

# Comparison of the effectiveness of cytodiagnosics, molecular identification of HPV HR and CINtecPLUS™ test to identify LG SIL and HG SIL

Porównanie efektywności cytodiagnostyki, molekularnej identyfikacji DNA i mRNA HPV HR oraz testu CINtecPLUS™ w wykrywaniu zmian LG SIL i HG SIL

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## Abstract

**Aim of the paper:** Comparison of conventional cytodiagnosics with molecular identification of DNA and mRNA HPV HR, immunocytochemical test for suppressor protein P16 and nuclear Ki 67 to detect cervical pathology screening of the division to LG SIL and HG SIL.

**Material:** 630 Pap smears were taken from women with suspected cervical pathology were submitted for analysis, together with 558 smears for the presence of DNA HPV HR, 421 swabs for the presence of mRNA HPV HR, 86 swabs for the presence of suppressor protein P16 and nuclear Ki 67. In all of the women standard colposcopy with biopsy and endocervical abrasion were performed.

**Method:** The study used a classic cytological smear, taken on the slide, rated in accordance with TBS classification, colposcopy implemented in accordance with the guidelines of the International Federation of Cervical Pathology and Colposcopy from 2003, molecular diagnostic tests based on identifying DNA, mRNA HPV HR and immunocytochemistry diagnostic test – CINtecPLUSTM.

**Results:** The sensitivity of Pap test identification of CIN 2+ was of 85% and specificity of 23%. Indicators PPV and NPV were respectively 39% and 72%. The accuracy of cytology reached a level of 46%. DNA HPV HR test obtained 91% sensitivity and 33% specificity of the diagnosis of CIN 2+. Its accuracy was 54%. The value of PPV and NPV for molecular diagnostics was respectively 43% and 87%. For mRNA HPV HR test sensitivity of the method was 79%, the specificity was 67%. CINtecPLUSTM test achieved 100% sensitivity and 67% specificity in the diagnosis of CIN 2+.

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### Conclusions:

1. Conventional cytodiagnosics are inferior in terms of both sensitivity and specificity of molecular test for DNA, mRNA HPV HR and immunocytochemical test for detecting of LG SIL and HG SIL.
2. Immunocytochemical technique shows maximum sensitivity and high specificity of detection of actual precancerous stages - CIN 2+.

Key words: **cervical intraepithelial neoplasia / DNA HR HPV / mRNA HPV HR / cytodiagnosics / immunocytochemistry / prevention / P16 / Ki67 /**

### Streszczenie

**Cel pracy:** Porównanie konwencjonalnej cytodiagnostyki z identyfikacją molekularną DNA HPV HR i mRNA HPV HR oraz immunocytochemicznym testem na wykrywanie białek supresorowych P16 i jądrowego Ki67 pod kątem wykrywania patologii szyjki macicy w skriningu z podziałem na rozpoznania histopatologiczne LG SIL i HG SIL.

**Materiał:** Analizie poddano 630 wymazów cytologicznych pobranych od kobiet z podejrzeniem patologii szyjki macicy, 558 wymazów na obecność DNA HPV HR, 421 wymazów na obecność mRNA HPV HR, 86 wymazów na obecność białek supresorowych P16 i jądrowego Ki67. U wszystkich badanych kobiet wykonano standardowe badanie kolposkopowe z pobraniem wycinków i abrazyj kanału szyjki macicy.

**Metoda:** W badaniach wykorzystano klasyczny wymaz cytologiczny pobierany na szkiełko podstawowe oceniany wg klasyfikacji TBS, kolposkopię realizowaną zgodnie z wytycznymi Międzynarodowej Federacji Patologii Szyjki Macicy i Kolposkopii z roku 2003, diagnostykę molekularną opartą o testy identyfikujące DNA i mRNA HPV HR oraz diagnostykę immunocytochemiczną, czyli test CINtecPLUS™.

