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PrOsPective observational COhoRt study on the performaNce of the WID-qEC test in patients undergoing hysterectomy (POPCORN-Study)

Research legislation:	Ordinance on human research with the exception of Clinical trials (HRO) [1].
Type of Research Project:	Research project involving human subjects
Risk Categorisation:	A
Project leader:	PD Dr. med. Franziska Siegenthaler Department of Obstetrics and Gynecology Bern University Hospital and University of Bern Friedbühlstrasse 19, 3010 Bern, Switzerland franziska.siegenthaler@insel.ch
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PROTOCOL SIGNATURE FORM

Study Title	***PrOsPective observational COhoRt study on the performaNce of the WID-qEC test in patients undergoing hysterectomy (POPCORN-Study)***

The project leader has approved the protocol version 1, 17.11.2024 and confirms hereby to conduct the project according to the protocol, the Swiss legal requirements [1, 2], current version of the World Medical Association Declaration of Helsinki [3] and the principles and procedures for integrity in scientific research involving human beings.

The project leader has received the ICF and consider it appropriate for use.

Project leader:

Department of Obstetrics and Gynecology
Bern University Hospital and University of Bern
Friedbühlstrasse 19, 3010 Bern, Switzerland

Name: PD Dr. med. Franziska Siegenthaler

Date: _____ Signature: _____

Sponsor:

Name: Prof. Dr. med. Michael D. Mueller

Date: _____ Signature: _____

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GLOSSARY OF ABBREVATIONS

BASEC	*Business Administration System for Ethical Committees*
CRF	*Case report form*
CV	*Coefficient of Variant*
FOPH	*Federal Office of Public Health*
HRA	*Human Research Act*
HRO	*Ordinance on Human*
LOB	*Limit of Blank*
SD	*Standard Deviation*
WID-qEC	*quantitative polymerase chain reaction test for endometrial cancer*

1 BACKGROUND AND PROJECT RATIONALE

Endometrial cancer is most common gynaecological cancer in the developed countries. Till now, there is no screening for it. The most common symptom are abnormal uterine bleeding. However, this symptom is unspecific, and most women undergo intervention which often confirm benign histology. To detect uterine cancer, simpler and more specific index tests are needed to triage women with abnormal uterine bleeding to avoid unnecessary diagnostic interventions such as endometrial biopsy, hysteroscopy, and curettage. Furthermore, the test also includes the detection of endocervical carcinoma with a sensitivity of 95%[11]. These cancers are often overseen as there is no visual suspicion and no increased endometrial thickness. The WID-qEC Test has been developed as a molecular test in women presenting with abnormal bleeding. It asses the sum of the percentage of fully methylated reference of the ZSCAN12 and GYPC regions. Concerning gender, the anatomic nature of the procedure (hysterectomy) is primarily affecting women. However, gender discussion define a broader definition than the anatomically defined woman. Therefore, we include people with a uterus, independently of their gender.

Specificity and sensitivity of the WID-qEC Test has been validated in numerous international clinical trials[10-13]. However, no study has evaluated the performance of this test including the hysterectomy specimen as reference histology. With this study, we aim to analyse the specificity and sensitivity of the WID-qEC test in women undergoing hysterectomy.

2 PROJECT OBJECTIVES AND DESIGN

2.1 Hypothesis and primary objective

Primary objective:

- To estimate the sensitivity and specificity of the WID-qEC test (obtained from the cervicovaginal region immediately prior to the hysterectomy) for the detection of endometrial/cervical cancers compared to the final hysterectomy specimen.

Secondary objectives:

- To assess the underlying pathology of the ecto-cervix, cervical canal, endometrium and Fallopian Tube (and if removed also the ovary) in patients whose WID-qEC test (obtained from the cervicovaginal region immediately prior to the hysterectomy) is false positive (i.e. WID-qEC test is positive in the absence of a cancer in the hysterectomy/adnexal specimen).
- To assess the underlying pathology of the ecto-cervix, cervical canal and endometrium in patients whose WID-qEC test (obtained from the cervicovaginal region immediately prior to the hysterectomy) is false negative (i.e. WID-qEC test is negative despite the presence of an invasive cancer in the cervix or the endometrial cavity).
- To compare the level of the SUM-PMR of the WID-qEC test (obtained from the cervicovaginal region immediately prior to the hysterectomy) with the immunohistochemical markers assessed in the cancerous endometrium (p53, MMR markers, Ki67) or in non-cancer patients (Ki67 only) in the normal endometrium or in the most advanced hyperplastic lesion.

2.2 Primary and secondary endpoints

Primary outcome:

- To estimate the Sensitvity and Specificity of WID-qEC test by comparing its performance to a hysterectomy specimen as reference histology.

