Reliable high risk HPV DNA testing by polymerase chain reaction: an intermethod and intramethod comparison

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Abstract

Background—The development of a reproducible, sensitive, and standardised human papillomavirus (HPV) polymerase chain reaction (PCR) test is required to implement HPV testing in cervical cancer screening programmes and for triaging women with mild to moderate dysplasia.

Aims—To determine the intermethod agreement between different GP5+/6+ and MY09/11 PCR based protocols for the detection and typing of high risk (HR) HPV DNA in cervical smears and to assess the intramethod reproducibility of the GP5+/6+ PCR enzyme immunoassay (EIA) for HR-HPV detection.

Methods—For the intermethod comparison, crude aliquots of 20 well characterised cervical smears comprising five HPV negative samples, and six and nine samples containing single and multiple HPV infections, respectively, were coded and sent from reference laboratory (A) to three other laboratories. One of these (laboratory B) used the GP5+/6+ PCR-EIA and was provided with standard protocols. Another laboratory (C) used GP5+/6+ PCR combined with sequence analysis and type specific PCR, whereas two laboratories (D and E) used MY09/11 PCR followed by restriction fragment length polymorphism (RFLP) analysis for the detection and typing of HR-HPV. The intramethod agreement of GP5+/6+ PCR-EIA was analysed in a subsequent study with four other laboratories (F to I) on crude aliquots of 50 well characterised cervical smears, consisting of 32 HR-HPV positive and 18 HPV negative samples. Standardised protocols, primers, and probes were also provided by the reference laboratory for HR-HPV detection.

Results—In the intermethod comparison, pairwise agreement of the different laboratories with reference laboratory A for the detection of HR-HPV varied between 75% and 100% (κ values: 0.5 to 1). Typing data revealed a broader range in pairwise agreement rates between 32% and 100%. The highest agreement was found between laboratories A and B using standardised protocols and validated reagents. In the intramethod evaluation, pairwise comparison of the laboratories F to I with reference laboratory A revealed excellent agreement rates from 92% to 100% (κ

values: 0.88 to 1.0) with an overall sensitivity of 97.5% (195/200) and specificity of 99.5% (199/200).

Conclusions—The detection of HR-HPV as a group is highly reproducible with GP5+/6+ PCR-EIA provided that standardised protocols and validated reagents are used.

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Keywords: human papillomavirus; polymerase chain reaction; intermethod agreement; intramethod agreement

Worldwide, cervical cancer is one of the most common forms of cancer among women. Although cytomorphological screening of cervical smears (the Papanicolaou test) has reduced the incidence of cervical cancer significantly, the test still has some limitations with respect to sensitivity and specificity. False negative rates for cervical premalignant lesions and cervical cancer between 15% and 50% and false positive rates of about 30% have been reported.^{1–3}

To date, it has been shown that high risk human papillomavirus (HR-HPV) genotypes are implicated in the aetiology of cervical cancer.⁴ Consequently, the inclusion of HR-HPV testing in cervical cancer screening programmes and the triaging of women with mild to moderate cervical dysplasia has been advocated.^{5–8}

As HPV cannot be cultured in vitro and no suitable serological assays are at present available, current methods are based on the detection of HPV DNA in exfoliated cervical cells. These methods include the hybrid capture assay (HCA), a simple direct HPV DNA detection assay using signal amplification,⁹ and the polymerase chain reaction (PCR) which is based on the in vitro amplification of target sequences.¹⁰ Both approaches seem robust and potentially suitable for routine. Concerning the PCR based methods, HPV type specific PCR are not suitable for large clinical trials owing to the wide variety of HPV genotypes infecting the genital tract. Broad spectrum detection has therefore been facilitated by consensus PCR assays, with general primers selected from highly conserved sequences of the majority of mucosal HPV genotypes.^{11–14} Moreover, subsequent detection steps are continuously amenable to modifica-

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tions to render these general primer PCR assays more feasible for large numbers of samples.

