WHO International Collaborative Study On The Detection of HPV DNA

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Study Group:

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- Reference laboratories
  C. Wheeler and W.G.V. Quint
- Production laboratory and neutral office
  P.N. Lelie
- Coordination WHO
  S. Pagliusi

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Composition of the HPV DNA Test Panel

Table 1. HPV DNA Standard Panel Composition

		Plasmid Dilution	Genome Equivalents per Milliliter								
Panel ID#	**HPV Types**	**Primary HPV/HR HPV Pool**	**6**	**16**	**18**	**31**	**33**	**35**	**45**	**52**	**C33A**
24	HPV 16	10^{-4}		10^{7}							10^{6}
8	HPV 16	10^{-5}		10^{6}							10^{6}
21	HPV 16	10^{-6}		10^{5}							10^{6}
16	HPV 16	10^{-7}		10^{4}							10^{6}
19	HPV 16	10^{-8}		10^{3}							10^{6}
23	HPV 16	10^{-9}		10^{2}							10^{6}
9	HPV 16	10^{-10}		10^{1}							10^{6}
5	HPV 16	10^{-11}		10^{0}							10^{6}
13	HPV 16 low/ HR	10^{-7} / 10^{-5}		10^{4}		10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}
10	HPV 16 high/ HR	10^{-5} / 10^{-5}		10^{6}		10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}
2	HR type pool	10^{-5}				10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}
7	HPV 6	10^{-7}	10^{4}								10^{6}
3	HPV 6/ HR	10^{-5}	10^{6}			10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}
14	C33A DNA	----									10^{6}
22	HPV 18	10^{-4}			10^{7}						10^{6}
1	HPV 18	10^{-5}			10^{6}						10^{6}
20	HPV 18	10^{-6}			10^{5}						10^{6}
11	HPV 18	10^{-7}			10^{4}						10^{6}
15	HPV 18	10^{-8}			10^{3}						10^{6}
18	HPV 18	10^{-9}			10^{2}						10^{6}
12	HPV 18	10^{-10}			10^{1}						10^{6}
4	HPV 18	10^{-11}			10^{0}						10^{6}
6	HPV 18 low/ HR	10^{-7} / 10^{-5}			10^{4}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}
17	HPV 18 high/ HR	10^{-5} / 10^{-5}			10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}	10^{6}

Initial characterization of the Panel and Organization of the Study

- Two reference laboratories performed PGMY Line Blot assay and SPF10 LiPa assay as well as qRT-PCR for HPV16 (E6), HPV18 (E7) and ß-globin
- Panels were distributed from the production laboratory (WHO International Laboratory for Biological Standards) to 29 laboratories worldwide; 115µl of each sample
- Each package contained an information sheet and a questionnaire to obtain technical information on the procedures used
- Results were submitted to neutral office and coded so that specific laboratories and HPV assays would not be linkable

Molecular Methods for HPV DNA Amplification and Detection

Hybridization-technique:

HC2 HPV DNA Assay (Digene)
FDA-approved

PCR-protocols:

Amplicor HPV MWP (Roche) CE
GP5+/GP6+ (de Roda et al. / Jacobs et al., 1995)

MY09/11 (Manos et al, 1989)
SPF (Kleter et al., 1998)
PGMY 09/11 (Gravitt et al. 2000)
Homebrew-PCR (??)

Screening

Genotyping

VALIDATION OF THE PANEL

- HPV16 was less efficiently amplified than HPV18 by qRT-PCR; Endpoint detection of 10E-6 for HPV16 (10E5 Geq/ml) and 10E-8 for HPV18 (10E3 Geq/ml)
- a subsequent analysis revealed a difference in dilution of at least one order of magnitude HPV18 > HPV16
- using qualitative assays both reference laboratories detected HPV16 at a dilution of 10E-7 (10E4 Geq/ml = 10 copies/10µl) and HPV18 at a dilution from 10E-7 to 10E-8 (10E3-10E4 Geq/ml)
- HC2 assay had a detection limit of 10.000-100.000 Geq per assay
- Amplicor MWP had a detection limit of 1 – 10 Geq per assay

Analysis of the performance of the participating labs

- Criteria for proficiency
  - Correct detection of the negative sample
  - Logical order in dilution series; for HPV16 and HPV18
    - ++---
    - +-+--
    - +--+- not logical
  - Correct HPV 16 and 18 detection in the background of human DNA with or without other HPV genotypes

- Four datasets without specific genotyping results were not analysed: HCII and MWP assay

