COMPARISON OF VARIOUS MOLECULAR METHODS FOR DETECTING HUMAN PAPILLOMAVIRUS FROM CLINICAL SPECIMENS

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Background

Introduction

1) Human Papillomavirus (HPV)
- Strictly species specific
- No animal reservoir

2) Infection
- Cutaneous and mucosal epithelium
  Skin, oral cavity, conjunctiva, anus and lower genital tract
- No systemic or blood borne phase

3) Clinical significance
- Majority: Subclinical and asymptomatic
- LR HPV infection
  Type: 6, 11(predominantly), 41, 42, 43 and 44
  Benign warts
- HR HPV infection
  Type: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 82
  High-grade squamous intraepithelial lesions (HSILs), invasive cancer

4) Detection
- Microscopy: cervical cytology based on the Bethesda system
- Antigen detection and ELISA
- Nucleic acid-based identification and typing
  hc2, real-time PCR, fragment analysis, DNA microarray, Sequencing, etc.

Purpose

Comparative evaluation of five molecular methods
- Hybrid capture
- Real-time PCR
- Fragment analysis
- Nucleic acid sequence based amplification
- Restriction fragment mass polymorphism

Methods

Study Population

Clinical specimens submitted for HPV screening tests
- Total 6322 specimens
- From 2010-04-15 to 2012-07-19

Methods

1) Hybrid capture
- For all specimens
- Using Hybrid Capture 2 High-Risk HPV DNA test (hc2, Qiagen)

2) Real-time PCR
- For all specimens
- Using Abbott RealTime High Risk test (Abbott)

3) Fragment analysis
- For positive cases in above two assays, considered as true
- Using Seeplex HPV 18 ASE genotyping kit (Seegene)

4) Restriction Fragment Mass Polymorphism (RFMP)
- From 2012-01-12 to 2012-06-07
- Using RFMP HPV PapilloTyper (Bruker)

5) Nucleic Acid Sequence Based Amplification (NASBA)
- Simultaneously performed with fragment analysis
- Using NucliSENS EasyQ HPV test (bioMerieux)

Detectable HPV types by various methods

Table 1. Detectable High Risk HPV types up to the detection methods

Detection methods	HPV types														
	16	18	31	33	35	39	45	51	52	56	58	59	66	68	82
hc2	+	+	+	+	+	+	+	+	+	+	+	+		+	
Real-time PCR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Fragment analysis	+	+	+	+	+	+	+	+	+	+	+	+		+	
RFMP	+	+	+	+	+	+	+	+	+	+	+	+		+	+
NASBA	+	+	+	+			+								

Results

Study Population

Table 2. Specimen sources

Department	Health promotion center	Obstetrics and Gynecology	Others	Total
Specimen No. (%)	6036 (95.5)	272 (4.3)	14 (0.2)	6322

Frequency of positivity

In 545 specimens, one or both of hc2 and real-time PCR showed positive results and fragment analysis was performed simultaneously.

Positivity for high risk HPV using fragment analysis was shown in 486 specimens (89.2%, 7.7% for total specimens).

The concordance rates of hc2 and real-time PCR in true positive results

hc2 with fragment analysis: 409 of 486 (84.2%)

Real-time PCR and fragment analysis: 481 of 486 (99.0%)

Table 3. Discordant results in 5 cases between real-time PCR and fragment analysis

Discordant case	hc2	real-time PCR	fragment analaysis
Case 1	Detected	Type 16 detected	Type 51
Case 2	Detected	Type 18 detected	Type 16 and 52
Case 3	Detected	HRC* detected	Type 16
Case 4	Not detected	HRC detected	Type 16
Case 5	Not detected	HRC detected	Type 16

* HRC; high risk common

Fragment analysis and NASBA

Type 16, 18, 31, 33 or 45 were detected in 167 cases

Table 4. Concordance between fragment analysis and NASBA (co-infected case is counted as 2 or more)

		fragment analysis				
		16	18	31	33	45
NASBA	16	43	1$	2	0	0
	18	1$	30	2	0	0
	31	0	0	13	0	0
	33	0	0	0	7	0
	45	0	2*	2	1	1
	Not detected	30	0	29	9	0
	Total	74	33	48	17	1

* In NASBA, type 18 and 45 were detected in 2 specimens.
$ In 1 case, coinfection of type 16 and 18 was identified.

Fragment analysis and RFMP

Table 5. Concordance among RFMP, real-time PCR, hc2 and fragment analysis

		fragment analysis	
		HR HPV detected	HR HPV not detected
RFMP	HR HPV detected	10	2
	HR HPV not detected	13	7
real-time PCR	HR HPV detected	23	9
	HR HPV not detected	0	0
hc2	HR HPV detected	13	1
	HR HPV not detected	10	8
	Total	23	9

Conclusions

1. In cytologically normal specimens, HPV screening and genotyping assays do not show the identical results frequently - Possibly, due to the differences of the limit of detection (LOD) or the sensitivity of each methods.
2. Fragment analysis: Based on the sequencing → appropriate for a confirmatory test
3. RFMP: Relatively poor performace