In order to consider HPV testing for cervical cancer screening programmes, issues like the reproducibility between different HPV methods and between different testing centres need further attention. The reproducibility of both HCA and the widely used general primer MY09/11 mediated PCR assay using different read-out protocols has already been evaluated.¹⁵⁻¹⁷ Recently, the read-out system of another widely used general primer mediated PCR system, the GP5+/6+ PCR, has been converted from conventional radioactive Southern blot hybridisation of the PCR products in a colorimetric enzyme immunoassay (EIA) (GP5+/6+ PCR-EIA).¹⁸ Like the latest version of HCA,¹⁹ it is a non-radioactive detection procedure in microtitre plate format which is easy to perform and generates objective numerical data. Although this new GP5+/6+ PCR-EIA system has already been evaluated on clinical specimens,²⁰ no interlaboratory reproducibility rates are yet available. We therefore assessed the reproducibility of the GP5+/6+ PCR-EIA in a multicentre intermethod and intramethod evaluation. The results of these evaluations are presented in this paper.

Methods

SELECTION OF REFERENCE SAMPLES AND COMPOSITION OF TEST PANELS

Cervical smears were selected from a group of women with abnormal cervical cytology ($\geq$ mild dysplasia) attending the outpatient clinics of the University Hospital Vrije Universiteit in Amsterdam, The Netherlands. The cervical smears were pretreated as described before.¹⁰ Selection of study samples was based on the following criteria:

- (1) an adequate quality of the DNA for PCR amplification as determined by a PCR assay with primers spanning 509 base pairs of the β globin gene²¹;
- (2) the presence or absence of HPV DNA after GP5+/6+ PCR;
- (3) confirmation of HPV types by both GP5+/6+ PCR-EIA using HR-HPV oligo (cocktail) probes¹⁹ and HPV E7 type specific PCR assays²²;
- (4) samples comprised one or more of the following HPV types considered as high risk: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68.

Fifty cervical smears were selected consisting of 18 HPV negative and 32 HPV positive samples. The 32 HPV positive smears comprised 23 single and nine multiple HPV infections.

For the intermethod comparison study, a subset of the 50 selected cervical smears was used to prepare a test panel of 20 specimens consisting of five HPV negative samples and six and nine samples containing a single and multiple HPV infections, respectively. The HPV positive samples comprised together a diversity of 13 HR-HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, and 68).

The test panel for the intramethod comparison study consisted of all 50 selected cervical smears including the 13 different HR-HPV genotypes and in addition HR-HPV 56.

STUDY DESIGN

One laboratory served as the reference laboratory (A) and established the test panel of cervical smears. Aliquots of these specimens (50 μ l) were coded and distributed by laboratory A to different laboratories. In the test panels, the HPV negative samples were randomly divided between the HPV positive samples; 10 μ l of the study samples had to be used for HPV testing. All participating laboratories had experience with HPV PCR technology. The reference laboratory did not participate in the HPV testing but collected and compared the HPV PCR test results from the different laboratories with its own reference data. The GP5+/6+ PCR-EIA results from the reference laboratory (A) were used as the gold standard as these results were confirmed by an alternative HPV E7 TS-PCR system and were therefore considered conclusive.

INTERMETHOD COMPARISON

Four laboratories (B to E) participated in the intermethod evaluation for the detection and typing of HR-HPV in cervical smears. These four laboratories applied their own in-house HPV PCR assays.

One of these laboratories (B) used the same method as the reference laboratory (A)—that is, GP5+/6+ PCR-EIA with a high risk oligo cocktail probe for the detection of HR-HPV and individual oligo probes for HPV typing and was provided with standardised protocols after an extensive training period.

Another laboratory (C) applied the GP5+/6+ PCR followed by agarose gel electrophoresis to detect the presence of HPV DNA and used type specific (TS) PCR for HPV 6, 11, 16, and 18 combined with direct sequence analysis of GP5+/6+ PCR products in cases of GP5+/6+ PCR positive and TS-PCR negative samples for HPV typing.

Two other laboratories (D and E) used MY09/11 primer mediated PCR. The presence of HPV DNA was analysed by agarose gel electrophoresis of the MY09/11 generated PCR products while typing was performed by restriction fragment length polymorphism (RFLP) analysis and hybridisation of the RFLP products with a generic oligonucleotide probe mixture.