**Wyniki:** Czulość badania cytologicznego identyfikującego zmiany CIN 2+ wyniosła 85%, a specyficzność 23%. Wskaźniki PPV i NPV wyniosły odpowiednio 39% i 72%. Dokładność cytologii osiągnęła poziom 46%. Test DNA HPV HR uzyskał 91% czulość i 33% specyficzność w diagnostyce zmian CIN 2+. Jego dokładność wyniosła 54%. Wartość PPV i NPV dla diagnostyki molekularnej wyniosła odpowiednio 43% i 87%. Dla mRNA HPV czulość metody wyniosła 79%, specyficzność 67%. Test CINtecPLUS™ osiągnął 100% czulość i 67% swoistość w rozpoznawaniu CIN 2+.

### Wnioski:

1. Cytodiagnostyka konwencjonalna ustępuje pod względem czulości i swoistości zarówno testom molekularnym DNA HPV jak i technice immunocytochemicznej w procesie wykrywania LG SIL i HG SIL.
2. Maksymalną czulość i wysoką swoistość wykrywania rzeczywistych stanów przedrakowych czyli zmian CIN 2+ wykazuje technika immunocytochemiczna.

Słowa kluczowe: **śródnabłonkowa neoplazja / DNA HPV HR / mRNA HPV HR / cytodiagnostyka / immunocytochemia / profilaktyka / P16 / Ki67 /**

### Introduction

Population-based prevention programs for cervical cancer brought tangible results in the reduction of morbidity and mortality in women. However, implementation of these programs over the past decade has proved that a nearly 100% reportability to research and repeat at intervals of 3-5 years, does not completely eliminate the incidence of cervical cancer. Particularly disturbing is occasional detection of cervical cancer in women who have had regular Pap tests. The cause of this problem is the relatively low sensitivity of cytodiagnosics and low efficiency of this method in identifying pathology of glandular epithelium of the cervix.

Currently there is a need for new diagnostic tests that would either supplement or replace cytodiagnosics as a screening tool. These methods should comply with WHO standards specified for screening tests, be competitive with conventional cytodiagnosics and levied on liquid based cytology (LBC) in the detection of precancerous lesions, squamous cervical cancer and adenocarcinoma.

According to the current views the main purpose of screening is to detect changes known as HG SIL (high grade squamous intraepithelial lesion), which correspond in terms of histological

view with CIN 2+ and the identification of LG SIL (low grade squamous intraepithelial lesion) or CIN 1 being exponent of infection with oncogenic types of HPV.

According to new methods such as molecular tests for DNA and mRNA HPV HR and immunocytochemical tests of suppressor protein P16 and nuclear Ki 67 involved in the development of CIN, sensitivity and accuracy of diagnostic tests resembling the final histopathological diagnosis are most important.

### Aim of the paper

Comparison of conventional cytodiagnosics with molecular identification of DNA and mRNA HPV HR, immunocytochemical tests for P16 and Ki67 to detect cervical pathology screening of the division to LG SIL and HG SIL.

### Materials

The study included 630 women aged 25-65 years (mean 45 years + /-SD) directed for further diagnostics because of the extensive evaluation of abnormal Pap smears performed in the screening.

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**Table I.** Statistical analysis of the relation between the final histopathological results and PAP test, DNA HPV HR test, mRNA HPV HR test and CINtecPLUSTM,  $p < 0.05$ .

Type of study	Result	Histopathological result			Statisticals analysis
		Standard	LG SIL	HG SIL	
PAP test	Standard	51 (23,0%)	39 (14,1%)	20 (15,3%)	$\chi^2=69,7$ $p<0,00001$ $V = 0,24$
	ASC-US	102 (45,9%)	87 (31,4%)	33 (25,2%)	
	LSIL	61 (27,5%)	139 (50,2%)	52 (39,7%)	
	HSIL	8 (3,6%)	12 (4,3%)	26 (19,8%)	
	PAT	171 (77,0%)	238 (85,9%)	111 (84,7%)	
DNA HPV HR test	Positive	140 (67,0%)	212 (91,4%)	107 (91,5%)	$\chi^2=53,41$ $P<0,00001$ $V = 0,31$
	Negative	69 (33,0%)	20 (8,6%)	10 (8,5%)	
mRNA HPV HR test	Positive	42(33,3%)	148(69,5%)	65(79,3%)	$\chi^2=58,23$ $P<0,00001$ $V = 0,37$
	Negative	84(66,7%)	65(30,5%)	17(20,7%)	
CINtecPLUS (P16 Ki 67)	Positive	10(33,3%)	11(47,8%)	15(100%)	$\chi^2=18,20$ $P=0,0001$ $V = 0,52$

