

Quality control for normal liquid-based cytology: Rescreening, high-risk HPV targeted reviewing and/or high-risk HPV detection?

Christophe E. Depuydt^{a, *}, Marc Arbyn^b, Ina H. Benoy^a, Johan Vandepitte^a,
Annie J. Vereecken^a, Johannes J. Bogers^{a, c}

^a Laboratory for Clinical Pathology (Labo Lokeren, campus RIATOL), Amerikalei, Antwerp, Belgium

^b European Network for Cervical Cancer Screening, Scientific Institute of Public Health, Juliette Wytsmanstraat, Brussels, Belgium

^c Laboratory of Cell and Tissue Research, Faculty of Medicine, University of Antwerp, Antwerp, Belgium

Received: November 8, 2007; Accepted: May 24, 2008

Abstract

The objective of this prospective study was to compare the number of CIN2+ cases detected in negative cytology by different quality control (QC) methods. Full rescreening, high-risk (HR) human papillomavirus (HPV)-targeted reviewing and HR HPV detection were compared. Randomly selected negative cytology detected by BD FocalPoint™ (NFR), by guided screening of the prescreened which needed further review (GS) and by manual screening (MS) was used. A 3-year follow-up period was available. Full rescreening of cytology only detected 23.5% of CIN2+ cases, whereas the cytological rescreening of oncogenic positive slides (high-risk HPV-targeted reviewing) detected 7 of 17 CIN2+ cases (41.2%). Quantitative real-time PCR for 15 oncogenic HPV types detected all CIN2+ cases. Relative sensitivity to detect histological CIN2+ was 0.24 for full rescreening, 0.41 for HR-targeted reviewing and 1.00 for HR HPV detection. In more than half of the reviewed negative cytological preparations associated with histological CIN2+ cases no morphologically abnormal cells were detected despite a positive HPV test. The visual cut-off for the detection of abnormal cytology was established at 6.5 HR HPV copies/cell. High-risk HPV detection has a higher yield for detection of CIN2+ cases as compared to manual screening followed by 5% full review, or compared to targeted reviewing of smears positive for oncogenic HPV types, and show diagnostic properties that support its use as a QC procedure in cytologic laboratories.

Keywords: human papillomavirus (HPV) • cervical cancer screening • quality assurance • Belgium • Flanders

Introduction

Cytological investigation of the cervix has proven to be a valuable tool in the early detection of cervical cancer. However, one of the problems limiting the value of cytological screening is the occurrence of false-negative test results [1]. Therefore, setting up quality control (QC)-systems to reduce this false-negative rate is a major issue in laboratories performing cervical cytology screening. QC of cervical cytology remains a matter of debate since there are wide differences in practice between laboratories and there has been little attempt to standardize the technique [2]. Many published studies also use a cytological or ill-defined end-point.

Clinical testing procedures designed to identify pre-invasive, and invasive neoplastic disease should be evaluated for the detection of clinically relevant disease (high-grade lesions) and should not merely be judged on the increase of detection of low-level abnormalities [3].

Rescreening slides interpreted as negative is a QC method specially designed to address this sensitivity problem inherent to the interpretation of cervical cytology [4].

Full rescreening of a 10% random fraction of smears reported as being within normal limits is a mandatory QC procedure in the United States [5, 6]. This QC method is criticized for its inefficiency and lack of statistical power [7, 8]. In the United Kingdom, rapid or partial reviewing (RR) of all smears initially interpreted as being non-abnormal has been introduced as an alternative and more useful QC standard [9–13]. Meta-analysis showed that RR of all negative slides of rapid prescreening (RPS) of the full workload, resulted in the detection of more additional abnormalities in

*Correspondence to: Christophe DEPUYDT,
Laboratory for Clinical Pathology (Labo RIATOL),
Amerikalei 62-64, B-2000 Antwerp, Belgium.
Tel.: +32 3 259 0300
Fax: +32 3 216 16 53
E-mail: Christophe.Depuydt@riatol.be

comparison with full rescreening of only 10% of the negative workload [14].

It could be hypothesized that limiting the rescreening to high-risk human papillomavirus (HR HPV) positive slides (HR HPV-targeted reviewing) the efficiency would increase. In this way, the size of the rescreening target group could be reduced, limiting the additional workload.

BD FocalPoint™ (previously AutoPap) is a computerized scanning system for the primary screening of cervical smears (BD Diagnostics-Tripath; Burlington, NC, USA). The system classifies smears into three different categories: (1) no further review (NFR), these smears can be stored with confidence as 'No Intra-epithelial Lesion or Malignancy' without being evaluated by a cytologist; (2) guided review or guided screening (GS), and (3) process review (PR), smears needing to be reviewed manually because of technical problems. The system provides a fixed proportion of 25% of NFR smears, as recommended by the manufacturer and approved by the Food and Drug Administration (FDA).

BD FocalPoint™ ranking system, classifying approximately 75% non-NFR slides in five quintiles, has been shown to correlate with the probability that a significant epithelial abnormality will be found [15]. Previously reported clinical trials have shown superior performance for the detection of all abnormal cases when compared with manual screening [16]. BD FocalPoint™ can be described as a system of RPS, defined as an initial, rapid microscopic inspection of a slide before a full routine evaluation [17]. The essential difference between RPS and GS is that in the latter all slides are submitted to a thorough uniform scanning by BD FocalPoint™ while in RPS there is only a quick scanning by a cytotechnologist.