INTRAMETHOD COMPARISON

For the intramethod comparison, four other laboratories (F to I) without previous experience with GP5+/6+ PCR-EIA participated in the studies. The reference laboratory (A) provided a standard operating procedure, digoxigenin labelled high risk oligo cocktail probes, and the GP5+/6+ PCR primers. The quality of this material was first validated and a sensitivity of between 10 and 200 copies of the HPV genome, depending on the HPV type, could be attained.¹⁷ Other reagents and equip-

Table 1 Intermethod agreement between different polymerase chain reaction (PCR) protocols (B–D) performed in different laboratories and the reference data (A) for the detection of high risk human papillomaviruses (HR-HPV) in 20 cervical smears

Method pair	Agreement obtained for:		% Overall agreement (No of identical / No tested)	κ Statistic
	Positives (n=15)	Negatives (n=5)		
A and B	15	5	100% (20/20)	1
A and C	13	5	90% (18/20)	0.76
A and D	14	5	95% (19/20)	0.88
A and E	10	5	75% (15/20)	0.50

B: GP5+/6+ PCR-EIA using HR (high risk) oligococktail probe for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. C: GP5+/6+ PCR and agarose gel electrophoresis. D, E: MY09/11 PCR and agarose gel electrophoresis.

Table 2 Comparison between different methods and the reference data for typing of high risk human papillomaviruses (HR-HPV)

Sample	Reference data	Laboratory*			
		B	C	D	E
1	Neg	Neg	Neg	Neg	Neg
2	Neg	Neg	Neg	Neg	Neg
3	Neg	Neg	Neg	Neg	Neg
4	Neg	Neg	Neg	Neg	Neg
5	Neg	Neg	Neg	Neg	Neg
6	18	16, 18	18	18	18
7	39	39	Neg	39	Neg
8	51	51	HPV pos†	51	Neg
9	52	52	52	52	52
10	56	56	56	56	56
11	58	33, 58	58	58	58

Typing agreement compared with reference data for single HPV infections:

12	16, 35	6/6 (100%)	4/6 (67%)	6/6 (100%)	4/6 (67%)
13	16, 68	16, 35	Neg	Neg	16, 61
14	31, 35	16, 68	16	16, 68	16
15	33, 35	31, 35	31	35	31
16	33, 45	33, 35	35	35	Neg
17	33, 58	18, 33, 45	45	33, 52	Neg
18	35, 59	33, 58	33	33	33
19	52, 68	35, 59, 66	59	31	31
20	31, 59, 66	52, 68	68	HPV pos†	Neg
		31, 59, 66	66	18	18

Typing agreement per HPV type compared with reference data for multiple HPV infections:

19/19 (100%)	8/19 (42%)	6/19 (32%)	4/19 (21%)
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Overall typing agreement compared with reference data:

25/25 (100%)	12/25 (42%)	12/25 (48%)	8/25 (32%)
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*Lab B: GP5+/6+ PCR-EIA using individual internal oligoprobes for HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68; Lab C: TS-PCR for HPV 6/11, 16, and 18 and direct sequence analysis of GP5+/6+ PCR products; Lab D, E: restriction fragment length polymorphism analysis of MY09/11 PCR products followed by hybridisation with an oligonucleotide probe mixture.

†The HPV type could not be identified by the typing procedure used, but the sample contained HPV DNA after hybridisation of the PCR products with a general HPV probe (method C) or after agarose gel analysis (method E).

For simplicity of the table, the samples have been sorted according to HPV type.

p Value for differences in typing between single and multiple HPV infections < 0.001 (χ^2 , $df=0.05$).

ment had to be purchased from their local distributors.

STATISTICAL ANALYSIS

The intermethod and intramethod agreement for HR-HPV detection was assessed by pairwise comparison of the test results with the reference data using percentage of agreement and the kappa (κ) statistic. κ Values express the proportion of possible agreement beyond chance. A κ estimate of less than 0.4 represents poor agreement, a κ estimate between 0.4 and 0.75 is fair to good agreement, and a κ estimate of more than 0.75 is excellent agreement.²³ For HPV typing, the intermethod agreement was assessed by pairwise comparison of the typing results with the reference data by the percentages of agreement. The χ^2 test was used to

indicate significant differences between typing of single and multiple HPV infections.

