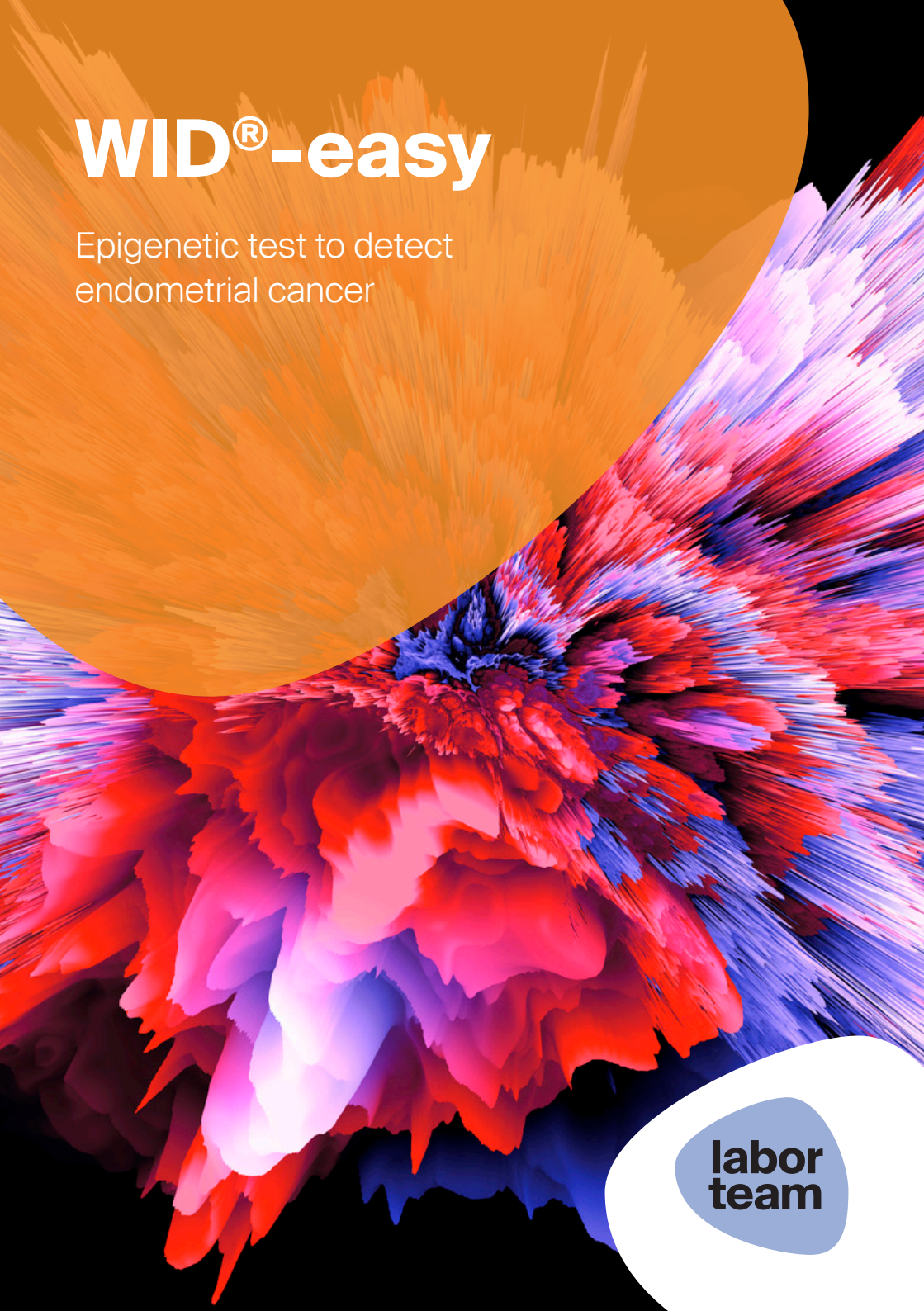
WID®-easy

Epigenetic test to detect endometrial cancer



labor team

Unusual bleeding is the main symptom in 90% of women with endometrial cancer. However, endometrial cancer can be determined as the cause in only 3% of peri- and postmenopausal cases of bleeding [1]. The current S3 Guideline [2] recommends subjective procedures such as clinical examinations, cytology, and transvaginal ultrasounds for the differential diagnosis of endometrial cancers with unusual bleeding [3]. Transvaginal ultrasound has a very low positive predictive value of 4.9% [4]. As a result, most women who undergo a surgical diagnostic procedure do not actually have endometrial cancer.