In the current prospective study, three different QC methods for the detection of relevant disease (histologically proven CIN2+) in negative cytology are compared (full rescreening, HR HPV-targeted reviewing and detection of HR HPV). To test the relative sensitivity of the three different QC methods in a highly negative population, the number of CIN2+ cases identified by each method after 36 months of follow-up was investigated. The negative cytology samples were derived from three sources: NFR, GS and manual screening (MS).

From the results of the analysis of the tested schemes new recommendations for QC are formulated.

All investigations were conducted in the laboratory of clinical pathology, a private laboratory member of the AML-Riatol group. Riatol has used liquid-based cytology since 1998, analyzing approximately 100,000 slides per year.

Material and methods

Cervical sample processing and cytological procedure

Cervical cells were collected using the Cervex-Brush® (Rovers, Oss, The Netherlands). After collection, brush heads were transferred directly into

alcohol-based preservative (BD SurePath™, BD Diagnostics-TriPath), and the vials were transported to the laboratory. Thin layer slide preparations were made with the fully robotic AutoCyte® PREP System (AutoCyte®, BD Diagnostics-TriPath) [18], and were prepared as described elsewhere [19].

The cytological results were classified according to the Bethesda system 2001 [20], using the classes negative for intraepithelial lesions (NEG), atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesions (L-SIL), ASC-US cannot exclude high-grade (ASC-H) and high-grade SIL (H-SIL).

Screening procedure and patients

Samples were collected during routine gynaecological health checks from women resident in the Flemish region (Belgium) by general practitioners or gynaecologists. Between April 2003 and August 2003, the first 230 slides of the daily load were loaded onto BD FocalPoint™, and the rest of the slides were screened manually. Smears that could not be interpreted by BD FocalPoint™ for technical reasons (PR) were also screened manually (MS). During that period full manual rescreening (FRE) was performed on an approximately 5% random sample of the smears labelled by BD FocalPoint™ as 'NFR' and of the samples screened manually and interpreted as being negative (Fig. 1). All rescreen-positive cases (ASC-US+) were checked by a cytopathologist and a pathologist. The rescreening results were blinded from the HPV results. From January 2004 until December 2004 one random slide per screener per day (1/80–90) was taken from normal cytology after prescreening and GS with BD FocalPoint™ (GS). All slides from the GS group were also rescreened. All samples from the three groups (NFR, MS and GS) were tested for the presence of high-risk (HR) HPV types, using the liquid-based cytology leftover, after the preparation of the SurePath smear. All slides positive for one or more oncogenic HPV types were rescreened by a cytotechnician and reinterpreted by the pathologist (aware of HPV type but blinded for viral load and for the study population (NFR, GS, MS)). The algorithm of the study design is given in Figure 1. Slides positive for unidentified (HPVX) HPV types were not rescreened.

Patient identifiers were coded to preserve confidentiality.

BD FocalPoint™-guided screening

All slides were scanned by BD FocalPoint™ system and categorized as qualified or PR. PR slides were screened manually. Qualified slides were categorized into five quintiles (75%, review slides) or as NFR, 25%, based on slide score. Slides in quintile 1 (Q1) have the highest probability of containing cytological abnormalities and Q5 the category the lowest probability. Slides classified as NFR immediately were designated as 'negative for intraepithelial lesions' with no manual review performed (except for slides with history, which were always manually reviewed, slides with a clinical history were excluded from the study population). 'Review' slides were screened using SlideWizards™ (BD-Diagnostics-Tripath) with GS by cytotechnologists with knowledge of the relative score ranking of each slide, given as quintiles within the 'Review' category, using the 15 PapMaps. After looking at the 15 PapMaps, each cytotechnician rapidly reviewed the entire slide (15 sec). BD FocalPoint™ and SlideWizards™ were used according to the manufacturer's instruction. In addition, as a positive control, a known slide previously diagnosed as H-SIL (CIN 3 biopsy confirmed and positive for HPV 16) was loaded in between the routine samples each day onto BD FocalPoint™. This control slide was always classified in the first quintile of the review category.