Results

INTERMETHOD COMPARISON BETWEEN GP5+/6+ AND MY09/11 PCR BASED PROTOCOLS

HPV detection analysis on cervical smears

Aliquots of 20 well characterised cervical smears were subjected to different PCR protocols employed in the different laboratories and compared with the reference data (table 1). Laboratory B correctly identified all 15 HPV positive smears and all five HPV negative samples, resulting in an overall agreement of 100% (20 of 20). Laboratory C failed to detect two specimens of the 15 HPV positive samples, but identified all five HPV negative smears. This resulted in an overall agreement of 90% (18 of 20). Laboratories D and E correctly identified all HPV negative samples, but both laboratories failed to identify one and five HPV positive smears, respectively. This revealed an overall agreement of 95% (19 of 20) for laboratory D and 75% (15 of 20) for laboratory E. The κ values ranged from 0.50 for agreement between laboratories A and E, to 0.76 for laboratories A and C, to 0.88 for laboratories A and D, to 1 for laboratories A and B.

HPV typing analysis on HPV positive cervical smears

Subsequently, to determine differences in HPV typing of six single and nine multiple HPV infections by the different procedures, HPV positive samples were subjected to typing analysis and results were compared with the reference data. The results are shown in table 2.

Evaluation of the single HPV infections showed that laboratory B identified the correct HPV type in all the six samples (samples 6 to 11). However, in two samples (sample 6 and 11) an additional HR-HPV type was detected compared with the reference data. Laboratory C detected the correct HR-HPV type in four of the six single HPV infections (samples 6, 9, 10, and 11). In one single HPV infection (sample 8) the HR-HPV type (HPV 51) could not be identified, while the remaining sample (sample 7) was tested HPV DNA negative. Only laboratory D identified the correct HPV type in all the six single HPV infections (samples 6 to 11) whereas laboratory E correctly identified the HR-HPV type in four of the six single HPV infections (samples 6, 9, 10, and 11). The two remaining samples (samples 7 and 8) were tested HPV DNA negative.

Evaluation of the multiple HPV infections showed that laboratory B correctly typed the HR-HPVs present in all the nine samples (samples 12 to 20). However, in two samples (samples 16 and 18) an additional HR-HPV type was detected compared with the reference data. Only a single HPV type was detected in all nine multiple HPV infections by laboratory C. In all these nine cases the HR-HPV type detected corresponded with one of the multiple HPV types present in the sample according to the reference data. Laboratory D detected single HPV types in five of the nine multiple HPV infections. In three of these (samples 14, 15,

Table 3 GP5+/6+ PCR-EIA results obtained by the different laboratories for the detection of high risk (HR) human papillomaviruses (HPV) in 50 cervical smears compared with the reference data

Sample	Reference data	HR-HPV			
		Lab F	Lab G	Lab H	Lab I
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	+
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	–
7	–	–	–	–	–
8	–	–	–	–	–
9	–	–	–	–	–
10	–	–	–	–	–
11	–	–	–	–	–
12	–	–	–	–	–
13	–	–	–	–	–
14	–	–	–	–	–
15	–	–	–	–	–
16	–	–	–	–	–
17	–	–	–	–	–
18	–	–	–	–	–
19	16	+	+	+	+
20	16	+	+	+	+
21	16	+	+	+	+
22	16	+	+	+	+
23	16	+	+	+	+
24	16	+	+	+	–
25	18	+	+	+	+
26	18	+	+	+	+
27	18	+	+	+	–
28	31	+	+	+	+
29	33	+	+	+	+
30	39	+	+	+	+
31	45	+	+	+	+
32	51	+	+	+	+
33	51	+	+	+	+
34	52	+	+	+	+
35	52	+	+	+	+
36	56	+	+	+	+
37	56	+	+	+	+
38	58	+	+	+	+
39	58	+	+	+	+
40	59	+	+	+	+
41	59	+	–	+	+
42	33, 35	+	+	+	+
43	16, 68	+	+	+	+
44	33, 45	+	+	+	+
45	35, 59	+	+	+	+
46	52, 68	+	+	+	+
47	16, 35	+	+	–	–
48	31, 35	+	+	+	+
49	33, 58	+	+	+	+
50	31, 66, 59	+	+	+	+

PCR-EIA, polymerase chain reaction–enzyme immunoassay.

