PAPNext

A Pap-Based DNA Test for Early Detection of Endometrial and Ovarian Cancers

TECHNICAL REPORT

Incidence and mortality of g

Despite the many recent advances in cancer diagnosis and treatment, g in women in the United Statesin part because there are no accurate screening methods for these cancers and they are often diagnosed at a late stage.**Endometrial cancer** is the most common gynecologic malignancy,deathsIt is often diagnosed at a late stage, when the 5-year survival rate is less than 30%.Worldwide, more than **200.000** deaths per year from g are expected[2,3].
undetected gynecologic malignancies has made the development of an effective screening tool a high priority.

Cervical fluid samples gathered during routine Pap tests are the basis of a screening test for gynecological cancers

Prevention and early detection remain essential to decreasing cancer mortality. For many years, researchers have strived to develop a feasible and reliable way to detect early-stage gynecological cancers.
The introduction of routine screening for cervical cancer with cytology (the Papanicolaou test, otherwise known as "**Pap smear**") has dramatically decreased the incidence and mortality of **cervical cancer** in the screened population, by permitting the detection of early-stage, surgically curable cervical tumors and their precursors. Unfortunately, the identification of malignant cells from **endometrial** and **ovarian** carcinomas in cervical cytology specimens is relatively uncommon. Microscopic examination cannot always distinguish them from one another, from cervical carcinomas, or from more benign conditions.
Screening DNA in Pap smears has the potential to increase the rate of **early-stage detection of endometrial and ovarian cancer**s in women who do not have any symptoms.
This DNA could be exploited to detect somatic mutations in tumor DNA released from endometrial and ovarian cancers shed cells accumulating in the cervix. These cells are sampled during routine Pap tests with a brush (a "Pap brush") that is inserted into the endocervical canal to scrape the surface of the cervix and then rinsed in a liquid-filled vial containing preservative fluid.
For the detection of cervical cancers, cells from the fluid are applied to a slide for cytological examination (the classic Pap smear). From the remaining sample, somatic mutations could be detected in tumor DNA of women with ovarian or endometrial cancers.

PAPnext™ test: a new dimension of screening for gynecological cancers

PAPnext™ is a screening test that identifies cancer-related alterations in DNA obtained from cervical fluids gathered during a routine Pap test.
PAPnext™ can detect endometrial and ovarian cancers at their early stage. Earlier detection of cancer could lead to earlier treatment and potentially better outcomes for patients.
PAPnext™ test leverages the existing cervical screening strategy with an advanced sequencing technology to assess for DNA mutations in **30 genes** (Table 1) that are commonly mutated in endometrial and ovarian cancers, providing a cost-effective screening approach for these gynaecologic malignancies.
PAPnext™ test is able to detect trace amounts of DNA from endometrial and ovarian cancers cells in Pap test samples, without previous knowledge of the tumor's genotype.

PAPnext™: Science behind the test

A recent study[4] demonstrated based on genetic analysis of DNA recovered from fluids obtained during a routine Papanicolaou (Pap) test.
When an advanced sequencing technology was used to screen Pap test samples, gathered from women endometrial and ovarian cancers, for somatic mutations in DNA, the assay identified cancer-related alterations in **93%** of women with **endometrial cancer** and **45%** of women with **ovarian cancer** . In addition, no cancer-related alterations were detected in samples collected from women without cancer, yielding a **very high specificity** (**>99.9%**)[5].

Eurofins Genoma Group S.r.l a socio unico

Legal Head Office
00138 Roma - Via di Castel Giubileo, 11
C.F. e P. Iva: 05402921000
REA: 883.955
Iscr. Reg. Impr. 369761/1997

Lab and Medical Practice Rome
00138 Roma - Via di Castel Giubileo, 11
Tel. +39 06 881 1270 (12 linee PBX) - Fax +39 06 6449 2025
Web: www.laboratoriogenoma.eu
E-mail: info@laboratoriogenoma.eu

Lab and Medical Practice Milan
20161 Milano - Affori Centre,Via Enrico Cialdini, 16
Tel. : + 39 02 3929 7626 - Fax : + 39 02 3929 76261
Web: www.genomamilano.it
E-mail info@genomamilano.it