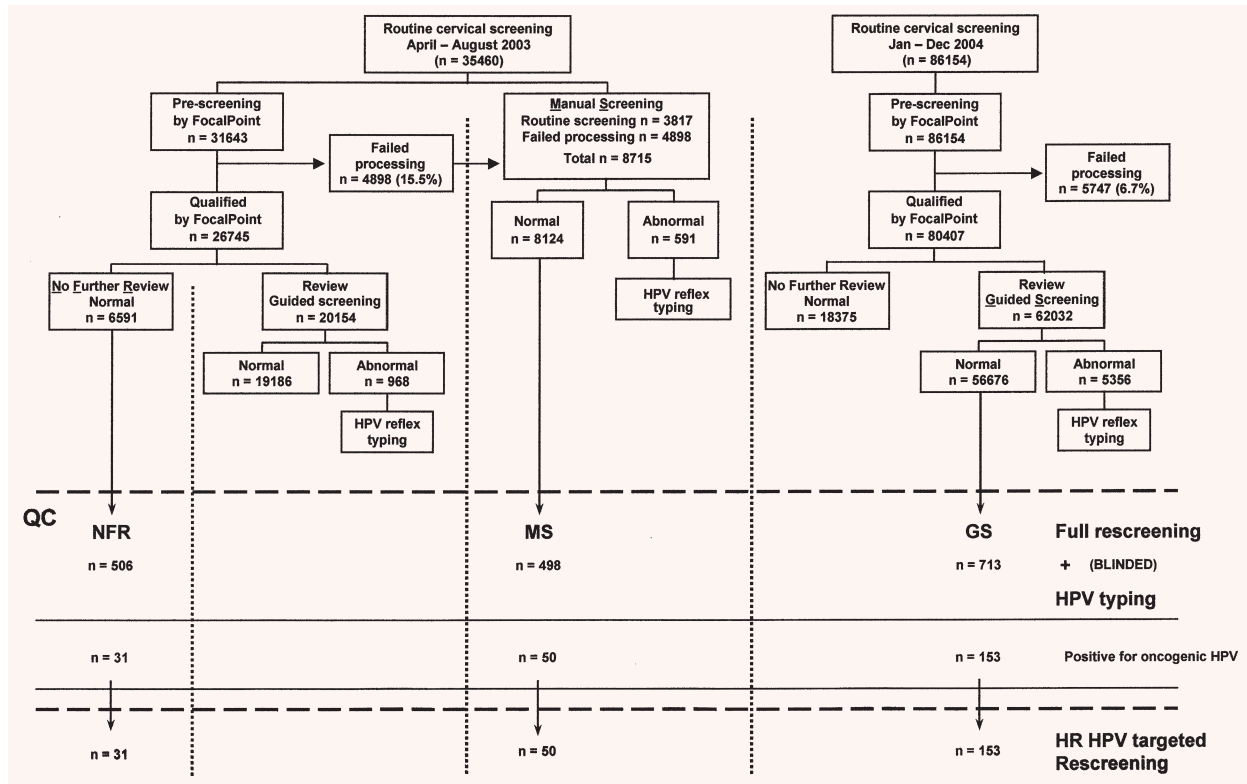


Fig. 1 Algorithm study design.

Isolation of DNA from cervical cells

DNA isolation from liquid-based cytology was performed as previously described [21–23]. Briefly, half of the 800 µl liquid-based preparations left-over was used for DNA extraction by Proteinase K. This was to ensure that there is still enough material left to prepare an extra slide, or do a second DNA extraction. The DNA extracts were stored at –20°C until PCR was performed.

PCR analysis of HPV DNA

Each sample was subjected to a quantitative real-time PCR amplification for the detection of β-Globin. This was done to confirm that the DNA quality was still suitable for PCR analysis, and to be able to calculate the viral load. All samples were tested with the MY 9/11 consensus PCR [21, 23], and with type-specific quantitative real-time PCR for oncogenic HPV types. The copy number and viral load of HPV type 16 E7, 18 E7, 31 E6, 33 L1, 33 E6, 35 E4, 39 E7, 45 E7, 51 E6, 52 L1, 52 E7, 53 E6, 56 E7, 58 L1, 58 E6, 59 E7, 66 E6 and 68 E7 was determined using a TaqMan-based real-time qPCR analysis as previously described [22, 23].

The amount of β-globin DNA (in nanograms) present in each sample was divided by the weight of 1 genome equivalent (i.e. 6.6 pg/cell) and a factor of 2 (since there are two copies of β-globin DNA/cell) to obtain the

number of genome equivalents in the sample. Viral loads in each specimen were expressed as the number of HPV copies/cell.

CIN 2+ cases and follow-up period

For all samples included in the QC series, a 36-month follow-up period was used. Our computer system was searched to identify cases with biopsy-proven CIN 2+. As golden standard a CIN2+ histological outcome was considered as a positive follow-up result. Cytological follow-up was only included when minimum two consecutive negative smears were available, which was considered as absence of CIN 2+. For 1215 out of 1717 samples follow-up was available (70.8%). An overview of the number of follow-up cytology, of cytology with HPV status and of biopsies taken in each of the QC series is given in Table 1.

Statistical analysis

Comparisons of means were studied by analysis of variance (ANOVA). The Chi-square statistics for trend was used to verify the existence of a trend across ordered groups, such as increase in HPV positivity according to the degree of cytological abnormality. Statistical tests were considered significant at $P < 0.05$.