and 17), the type detected corresponded with one of the HR-HPV in the sample. In the two other cases (samples 18 and 20), an HR-HPV type was found which did not represent one of the types identified by the reference laboratory. Two multiple HPV infections were detected where in one sample (sample 13) the HPV types were correctly identified and in the other sample (sample 16) the HPV types were partly identified. Of the two remaining samples, one (sample 19) was found HPV positive but could not be identified, while the other sample was

Table 4 Intramethod agreement between laboratories (E–H) and reference laboratory (A) for the detection of high risk human papillomaviruses (HR-HPV) as a group in 50 cervical smears by GP5+/6+ PCR-EIA

Laboratory pair	Agreement obtained for:		Percent overall agreement (n identical/n tested)	κ Statistic
	Positives (n=32)	Negatives (n=18)		
A and F	32	18	100% (50/50)	1
A and G	31	18	98% (49/50)	0.96
A and H	31	18	98% (49/50)	0.96
A and I	29	17	92% (46/50)	0.83

PCR-EIA, polymerase chain reaction–enzyme immunoassay.

scored HPV negative (sample 12). Laboratory E also identified single HPV types in five of the nine multiple HPV infections (samples 13, 14, 17, 18, and 20). In two of these five cases (samples 18 and 20), the HPV type did not correspond with either of the HPV types present in the sample. One double HPV infection was found (sample 12) including HPV 61, which the reference laboratory had not tested for. The remaining three samples (samples 15, 16, and 19) were tested HPV DNA negative.

Taking the typing data together, the percentages of overall agreement with the reference laboratory were 100%, 48%, 48%, and 32% for the methods employed in laboratories B, C, D, and E, respectively. Moreover, the typing agreement of all laboratories together was significantly higher ($p < 0.001$) for single HPV infections (83%; 20/24) than for multiple HPV infections (49%; 37/76).

INTRAMETHOD COMPARISON OF HR-HPV GP5+/6+ PCR-EIA

Crude cell suspensions of 50 cervical smears were analysed in different laboratories by GP5+/6+ PCR-EIA and compared to the reference data.

As shown in table 3, among the HPV negative samples, one sample (3) was tested HR-HPV positive by laboratory I only. Among the HR-HPV positive samples, four samples (24, 27, 41, and 47) were tested false negative. One of these samples (47) was tested HR-HPV negative by two independent laboratories (H and I), while the remaining three samples were scored HR-HPV negative by only one of the laboratories.

Laboratory F correctly identified all 32 HPV positive and all 18 HPV negative specimens of the test panel, while both the laboratories G and H identified all the HPV negative samples but failed to identify one HR-HPV positive sample. Laboratory I correctly identified 29 of the 32 HPV positive samples and additionally tested one sample HR-HPV positive among the 18 HPV negative specimens of the test panel. The percentages of agreement between the different laboratories and the reference laboratory varied from 92% (46 of 50; laboratory I) to 98% (49 of 50; laboratories G and H) to 100% (50 of 50; laboratory F). Likewise, κ values ranged from 0.83 to 0.96 to 1 for laboratories I, G and H, and F, respectively. The results are summarised in table 4.

Discussion

In view of potential applications of HR-HPV PCR assays in cervical cancer screening programmes,²⁴ the intermethod agreement of GP5+/6+ and MY09/11 consensus PCR based protocols was investigated by multiple test centres. A higher reproducibility for HR-HPV detection than for HPV typing was found. Among the different protocols, GP5+/6+ PCR amplification followed by hybridisation of the PCR products with a cocktail probe for HR-HPV types in an EIA format revealed the highest agreement with the reference data. Moreover, excellent intramethod agreement between other test centres was obtained with

this method in a subsequent study. These data indicate that HR-HPV GP5+/6+ PCR-EIA has outstanding reproducibility.