Secondary outcome:

- Specimen which scores false positive in the WID-qEC test: analysis of pathology of the full specimen to objectify the underlying cause for false-positive result
- Specimen which score false negative in the WID-qEC test: analysis of pathology of the full specimen to objectify the underlying cause for false-negative result
- SUM-PMR of WID-qEC test compared to immunohistochemical markers in cancer of the endometrium (p53, MMR markers, Ki67), or in non-cancer patients (Ki67 only)

2.3 Project design

The proposed study is a prospective diagnostic accuracy study designed to assess the sensitivity and specificity of the WID-qEC test in detecting endometrial and cervical cancers. This design is appropriate because it allows for the direct comparison of the WID-qEC test results with the definitive histopathological diagnosis following hysterectomy. Given the study's objectives, a blinded design is employed, where the investigators analyzing the WID-qEC test results are blinded to the histopathological outcomes, reducing bias and ensuring the objectivity of the results. The study is non-randomized because it involves a single-arm cohort of patients undergoing hysterectomy, making randomization unnecessary. The framework is exploratory as it aims to evaluate the diagnostic performance of a relatively new IVD device in a clinical setting. An interventional study design is justified as it allows for the collection of cervicovaginal samples immediately before hysterectomy and before disinfection, which is crucial for evaluating the WID-qEC test's performance in a real-world setting. The collection of these specimens is necessary to provide data that cannot be obtained retrospectively or from pre-existing samples. Although this introduces minimal additional risks as the patients will be already under general anesthesia, these are outweighed by the potential clinical benefits of validating a new diagnostic tool that could improve cancer detection rates.
Potential limitations include the risk of selection bias, as the study population is limited to women undergoing hysterectomy no matter the reason. To reduce this, efforts will be made to include a diverse patient population. Another limitation is the reliance on histopathology as the gold standard, which, while highly accurate, is not infallible.
Measures to avoid bias are blinding of investigators of the laboratory team towards the histopathological outcomes. This is to prevent interpretative bias. Also we establish a standardized protocol to reduce variability. Also, the histopathological analysis will be conducted by pathologist using standardized criteria. The statistical analysis will be pre-specified and conducted by statisticians, to reduce potential bias.
The patients will not be blinded towards the result of the WIDqEC test. However, this has no specific influence on the patients' history as the final pathology report will be decisive for the patients follow up care and diagnosis. The test result of the WIDqEC test will not influence the further care of the patient.

3 PROJECT POPULATION AND STUDY PROCEDURES

3.1 Project population, inclusion and exclusion criteria

The study will include all women undergoing a hysterectomy at our institution, regardless of the underlying indication for the surgery. By including all women undergoing hysterectomy, the study aims to capture a broad spectrum of cases, ranging from benign conditions to suspected or confirmed malignancies (endometrial and cervical cancers). This approach ensures that the study evaluates the WID-qEC DNA methylation test's performance across various clinical scenarios, providing a robust assessment of its sensitivity and specificity.

Women undergoing hysterectomy are already indicated for surgical intervention, making them an appropriate and relevant population for evaluating a diagnostic test that could potentially alter surgical planning or follow-up care. Given that approximately 70 of the anticipated 300 cases are

expected to involve endometrial or cervical cancer, the study will focus on a population where accurate preoperative diagnosis is crucial for optimal patient management.

The study population is representative of the broader target population of women who may undergo hysterectomy for various gynecological conditions, including benign and malignant diseases. The inclusion of a diverse range of indications for hysterectomy ensures that the findings are generalizable to clinical practice. By evaluating the test in a real-world setting with patients presenting for routine care, the study aims to reflect the test's utility in a broad, representative population.

Given that the study involves women undergoing hysterectomy, the population is inherently female. However, attention will be given to ensure that the study includes a diverse range of women in terms of age, race, and socioeconomic background. Recruitment will be conducted without bias, and efforts will be made to include women from various demographic groups to ensure that the results are applicable to a wide patient population. We will collect demographic details in order to identify any bias.

While the study population is exclusively female due to the nature of the procedure (hysterectomy), the principles of sex and gender equity are relevant in ensuring that women of different ages and backgrounds are included. This is important for the scientific validity of the results, as it ensures that the test's performance is evaluated across a range of physiological and demographic variables. Again, demographic details will be collected and are under investigation.

This study does not specifically target vulnerable subjects, such as minors or those incapable of judgment. The inclusion criteria are based solely on the indication for hysterectomy, and all participants will be capable of providing informed consent. If not, they will be excluded from the Study.

If a patient who could be considered vulnerable (due to advanced age or cognitive impairment) is eligible for the study, their participation will be evaluated by tests to evaluate the capacity to consent and understand the study's purpose. The inclusion of such individuals will be done with careful consideration, ensuring that their participation is ethically justified and that comparable results could not be obtained from non-vulnerable subjects.

Inclusion Criteria:

- 18 years or older
- Undergoing a total hysterectomy at the Dept. of Gynecology at the University Inselspital Bern, irrespective of the underlying reason for the hysterectomy
- Signed written informed consent

Exclusion Criteria:

- Lack of capacity to provide a written informed consent
- Refusal to participate in the study
- Presence of medical conditions contraindicating general anesthesia

3.2 Recruitment, screening and informed consent procedure

The project leader explains to each participant the nature of the research project, its purpose, the procedures involved, the expected duration, the potential risks and any discomfort it may entail. Each participant is informed that the participation in the research project is voluntary and that he/she may withdraw from the research project at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The participants are informed that he/she can ask any question. Enough time is given to the participant.

All participants are given an information document and a consent form describing the research project. The formal consent of a participant, using the approved consent form, is obtained before the participant is enrolled in the research project.

The participant should read, understand, and voluntarily agree before signing and dating the informed consent form, and is given a copy of the signed document. The consent form is signed and dated by the participant and the project leader (or her/his designee). The signed consent form it is retained as part of the investigation records.

