

## ORIGINAL ARTICLE

# Primary screening for high risk HPV by home obtained cervicovaginal lavage is an alternative screening tool for unscreened women

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**Background/Aims:** Self sampling is considered an adjuvant tool to facilitate the participation of women in cervical cancer screening programmes. This study aimed to evaluate whether cervicovaginal lavage could be an alternative for the cervical smear in cytology and human papillomavirus (HPV) testing and to assess the acceptance of the self sampling device by women.

**Methods:** Fifty six women with abnormal cervical cytology (very mild dyskaryosis or worse) and 15 women with normal cervical cytology obtained a self collected cervicovaginal lavage at home and filled in a questionnaire on the use of the device. At the colposcopy clinic the gynaecologist performed the same procedure followed by a cervical smear for cytology and HPV DNA testing.

**Results:** The self sampling device was acceptable to 88% of the women. The concordance between the cytology results in the smear and the lavage by the doctor and the patient was 54% and 41%, respectively ( $\kappa = 0.28$  and  $0.14$ ). The concordance between high risk HPV detection in the smear and the lavage by the doctor and the patient was 93% and 78%, respectively ( $\kappa = 0.82$  and  $0.53$ ). Ninety one per cent of the women with high grade cervical intraepithelial neoplasia (CIN) had a high risk HPV positive test in the smear, compared with 91% and 81% in the lavages taken by the doctor and the patient, respectively.

**Conclusions:** HPV DNA testing by home obtained samples is useful as a screening tool for cervical cancer, whereas cervical cytology by self sampling is not. Although the sensitivity for high grade CIN by high risk HPV testing in the lavage by the patient is not significantly lower than that in the cervical smear, self sampling for HPV DNA is a feasible alternative method in women who decline to participate in population based cervical cancer screening programmes. However, participation in the screening programme remains the best option.

Cervical cancer is a preventable disease. Its development through premalignant stages, detectable by cervical cytology years before cervical cancer appears, has resulted in the organisation of population based cervical cancer screening programmes. These screening programmes have contributed to a reduction in the incidence and mortality of cervical cancer.<sup>1,2</sup> However, there are some drawbacks including low attendance and the limited sensitivity of cytological screening.<sup>3–5</sup> Moreover, it is known that 50% of the cases with invasive cervical cancer arise in women who are not adequately screened.<sup>6,7</sup>

The screening method used in cervical cancer screening is the classic Papanicolaou smear (Pap smear), taken directly from the cervix. Self sampling is regarded as a possible alternative to facilitate the screening of women who refuse to participate in cervical cancer screening programmes.<sup>8,9</sup> A sampling method performed by the woman herself, without intervention by a doctor, could lower the threshold and increase the uptake of screening.

Several studies have established that an infection with high risk human papillomavirus (HPV) is the main cause for the development of cervical cancer. High risk HPV types can be identified in nearly all cervical carcinomas.<sup>10</sup> Women with normal cervical cytology and a high risk HPV positive test are more at risk of developing severe cervical dysplasia than women without high risk HPV<sup>11,12</sup> and, moreover, in women with abnormal cervical cytology a persistent high risk HPV infection is required for the development and maintenance of severe dysplastic cervical lesions.<sup>13–15</sup> Thus, testing for high risk

HPV, as an adjunct to cervical cytology, has been recommended for screening to determine a high risk group.<sup>15</sup>

“It is known that 50% of cases with invasive cervical cancer arise in women who are not adequately screened”

The aim of our study was to evaluate testing for HPV DNA and cervical cytology in home obtained, self collected material by cervicovaginal lavage as an alternative to the Pap smear. A lavage taken by the doctor was included in the study as a control for the lavage taken by the patient. We were also interested in the acceptance of the self sampling device as an alternative screening tool.