**Table II.** The values of sensitivity (SENS), specificity (SPEC), positive (PPV) and negative (NPV) predictive value and accuracy (ACC) as well as reliability indicators (LR +, LR-) for PAP test, DNA HPV HR test, mRNA HPV HR test and CINtecPLUSTM in women with LG SIL. Abbreviations in the table are as follows: TP- true positive results, FP-false positive results, FN false-negative results, TP true-negative results.

LG SIL	TP	FP	FN	TN	SENS (%)	SPEC (%)	LR+	LR-	PPV (%)	NPV (%)	ACC (%)
ASC-US	87	102	39	51	69	33	1,04	0,93	46	57	49
HSIL	12	8	39	51	24	86	1,74	0,88	60	57	57
LSIL	139	61	39	51	78	46	1,43	0,48	70	57	66
PAPA	238	171	39	51	86	23	1,12	0,61	58	57	58
DNA HPV	212	140	20	69	91	33	1,36	0,26	60	78	64
mRNA HPV	148	42	65	84	69	67	2,08	0,46	78	56	68
CINtecPLUS	11	10	12	20	48	67	1,43	0,78	52	63	58

**Table III.** The values of sensitivity (SENS), specificity (SPEC), positive (PPV) and negative (NPV) predictive value and accuracy (ACC) as well as reliability indicators (LR +, LR-) for PAP test, DNA HPV HR test, mRNA HPV HR test and CINtecPLUSTM in women with HG SIL.

HG SIL	TP	FP	FN	TN	SENS (%)	SPEC (%)	LR+	LR-	PPV (%)	NPV (%)	ACC (%)
ASC-US	33	102	20	51	62	33	0,93	1,13	24	72	41
HSIL	26	8	20	51	57	86	4,17	0,50	76	72	73
LSIL	52	61	20	51	72	46	1,33	0,61	46	72	56
PAPA	111	171	20	51	85	23	1,10	0,66	39	72	46
DNA HPV	107	140	10	69	91	33	1,37	0,26	43	87	54
mRNA HPV	65	42	17	84	79	67	2,38	0,31	61	83	72
CINtecPLUS	15	10	0	20	1,00	67	3,00	0,00	60	100	78

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The study included:

- repeat Pap test taken with Cervex Brush™ (630 women).
- material taken with Cervex Brush™ on liquid medium for molecular identification of DNA (558 women) and mRNA HPV HR (421 women).
- material taken with Cervex Brush™ in order to perform tests for the presence of immunocytochemical P16 and Ki67 (86 women).
- Colposcopy with punch biopsy of suspicious focal sites or transition zone and diagnostic abrasion of cervix (630 women).

## Methods

Cytodiagnosics - Sampling technique, fixation, staining and evaluation of smears by The Bethesda System was in compliance with the procedure in force in the European Screening Programs and Population Prevention and Early Detection of Cervical Cancer implemented in Poland since 2005.

Colposcopy – performed using Olympus optical colposcopic OCS-500 and Leisegang 3MLW and documented by the classification of the International Federation of Cervical Pathology and Colposcopy in 2003. Verified cytological diagnosis as well as molecular and immunocytochemical study was carried out in the Laboratory of Cervical Pathophysiology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences, Poznan and the Department of Gynecology and Obstetrics Hospital, NZO of St. Alexandra, Kielce.