INTERMETHOD COMPARISON

Comparison of different in-house HPV PCR methods with the reference data showed that the agreement was fair to excellent (κ values: 0.5 to 1) for the detection of HR-HPV DNA in cervical smears (table 1). A markedly lower agreement rate (75%) for HPV detection was observed for laboratory E using MY09/11 PCR and agarose gel analysis compared with other laboratories (90% to 100%). It has to be noted that the quality of the DNA in all samples was appropriate for efficient PCR amplification of at least 500 base pair fragments. As the MY09/11 PCR amplifies a shorter fragment of 450 base pairs in the HPV L1 open reading frame, the disagreement cannot simply be explained by inadequate DNA quality of the specimens. This is further supported by the observation that laboratory D, using the same MY09/11 PCR assay including the read-out protocol, obtained an excellent agreement (95%) with the reference data for the detection of HPV DNA. In addition, the agreement between both laboratories (D and E) using MY09/11 PCR was only fair (κ value: 0.40). The discrepancies were restricted to false negative test results. Moreover, since all HPV positive samples included in the test panel contained high amounts of HPV DNA according to the optical density values of the reference data, the false negative test results are also unlikely to be a result of sampling errors.

Furthermore, a broader range in agreement was found for HPV typing compared with the detection of HPV DNA. Moreover, the HPV typing results varied more strongly for the multiple infections compared with the single infections ($p < 0.001$; table 2). In our study it was shown that differences in read-out systems make a large contribution to variations in HPV typing. Direct sequencing of GP5+/6+ PCR products apparently failed to identify under-represented HPV types in the multiple HPV infections, in contrast to hybridisation of GP5+/6+PCR products with digoxigenin labelled oligo probes in EIA. In addition, this latter method detected some additional HPV types compared with the reference laboratory owing to differences in the interpretation of weak signals. That variations in HPV typing may occur using different protocols is further substantiated by the observation that both laboratories using the same MY09/11 PCR-RFLP procedures had a lower detection rate for multiple HPV infections than the laboratory using GP5+/6+ PCR-EIA. However, in another study,²⁵ the reverse was found when a similar dot blot procedure was used for HPV typing of both GP5+/6+ and MY09/11 PCR products derived from the same series of samples. These data suggest that the efficiency of HPV testing by consensus PCR is not only dependent on the specificity of the primers but also on the read-out system applied. Moreover, these results strongly reinforce the need for

standardisation of read-out systems employed in different laboratories.²⁶

INTRAMETHOD COMPARISON

In the intramethod comparison, the reproducibility of the HR-HPV GP5+/6+ PCR-EIA—tested on 50 well characterised specimens—was fairly uniform among different laboratories, as shown by the small differences in the agreement rates (92% to 100%; table 4). Except for one case, the few observed discrepancies comprised false negative test results of HPV positive samples (table 3). Nevertheless, the five false negative test results among a total of 200 tests in the four laboratories show a high overall sensitivity of 97.5% (195/200). Likewise, the specificity of GP5+/6+ PCR for HR-HPV detection was excellent. There was only one single false positive test result obtained among the 200 tests conducted in the four laboratories, resulting in an overall specificity of 99.5% (199/200).

Finally, the main goal of this study was the recognition that HPV testing can be performed reliably by consensus HPV PCR based protocols and between different testing centres. Smits *et al* already showed that agreement between CPI/II PCR and MY09/11 PCR for the detection of HPV DNA in cervical smears was excellent (κ values between 0.82 and 0.84).²⁷ Moreover, high interlaboratory reproducibilities for the detection of HPV DNA with MY09/11 PCR in clinical specimens of about 88% to 97% have been found previously.^{16, 17} The results of our study are in line with these reports and suggest that the variation in HR-HPV detection by different consensus HPV PCR based protocols can be quite small. Most importantly, however, testing for HR-HPV as a group appears to be more reproducible than testing for individual HPV types. Since results from recent case-control studies show that the risk for women of getting cervical cancer does not differ significantly for the different HPV genotypes,^{28, 29} individual HPV typing is unlikely to be more relevant clinically than HR-HPV group specific testing. With the data obtained in this study, this argues that HR-HPV group specific detection should be the strategy of choice in cervical cancer screening programmes. It has additionally been shown that the HR-HPV GP5+/6+ PCR-EIA has high reproducibility for the detection of HR-HPVs and can easily be transferred to other laboratories provided that standardised protocols and validated reagents are used. Therefore, this test could be used in large clinical trials. Recently, a trial of 44 000 women was started to evaluate HR-HPV testing with the GP5+/6+ PCR-EIA in population based cervical cancer screening.

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