The WID®-easy test offers the possibility of improving this situation and very quickly arriving at a diagnosis. The test demonstrates sensitivity comparable to that of transvaginal ultrasound, but reduces the rate of false positive results by 94%.

Aside from breast cancer, endometrial cancer is the most common gynecological malignancy [2], and the incidence – especially of the less favorable non-endometrioid cancers of the uterine corpus, which are even more difficult to detect with ultrasound [5] – is increasing rapidly [6]. In Switzerland, around 950 women every year develop new cases of endometrial cancer. There has been no established screening procedure so far for this type of cancer.

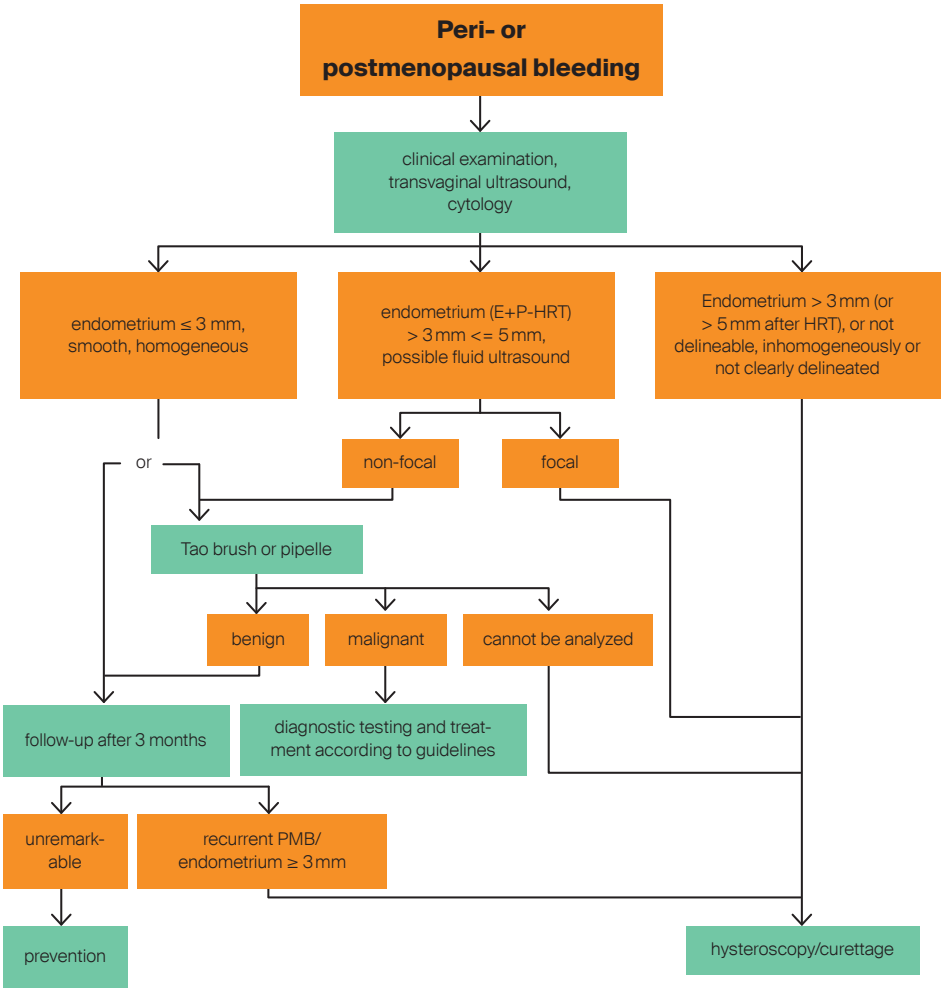
Of all gynecological malignancies, a delayed diagnosis of corpus cancer has the most drastic negative impact on survival rate [7]. Therefore, early and precise diagnosis of endometrial cancer is crucial.