Subjects for the performance study will be screened during their preoperative visit, scheduled between one week and two days before the procedure. During this visit, trained personnel, including doctors, will provide a thorough explanation of the study and address any questions the patients may have. Patients will then be given the opportunity to consider participation, and if they meet the inclusion criteria, they can sign the consent form and be enrolled in the study. Recruitment and enrolment will take place exclusively at the University Hospital Inselspital in Bern.

The recruitment period is planned from January 1st, 2024, to December 31st, 2026. If the enrolment goals are not met within this timeframe, the recruitment period may be extended by an additional year.

3.3 Study procedures

Study Periods	***Screening***	***Participant information and consent***	***Test***	***Evaluation***
Visit	***0***	***1***	***2***	
Time (day)	***-7 to -2***	***-2***	***0***	***4-7***
Duration (hour)	***0.5***	***1***	***1-6***	***2***
Participant Information		***x***		
Participant consent (ICF)		***x***		
Demographics	***X***			
Medical History	***X***			
In- /Exclusion Criteria	***X***		***x***	
Physical Examination		***x***		
Vital Signs		***x***	***x***	
WID-qEC Test			***x***	
Hysterectomy			***x***	
Specimen collection			***x***	
Obtain histological results				***x***
Obtain immunhistological results				***x***

During the performance study, subjects will undergo both medical and non-medical procedures that are essential for collecting the data needed to evaluate the IVD device. These procedures will include:

Preoperative Screening:

- Medical: A standard preoperative visit will occur between 1 week and 2 days before the hysterectomy. During this visit, trained personnel (doctors) will conduct a comprehensive

medical evaluation, which includes reviewing the subject's medical history, confirming the diagnosis, and ensuring eligibility according to the study's inclusion criteria.

- Non-Medical: The study will be explained in detail to each subject, and informed consent will be obtained. The subject will also be given adequate time to ask questions and consult with family or other advisors.

Specimen Collection:

- Medical: Immediately prior to the hysterectomy, a cervicovaginal specimen will be collected using the WID-qEC test. This collection is non-invasive and does not pose additional risks beyond those associated with standard clinical care.

Hysterectomy and Specimen Processing:

- Medical: The hysterectomy will be performed according to standard clinical practice. Following the procedure, the specimens (including the cervix, endometrium, and adnexal tissues) will undergo histopathological examination to determine the presence or absence of cancer. This analysis serves as the reference standard for evaluating the WID-qEC test results
- Non-Medical: Data from the WID-qEC test and the histopathological examination will be collected and documented for comparison and analysis.

The administration of the WID-qEC test immediately before the hysterectomy is a study-specific procedure that deviates slightly from normal clinical practice. Typically, such a test might not be performed right before surgery. However, this deviation is necessary to evaluate the test's performance in detecting endometrial and cervical cancers.

After collection, the cervicovaginal specimens will undergo analysis using the WID-qEC test to assess the presence of cancer-related biomarkers. This procedure is critical for evaluating the test's sensitivity and specificity.

The hysterectomy specimens will be processed and examined by pathologists. This will include standard procedures for tissue fixation, sectioning, staining, and microscopic evaluation to confirm the presence or absence of malignancy as well as immunohistological parameters.

The results of the WID-qEC test will be compared with the histopathological findings to assess the test's diagnostic accuracy. Data from these procedures will be analysed to determine the test's sensitivity, specificity, and overall performance.

As the WID-qEC test is performed by healthcare professionals rather than the subjects themselves, there is no need for subjects to be trained on the use, handling, storage, or return of the IVD device. All test-related procedures will be conducted by trained medical staff in a controlled clinical setting.

If any new significant findings arise during the performance study that could affect the subjects' health or safety, these will be promptly communicated to the subjects. This may include discoveries that necessitate additional medical care or changes in the study protocol. Any incidental findings identified during the study (e.g., unexpected pathological results) will be immediately reported to the Principal Investigator (PI). The PI will then determine the clinical relevance of these findings and decide on the appropriate course of action. Subjects will be informed of these findings and offered additional follow-up or treatment as necessary.

This comprehensive approach ensures that all study-related procedures are conducted safely and ethically while maintaining the integrity of the study data and protecting the well-being of the subjects.

Minimising bias: To ensure the reliability and validity of the study results, several measures will be implemented to minimize or avoid bias.

The study will include a diverse population of women undergoing hysterectomy for various indications, ensuring that the findings are generalizable across different patient subgroups. Selection bias will be minimized by including all eligible women consecutively, without preference or exclusion based on demographic or clinical characteristics.

The histopathological evaluations of the hysterectomy specimens will be conducted by pathologists who are blinded to the WID-qEC test results. This will prevent any bias in the interpretation of the histological findings based on the test outcome.

The WID-qEC test will be performed using a standardized protocol. This includes uniform specimen collection, processing, and analysis procedures.

Data will be collected using validated case report forms (CRFs), ensuring consistency and accuracy in capturing relevant information. Statistical analysis will be pre-specified in the study protocol to avoid data-driven bias.

Potential confounders such as age, prior medical history, and other relevant clinical factors will be recorded and controlled for during the statistical analysis to ensure that the associations observed are attributable to the WID-qEC test performance and no other variables.

3.4 Withdrawal and discontinuation

Subjects have the right to withdraw their consent and discontinue their participation in the study at any time, without providing a reason and without any negative consequences for their ongoing or future medical care.