Table 1 Follow-up of negative cytology from: prescreening with BD FocalPoint™ (NFR), review of the prescreened slides by guided screening with SlideWizard (GS), and manual screening (MS)

Study group	Follow-up by								
	cytology			Cyto + HPV		Biopsy		None	
<i>n</i>	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	
NFR	506	332	65.6	21	4.2	33	6.5	150	29.6
MS	498	327	65.7	26	5.2	21	4.2	143	28.7
GS	713	79	11.1	425	59.6	51	7.2	209	29.3
Total	1717	738	43.0	472	27.5	104	6.1	502	29.2

Results

From April until August 2003, 35,460 liquid-based preparations were prepared for routine cervical screening (Fig. 1). During that 5-month period, the first 230 slides received that day were loaded onto BD FocalPoint™ for prescreening ($n = 31,643$). Each day the rest of the available slides were screened manually ($n = 3817$). Also slides that failed processing by BD FocalPoint™ were screened manually ($n = 4898$). During that 5-month period 26,745 slides were qualified by BD FocalPoint™, 6591 slides were classified as NFR (24.6%) and 20,154 slides were classified as Review (75.4%). From the qualified slides, selected by BD FocalPoint™ as NFR ($n = 6591$) 506 slides were randomly selected for manual rescreening and HPV typing. From the manually screened slides with normal cytology ($n = 8124$), 498 slides were randomly selected for manual rescreening and HPV typing (MS).

In 2004, BD FocalPoint™ processed 86,154 slides, 5747 slides were excluded because of failed processing (1233 rerun and 4514 PR). From the remaining 80,407 qualified slides, 18,375 slides were classified as NFR (22.85%) and 62,032 slides were classified as Review (77.15%). From the review slides, 713 slides (1.15%), which were cytologically negative after GS, were randomly included for QC with oncogenic HPV detection and rescreening (GS).

A thorough search of our laboratory result database for the 1717 cytological normal slides included in the study, revealed 17 CIN 2+ cases (Table 2).

Full rescreening of NFR, GS and MS

After full manual rescreening (FRE) of NFR, GS and MS, in total 19 slides were diagnosed as abnormal, 3 LSIL in the NFR group (0.59%), 11 in the GS group (1.54%) 7 ASC-US and 4 L-SIL, and 5 in the MS group (1.00%), 1 HSIL, 2 LSIL and 2 ASC-US. There was no significant difference between the numbers of abnormal slides detected in each group. The results from the rescreening, HPV typing and rescreening of the slides positive for oncogenic HPV types in the NFR, GS and MS groups is given in Table 3.

In this study, a false positive rescreen result was defined as a case classified as abnormal (\geq ASC-US) at rescreening by the cytotechnologist, but HPV negative and confirmed by the pathologist as being negative for intraepithelial neoplasia. The false positive rate of full rescreening was 0% in NFR and GS and 40% in MS.

Yield of additional abnormal slides picked up by high-risk (HR) HPV-targeted rescreening

The HR HPV rate was 6.1%, 10.0% and 21.6% in, respectively, the NFR, MS and GS group.

By targeted HR HPV rescreening four additional cytologically abnormal cases were identified in the NFR group (200%), 24 in the GS group (267%) and 16 in the MS group (533%; for details see Table 3).

Comparison of the relative sensitivity of FRE versus HR HPV-targeted rescreening

The relative sensitivity is calculated as the detection rate of confirmed ASC-US+ CIN2+ cases picked up by full rescreening over that picked up by HR HPV-targeted rescreening. Since none of the CIN2+ cases were picked up by full rescreening in the NFR group, no relative sensitivity could be calculated for the NFR group. The relative sensitivity was 50% in the GS group and 100% in the MS group. Overall relative sensitivity of general rescreening *versus* HR HPV-targeted screening was 50%.

Specificity and PPV of full rescreening, HR HPV-targeted reviewing and HR HPV detection

The specificity to detect CIN2+ by full rescreening was 99.1%, 76.5% by HR HPV-targeted reviewing and 87.2% by detection of HR HPV. PPV for finding an underlying CIN2+ lesion was 21%,

Table 2 Overview of biopsy confirmed CIN 2+ cases

Case #	Study group	FRE	ONC+RE	HPV	Viral load	Histology
A	GS	NEG	NEG	16	0.0429	CIN 3
B	GS	NEG	NEG	16	10.0861	CIN 2
C	GS	L-SIL	ASC-US	16	8.3974	CIN 3
D	GS	ASC-US	L-SIL	16*,39	1186.5076	CIN 3
E	GS	ASC-US	H-SIL	18*,31,33,58	71.6029	CIN 3
F	GS	NEG	NEG	39	0.7781	VIN 3
G	MS	H-SIL	ASC-US	31	0.5285	CIN 3
H	MS	NEG	NEG	31*,35	0.0144	CIN 2
I	MS	NEG	NEG	18	6.6268	CIN 3
J	NFR	NEG	NEG	16*,58	50.5446	CIN 3
K	NFR	NEG	ASC-H	16	26.6882	CIN 3
L	GS	NEG	NEG	16	0.3359	CIN 3
M	GS	NEG	L-SIL	16	13.6599	CIN 3
N	GS	NEG	ASC-R	16*,33	18.7129	CIN 3
O	GS	NEG	ASC-H	16	2.9539	CIN 2
P	GS	NEG	NEG	16	0.1970	CIN 2
Q	NFR	NEG	NEG	16,33*,53	186.7860	CIN 3

FRE = full manual rescreening without knowledge of quintile ranking or HPV status, ONC+RE = HR HPV targeted rescreening, e.g. with knowledge of oncogenic HPV type but without knowledge of the viral load, * = only the viral load for the HPV type with the highest load is given. Viral load is expressed as HPV copies/cell.