A major risk factor for endometrial cancer is an elevated estrogen concentration for many years. Women with menstrual irregularities, later menopause, childlessness, or certain hormone replacement therapies have an increased risk of developing endometrial cancer. In addition, being overweight and having high blood pressure and type 2 diabetes mellitus can increase the tumor risk. It is known that obesity increases estrogen production. It has not yet been determined whether there is a risk from phytoestrogens (estrogen-like substances in foods). However, it is confirmed that hormone therapy exclusively with estrogen increases the risk.

High false-positive rate in previous diagnostic testing

The current S3 Guideline [2] recommends subjective and experience-based procedures such as clinical examinations, cytology, and transvaginal ultrasounds to rule out or diagnose endometrial cancer when there is abnormal bleeding.

94% reduction in the rate of false positives compared to ultrasound



Current diagnostic pathway for peri- and postmenopausal bleeding according to the S3 Guideline [2]. E, estrogen; P, progestogen; HRT, hormone replacement therapy; PMB, postmenopausal bleeding

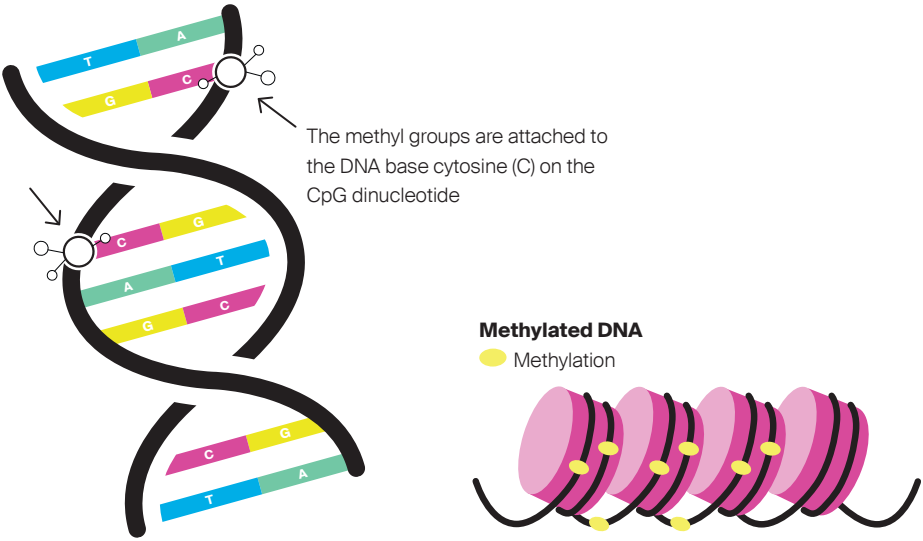
The sensitivity of cytology in detecting endometrial cancer is inadequate, at 45% in symptomatic women [8] and only 26% in asymptomatic women who have had a Pap test up to three years before diagnosis [3].

Transvaginal ultrasound measures the endometrial thickness, with a thickness of more than 3 mm being considered pathological in postmenopausal women. The detection rates vary between white women (89.5%) and black women (51.1%) [9]. The specificity of this measurement varies between 25.7% [9] and 42.1% [10], and the positive predictive value is only 4.9% to 7.3% [4, 10]. Additionally, the ability of ultrasound to detect serous endometrial cancers is particularly poor, as around 25% of these aggressive cancers are not accompanied by increased endometrial thickness [5].

This means that many women who undergo a surgical diagnostic examination do not have cancer. In this light, the development of a test for improved diagnosis of endometrial cancer is of great significance. This test should be easy to perform, enable objective and fast, automated analyses, have sensitivity comparable to that of ultrasound, and at the same time offer significantly increased specificity.