## METHODS

From December 1998 until March 2000, 75 women referred to the colposcopy clinic of the University Hospital Rotterdam ( $n = 63$ ) and the University Hospital Vrije Universiteit in Amsterdam ( $n = 12$ ) were asked to participate in our study. Four women with abnormal cervical cytology refused to participate. Of the 71 women enrolled in the study, 56 had abnormal cervical cytology (very mild dyskaryosis or worse)

**Abbreviations:** CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; Pap, Papanicolaou; PBS, phosphate buffered saline; PCR, polymerase chain reaction

**Table 1** Cytology results of the lavages taken by the doctor and the patient compared with the Papanicolaou (Pap) smear

Cytology result	Pap smear		
	Normal	Very mild dyskaryosis	Mild dyskaryosis or worse
Lavage taken by doctor (n=71)			
Normal	15	4	23
Very mild dyskaryosis	–	–	3
Mild dyskaryosis or worse	–	1	22
Unsatisfactory	–	–	3
Lavage taken by patient (n=71)			
Normal	11	4	28
Very mild dyskaryosis	–	–	1
Mild dyskaryosis or worse	–	–	12
Not done	4	1	10

Unsatisfactory indicates no or too few cervical cells detectable. Agreement between lavage taken by doctor and Pap smear:  $\kappa=0.28$ ; agreement between lavage taken by patient and Pap smear:  $\kappa=0.14$ ; agreement between lavage taken by doctor and lavage taken by patient:  $\kappa=0.37$ .

and 15 women had normal cervical cytology. The mean age of the participating women was 35 years (range, 20 to 63 years). After an explanation of the study and the use of the self sampling device by the study coordinator, written informed consent, approved by the ethics review boards of both participating hospitals, was obtained from each participant.

At intake, women received a cervicovaginal self sampling device, a form with detailed instructions, and a questionnaire on the use of the device. The self sampling device consisted of an irrigation syringe (50 ml; Bard Inc, Covington, UK), a disposable female urine catheter (single use female urine catheter ch.16; Astra Tech, Mölndal, Sweden), and a container with 15 ml sterile phosphate buffered saline (PBS) for irrigation. According to the instructions, the catheter had to be attached to the syringe to aspirate the irrigation fluid from the container. After aspiration, a cervicovaginal specimen was obtained by inserting the tip of the catheter as deep as possible into the vagina and pressing and releasing the balloon of the syringe three times, to flush the irrigation fluid into the vagina and back into the syringe. After removal of the catheter, the syringe, which contained the cervicovaginal specimen, had to be emptied in the container. Women were asked to obtain a cervicovaginal lavage the day before their return visit to the colposcopy clinic. At colposcopy, after introducing a vaginal speculum, the gynaecologist performed a cervicovaginal lavage with a similar device by irrigating the cervix and aspirating the fluid pooled in the posterior vaginal fornix. This was followed by a cervical smear obtained with a Cervex® brush (International Medical Products, Zutphen, the Netherlands). After a smear was made on to a glass slide the brush was placed in a buffer solution (PBS) and sent to the laboratory for HPV detection. Colposcopic examination followed and biopsy samples were taken for histological verification of suspected lesions. If necessary, women were treated according to a standard protocol. When no lesions were seen at colposcopy the cervix was considered to be free of disease (no cervical intraepithelial neoplasia (CIN)) and no biopsies were taken. The lavages, the cervical smear, and the brush for HPV detection were processed at the department of pathology at the Vrije Universiteit Medical Centre in Amsterdam. The lavages were vortexed and divided into two specimens. The first was used for cervical cytology reading, the second for HPV DNA testing.

### Questionnaire

All participants were asked to fill in a questionnaire on the use of the self sampling device including the following questions: (1) What is your opinion about the use of the self sampling device? Answer: easy/difficult, and for what reason? (2) What screening tool would you prefer for your next screening

round—self sampling or Pap smear? Answer: self sampling/Pap smear, and for what reason?