If a subject experiences adverse events or other safety concerns that, in the judgment of the investigator, may pose a risk to their health, they may be withdrawn from the study.

The decision to withdraw a subject will be documented in the study records, including the reason for withdrawal and the date of withdrawal. Subjects who withdraw voluntarily will be asked, but not required, to provide the reason for their decision. In cases of withdrawal due to safety concerns or protocol deviations, the subject will be informed, and a final study visit may be conducted to ensure their well-being.

3.5 Identification and description of the In Vitro Diagnostic (IVD) device under investigation

The WID®-easy test is used in accordance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) Chapter II, Article 5 (5).

The legal manufacturer of the RUO WID®-easy PCR Kit is Sola Diagnostics GmbH. The WID®-easy test is used in accordance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices (IVDR) Chapter II, Article 5 (5). The PCR kit was verified and manufactured in collaboration with BIOTYPE GmbH in Dresden (Germany). BIOTYPE has been a renowned German solution provider for precision molecular diagnostics, contract development and production for 25 years. BIOTYPE is certified according to the standards DIN EN ISO 13485 and DIN EN ISO 9001 in the area of sales, development, production and services of molecular biological tests for in-vitro diagnostics (ISO 13485:2016) and for research and diagnostics (ISO 9001:2015).

The WID®-easy test is a non-invasive diagnostic test for simple and rapid screening of women presenting with symptoms suggestive of endometrial cancer, particularly peri- and postmenopausal women with abnormal uterine bleeding. The test is based on quantitative, methylation-specific polymerase chain reaction targeting the GYPC and ZSCAN12 genes, which are known to be highly methylated in endometrial cancer.

List of detected methylation sites:

- Primer Mix A: ZSCAN12 (Chr6: 28367535-28367599)
- Primer Mix B: GYPC (Chr2: 127413797-127413902)
- Primer Mix B: COL2A1 (Chr12: 48381229-48381320)

Kit content:

- Master Mix: Black 1 x 1 mL
- Positive Control: White 1 x 60 µL
- Primer Mix A: Red 1 x 50 µL

- Primer Mix B: Yellow 1 x 50 µL
- Standard 1: Green 1 x 60 µL
- Standard 2: Green 1 x 60 µL
- Standard 3: Green 1 x 60 µL
- Standard 4: Green 1 x 60 µL

Reagents required but not provided:

- Mag-Bind ® Blood & Tissue DNA Kit or NucleoMag Blood 200 µL Kit
- EZ DNA Methylation-Lightning Kit Zymo Research D5030-E
- QubitTM dsDNA BR Assay or QuantiFluor ONE dsDNA System
- TE Buffer pH 8.0 (Ambion) or Nuclease-Free Water

Instruments and software:

LightCycler® 480 II, 96-well (05015278001, Roche Diagnostics (Schweiz) AG)

DNA methylation is a modification of the DNA which is essential in the epigenetic regulation of gene expression. It involves the addition of a methyl group to the 5th position of the pyrimidine ring of the cytosine nucleotide in the regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide (CpG) in the linear sequence of bases along its 5' → 3' direction. These CpG sites occur with high frequency in genomic regions called CpG islands, which are frequently located in the promoter region of genes. During carcinogenesis, several of these CpG islands become methylated[13-15], including CpG islands of the ZSCAN12 and GYPC genes in endometrial and cervical cancer[8,10]. The WID®-easy PCR assay allows the detection of hypermethylation of the genes ZSCAN12 and GYPC on bisulfite-converted DNA isolated from cervico-vaginal specimens using COL2A1 as an internal control for DNA input and efficacy of bisulfite-conversion. A standard curve is used to calculate the input amount for each reaction of each sample and a positive control is included to enable quantification of DNA methylation. Both the positive control and standard curve are based on the information provided by an artificial gBlock fragment containing all three fully methylated and bisulfite-converted amplicon sequences at a distinct copy number per reaction. The gBlock standard curve (slope and intercept) and the gBlock positive control are required to calculate the level of methylation per sample as the “percentage of fully methylated reference” (PMR)[14] for each target.

PMR is calculated by dividing the target (GYPC or ZSCAN12) to the reference (COL2A1) input amount ratio of each sample by the target to reference input of a positive control sample that represents a fully methylated sample.

Where t refers to the mean input amount of GYPC or ZSCAN12, c refers to the mean input amount of COL2A1, s refers to the sample, and g refers to the gBlock positive control. The final test result is calculated as the Σ (PMR) of both target PMRs.

The WID®-easy test is a cervicovaginal swab-based in-vitro test for triaging woman ≥ 45 years of age with abnormal uterine bleeding for further invasive histological evaluation.

The WID®-easy test is characterized by extremely high values for sensitivity (90.9%), specificity (97.3%) and NPV (99.7%). Above all, the PPV of the WID®-easy test is ten times higher at 50% than that of sonography, whose PPV is only 5%.[11]

Analytical specificity

Non-bisulfite-converted human DNA was tested with the assay. There is no amplification on human DNA for ZSCAN12 and GYPC.