Epigenetic analysis to detect endometrial cancer

The WID-qEC test (**W**omen's Cancer **ID**entification utilizing **q**uantitative **P**CR for **E**ndometrial **C**ancer, also called the WID®-easy test) was developed by the research team of Professor Martin Widschwendter at renowned universities and institutes (University College London (UCL), Karolinska Institute in Sweden, EUTOPS Institute of the University of Innsbruck). In 716 cervicovaginal swabs, a total of 850,000 DNA regions, known as CpG dinucleotides, were examined for their DNA methylation. Two regions in the *ZSCAN12* and *GYPC* genes were identified from the analysis of this enormous amount of data, which comprises over 600 million data points.



Methylated DNA

These regions show elevated methylation in the presence of endometrial cancers, but are not methylated in their absence [11].

Using real-time PCR, the methylation status of these gene regions can be determined, with the reference gene *COL2A1* used for normalization.

To perform WID®-easy, a cervicovaginal swab is first sent from the patient to the laboratory where, after DNA extraction and bisulfite sequencing, a quantitative PCR reaction is carried out to determine the methylation status of DNA regions of the *ZSCAN12* and *GYPC* genes.

Significantly improved specificity thanks to WID®-easy

Thanks to this new test procedure, the false-positive results that frequently occur with ultrasound can be significantly reduced. The study results for WID®-easy are convincing, meaning that patients can be diagnosed more effectively and treated earlier, and unnecessary interventions and healthcare costs can be reduced.

The study, conducted with over 700 cervical swabs, showed that the epigenetic test offered a sensitivity similar to ultrasound, but had a significantly higher specificity compared to qualitative ultrasound assessment. The detection of endometrial cancers in the cohorts studied here did not seem to

depend on histology, grade, stage, age, ethnicity, or menopause status. Early stages of endometrial cancer and even non-endometrioid cancers can be reliably detected thanks to WID®-easy. Above all, serous endometrial cancers can be identified with a sensitivity of 97% [11]. Furthermore, the sensitivity of the WID®-easy test is significantly superior to cytology, and endocervical cancers can also be more easily detected with it [12].

In another study, the test was validated in a cohort of women ≥ 45 years of age with abnormal bleeding. Of the 474 symptomatic women, 400 agreed to participate in the study. Transvaginal ultrasound alone was conclusive in 62% of patients, while 38% of patients required additional imaging tests. Histological work-up was recommended in 47% of study participants, with 3% being diagnosed with cancers. WID®-easy demonstrated a sensitivity of 91% and a high specificity of 97%, while ultrasound had a sensitivity of 91% and a lower specificity of 46%. Interestingly, two cancers were not detected by hysteroscopy and curettage (only later by hysterectomy or biopsy of a liver metastasis), while the WID-qEC test was positive [4].

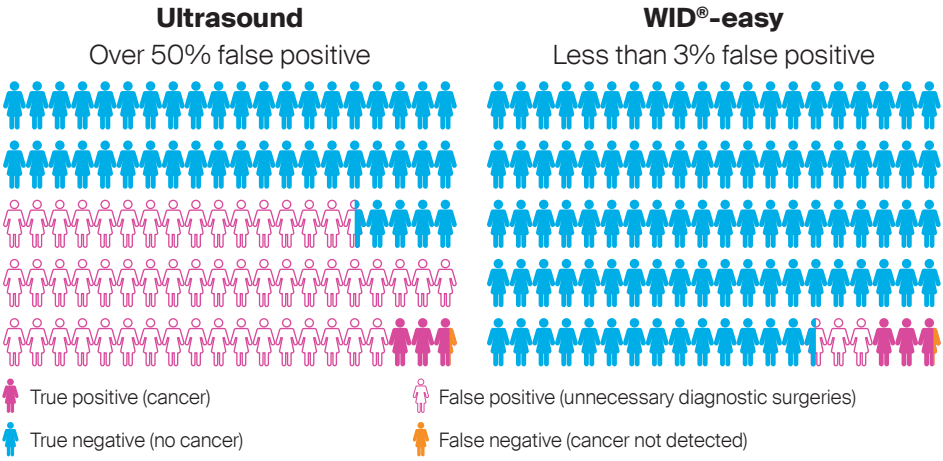
WID®-easy and its advantages

WID®-easy demonstrates sensitivity comparable to transvaginal ultrasound but reduces the rate of false positive results by 94%.