Molecular diagnostics of HPV HR – molecular diagnostics of DNA HPV HR – performed with the use of the Roche Diagnostics COBAS equipment X 480, COBAS Z 480 which identifies 14 types of DNA HPV HR. Molecular diagnostics of mRNA HPV HR – performed with the use of the Biomerieux EAZY Q, EAZY MAG which identifies 5 types of mRNA HPV HR (16, 18, 31, 33, 45). All stages of molecular tests were performed in the Laboratory of Cervical Pathophysiology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences, Poznan.

Immunocytochemistry diagnostics – performed for cytological preparations fixed and stained with Papanicolaou method together with CINTecPLUS™ procedures of mtm-Cytology Laboratories AG. Suppressor protein P16 and nuclear Ki 67 were being identified. All stages of tests were performed in the Laboratory of Cervical Pathophysiology, Gynecology and Obstetrics Clinical Hospital, Karol Marcinkowski University of Medical Sciences, Poznan.

## Statistical analysis

The values of the analyzed parameters due to the nominal measurement scale were characterized by cardinality and percentage. Differences between the analyzed non-measurable parameters were assessed in multi-way tables and test for homogeneity or independence  $\chi^2$ . To evaluate the existing relation,  $\Phi$  factor was applied or Cramer's V (multi-way table), considering values from 0 (no relation) to 1 (total dependence). The usefulness of diagnostic tests by calculating the sensitivity, specificity, positive predictive value, negative predictive value and accuracy was evaluated. 5% inferential error was accepted together with the associated significance level  $p < 0.05$  indicating a statistically significant difference or relation.

## Results

The sensitivity of Pap test identification of CIN 2 + was of 85% and specificity of 23%. Indicators PPV and NPV were respectively 39% and 72%. The accuracy of cytology reached a level of 46%. DNA HPV HR test obtained 91% sensitivity and 33% specificity of the diagnosis of CIN 2 +. Its accuracy was 54%. The value of PPV and NPV for molecular diagnostics was respectively 43% and 87%. For mRNA HPV HR test sensitivity of the method was 79%, the specificity was 67%. CINTecPLUS™ test achieved 100% sensitivity and 67% specificity in the diagnosis of CIN 2 + (Table I, Table II, Table III).

## Discussion

The main parameter determining the usefulness of a screening test is its sensitivity. After many years of screening in selected countries of Western Europe, 70% - 90% of the target population was covered by regular cytological examination [11]. Recent decrease in morbidity and mortality due to cervical cancer has been observed mainly in countries employing extensive screening programs. Nowhere, however, the problems associated with the development of cervical pathology have been completely solved. An example is the Netherlands where, since 2002, despite continuous and active screening programs, there is no further reduction of morbidity and mortality due to cervical cancer, which has consistently been observed in previous years [11]. Incidence rate "has stopped" at the value of 7.3 / 100,000 women and mortality rate at 2.3 / 100,000 women.

The limitation of cervical cancer screening tests is their low sensitivity. Our research based on standards associated with Polish programs of prevention and early detection of cervical cancer. Obtained in the course of the present study, the sensitivity of conventional cytodiagnosis used for detection of CIN 2 + reached 85%.

This result is comparable or even higher than that reported by other laboratories, where the sensitivity of identification of CIN 2 + ranges from 30% to 70% [2, 3]. Without a doubt, the effectiveness of cytodiagnosis detection system decreases significantly for cytological diagnosis endowed with a higher risk of misidentification of precancerous changes such as ASC-US. For this cytological diagnosis, sensitivity of detection of cervical pathology, regardless of its degree of severity was only 67% and specificity was 33%.

Broad, multi-center meta-analysis published in 2006 concluded that the sensitivity of pathology detection for the cytological diagnosis of ASC-US was 53% while specificity was similar to that obtained in the present study [2, 3].

The obtained cytodiagnostic parameters of sensitivity and specificity of cervical pathology indicate a significant risk of false negative and false positive results. The low specificity (not exceeding 33%) of ASC-US diagnosis is associated with excessive number of unnecessary and expensive verifying medical diagnostic procedures – colposcopies. A solution is problem currently implemented for screening in the world, is the use of objective molecular diagnostic of HPV infection.