12% and 7% for, respectively, full rescreening, HR HPV-targeted reviewing and HR HPV detection; an overview for each individual QC group is given in Table 4.

Efficiency of full rescreening versus HR HPV-targeted reviewing and detection of HR HPV

Finally, general full rescreening of 1717 slides allowed detection of only four histological confirmed CIN 2+ cases (0.23%), whereas HR HPV-targeted rescreening of only 234 HR HPV positive slides yielded detection of eight CIN2+ cases (3.42%). This means that HR HPV-targeted rescreening is 15 times more sensitive than full reviewing as QC method. All CIN 2+ cases detected in follow-up were positive for one or more oncogenic HPV types. Detection of HR HPV (7.3%) without screening was 32 times more sensitive than full reviewing.

Rank versus viral load

For slides in the GS group, BD FocalPoint™ assigned each slide a group ranking, ranging from 1 to 5, where a rank of 1 indicates the

group most likely to contain abnormalities. The group ranking in function of HPV positivity and cytology is given in Table 5. The difference in viral load between ASC-US+ and Neg cases is highest in the 1st and 2nd quintile, and non-existing in 3rd–5th quintile (ANOVA F-ratio = 2.298; *P* = 0.019).

HPV typing and viral load cut-off for the visual detection of abnormal cytology/detection of CIN2+

HPV typing revealed 31 slides positive for oncogenic HPV types in the NFR group (6.1%), 50 in the MS group (10.0%) and 153 in the GS group (21.5%) ($\chi^2 = 73.1$, *P* < 0.001). There was no difference in viral load between the different HR HPV types (*P* > 0.05). Therefore all HR HPV positives were pooled in the following analysis. Because all HPV HR positive slides (*n* = 234) were rescreened with knowledge of HPV type but without knowledge of viral load (blinded), we could calculate the viral load cut-off for the visual detection abnormal cytology (ASC-US+). The viral load of oncogenic positive slides, which were labelled ASC-US or higher (150.2 copies/cell), was significantly higher than the oncogenic positive slides, which were deemed negative after rescreening

Table 3 Comparison of negative cytology from: prescreening with BD FocalPoint™ (NFR), review of the prescreened slides by guided screening with SlideWizard (GS), and manual screening (MS)

HPV type	NFR			GS			MS		
	HPV	FRE	ONC+ RE	HPV	FRE	ONC+ RE	HPV	FRE	ONC+ RE
16,(18)	4		ASC-H ^K	25 ^{A,B,L,P} (2)	2L-SIL ^C	6ASC ^C ,2L-SIL ^M , ASC-H ^O	7	L-SIL	2ASC-H,ASC
16,31,(35)				2(2)	ASC	ASC			
16,33,(39)	1			1(1)		ASC			
16,33,53	1 ^Q								
16,(35),39	1			3	ASC ^D	L-SIL ^D			
16,51				1					
16,52				1					
16,58	1 ^J								
18,31				1		ASC			
18,(31,33,58)	6		ASC	8(1)	ASC,(ASC ^E)	L-SIL,(H-SIL ^E)	17 ^I		6ASC
18,39,(52)				1 ^N (1)	ASC	ASC-H			
18,51,52				1	L-SIL	H-SIL			
18,56				1		ASC			
18,58				1					
31				13			4	H-SIL ^G	2ASC ^G
31,33,(39)				1(1)			(1)		ASC
31,33,52				1					
31,35,39,58				1		ASC			
31,35,(53)	3		ASC,AGUS				1 ^H (1)		
31,35,56	1								
31,39				1		ASC	1		ASC
31,52				1					
33	1			5		ASC	1		
33,52				1		ASC			
35,(39)				4(1)		ASC	1		
35,52				1					
35,53				1					
35,58				1					
39,(52)	1	L-SIL	ASC	10 ^F	ASC	L-SIL,ASC	2(1)		ASC,(ASC)
45				1					
51,(53)	(1)			9(2)		ASC	2		
51,66				1					
52,(53)	3			7		2ASC	2(1)		L-SIL,(ASC)
52,58				2					
53	2			8		2ASC	1		
56,(66)	1			8(1)		ASC	1		
58	1			7		ASC,ASC-H	2		ASC

Table 3 Continued.

66	3	L-SIL	L-SIL	8			4	L-SIL	L-SIL
68				3					
X+	58	L-SIL	ND	258	ASC,L-SIL	ND	50		
Neg	417			302			398	2ASC	ND
Total	506	506	31	713	713	153	498	498	50
Abnormal/Onc+	31	3	6	153	11	33	50	5	19
%	6,1	0,6	19,4	21,5	1,5	21,6	10,0	1,0	38,0
Detected CIN2+	3	0	1	11	3	5	3	1	1
%	100	0	33,3	100	27,3	45,5	100	33,3	33,3

HPV = number of cases positive after HPV typing, FRE = full manual rescreening without knowledge of quintile ranking or HPV status, ONC+RE = HR HPV targeted rescreening, ND = not done, ^{A-Q} CIN 2+ biopsy confirmed cases.