### Cervical cytology

From each cytology specimen two cytopins were made and Pap stained. The cytology slides and biopsy samples were read by an expert pathologist who was unaware of the clinical findings. Cervical smears were classified according to the KOPAC classification, the standard classification in the Netherlands.<sup>16</sup> This is a modification of the Pap classification.<sup>17</sup> Cervical smears are cytomorphologically classified as Pap 1 (normal cytology), Pap 2 (very mild dyskaryosis), Pap 3a (mild to moderate dyskaryosis), Pap 3b (severe dyskaryosis), Pap 4 (suspected of carcinoma in situ), and Pap 5 (suspected of at least microinvasive carcinoma). Histology was classified as CIN0 (no dysplasia), CIN1 (mild dysplasia), CIN2 (moderate dysplasia), and CIN3 (severe dysplasia).

### High risk HPV testing

The specimens for HPV testing were centrifuged at 2719  $\times g$  for six minutes to pellet the cells. The supernatant was discarded and the pellet was suspended in 1 ml 0.01M Tris/HCl (pH 8.3) and stored at  $-80^{\circ}\text{C}$  until further analysis. A  $\beta$  globin polymerase chain reaction (PCR) was performed to ascertain the quality of the target DNA. Testing for HPV was done by PCR enzyme immunoassay, which used HPV general primer mediated PCR with the general primers GP 5+/6+ to detect a broad spectrum of mucosotropic HPV types.<sup>18, 19</sup> PCR products were used to identify in one assay all 14 high risk HPV types using enzyme immunoassay (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). This has been described previously and has been clinically validated.<sup>19, 20</sup>

### Statistical analysis

The concordance between the cytology (Pap smear classification) and HPV results from the smear, from the lavage taken by the doctor (lavage-doctor), and from the lavage taken by the patient (lavage-patient) was calculated. The lavage-doctor was included in the study as a control for the lavage-patient, because the first was performed under optimal conditions; that is, the cervix was visible during irrigation. The  $\kappa$  value was computed as a measure of overall agreement beyond chance. A  $\kappa$  estimate of less than 0.2 indicates poor agreement, a  $\kappa$  estimate between 0.2 and 0.8 fair to moderate agreement, and a  $\kappa$  estimate of more than 0.80 good agreement.<sup>21</sup> To compare the performances of HPV testing with cytology results in the smear and the lavages, we calculated for each test the sensitivity, specificity, and positive and negative predictive values to detect high grade CIN (CIN 2/3; moderate to severe dysplasia) and tested whether there were differences using

**Table 2** High risk human papillomavirus (HR-HPV) testing in the lavages taken by the doctor by the patient compared with HPV testing in the smear

Test result	Smear	
	HR-HPV positive	HR-HPV negative
Lavage taken by doctor (n=71)		
HR-HPV positive	48	2
HR-HPV negative	3	16
β Globin negative	1	1
Lavage taken by patient (n=71)		
HR-HPV positive	30	–
HR-HPV negative	12	12
β Globin negative	–	2
Not done	10	5

β Globin PCR negative indicates no amplifiable DNA for HPV testing in specimen. Agreement between HPV testing in lavage taken by doctor and smear:  $\kappa=0.82$ ; agreement between lavage taken by patient and smear:  $\kappa=0.53$ ; agreement between lavage taken by doctor and lavage taken by patient:  $\kappa=0.47$ .

the Mc-Nemar test. For each woman, the highest CIN grade in either the diagnostic cervical biopsy or the cervical tissue obtained at treatment was used as the reference for assessing test performance. Women with unsatisfactory cervical cytology—that is, no or too few cervical cells—or a β globin PCR negative test were not included in these analyses because we assumed that in a normal situation they would have been asked to repeat the self sampling or return for a repeat test.

## RESULTS

### Questionnaire on the use of the self sampling device

The use of the self sampling device was considered easy by 49 of the 56 (88%) women who performed a self sampling. The remaining seven women concluded that the device was difficult to use. They were uncertain about the amount of fluid they had aspirated and questioned the efficacy of the lavage. Three of them had normal cervical cytology.

At the next screening round, 13 of 56 (23%) of the women said that they would prefer the classic Pap smear to the self sampling. Their reasons were: (1) no problem with gynaecological examination ( $n = 8$ ), and (2) the self sampling device is not practical ( $n = 5$ ). The remaining group favoured the self sampling. All participating women regularly went to see their doctor for a Pap smear.