- Clear result
- Fast diagnosis
- High reliability
  (sensitivity 91%, specificity 97%)

Comparison of ultrasound and WID®-easy

In summary, considering the current guidelines and with optimal ultrasound diagnostic testing, for 100 women in the age group ≥ 45 years who present due to abnormal bleeding, around 56 women must undergo surgical diagnostic work-up in order to identify 3 cancers. In contrast, when using WID®-easy, only around 6 women with a positive test result need to undergo diagnostic surgery to identify the 3 cancers. As a result, WID®-easy reduces the rate of false-positive results by 94%.



Performance of ultrasound (endometrial thickness > 3 mm) and WID®-easy in 100 women ≥ 45 years of age who present with abnormal bleeding (adapted from Evans et al. 2023 [4]).

Indications for the WID®-easy test and recommended action

Analysis of all the currently available data shows that the WID®-easy test is indicated for women (≥ 45 years old) with abnormal bleeding. Women with a positive WID®-easy test result should undergo histological diagnosis as soon as possible in order to avoid needlessly delaying treatment that may be required. Women with a negative WID®-easy result can be treated conservatively at first and monitored with ultrasound. If necessary, repetition of the WID®-easy test can also be considered.

Information on findings

The laboratory report has a different comment depending on the methylation status. In the laboratory findings, you receive “abnormal” or “unremarkable” as a possible result, with “abnormal” indicating the presence of endometrial cancer and “unremarkable” indicating the opposite.
Like every medical test, the WID®-easy does not offer 100% certainty. An abnormal test result does not always mean that cancer is present: According to Evans et al. [4], fewer than 3 in 100 cases were false positive. Likewise, an unremarkable test result does not always mean that cancer is absent: According to Evans et al. [4], fewer than 1 in 100 cases were false negative.

Profile number	20574, laboratory order incl. informed consent*
Price	CHF 341.95, mandatory provision**
Material	Swab of the secretion from the cervix and from the posterior vaginal vault, test kit M900264.
Execution time	7 working days

*Before a genetic diagnosis, the patient must receive genetic counseling and be fully informed about the process. In addition, written informed consent for genetic testing in accordance with the current Swiss Law on Human Genetic Testing (GUMG) must be provided.

** As a rule, the health insurance company will cover the costs of the test. However, it is possible that the health insurance company will refuse to cover the costs as part of the basic insurance and/or any supplementary insurance. In this case, the patient must bear the costs themselves.

Sampling instructions

Please note

In the form of a swab (test kit M900264), a sample should be taken of the secretion around the cervix and from the posterior vaginal vault. Sampling for the WID®-easy test is performed:

- without the use of lubricant.
- without prior cleansing of the vagina, as the tumor DNA is located in the vaginal secretion.
- before sampling for another test, e.g. for a Pap smear.
- before introducing another substance into the vagina or near the cervix, e.g. acetic acid for visual inspection (VIA).
- before transvaginal ultrasound.
- or three days after these procedures at the earliest.

It is important that – in contrast to taking a Pap smear – the sample material is not rubbed forcefully against the cervix (the goal is not to obtain cervical cells), but that the sample picks up the secretions around the cervix and from the posterior vaginal vault.

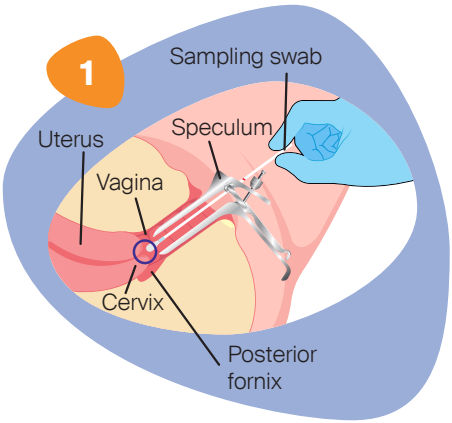
In order to avoid foreign DNA in the sample, unprotected sex should be avoided 24 hours before sample collection.