Analytical sensitivity

The analytical sensitivity of the WID®-easy PCR Kit is defined as the concentration (copies/µL of the eluate) of bisulfite-converted DNA molecules of the target regions of ZSCAN12 or GYPC that

can be detected with a positivity rate of 95 %. The analytical sensitivity was confirmed by analysis of dilution series of artificial DNA of the methylated targets ZSCAN12 and GYPC. The confirmation was carried out on five different days with 6 replicates per concentration. For the detection of ZSCAN12 and GYPC, the analytical sensitivity is < 1 copies/µL with a 95 % CI (Confidence Interval).

Precision

The precision of the WID®-easy PCR Kit was determined as inter-assay variability (variability between different experimental setups) and inter-lot variability (variability between different production lots).

For the inter-assay variability, the study was run on three different setups (three instruments, three operators) on five days and in five replicates. For the analysis, the SD (Standard Deviation) and Coefficient of Variation (% CV) of repeatability, of reproducibility, and of within-laboratory precision were calculated as per the CLSI EP05 (4.6.2) guidelines. With regard to the Cq-value results, the % CV of repeatability, reproducibility, and within-laboratory precision did not exceed 5 %, indicating that the performance of the assay is not affected by the testing procedure.

For the inter-lot variability, two different production lots were tested at two different cDNA concentrations. The SD and coefficient of variation (% CV) were calculated as per the CLSI EP05 (4.6.2) guideline. The ZSCAN12 and GYPC assays show a % CV < 5 % for all samples, including positive control and standards. In conclusion, the assay performance is not affected by different kit lots.

Limit of blank

This study followed CLSI EP-17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. The LOB (Limit of Blank) was performed using at least two kit lots with eight replicates measured on three different days; in total, 120 wells were measured. The LOB for ZSCAN12 is Cp 43.0 and for GYPC is Cp 42.4.

Linear range

The linear range of the WID®-easy PCR Kit was evaluated by analyzing a logarithmic dilution series of cDNA for both targets GYPC and ZSCAN12 using concentrations ranging from 10 to 100,000 copies/reaction, and each dilution was analyzed in four replicates.

The WID®-easy test was developed based on genome-wide screening of differentially methylated regions in cervicovaginal samples from 144 endometrial cancer cases and 572 cancer-free controls using the Infinium MethylationEPIC array (Illumina). MethyLight reactions were designed for the top differentially methylated positions and tested in a subset of samples and the top three reactions were selected for the WID®-easy test, which was validated in an independent group of 562 volunteers (all > 18 years) in three diagnostic and two predictive settings: (1) the FORECEE Validation set, consisting of cervicovaginal liquid-based cytology samples from symptomatic women attending the hospital diagnosed as ECs (n = 71), benign gynecologic patients (n = 29), or healthy volunteers (n = 37), matched to cases by age; (2) the Barcelona Validation set, consisting of cervicovaginal self-samples from consecutive incident EC cases (n = 131), hospital controls with benign conditions (n = 102), and women attending hospital for nongynecologic diseases (n = 18), frequency-matched to cases by age; (3) the postmenopausal bleeding (PMB) Cohort, consisting of vaginal swabs from consecutive women presenting with postmenopausal bleeding at University College London Hospital (N = 63); (4) the Lynch Cohort, consisting of cervicovaginal liquid-based cytology samples collected from consecutive women presenting to University College London Hospital because of Lynch syndrome (N = 25); and (5) the Karolinska Cohort, a nested case/control setting using cervicovaginal liquid-based cytology samples collected between 2011 and 2015 from a Swedish cervical sample cohort–based biobank from women diagnosed with EC up to 3 years after sample collection (n = 32) or women who did not develop EC by the end of the study period (2015) on the basis of the Swedish cancer registry (n = 54)[8].

In a further work, Schreiberhuber et al investigated whether the WID®-easy test can additionally identify women with cervical cancer. Moreover, we evaluate the test's applicability in a SurePath-based hospital-cohort by comparing its ability to detect endometrial and cervical cancer to cytology. In a set of 23 cervical cancer cases and 28 matched controls the receiver operating characteristic (ROC) area under the curve (AUC) is 0.99 with a sensitivity and specificity of 100% and 92.9%, respectively. Amongst the hospital-cohort (n = 330), the ROC AUC is 0.99 with a sensitivity and specificity of 100% and 82.5% for the WID®-easy test, respectively, and 33.3% and 96.9% for cytology (considering PAP IV/V as positive). Our data suggest that the WID®-easy test detects both endometrial and cervical cancer with high accuracy.

In a very recent work, carried out at the University College London, a prospective study was conducted with the presentation of 474 symptomatic women to compare the performance of the current improved version of the WID®-easy test with the standard procedure in the United Kingdom. 400/474 women (84.4%) agreed to participate, while one patient withdrew after providing consent. 399 women were included in the primary analysis cohort. Based on 603 index imaging tests, 186 (47%) women were recommended for a reference histology test (biopsy, hysteroscopy, or both). 12 women were diagnosed with cancer, 375 were not diagnosed with cancer, 12 had inconclusive clinical outcomes and were considered study dropouts. 198 reference histology test procedures detected nine cases of cancer and missed two; one further cancer was directly diagnosed at hysterectomy without a previous reference test. The AUC for detection of uterine cancer based on endometrial thickness in mm was 87.2% versus 94.3% based on WID®-easy. Endometrial thickness assessment on ultrasound scan was possible in 379 (95%) of the 399 women and a prespecified cut-off of 4.5 mm or more showed a sensitivity of 90.9%, a specificity of 79.1%, a positive predictive value of 11.8%, and a negative predictive value of 99.6%. The WID®-easy test was possible in 390 (98%) of the 399 patients with a sensitivity of 90.9%, a specificity of 92.1%, positive predictive value of 25.6%, and a negative predictive value of 99.7%, when the prespecified threshold of 0.03 ΣPMR or more was applied. When a higher threshold was applied (0.3 ΣPMR) the specificity increased to 97.3% without a change in sensitivity.