Fifteen women, five with normal and 10 with abnormal cervical cytology, did not perform the self sampling. Their reasons were: (1) they forgot to perform it ( $n = 10$ ) and (2) they were too nervous about the colposcopic examination ( $n = 5$ ). Three lavages by the doctor were unsatisfactory for cytological judgement. Four samples (lavage-doctor ( $n = 2$ ) and lavage-patient ( $n = 2$ )) were β globin PCR negative.

### Agreement of cytology and HPV testing between cervical smears, lavages, and self sampling specimens

There was fair agreement between the cytology results in the Pap smear and the lavage-doctor ( $\kappa = 0.28$ ; table 1) with a 54% concordance (37 of 69 satisfactory slides). The agreement between the cytology results in the Pap smear and the lavage-patient was poor ( $\kappa = 0.14$ ), with a concordance of 41% (23 of 56 slides). A fair agreement was obtained between the

**Table 3** The performance of human papillomavirus (HPV) detection and cervical cytology by Papanicolaou (Pap) smear and lavages taken by the doctor and the patient for the detection of high grade cervical intraepithelial neoplasia (CIN)

Test	Test result	CIN 2 or 3		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		Yes (n)	No (n)				
<i>Cervical cytology</i>							
Pap smear (n=71)	Normal (n=15)	–	15	100*	40	59	100*
	Very mild dyskaryosis (n=5)	3	2				
	Mild dyskaryosis (n=11)	3	8				
	≥Moderate dyskaryosis (n=46)	27	13				
Lavage taken by doctor (n=71)	Normal (n=42)	12	30	61	81	73	71
	Very mild dyskaryosis (n=3)	1	2				
	Mild dyskaryosis (n=6)	3	3				
	≥Moderate dyskaryosis (n=17)	15	2				
	Unsatisfactory	2	1				
Lavage taken by patient (n=71)	Normal (n=43)	15	28	42	93	85	65
	Very mild dyskaryosis (n=1)	1	–				
	Mild dyskaryosis (n=1)	1	–				
	≥Moderate dyskaryosis (n=11)	9	2				
	Unsatisfactory	–	–				
	Not done	7	8				
<i>HPV testing</i>							
Pap smear (n=71)	HR-HPV + (n=52)	30	22	91	42	58	84
	HR-HPV – (n=19)	3	16				
	β Globin negative	–	–				
Lavage taken by doctor (n=71)	HR-HPV + (n=50)	29	21	91	43	58	84
	HR-HPV – (n=19)	3	16				
	β Globin negative	1	1				
Lavage taken by patient (n=71)	HR-HPV + (n=30)	21	9	81	68	70	79
	HR-HPV – (n=24)	5	19				
	β Globin negative	–	2				
	Not done	7	8				

\*All women with abnormal cervical cytology underwent colposcopic examination and biopsy sampling. Differences in the performance of the tests were calculated. Mc-Nemar cytology smear versus lavage taken by doctor:  $\chi^2=10.1$ ;  $p < 0.001$ ; cytology lavage taken by doctor versus lavage taken by patient:  $\chi^2=0.2$ ; NS; cytology smear versus lavage taken by patient:  $\chi^2=13.1$ ;  $p < 0.001$ . HR-HPV testing smear versus lavage taken by doctor:  $\chi^2=0$ ; NS; HR-HPV testing lavage taken by doctor versus lavage taken by patient:  $\chi^2=1.3$ ; NS; HR-HPV testing smear versus lavage taken by patient:  $\chi^2=1.3$ ; NS. HR, high risk; NPV, negative predictive value; NS, not significant; PPV, positive predictive value.

cytology results of the lavage-doctor and the lavage-patient ( $\kappa = 0.37$ ; data not shown), with a 74% concordance between the cytology results performed on the two different samples.