Verification of ASC-US diagnosis by performing a test for the presence of at least 14 types of HPV is now the so-called standard of care, including extensive screening. In study tests, carried out in terms of this paper, the sensitivity of the molecular identification of DNA HPV in cervical pathology was 91%

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and was significantly higher compared to the sensitivity of cytodiagnostic. The results are consistent with reports published by other research centers. According to meta-analysis from 2002, the sensitivity of molecular detection of DNA HPV in cervical pathology CIN 2 + was of 96.1% and specificity of 90.7%. It should be noted that this specificity is considerably reduced if the study test includes women at the age of 30, and reaches 76.5% [1]. Specificity of molecular diagnostics increases with age of the population studied and reaches 95.5% for women over 30 years of age [9]. Very interesting is molecular diagnostic accuracy in the detection of cervical pathology. Its value is 70% and is higher than accuracy of cytodiagnosics in detecting the type of LG SIL (58%) and accuracy for detecting HG SIL (48%).

High value (NPV) of 87% for DNA HPV testing was also the highest obtained NPV score in all the studies, except for the immunocytochemical method. The present study demonstrates that the identification of transcripts of five oncogenic HPV types is associated with 72% pure sensitivity and 67% specificity in the detection of cervical pathology. The sensitivity of mRNA HPV test is higher for changes of HG SIL or CIN2 +, reaching a value of 79%.

Very similar results were obtained by Keegan et al, who assessed the sensitivity of determination of transcripts in detecting cervical pathology at 71.4% [5]. Lower sensitivity (63%) was described by Halfon et al, using the assay to detect transcripts of CIN 2 + [4]. Assuming the same assumptions, Sorby et al showed 81% sensitivity and 97% NP, for the diagnosis of mRNA HPV [13]. As a result of studies which represent the object of this paper, it was found that the CINTecPlus™ test has the highest accuracy in identify CIN 2 + (78%) and its sensitivity is set to 100%, compared to all analyzed methods.

These results are consistent with those of other published results, evaluating the sensitivity of CINTecPlus™ in the identification of CIN 3, from 81% to 100% and specificity from 60% to 75% [10]. Szarewski et al showed 92.7% sensitivity and 65.8% specificity of detection of CIN 2 + by CINTecPlus™ test [12]. In conclusions, the comparative analysis of different methods for the detection of cervical pathology showed a significant advantage for both molecular HPV testing and conventional immunohistochemistry on cytodiagnosics. These results are consistent with those of other published results on sensitivity of CINTecPlus™ in the identification of CIN 3 reaching 81% to 100% and specificity of 75% to 60% [10]. Szarewski et al showed 92.7% sensitivity and 65.8% specificity of detection of CIN 2 + by CINTecPlus™ test [12]. To conclude, the comparative analysis of different methods for the detection of cervical pathology showed a significant advantage for both molecular DNA HPV testing and conventional immunohistochemistry over cytodiagnosics.

It was apparent in detecting changes in LG SIL and HG SIL, the analysis of sensitivity, specificity and accuracy of particular method. Conventional cytodiagnosics has demonstrated its superiority over mRNA HPV test in terms of specificity for changes of LG SIL and HG SIL. There is evidence that testing only 5-five types of HPV is insufficient. Based on available studies, it can be concluded that in the near future screening should include modified cytodiagnosis and immunocytochemical detection markers of carcinogenesis with special emphasis on p16<sup>INK4a</sup> tumor suppressor protein and nuclear factor Ki-67. The results presented in this study, along with other relevant publications

create an opportunity for the immunocytochemical method to gradually replace traditional cytodiagnosics in cervical cancer screening.

## Conclusions

1. Conventional cytodiagnosics are inferior in terms of both sensitivity and specificity to molecular test for DNA, mRNA HPV HR and immunocytochemical tests for detecting LG SIL and HG SIL.
2. Immunocytochemical technique shows maximum sensitivity and high specificity of detection of actual precancerous stages - CIN 2 +.

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