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Labelling, supply (re-SUPPLY) and storage conditions

Storage is in room temperature.

The IVD device under investigation, along with any applicable comparators, will be provided to the study site by the Sponsor or a designated logistics partner. The shipment will include detailed records of all devices supplied, including identification codes, batch numbers, and quantities. If additional quantities are needed, a re-supply will be provided.

For any unused, expired, or malfunctioning IVD devices, the process for return or disposal will follow the procedures of standard care. The Sponsor will provide instructions for the return or destruction of these devices, and all actions will be documented appropriately.

Access to the storage area will be restricted to authorized study personnel only.

The labelling is conducted in the operating room with patient's name and birthdate. The labelling will comply with all regulatory requirements to ensure proper identification and usage of the device during the study.

Accountability of IVD device

To ensure the integrity and control of the IVD devices used in this performance study, several procedures will be implemented:

- Controlled Access to IVD Devices: The Principal Investigator (PI) or an authorized designee will be responsible for overseeing the access to and use of all IVD devices involved in the study. Devices will be securely stored in a restricted area, accessible only to authorized study personnel. Each device will be logged upon arrival at the study site, and its use will be documented to ensure that devices are utilized strictly according standard care procedures.
- Use of IVD Devices: The IVD devices will be used exclusively for the performance study. Before the initiation of the study, all participating personnel will receive thorough training on the correct use, handling, and storage of the IVD devices. Each use of the IVD device will be documented, including the date, the identification code of the device used, and the subject number.
- Unused Devices: Any IVD devices that remain unused at the end of the study or when no longer required will be returned to the Sponsor or disposed of following the Sponsor's instructions. The return of unused devices will be documented, including the quantity, identification codes, and the date of return.
- Expired or Malfunctioning Devices: If any IVD devices expire or are found to be malfunctioning during the course of the study, they will be promptly returned to the Sponsor.

The PI or authorized designee will document the identification code, batch number, and the date of return for each expired or malfunction

Return, Analysis or Destruction of the IVD Device

All used or unused IVD devices provided specifically for the study will be prepared for return to the Sponsor. Devices will be carefully packaged according to the Sponsor's guidelines to ensure safe transport, and a detailed inventory will be included with the shipment, listing all returned devices along with their identification numbers, batch numbers, and usage status. The shipment will be coordinated with the Sponsor to ensure proper handling and tracking until the devices are received.

If the devices are not required to be shipped back, they will be disposed of or destructed at the hospital. This process will comply with local regulations for medical waste disposal, ensuring that

all devices are handled safely and environmentally responsibly. Documentation of the disposal or destruction, including the method and date, will be maintained as part of the study records.

4 STATISTICS AND METHODOLOGY

4.1. Statistical analysis plan

The primary aim is to estimate sensitivity and specificity.

Determination of Sample Size

- The University Inselspital Bern does ~ 300 hysterectomies annually of which 70 are due to endometrial/cervical cancers and 230 are due to nonendometrial/cervical cancer reasons (i.e. ovarian cancer, fibroids, prolapse, urological)
- Assuming we detect 64 out of 70 cancer cases we would estimate a sensitivity of 91.4% with a 95% confidence interval of 84.9% to 97.9%.
- Assuming 224 out of 230 non-cancer cases have a negative WID-qEC test result we would estimate a specificity of 97.3% with a 95% confidence interval of 95.3% to 99.5%.

With 70 cases and 230 cancer-free individuals we could estimate an area under the receiver operating characteristic curve(ROC) of 0.95 with a 95% confidence interval of 0.914 to 0.987.

We will calculate the Sensitivity and Specificity of WID-qEC test along with 95% confidence intervals using the Wilson Method.

Secondary:

- Specimen which scores false positive in the WID-qEC test: analysis of pathology of the full specimen to objectify the underlying cause for false-positive result
- Specimen which score false negative in the WID-qEC test: analysis of pathology of the full specimen to objectify the underlying cause for false-negative result

This is descriptive and of no statistical nature.

- SUM-PMR of WID-qEC test compared to immunhistochemical markers in endometrial cancer (p53, MMR markers, Ki67), or in non-cancer patients (Ki67 only)
- SUM-PMR values will be compared to immunohistochemical markers using a Wilcoxon rank sum test and visually displayed using box plots. P-values will be computed and statistical significance assessed at a p=0.05 level.

4.2. Handling of missing data

We will report the missing data, this will not be used for the calculation as of above.

5 REGULATORY ASPECTS AND SAFETY

5.1 Local regulations / Declaration of Helsinki

This research project will be conducted in accordance with the protocol, the Declaration of Helsinki [3], the principles of Good Clinical Practice, the Human Research Act (HRA) and the Human Research Ordinance (HRO) [1] as well as other locally relevant regulations. The project leader acknowledges his responsibilities as both the project leader and the Sponsor.