Table 4 Overview of relative sensitivity, specificity and PPV for detection of CIN 2+ in the different QC groups for each of the QC screening methods

QC Method	QC group	Relative sensitivity (%)	Specificity (%)	PPV (%)
Full rescreening	all	23.5	99.1	21.1
	NFR	0	93.8	0
	MS	33.3	90.1	2.0
	GS	27.3	78.6	2.0
HR HPV targeted rescreening	all	41.2	76.5	12.1
	NFR	33.3	94.0	3.2
	MS	33.3	90.1	2.0
	GS	54.6	79.1	3.9
HR HPV detection	all	100	87.2	7.3
	NFR	100	94.4	9.7
	MS	100	90.5	6.0
	GS	100	79.8	7.2

PPV = positive predictive value.

(25.9 copies/cell) (ANOVA; F-ratio = 8.894; *P* = 0.003). Receiver operating characteristic (ROC) curve analysis revealed a cut-off for ASC-US+ at 6.4508 copies/cell with a sensitivity of 54.4 and specificity of 82.4 with an area under the ROC curve of 0.698 and a 95%CI between 0.635–0.757 (Fig. 2 dotted line). For the detection of CIN2+ by HR HPV testing ROC curve analysis revealed a cut at 0.0142 copies/cell with a sensitivity of 100% and specificity of

88.3% with an area under the ROC curve of 0.956 and a 95%CI between 0.945 and 0.965 (Fig. 2 full line).

Discussion

One of the advantages of using liquid cytology for the collection of cervical specimens is that multiple diagnostic tests can be performed on a single sample avoiding recall of women for additional testing. Additionally, the technique can easily be automated and combined with HPV testing on a large number of samples. In the present study, 1717 samples were analyzed, in all samples β-globin DNA could be amplified after proteine K digestion. Therefore the SurePath fixative provides an excellent preservation fluid allowing a simple DNA extraction.

Real-time PCR has the advantage of being highly specific for HR HPV detection, reproducible and capable of detecting viral load up to eight orders of magnitude in a linear range [23, 24]. In this study, 16 type-specific real-time PCRs were used to detect oncogenic HPV [22, 23]. Using the same real-time PCRs, Moberg and coworkers showed that the risk of developing cervical CIS or CIN 3 increases with higher viral load for most of the HPV types studied [25] and that HPV load is a type-dependent risk marker for invasive carcinoma [26]. Several studies have proven the prognostic value of HPV viral load in the evolution to cervical intraepithelial neoplasia [27–29].

Moreover, HPV viral load detection and typing by real-time PCR in cases of negative cytology has the possibility to calculate odd ratios for developing cancer for each individual slide.

In the present paper, HR HPV detection in cytologically negative samples was used in a QC setting. Our results show that the probability of detecting abnormal cell(s) in an LBC preparation is a function of the associated HPV viral load and that HR HPV detection has a higher sensitivity to detect CIN2+ than cytological rescreening. These results are concordant with the work of

Table 5 Quintile ranking in function of HPV positivity and cytology after HR targeted rescreening of samples from the GS group

ONC+RE	HPV	Quintile					Total <i>n</i>
		1	2	3	4	5	
NEG	-	52	53	61	73	63	302
	X+	37	50	59	55	57	258
	Onc+	19 ^A	28 ^P	31 ^F	29 ^{B,L}	18	125
ASC-US	Onc+	2	6 ^{C,N}	4	2	4	18
ASC-H	Onc+	2		10			3
L-SIL	Onc+	2 ^M			2 ^D	1	5
H-SIL	Onc+	2 ^E					2
Total		116	137	156	161	143	713

^{A-F,L-P} CIN 2+ biopsy confirmed, ONC+RE = HR HPV targeted rescreening.

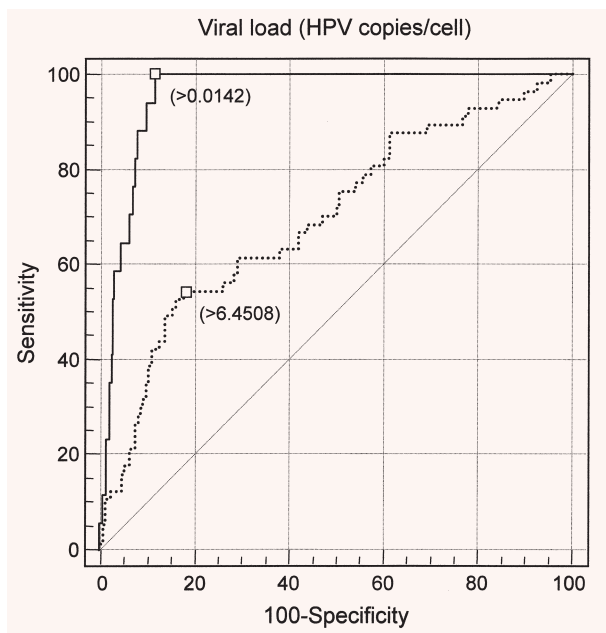


Fig. 2 ROC curve analysis for detection of CIN 2+ by cytology (ASC-US+ cases, dotted line) and by HR HPV viral load detection (full line).