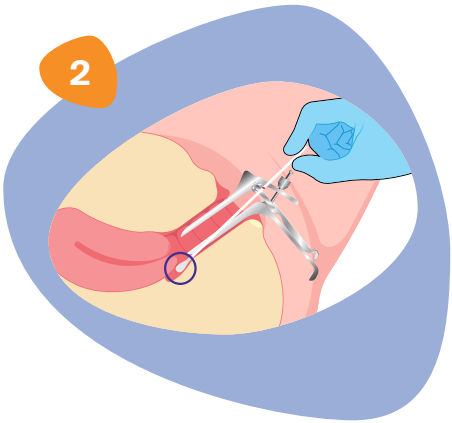
Conditions that restrict the outflow of tumor DNA from the uterine cavity into the vagina, such as large endocervical polyps or fibroids, can impair the sensitivity of the test.

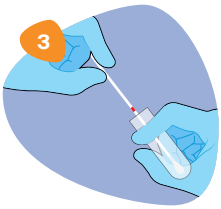
Instructions

1) Insert the speculum to visualize the cervix and the posterior fornix.
2) Insert the sampling swab into the vagina and first take a sample in the upper vaginal region, ideally without touching the cervix (Fig. 1).
3) Hold the sampling swab in this position and slowly rotate it once 360 degrees (for 2–3 seconds).

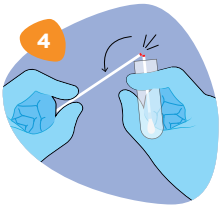
4) Insert the sampling swab into the posterior fornix of the vagina (Fig. 2).
5) Hold the sampling swab in position here as well and slowly rotate it 360 degrees for 2–3 seconds.
6) Remove the sampling swab from the vagina.







7) Unscrew the cap from the eNAT® tube.
8) Insert the sampling swab into the tube until the predetermined breaking point (red mark) reaches the level of the tube opening (Fig. 3).



9) Bend the shaft of the sampling tube at an angle of 180 degrees to break it at the predetermined breaking point, holding the tube away from your face (Fig. 4).
If necessary, carefully rotate the shaft of the sampling swab to make the break easier. Dispose of the upper portion of the swab.

10) Replace the cap on the tube and close it tightly (Fig. 5).
11) Mix the tube 10 times by rotating it 180 degrees and store the sample in an upright position at room temperature for at least 5 minutes.
12) Store the sample at room temperature for a maximum of 48 hours before it is sent to the laboratory.

References

(1) Clarke MA, Long BJ, Del Mar Morillo A, Arbyn M, Bakkum-Gamez JN, Wentzensen N. Association of endometrial cancer risk with postmenopausal bleeding in women: A systematic review and meta-analysis. JAMA Intern Med. 2018; 178(9):1210-1222. https:\\doi.org/10.1001/jamainternmed.2018.2820

(2) Emons G, Steiner E, Vordermark D, Uleer C et al. Endometrial Cancer. Guideline of the DGGG, DKG and DKH (S3-Level, AWMF Registry Number 032/034-OL, September 2022). Part 1 with recommendations on the epidemiology, screening, diagnosis and hereditary factors of endometrial cancer, geriatric assessment and supply structures. Geburtshilfe Frauenheilkd [Obstetrics and Gynaecology]. 2023; 83(8):919-962. https:\\doi.org/10.1055/a-2066-2051

(3) Frias-Gomez J, Tovar E, Vidal A, Murgui L, Ibáñez R, Peremiquel-Trillas P, Paytubi S, Baixeras N, Zanca A, Ponce J, Pineda M, Brunet J, de Sanjosé S, Bosch FX, Matias-Guiu X, Alemany L, Costas L; Screenwide Team. Sensitivity of cervical cytology in endometrial cancer detection in a tertiary hospital in Spain. Cancer Med. 2021; 10(19):6762-6766. https:\\doi.org/10.1002/cam4.4217

(4) Evans I, Reisel D, Jones A, Bajrami A, Nijjar S, Solangon SA, Arora R, Redl E, Schreiberhuber L, Ishaq-Parveen I, Rothärmel J, Herzog C, Jurkovic D, Widschwendter M. Performance of the WID-qEC test versus sonography to detect uterine cancers in women with abnormal uterine bleeding (EPI-SURE): a prospective, consecutive observational cohort study in the UK. Lancet Oncol. 2023; 24(12):1375-1386. https:\\doi.org/10.1016/S1470-2045(23)00466-7