5.2 Notification of safety and protective measures (HRA Art. 15, HRO Art. 20)

If, during the research project, circumstances arise which could jeopardise the safety or health of the participants or lead to a disproportionate relationship between the risks and burdens and the benefits, all the measures required to ensure protection are to be taken without delay.
The project leader and the Sponsor) is promptly notified (within 24 hours) if immediate safety and protective measures must be taken during the conduct of the research project. The Ethics Committee will be notified via BASEC of these measures and of the circumstances necessitating them within 7 days.

5.3 Serious events (HRO Art. 21)

If a serious event occurs, the research project will be interrupted and the Ethics Committee notified on the circumstances via BASEC within 7 days according to HRO Art. 21[1].

The project leader reports to the ethics committee on the connection between the event and the collection of health-related personal data or the sampling of biological material. At the same time, the project leader submits proposals concerning the next steps to be taken.

Any new relevant information and the outcome to the original Serious Event is reported to the ethics committee via BASEC.

5.4 Amendments

Substantial changes to the project set-up, the protocol and relevant project documents will be submitted to the Ethics Committee for approval according to HRO Art. 18 before implementation. Exceptions are measures that have to be taken immediately in order to protect the participants.

The following are considered to be substantial changes:

a. changes affecting the participants' safety and health, or their rights and obligations;
b. changes to the protocol which concern the objectives of the research project;
c. a change of research site or conducting the research project at an additional site; or
d. a change of project leader or Sponsor.

5.5 End of project

Upon project completion or discontinuation, the Ethics Committee is notified within 90 days.

The completion of the research project is defined by the last collection of the last sampling of biological material.

All biological materials and health-related data are anonymized upon termination of data analysis.

The Sponsor and the PI affirm and upholds the principle of the subject's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the subjects shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

The assignment to each subject of a unique subject identification number ensures subject confidentiality. The identification number is generated by a rising number, starting with 001, 002 etc. For data verification purposes (monitoring) the PI or institution provide direct access to source data during and after the performance study to the Sponsor's personnel, or to other personnel designated and authorized by the Sponsor.

The PI or institution provide direct access to source data during and after the performance study for Sponsor audits, CEC review and RA inspections.

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[1] A serious event is defined as any adverse event where it cannot be excluded, that the event is attributable to the sampling of biological material or the collection of health-related personal data, and which:
a. requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
b. results in permanent or significant incapacity or disability; or
c. is life-threatening or results in death.

We also refer to chapter 3.4.

4.6 Insurance

In the event of project-related damage or injuries, the Sponsor will be liable. The liability coverage covers damage occurring up to 10 years after the completion of the research project

6 FURTHER ASPECTS

6.1 Overall ethical considerations

Also see chapter 1. As we include any patient being scheduled an hysterectomy, we achieve a fair balance of study participants. As patient do anyways are being informed by their final histopathology, we do not withhold any incidental findings that would anyway have been presented by them without this study. There is no surplus information to be found compared to a standard anamnesis and lab work before a standard hysterectomy outside of this study.

6.2 Risk-Benefit Assessment

There is no additional risk for patients participating in this study, as the procedure is exactly the same and the risk of a vaginal swab compared to the anyway scheduled hysterectomy is not big enough to be considered.
Patients participating can help future patients in understanding the disease better, and enable an easier and less dangerous way in diagnosing endometrial cancer.
The risk of a project includes the risk of unauthorized data access and/or unwanted identification of project participants.

6.3 Rationale for the inclusion of vulnerable participants

This study does not specifically target vulnerable subjects, such as minors or those incapable of judgment. The inclusion criteria are based solely on the indication for hysterectomy, and all participants will be capable of providing informed consent. If not, they will be excluded from the Study.
If a patient who could be considered vulnerable (due to advanced age or cognitive impairment) is eligible for the study, their participation will be evaluated by tests to evaluate the capacity to consent and understand the study's purpose. The inclusion of such individuals will be done with careful consideration, ensuring that their participation is ethically justified and that comparable results could not be obtained from non-vulnerable subjects.

7 QUALITY CONTROL AND DATA PROTECTION

7.1 Quality measures

The Sponsor is responsible for ensuring that quality assurance (QA) and quality control (QC) systems are in place and maintained throughout the performance study. This is achieved through the development and implementation of written Standard Operating Procedures (SOPs) and Working Instructions (WIs). These documents are applied to standardize procedures, minimize variability, and ensure that the study is conducted in compliance with regulatory requirements and the study protocol.

The QA system is designed to systematically monitor and evaluate various aspects of the study to ensure that it meets the predefined quality standards. The Sponsor conducts regular audits and inspections to verify compliance with SOPs, protocol adherence, and regulatory requirements.

QC activities involve the operational techniques and activities employed to fulfill quality requirements. These include routine checks and verifications at various stages of the study to ensure data accuracy, integrity, and completeness. QC measures are applied to all critical processes, from subject recruitment and data collection to data analysis and reporting.

The Sponsor employs specific software tools for data management, monitoring, and analysis to ensure the integrity and quality of the data. For example, electronic data capture (EDC) systems, clinical trial management systems (CTMS), and statistical analysis software are used. These systems are validated and regularly updated to comply with the latest industry standards.