Slecht *et al.* [30] and Bargas and de Marval [31]. Because HPV is an intracellular virus, the viral load is proportional to the number of infected cells. Therefore, the higher the viral load, the greater the number of infected cells on the slide and the greater the probability for the LBC test to be abnormal. Rescreening slides classified as NFR without knowledge of HR HPV status, revealed to be very insensitive. None of the CIN2+ cases were detected by

full rescreening in this group. Only after HR targeted reviewing one of the three CIN 2+ cases could be detected. A possible explanation could be that the majority of NFR slides do not contain abnormal cells, and that only a limited number of NFR slides contain very few abnormal cells that can be detected more easily with knowledge of HR HPV positivity. Also, CIN2+ cases in the NFR group that were missed by full- or HR HPV-targeted rescreening had a high viral load, suggesting that no abnormal cells were present in these slides.

Considering that only 1.15% of negative cytology slides after GS in 2004 were included in this study, and that 11 CIN2+ cases were detected in negative cytology, a substantial number of CIN 2+ cases could have been missed by screening in 2004. None of the missed CIN 2+ cases by screening were HPV negative. The CIN 2+ cases missed by HR HPV-targeted rescreening were the cases with the lowest viral load. The viral load of five out of nine of these CIN 2+ cases was below the 6.4 copies/cell threshold. BD FocalPoint™ has categorized all confirmed CIN 2+ cases in the review group, with 50% of cases in the first two quintiles. Despite knowledge of quintile rank and HPV status, in 5 out of 11 of reviewed negative cytological preparations associated with histological CIN2+ the cytotechnicians/pathologists could not detect morphologically abnormal cells. Since three CIN2+ cases were categorized to the 4th quintile, quintile ranking is not a useful criterion for increasing efficiency of full rescreening. A recent Swiss study showed that the probability for a PAP test to be abnormal is directly proportional to HPV viral load [31], and they suggested a theoretical limit for cytology sensitivity. Because rescreening of HPV ONC+ slides was blinded from the viral load results, the visual limit for the detection of cytological abnormal cells (ASC-US+) could be calculated. The most sensitive method for the detection of CIN2+ cases in normal cytology was oncogenic HPV detection by quantitative real-time PCR (100%). This was also a very specific method (87.2%). The specificity could further be

increased to 88.3% by setting the viral load cut-off above 0.0142 HR HPV copies/cell. Only full rescreening was more specific, but only 23.5% of CIN2+ cases could be detected.

We therefore propose a new QC method for normal cytology: starting with the detection of oncogenic HPV by real-time PCR, which is both very sensitive and specific. HPV HR positive, cytologically negative cases should be rescreened or should be retested after 6 to 12 months. Cases with persistent HPV infection should be referred for further investigation. An additional advantage of this new QC method would be the elimination of false positive cytology since HPV negative cases would not be rescreened. Additionally, for slides positive for oncogenic HPV types with a viral load below the 6.5 copies/cell cut-off retesting the viral load after 6–12 months could determine if the HPV infection is transient or persistent and colposcopy is warranted. Women with a persistent HPV infection could be directed to colposcopy. Another advantage is the knowledge of the exact type of oncogenic HPV present. HPV 16 and 18 positive samples have a greater risk of developing cancer compared to intermediate oncogenic HPV types such as HPV 53, 66 and 67 [32].

The use of HR HPV detection as QC method instead of full rescreening would result in a 4.25 fold increase in CIN 2+ detection

compared to full rescreening (17 instead of 4). The percentage of sample required for QC by HR HPV testing could be lowered to 2.5% compared to 10% in full rescreening as stated by guidelines, with a better result.

Acknowledgements

We thank the cytotechnologists, Els Dewulf, Karin Francken, Kristin Van Belle, Sabrina Van Belle, Tamara Van Den Broeck, and laboratory technicians, Rachel Baveco, Katrien Beerden, Sarah Berghmans, Ludo Boels, Carmen De Maesschalck, Isabel De Brabander, Brenda Gabriels, Inge Goegebeur, Karen Illegheems and Miranda Vervoort. We also thank Dr. Kristl Claeys for invaluable comments on the manuscript.

MA received financial support from the European Commission (Directorate of SANCO, Luxembourg, Grand-Duché du Luxembourg) through the ECCG (European Cooperation on development and implementation of Cancer screening and prevention Guidelines, IARC, Lyon, France), the DWTC/SSTC (Service for Science, Culture and Technology, Brussels, Belgium), and IWT (Institute for the Promotion of Innovation by Science and Technology in Flanders (through the Unit of Health Economics and Modeling Infectious Diseases, Vaccine & Infectious Disease Institute, University of Antwerp; project number 060081).