Ability to detect HPV16 in 24 samples

Detection method			1	2	3	4	5	6	7	8	9	10	11	12	13	14	14	14	14	14	14	14	15	16	17	17	17	18	19
Code	Sample	Dilution	Ref 1	Ref 2	Ref 2	A	B	C	A	D	E	F	G	H	I	F	J	K	L	M	N	O	P	Q	R	S	O	T	U
24	HPV 16	10^{-4}																											
8	HPV 16	10^{-5}																											
21	HPV 16	10^{-6}																											
16	HPV 16	10^{-7}																											
19	HPV 16	10^{-8}																											
23	HPV 16	10^{-9}																											
9	HPV 16	10^{-10}																											
5	HPV 16	10^{-11}																											
13	HPV 16 low + HR	10^{-7}																											
10	HPV 16 high + HR	10^{-5}																											
2	HR types	10^{-5}																											
7	HPV 6	10^{-7}																											
3	HPV 6 + HR	10^{-5}																											
14	C33A DNA	N.A.																											
22	HPV 18	10^{-4}																											
1	HPV 18	10^{-5}																											
20	HPV 18	10^{-6}																											
11	HPV 18	10^{-7}																											
15	HPV 18	10^{-8}																											
18	HPV 18	10^{-9}																											
12	HPV 18	10^{-10}																											
4	HPV 18	10^{-11}																											
6	HPV 18 low + HR	10^{-7}																											
17	HPV 18 high + HR	10^{-5}																											

Ability to detect HPV18 in 24 samples

Detection method			1	2	3	4	5	6	7	8	9	10	11	12	13	14	14	14	14	14	14	14	15	16	17	17	17	18	19
Code	Sample	Dilution	Ref 1	Ref 2	Ref 2	A	B	C	A	D	E	F	G	H	I	F	J	K	L	M	N	O	P	Q	R	S	O	T	U
24	HPV 16	10^{-4}																											
8	HPV 16	10^{-5}																											
21	HPV 16	10^{-6}																											
16	HPV 16	10^{-7}																											
19	HPV 16	10^{-8}																											
23	HPV 16	10^{-9}																											
9	HPV 16	10^{-10}																											
5	HPV 16	10^{-11}																											
13	HPV 16 low + HR	10^{-7}																											
10	HPV 16 high + HR	10^{-5}																											
2	HR types	10^{-5}																											
7	HPV 6	10^{-7}																											
3	HPV 6 + HR	10^{-5}																											
14	C33A DNA	N.A.																											
22	HPV 18	10^{-4}																											
1	HPV 18	10^{-5}																											
20	HPV 18	10^{-6}																											
11	HPV 18	10^{-7}																											
15	HPV 18	10^{-8}																											
18	HPV 18	10^{-9}																											
12	HPV 18	10^{-10}																											
4	HPV 18	10^{-11}																											
6	HPV 18 low + HR	10^{-7}																											
17	HPV 18 high + HR	10^{-5}																											

Conclusions from this study

- All participants could detect oncogenic HPV 16 and 18 at the highest concentrations available in the panel, corresponding to 100.000 copies per 10 µl sample
- Most laboratories showed a good level of sensitivity and could detect dilutions up to 1.000 copies per 10 µl sample
- The single negative control was negative by all labs except for one which detected HPV6
- The lower level of HPV16 detection varied 1000-fold among participants
- The lower level of HPV18 detection varied 100.000 fold
- Detection of HPV31 had an overall proficiency below 50% because of 12 false negative results at 10.000 copies/10µl
- All except one laboratory were proficient in the detection of HPV33 and 45
- HPV6 had a high number of false positives
- HPV35 and 52 had intermediate proficieny results
- False positives were mostly clustered among laboratories that used "in house" PCR methods

Conclusion from this study

- Harmonized SOPs may help improve quality of assays when inaccurate results are frequent in individual laboratories
- International standard reagents provide a helpful tool for high quality HPV amplification, detection and genotyping
- This strategy allows laboratories to measure their results in relation to other groups globally and improve their performance for molecular HPV detection in the future
- Specificity and sensitivity of methods are crucial for both epidemiological surveys and vaccine evaluation and future monitoring of vaccine performance upon deployment in populations
- Sample collection and handling, including DNA extraction procedures are a further step in the harmonization of laboratory procedures for HPV typing