The PI at each study site plays a critical role in maintaining the quality of the study by ensuring that all personnel involved are adequately trained and qualified to perform their assigned tasks. The PI is responsible for ensuring that all study team members possess the necessary experience, scientific knowledge, and clinical expertise to conduct the study effectively. This includes both general qualifications and specific training related to the performance study, such as training on the IVD device under investigation, study protocol, SOPs, and GCP (Good Clinical Practice) guidelines.

Each member of the study team receives detailed training on the performance study, tailored to their role. This may include training on patient recruitment, data collection, IVD device handling, and safety reporting.

The PI ensures that team members remain competent throughout the study by providing continuous training and assessments as necessary. This ongoing training ensures that the team can adapt to any changes or updates in the study protocol or procedures.

For quality assurance the Ethics Committee may visit the research sites. Direct access to the source data and all project related files and documents must be granted on such occasions.

The project leader has appropriate knowledge and skills in the areas of data security and data protection or is able to ensure compliance by calling in appropriate expertise (Art. 4 HRO).

7.2 Data recording and source data

In this performance study, source data and source documents play a crucial role in ensuring the traceability, validity, and integrity of the study findings. As defined by ISO 20916, source data encompasses all original records or certified copies necessary for the evaluation of the clinical performance study. Source documents are the printed or electronic materials that contain this source data.
For each type of data collected in the Case Report Form (CRF), specific source documents are identified as follows:
Hospital Records:

- Source Data: Medical histories, physical examination notes, and other clinical observations recorded during patient visits.
- Example Data: Patient demographics, medical history, and treatment records.
- Location: Stored securely in the hospital's electronic health record (EHR) system EPIC® or in physical medical files at the study site.

Laboratory Notes:

- Source Data: Results and observations from laboratory tests conducted during the performance study.
- Example Data: Blood test results, biochemical analyses, and other diagnostic tests as well as histopatholocigal findings or immunhistochemistry.
- Location: Stored in the laboratory information management system or in physical laboratory logs.

Test Results:

- Source Data: Results generated by the IVD device under investigation.

- Example Data: Device outputs, such as numerical test results or qualitative assessments.
- Location: Stored within the device's data management system, printed records, or transferred to the hospital's EPIC® system.

Stored on secure servers with regular backups to prevent data loss called SharePoint 2013 platform that is centrally set up by the Inselspital. Access is restricted to the PI and authorized study personnel. Authorized staff can access the data through secure login credentials. Audit trails are maintained to track any access or modifications to the data. The physical location of the servers is in Zollikofen BE.
All source data is kept securely at the study site in compliance with local regulations and good clinical practice (GCP) guidelines. This includes Electronic Data. Stored on secure, access-controlled servers with regular audits and data integrity checks, mainly EPIC® and Synedra® and Sharepoint.
The SharePoint server is kept in a locked server-room. Only system administrators have direct access. An anonymous access is not possible, you will need personal credentials to access. There are also role concepts (read, write, administrator) for each user to be tagged. Any change will be tracked with the personal data of the specific worker.
Only study conductors are listed above have access to the data. Backup is done once a week. Retention time is 30 days.
Physical Documents are kept in locked cabinets within a secure, restricted-access area of the study site. Throughout the study, these source documents will be maintained in an organized manner to ensure they are easily retrievable for monitoring, auditing, or inspection purposes. At study termination, the source documents will be archived according to regulatory requirements, ensuring long-term data integrity and compliance with applicable standards.

7.3 Confidentiality and coding

Project data will be handled with uttermost discretion and is only accessible to authorized personnel who require the data to fulfil their duties within the scope of the research project. On the CRFs and other project specific documents, participants are only identified by a unique participant number. Coding is done using a method based on the current state of the art that must be based on the current state of the art (Art. 26 HRO).

We also refer to chapter 7.4.

The participant identification list is stored by the study nurse under protection. Considering the storage protection, we refer to chapter 7.2.

A comprehensive security system is implemented to prevent unauthorized access to the electronic data. This includes encryption of data during transfer and storage, secure login procedures, and regular audits of access logs. Access to the electronic data system will be restricted to authorized personnel only. A list of users with their access levels and dates of access will be maintained and regularly reviewed. The system will maintain a complete audit trail to track all data entries, modifications, and deletions, ensuring transparency and accountability in data handling. A robust security system of the Inselspital Bern implemented to protect the data from unauthorized access, with specific protocols for internal and external threats. Backup procedures will be in place to ensure data is regularly saved and can be retrieved in case of system failures.

The Sponsor and the PI affirm and upholds the principle of the subject's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the subjects shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals. Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited.

The PI or institution provide direct access to source data during and after the performance study for Sponsor audits, CEC review. Biological material in this project is not identified by participant name but by a unique participant number. Coding is done using a method based on the current state of the art (Art.26 HRO).

If no further use of specimens or personal data is planned, the specimens will be destroyed, and the personal data will be anonymized after the storage period has ended.

7.4 Retention and destruction of project data and biological material

The project leader retains all the research project data for a period of at least ten years after the completion or early termination of the research project.

9 FUNDING / PUBLICATION / DECLARATION OF INTEREST

A final report will be handed in to the CEC. In addition, the report will be submitted for publication.

Costs are covered by the research fund of the Frauenklinik Bern managed by Prof. Mueller. We declare no conflict of interest.

The test kits as well as the transportation cost and laboratory costs are covered by the labor team as co-author of the study.

10 REFERENCES

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