 196 samples were collected in PreservCyt and DNA/RNAs were prepared using Qiagen's MagAttract(r) RNA Cell Mini M48 Kit. After comparing the data, discordant results between the HCII and FS were assessed by the Amplicor HPV Detection Kit. All tests were executed as indicated by the manufacturers.
Conclusions: First, we compared the results obtained by HPV-Proofer and FS systems. The FS system detected more positive samples when comparing the five HPV types detected by the HPV-Proofer system, indicating the presence of HPV DNA in more samples (HPV 16: 35 v. 16 cases, HPV18: 19 v. 10 cases, HPV31: 9 v. 0 cases, HPV33: 5 v. 3 cases, HPV45: 9 v. 5 cases). All samples found positive by HPV-Proofer were also detected by FS. The comparison between HCII and FS resulted in a concordance of 76.53%. There were 42 samples found positive by FS and negative by HCII. Five of these were single infections with HPV66, which is not detected by HCII. The remaining 37 samples were analyzed by the Amplicor HPV Detection Kit, and 30 samples were confirmed positive. Four further cases were found positive by HCII and negative by FS. Two of these samples were found positive and 2 samples negative by the Amplicor HPV Detection Kit.
We propose the FS system as a diagnostic tool based on its overall sensitivity and reliable specificity. Moreover, based on its high sensitivity for types targeted by HPV vaccines, we propose it as a pre-vaccination test.

Objectives:

Given the complexity of multiple infections, viral persistence, vaccination and implementation of interventions, the accurate detection of Human Papillomaviruses (HPV) is essential in cervical cancer patient management, screening and follow-up. HPV typing has a growing significance for clinical practice. HPV type is important in diagnosing persistent infections, the main risk factor for cervical cancer. Multiplex HPV infections also need typing for the accurate diagnosis. The follow-up after therapy also requires typing for prognosis assessment. The introduction of HPV vaccines also involves the detection of the types included in the respective vaccines, since it is advised to exclude the infections with the types included in the vaccines.
We wished to evaluate the clinical performance of three HPV detection systems: Hybrid Capture II (HCII), PreTect HPV-Proofer Kit (HPV-Proofer) and Full Spectrum HPV Amplification and Detection System (FS, published as the L1F/L1R PCR and hybridization based HPV detection and typing system).
The HPV types detected by the individual tests are presented in Table 1. HCII and Amplicor detect 13 HR types without a typing result, whereas the HPV Proofer detection and typing includes the five most frequent HPV types causing cervical cancer. The FS system types for 14 HR HPVs and also features a general LR detection.

Methods:

Clinical specimens were collected at outpatient clinics in London, England. 196 samples were collected in PreservCyt and DNA/RNAs were prepared using Qiagen's MagAttract(r) RNA Cell Mini M48 Kit. After comparing the data, discordant results between the HCII and FS were assessed by the Amplicor HPV Detection Kit. All tests were executed as indicated by the manufacturers.

Results:

First, we compared the results obtained by HPV-Proofer and FS systems (Table 2). The FS system detected more positive samples when comparing the five HPV types detected by the HPV-Proofer system, indicating the presence of HPV DNA in more samples (HPV 16: 35 v. 16 cases, HPV18: 19 v. 10 cases, HPV31: 9 v. 0 cases, HPV33: 5 v. 3 cases, HPV45: 9 v. 5 cases). All samples found positive by HPV-Proofer were also detected by FS. The lowest positivity rate was detected at the HPV-Proofer test, a result anticipated since the HPV-Proofer test detects only the persistent transforming infections expressing E6/E7 oncogenes.
Next we examined the hybridization signals obtained by the FS system by comparing these to the results yielded by the HPV-Proofer for the detected five types. The hybridization signals for the cases found positive by both systems were in the range of 40,000-60,000, in 34% of cases, while samples presenting FS positivity and HPV-Proofer negativity involved different, higher or lower hybridization signals equally distributed. Samples yielding low hybridization signals (0-20000) were HPV-Proofer positive in only 8 % of cases. These results suggest that the lower hybridization signals correspond to HPV-Proofer negativity, while the higher signals obtained by the FS system corresponded to higher HPV-Proofer positivity. However, a marked percentage of samples in this group presented HPV-Proofer negativity, therefore we found that the high FS hybridization signal does not necessarily correlate with HPV E6-E7 expression. (Figure 1A, 1B)
The comparison between HCII and FS resulted in a concordance of 76.53%. (Table 3) There were 42 samples found positive by FS and negative by HCII. Five of these were single infections with HPV66, which is not detected by HCII. The remaining 37 samples were analyzed by the Amplicor HPV Detection Kit, and 30 samples were confirmed positive. Four further cases were found positive by HCII and negative by FS. Two of these samples were found positive and 2 samples negative by the Amplicor HPV Detection Kit.
Finally we compared the distribution of HR HPV types in the English and Hungarian samples encountered in our practice. We analyzed HR data obtained from 9000 positive samples from Hungarian patients and 277 samples from English patients analyzed in 2007. We concluded that the general prevalence of HPV types corresponds to data from previous assessments, presenting some minor differences in the case of some types. (Figure 2)