Molecular methods for evaluation of HPV infections

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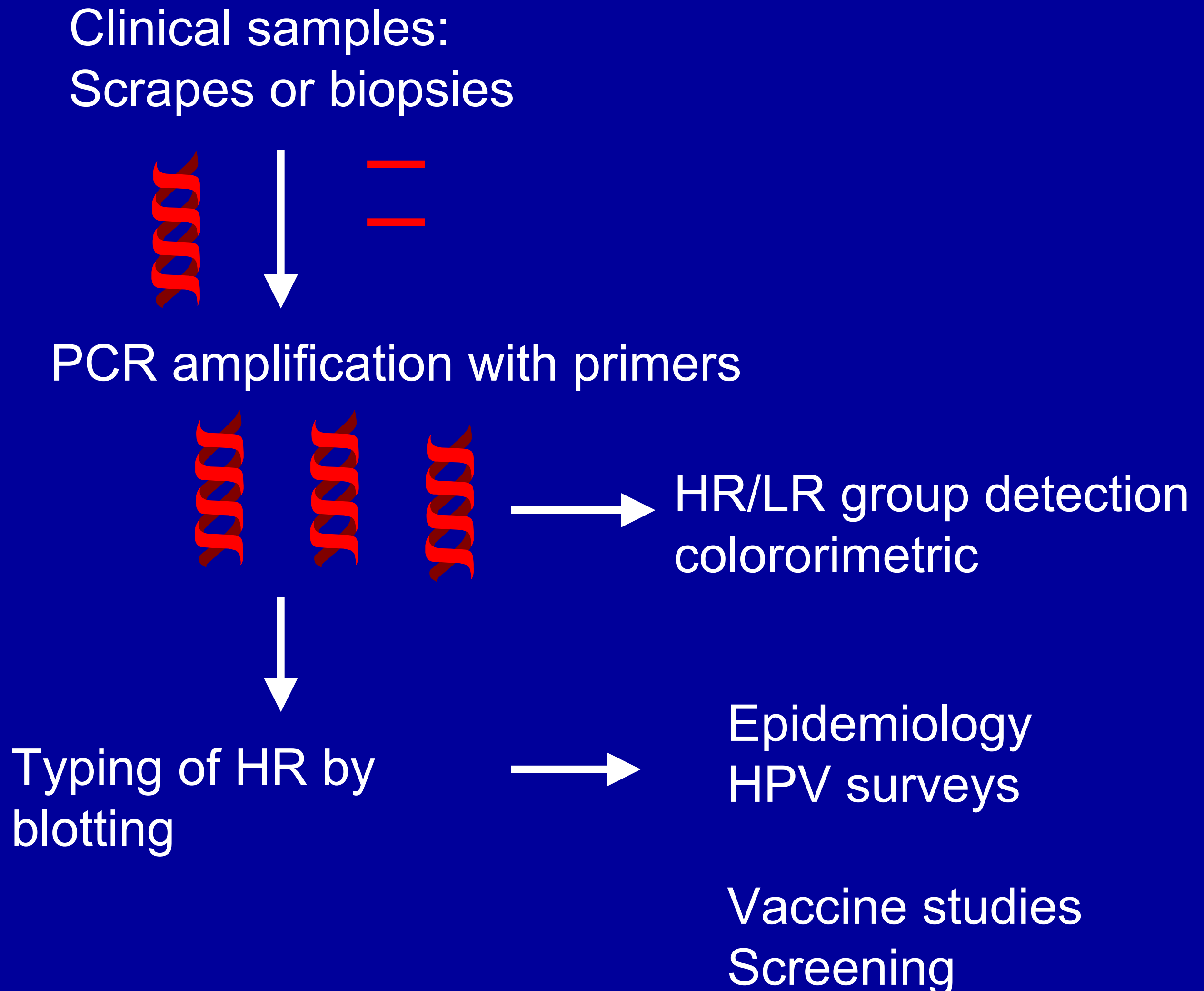
Molecular Methods for HPV DNA amplification and detection



Molecular Methods for HPV DNA Amplification and Detection

Digene Hybrid Capture Assay	Luminescence
Roche Multiple Well Plate assay	Colorimetric
degGP5+/6+	BioMedlab DNA chip
GP5+/6+ (150 bp)	Reverse Line Blot Assay EIA and 2-D Array
MY09-11 and PGMY (450 bp)	Reverse Line Blot Assay
SPF10 (65 bp)	Reverse Line Blot Assay
Other PCR typing methods	
Type specific HPV 16, 18, or…	Southern blot or dot blot

Test Panel samples (1): geq/ml

Code	Dilution series (geq/ml) HPV 16	HPV 18	C33A DNA genomes/ml
24	10^7		10^6
8	10^6		10^6
21	10^5		10^6
16	10^4		10^6
19	10^3		10^6
23	10^2		10^6
9	10^1		10^6
5	10^0		10^6
22		10^7	10^6
1		10^6	10^6
20		10^5	10^6
11		10^4	10^6
15		10^3	10^6
18		10^2	10^6
12		10^1	10^6
4		10^0	10^6
14	Background DNA		10^6