(5) Kiff JM, Williams-Weisenberger M, Spellacy D, Garg B, Munro EG, Bruegl AS. Ultrasonographic evaluation of endometrial stripe thickness is insufficient to rule out uterine serous carcinoma. Cancer Causes Control. 2023; 34(12):1133-1138. https:\\doi.org/10.1007/s10552-023-01759-y

(6) Clarke MA, Devesa SS, Harvey SV, Wentzensen N. Hysterectomy-corrected uterine corpus cancer incidence trends and differences in relative survival reveal racial disparities and rising rates of nonendometrioid cancers. J Clin Oncol. 2019; 37(22):1895-1908. https:\\doi.org/10.1200/JCO.19.00151

(7) Sud A, Torr B, Jones ME, Broggio J, Scott S, Loveday C, Garrett A, Gronthoud F, Nicol DL, Jhanji S, Boyce SA, Williams M, Riboli E, Muller DC, Kipps E, Larkin J, Navani N, Swanton C, Lyratzopoulos G, McFerran E, Lawler M, Houlston R, Turnbull C. Effect of delays in the 2-week-wait cancer referral pathway during the COVID-19 pandemic on cancer survival in the UK: a modelling study. Lancet Oncol. 2020; 21(8):1035-1044. https:\\doi.org/10.1016/S1470-2045(20)30392-2

(8) Frias-Gomez J, Benavente Y, Ponce J, Brunet J, Ibáñez R, Peremiquel-Trillas P, Baixeras N, Zanca A, Piulats JM, Aytés Á, Matias-Guiu X, Bosch FX, de Sanjosé S, Alemany L, Costas L; Screenwide Team. Sensitivity of cervico-vaginal cytology in endometrial carcinoma: A systematic review and meta-analysis. Cancer Cytopathol. 2020; 128(11):792-802. https:\\doi.org/10.1002/cncy.22266

(9) Doll KM, Romano SS, Marsh EE, Robinson WR. Estimated performance of transvaginal ultrasonography for evaluation of postmenopausal bleeding in a simulated cohort of black and white women in the US. JAMA Oncol. 2021; 7(8):1158-1165. https:\\doi: 10.1001/jamaoncol.2021.1700

(10) Long B, Clarke MA, Morillo ADM, Wentzensen N, Bakkum-Gamez JN. Ultrasound detection of endometrial cancer in women with postmenopausal bleeding: systematic review and meta-analysis. Gynecol Oncol. 2020; 157(3):624-633. https:\\doi.org/10.1016/j.ygyno.2020.01.032

(11) Herzog C, Marín F, Jones A, Evans I, Reisel D, Redl E, Schreiberhuber L, Paytubi S, Pelegrina B, Carmona Á, Peremiquel-Trillas P, Frias-Gomez J, Pineda M, Brunet J, Ponce J, Matias-Guiu X, de Sanjosé S, Alemany L, Olaitan A, Wong M, Jurkovic D, Crosbie EJ, Rosenthal AN, Bjørge L, Zikan M, Dostalek L, Cibula D, Sundström K, Dillner J, Costas L, Widschwendter M. A simple cervicovaginal epigenetic test for screening and rapid triage of women with suspected endometrial cancer: validation in several cohort and case/control sets. J Clin Oncol. 2022; 40(33):3828-3838. https:\\doi.org/10.1200/JCO.22.00266

(12) Schreiberhuber L, Herzog C, Vavourakis CD, Redl E, Kastner C, Jones A, Evans I, Zikan M, Cibula D, Widschwendter P, Pfau K, Math B, Seewald M, Amory S, Obrist P, Widschwendter M. The WID-qEC test: Performance in a hospital-based cohort and feasibility to detect endometrial and cervical cancers. Int J Cancer. 2023; 152(6):1269-1274. https:\\doi.org/10.1002/ijc.34275

labor team w ag
Blumeneggstrasse 55
9403 Goldach
+41 71 844 45 45
info@team-w.ch
www.laborteam.ch

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