Conclusions:

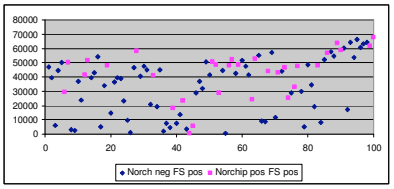
We propose the FS system as a diagnostic tool based on its overall sensitivity and reliable specificity. Moreover, based on its high sensitivity for types targeted by HPV vaccines, we propose it as a pre-vaccination test.

	FS	HC II HR	HPV-Proofer	Amplicor
HPV-16	√	√	√	√
HPV-18	√	√	√	√
HPV-31	√	√	√	√
HPV-33	√	√	√	√
HPV-35	√	√		√
HPV-39	√	√		√
HPV-45	√	√	√	√
HPV-51	√	√		√
HPV-52	√	√		√
HPV-56	√	√		√
HPV-58	√	√		√
HPV-59	√	√		√
HPV-66	√	√		
HPV-68	√	√		√
result	typing (14HR)	HR	typing (5 HR)	HR

Table 1. HPV types detected by the different HPV diagnostic systems

total 77	16	18	31	33	45	total	percentage
HPV-Proofer positive	16	10	0 (18)	3	4	33	**43%**
HPV-Proofer negative	19	10	9	3	3	44	57%
HCII positive	20	12	4	5	6	47	**61%**
HCII negative	15	8	5	1	1	30	39%
FS typing system positive	35	19	9	5	7	75	**97%**
FS typing system negative	0	1 (31)	0	1 (56)	0	2	3%
total	35	20	9	6	6		

Table 2. Comparison of the positivity rate of the tests for the 5 HPV types (16, 18, 31, 33, 45) detected by the HPV-Proofer kit among 77 samples



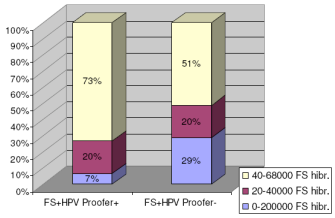


Figure 1A, 1B. Distribution of FS and HPV-Proofer positivity based on FS hybridization signals (FS+ HPV-Proofer- 80 samples, FS+ HPV-Proofer+ 30 samples)

total 196	HCII positive	HCII negative	HPV Proofer poz	HPV Proofer neg
FS positive	58	42	30	69
FS negative	4	92	2	96
HCII positive			31	31
HCII negative			0	134

Table 3. The distribution of the positivity among the 196 samples detected by the FS, HCII and HPV Proofer systems.

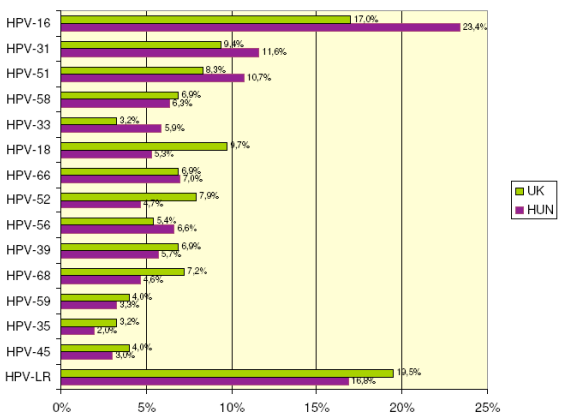


Figure 2. Comparison of the type specific positivity among the HR HPV positive samples and LR positive samples originated from Hungary (n:9253; 2004-2007) and UK (n: 277 in 2007)