The concordance between the high risk HPV test results in the smear and the lavage-doctor was 93% (64 of 69 women with  $\beta$  globin positive PCR tests), which is a good agreement ( $\kappa = 0.82$ ; table 2). A 78% (39 of 48 women with  $\beta$  globin positive PCR tests) concordance between the HPV test results in the smear and those in the lavage-patient was seen, indicating moderate agreement ( $\kappa = 0.53$ ). There was moderate agreement between the results obtained by HPV testing of the lavage-doctor and the lavage-patient ( $\kappa = 0.47$ ; data not shown), with a 75% concordance.

### Detection rate for high grade CIN

High grade CIN was detected in 33 women (46%; table 3). In two of these women the lavage-doctor samples were unsatisfactory for cytological reading. The cytology results from the lavage-doctor would have identified 19 of the detectable 31 high grade CIN lesions (61%), with a specificity, positive, and negative predictive value of 81%, 73%, and 71%, respectively. Seven patients with a high grade CIN lesion did not perform the self sampling. The cytology results in the lavage-patient would have identified 11 of the 26 eligible patients with high grade CIN (42%), with a specificity, positive, and negative predictive value of 93%, 85%, and 65%, respectively, which is not significantly different to the performance of the lavage-doctor (Mc-Nemar  $\chi^2 = 0.2$ ).

Thirty of 33 (91%) high grade CIN lesions would have been identified when colposcopic examination was performed in the case of a positive high risk HPV test result in the smear. In one woman with a high grade CIN lesion the  $\beta$  globin PCR was negative in the lavage-doctor. Thus, a high risk HPV positive test result in the lavage-doctor would identify 29 women out of 32 (91%) women with high grade CIN lesions, whereas a high risk HPV positive test result in the lavage-patient would identify 21 of 26 (81%) eligible women with high grade CIN. The performance of HPV testing in the smear and in the lavage-patient was not significantly different (Mc-Nemar  $\chi^2 = 1.3$ ) The specificity for high grade CIN of high risk HPV testing in the smear and in the lavage-patient was 42% and 68%, respectively, with positive predictive values of 58% and 70%, respectively, and negative predictive values of 84% and 79%, respectively.

No significant difference in the detection of high grade CIN could be found between the HPV test results in the lavage-doctor and the lavage-patient (Mc-Nemar  $\chi^2 = 1.3$ ).

## DISCUSSION

Our results indicate that cytological screening for cervical cancer by self sampling is no alternative for cytological screening by the classic Pap smear. The agreement between the Pap smear and the lavage by the patient was low, and less women with high grade CIN would be identified by cytology in the lavage than in the Pap smear. In contrast, high risk HPV testing in self obtained cervicovaginal lavage is a feasible alternative method. The sensitivity for high grade CIN in women with a high risk HPV positive test result in the lavage by the patient was lower, although not significantly lower, than in the smear (81% *v* 91%). The specificity of the HPV test in self sampled material was higher than in the smear (68% *v* 42%).

We included women with abnormal and normal cervical cytology to evaluate the use of the self sampling device in these two groups. The self sampling device was acceptable to 88% of the participating women. No differences in acceptability were seen in women with or without abnormal cervical cytology. Seventy seven per cent of the participating women would choose self sampling by vaginal lavage above the classic Pap smear as an alternative screening tool for their next

### Take home messages

- Cytological screening for cervical cancer by self sampling is not an alternative for cytological screening by the classic Papanicolaou (Pap) smear
- The agreement between the Pap smear and the lavage by the patient was low, and less women with high grade cervical intraepithelial neoplasia (CIN) would be identified by cytology in the lavage than in the Pap smear
- However, high risk human papillomavirus (HPV) testing in self obtained cervicovaginal lavage is a feasible alternative method—there was no significant difference for the detection of high grade CIN between HPV testing of self sampled lavage material or physician obtained cervical brush
- Although participation in the screening programme remains the best option, self sampling for HPV DNA is a feasible alternative method in women who decline to participate in population based cervical cancer screening programmes

screening round, on condition that both screening methods obtain equal results. Seven women questioned the efficacy of the self sampling. Their main problem was the uncertainty about the amount of fluid they aspirated. However, we only found one  $\beta$  globin negative sample in these samples, indicating that the perception of the efficacy of this method by the women was different to the reality. In future studies we are planning to modify the instructions about using the device.