References

1. **Van der Graaf Y, Vooijs GP, Gaillard HL, et al.** Screening errors in cervical cytology screening. *Acta Cytol.* 1987; 31: 434–8.
2. **Krieger P, Naryshkin S.** Random rescreening of cytologic smears. A practical and effective component of quality assurance programs in both large and small cytology laboratories. *Acta Cytol.* 1994; 38: 291–8.
3. **Wilbur DC, Prey MU, Miller WM, et al.** The AutoPap system for primary screening in cervical cytology: comparing the results of a prospective, intended-use study with routine manual practice. *Acta Cytol.* 1998; 42: 214–20.
4. **Melamed MR.** Rescreening for quality control in cytology. *Acta Cytol.* 1996; 40: 12–3.
5. **United States Department of Health and Human Services HCFA.** Medicare and CLIA programs. Amended 1988. *Fed Regist.* 1992; 57: 493–1257.
6. **CLIA.** Rules and regulations. *Fed Regist.* 1990; 55: 9538–74.
7. **Melamed MR, Flehinger BJ.** Reevaluation of quality assurance in the cytology laboratory. *Acta Cytol.* 1992; 36: 461–5.
8. **Hutchinson ML.** Assessing the costs and benefits of alternative rescreening strategies. *Acta Cytol.* 1996; 40: 4–8.
9. **Farker CA.** Partial rescreening of all negative smears: an improved method of quality assurance in laboratories undertaking cervical screening. *Cytopathology.* 1993; 4: 47–50.
10. **Herbert A, Johnson J, Patnick J.** Achievable standards, benchmarks for reporting and criteria for evaluating cervical cytopathology. *Cytopathology.* 1995; 6: 301–3.
11. **The Scottish Office.** Report of the Working Party on the Internal Quality Control for Cervical Cytopathology Laboratories. Edinburgh: The Scottish Office; 1995.
12. **NHSCSP.** Achievable standards, benchmarks for reporting, and criteria for evaluating cervical cytopathology. 2nd ed. Sheffield: NHSCSP publication no. 1; 2000.
13. **Arbyn M, Schenck U.** Detection of false negative Pap smears by rapid reviewing: a meta-analysis. *Acta Cytol.* 2000; 44: 949–57.
14. **Arbyn M, Schenck U, Ellison E, et al.** Metaanalysis of the accuracy of rapid prescreening relative to screening of Pap smears. *Cancer.* 2003; 99: 9–16.
15. **Bibbo M, Hawthorne C, Zimmerman B.** Does use of the AutoPap assisted primary screener improve cytologic diagnosis? *Acta Cytol.* 1999; 43: 23–6.
16. **Wilbur DC, Prey MU, Miller WM, et al.** Detection of High Grade Squamous Intraepithelial Lesions and Tumors using the AutoPap System. *Cancer.* 1999; 87: 354–8.
17. **Baker A, Melcher DH.** Rapid cervical cytology screening. *Cytopathology.* 1991; 2: 299–301.
18. **Vassilakos P, Saurel J, Rondez R.** Direct-to-vial use of the AutoCyte PREP liquid-based preparation for cervical-vaginal specimens in three European laboratories. *Acta Cytol.* 1999; 42: 65–8.
19. **Vassilakos P, Griffin S, Megevand E, et al.** CytoRich liquid-based cervical cytology test: Screening results in a routine cytopathology service. *Acta Cytol.* 1998; 42: 198–202.
20. **Solomon D, Davey D, Kurman R, et al.** The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA.* 2002; 287: 2114–9.
21. **Depuydt CE, Vereecken AJ, Salembier GM, et al.** Thin-layer liquid-based cervical cytology and PCR for detecting and typing human papillomavirus DNA in Flemish women. *Br J Cancer.* 2003; 88: 560–6.
22. **Depuydt CE, Benoy IH, Bailleul EJ, et al.** Improved endocervical sampling and HPV viral load detection by

- Cervex-Brush® Combi. *Cytopatology*. 2006; 17: 374–81.
23. **Depuydt CE, Boulet GA, Horvath CA, et al.** Comparison of MY09/11 consensus PCR and type-specific PCRs in the detection of oncogenic HPV types. *J Cell Mol Med*. 2007; 11: 881–91.
 24. **Tucker RA, Unger ER, Holloway BP, et al.** Real-time PCR-based fluorescent assay for quantitation of human papillomavirus types 6, 11, 16, and 18. *Mol Diagn*. 2001; 6: 39–47.
 25. **Moberg M, Gustavsson I, Gyllensten U.** Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma *in situ*. *Int J Cancer*. 2004; 112: 854–9.
 26. **Moberg M, Gustavsson I, Wilander E, et al.** High viral loads of human papillomavirus predict risk of invasive cervical carcinoma. *Br J Cancer*. 2005; 92: 891–4.
 27. **Ylitalo N, Josefsson A, Melbye M, et al.** A prospective study showing long-term infection with human papillomavirus 16 before the development of cervical carcinoma *in situ*. *Cancer Res*. 2000; 60: 6027–32.
 28. **Ylitalo N, Sorensen P, Josefsson A, et al.** Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma *in situ*: a nested case-control study. *Lancet*. 2000; 355: 2194–8.
 29. **Josefsson AM, Magnusson PK, Ylitalo N, et al.** Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma *in situ*: a nested case-control study. *Lancet*. 2000; 355: 2189–93.
 30. **Schlecht NF, Trevisan A, Duarte-Franco E, et al.** Viral load as a predictor of the risk of cervical intraepithelial neoplasia. *Int J Cancer*. 2003; 103: 519–24.
 31. **Bigras G, de Marval F.** The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13,842 women. *Br J Cancer*. 2005; 93: 575–81.
 32. **Muñoz N, Bosch FX, de Sanjosé S, et al.** Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*. 2003; 348: 518–27.