We included the lavage taken by the doctor as a control for the lavage taken by the patient. No significant difference in the detection of high grade CIN was found between the test results in the lavage-doctor and lavage-patient, indicating that the conditions of the performances—that is, irrigating the cervix directly or indirectly—of the two different lavages did not differ. However, the concordance between HPV testing in the lavage-doctor and the Pap smear appeared to be higher than that in the lavage-patient.

“77% of the participating women would choose self sampling by vaginal lavage above the classic Pap smear as an alternative screening tool for their next screening round, on condition that both screening methods obtain equal results”

Fifteen women (21%) did not perform a home obtained lavage sample. Ten of them had an abnormal cervical smear. In one third of the cases emotional stress with regard to the examination at the colposcopy clinic was the reason for not doing the self sampling at home.

In our study women were asked to obtain a sample at home. This contrasts with other studies that evaluated self sampling under optimal conditions—at the outpatient clinic just after being provided with extensive information.<sup>9, 22</sup> In our study a more realistic condition was investigated. Our participation rate of 79% was high when compared with other studies evaluating home obtained self samples. A participation rate of 68% was seen in a study involving 25 women with abnormal cervical cytology who performed a self administered vaginal lavage at home and returned the sample by mail.<sup>23</sup>

The high performance of high risk HPV testing in the lavage in our study is in agreement with other studies. Wright *et al* found a high risk HPV positive test in patient obtained vaginal swabs in 66% of high grade dysplastic cervical lesions or worse in an unscreened population known to have a high incidence of premalignant lesions.<sup>22</sup> Nurse obtained swabs revealed an 84% correlation. They concluded that self testing was as sensitive as a Pap smear performed by a health care provider, and proposed self testing for HPV DNA in areas where access to care is limited. In other studies an 85–93% correlation was



found for high grade CIN in patient obtained vaginal swabs,<sup>9,24</sup> indicating that self sampling for HPV is also adequate when other techniques are used. In a future study, we are planning to compare the acceptability and efficacy of self sampling by these vaginal swabs with the lavage device.

We found no significant difference in detecting high grade CIN between HPV testing in self sampled lavage material or physician obtained cervical brushes. Moreover, we found a higher specificity and positive predictive value for HPV testing in self sampled material. As long as proper instructions are given to the women, self sampling for HPV DNA testing seems suitable as an alternative screening tool. Although the Pap smear (with additional high risk HPV testing) remains the best screening tool for cervical cancer and its precursors, the high sensitivity for high grade CIN of high risk HPV testing in self sampled material allows us to advise self sampling in women who decline to participate in such programmes because it could largely reduce the risk of cervical cancer associated with not participating in a screening programme.<sup>6,7</sup> In women who do participate the Pap smear remains the best option.

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### REFERENCES

- 1 **Quinn M**, Babb P, Jones J, *et al* on behalf of the United Kingdom Association of Cancer Registries. Effect of screening on incidence of and mortality from cancer of cervix in England: evaluation based on routinely collected statistics. *BMJ* 1999;**318**:904–8.
- 2 **Sigurdsson K**. The Icelandic and Nordic cervical screening programs: trends in incidence and mortality rates through 1995. *Acta Obstet Gynecol Scand* 1999;**78**:478–85.
- 3 **Koss LG**. The Papanicolaou test for cervical cancer detection. *JAMA* 1989;**261**:737–43.
- 4 **Cuzick J**, Szarewski A, Terry G, *et al*. Human papillomavirus testing in primary cervical screening. *Lancet* 1995;**345**:1533–6.
- 5 **Raffle AE**, Alden B, Mackenzie EFD. Detection rates for abnormal cervical smears: what are we screening for? *Lancet* 1995;**345**:1469–73.

- 6 **Van der Graaf Y**, Zielhuis GA, Peer PGM, *et al*. The effectiveness of cervical screening: a population-based case-control study. *J Clin Epidemiol* 1988;**41**:21–6.
- 7 **Sasieni PD**, Cuzick J, Lynch-Farmery E, and The National Co-ordinating Network for Cervical Screening Working Group. Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. *Br J Cancer* 1996;**73**:1001–5.
- 8 **Fischer S**. Inexpensive population screening for abnormal cells from the uterine cervix and valid results using the vaginal cytospinette. *Gynecol Oncol* 1992;**46**:62–4.
- 9 **Hillemanns P**, Kimming R, Hütteman U, *et al*. Screening for cervical neoplasia by self-assessment for human papillomavirus DNA. *Lancet* 1999;**354**:1970.
- 10 **Walboomers JMM**, Jacobs MV, Manos MM, *et al*. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol* 1999;**189**:12–19.
- 11 **Rozendaal L**, Walboomers JMM, Van der Linden JC, *et al*. PCR based high risk HPV test in cervical cancer screening gives objective risk assessment of women with cytologically normal cervical smears. *Int J Cancer* 1996;**68**:766–9.
- 12 **Rozendaal L**, Westerga J, van der Linden JC, *et al*. PCR based high risk HPV testing is superior to neural network based screening for predicting incident CIN III in women with normal cytology and borderline changes. *Int J Cancer* 2000;**53**:606–11.
- 13 **Remmink AJ**, Walboomers JMM, Helmerhorst ThJM, *et al*. The presence of persistent high-risk HPV genotypes in dysplastic cervical lesions is associated with progressive disease: natural history up to 36 months. *Int J Cancer* 1995;**61**:306–11.
- 14 **Ho GYF**, Burk RD, Klein S, *et al*. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;**87**:1365–71.
- 15 **Nobbenhuis MAE**, Walboomers JMM, Helmerhorst ThJM, *et al*. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *Lancet* 1999;**354**:20–5.
- 16 **Hanselaar AGJM**. Het bevolkingsonderzoek op baarmoederhalskanker. Een uniform model voor cytopathologisch onderzoek. *Medisch Contact* 1995;**50**:1590–2.
- 17 **Vooijs GP**. De advisering bij afwijkende bevindingen van cytologisch onderzoek van de cervix uteri. *Ned Tijdschr Geneesk* 1987;**131**:1662–3.
- 18 **De Roda Husman AM**, Walboomers JMM, van den Brule AJC, *et al*. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995;**76**:1057–62.
- 19 **Jacobs MV**, van den Brule AJC, Snijders PJF, *et al*. A general primer GP5+/6+ mediated PCR-EIA for rapid detection of high- and low-risk HPV DNA in cervical scrapes. *J Clin Microbiol* 1997;**35**:791–5.
- 20 **Jacobs MV**, Zielinski D, Meijer CJLM, *et al*. A simplified and reliable HPV testing of archival Papanicolaou-stained cervical smears: application to cervical smears from cancer patients starting with cytologically normal smears. *Br J Cancer* 2000;**82**:1421–6.
- 21 **Altman DG**. *Practical statistics for medical research*. Chapman and Hall, 1991, London.
- 22 **Wright TC**, Denny L, Kuhn L, *et al*. HPV DNA testing of self-collected vaginal samples compared with cytologic screening to detect cervical cancer. *JAMA* 2000;**283**:81–6.
- 23 **Morrison EA**, Goldberg GL, Hagan RJ, *et al*. Self-administered home cervicovaginal lavage: a novel tool for the clinical-epidemiologic investigation of genital human papillomavirus infections. *Am J Obstet Gynecol* 1992;**167**:104–7.
- 24 **Sellers JW**, Lorincz AT, Mahony JB, *et al*. Comparison of self-collected vaginal, vulvar and urine samples with physician-collected cervical samples for human papillomavirus testing to detect high-grade squamous intraepithelial lesions. *Can Med Assoc J* 2000;**163**:513–18.