PAPnext™: Benefits

PAPnext™ can be easily implemented as routine gynecological screening performed at the same time women undergo a Pap test for cervical cancer. The additional DNA analysis provided with **PAPnext™** test is noninvasive and easily administered in the context of an annual gynecologic examination.
PAPnext™ testing has the potential to improve the conventional cytology screening for cervical cancer through the unambiguous detection of DNA from endometrial and ovarian carcinomas
Most of the deaths are caused by tumors that metastatize prior to the onset of symptoms. **PAPnext™** test allows early detect endometrial and ovarian cancers at either a precancerous or early cancerous stage, when the disease is most curable
PAPnext™

PAPnext™:

PAPnext™ test is meant for preventative surveillance of high-risk populations. It may be beneficial for, but not limited to: a significant family history of endometrial and ovarian cancer.

- endometrial and ovarian cancer.

PAPnext™: Assay Method

The **PAPnext™** test is performed using highly advances Next Generation Sequencing (NGS) technology to screen for tumor DNA mutations in **30 genes** that are commonly mutated primarily in **endometrial, ovarian** and **cervical** cancer.
Cancer cells are sampled during routine Pap tests with a brush (a "**Pap brush**") that is inserted into the endocervical canal to scrape the surface of the cervix and then rinsed in a liquid-filled vial containing preservative fluid. The assay requires DNA extracted from cervical cells, gathered through a PAP brush, which is used for PCR amplification of both the wild type and mutant DNA. After NGS sequencing, tumor DNA mutation screening in the targeted genes is performed using an advanced bioinformatic analysis. Databases queried include Catalogue of Somatic Mutations in Cancer (COSMIC), The Cancer Genome Atlas (TCGA), cBioPortal, National Center of Biotechnology Information (NCBI), locus specific databases and other public databases.
PAPnext™ uses powerful custom-built bioinformatics solutions to support variant analysis that enables fast, reliable and highly accurate results. When a variant is detected during the sequencing process, its pathogenicity will be investigated using a sophisticated software.
The gene content was carefully selected to include content cited by **COSMIC** (***Catalogue Of Somatic Mutation In Cancer***) **database**. These genes and gene regions include single nucleotide variants (SNV) and insertions and deletions (indels) that have demonstrated involvement in tumors.

Target Coverage

Coverage is the number of times a region is sequenced (the number of reads) within a single run. In general, the deeper the coverage of a targeted region, the more sensitive and reliable the assay is. For variant calling, 8.000x coverage is required for reliable detection of mutations occurring at frequencies as low as 1%. To pass quality control (QC) metrics for the **PAPnext™** test, samples should yield > 8.000x coverage on > 93.5% of bases targeted by the assay.

Mutant Allele Fraction (MAF)

The mutant allele fraction is the frequency of the mutant allele identified in the sample and is reported for base substitutions, insertions and deletions.

Understanding PAPNext™ results

GENOMA's **PAPnext™** test reports on the absence or presence of somatic mutations in the genes screened. Mutant DNA percentage is also reported.
POSITIVE RESULT: This result shows that the test **identified clinically relevant somatic mutation(s)** in tumor DNA, in one or more of the targeted genes screened. A patient with a **positive test** result should be referred for genetic counselling before any medical decisions are made.
NEGATIVE RESULT: This result shows the test **has not detected any clinical relevant mutation** in the targeted genes screened. A single test cannot always detect all possible genetic changes that cause a particular cancer condition, hence a negative results do not completely rule out the presence of malignancies screened.

Eurofins Genoma Group S.r.l a socio unico

Legal Head Office
00138 Roma - Via di Castel Giubileo, 11
C.F. e P. Iva: 05402921000
REA: 883.955
Iscr. Reg. Impr. 369761/1997

Lab and Medical Practice Rome
00138 Roma - Via di Castel Giubileo, 11
Tel. +39 06 881 1270 (12 linee PBX) - Fax +39 06 6449 2025
Web: www.laboratoriogenoma.eu
E-mail: info@laboratoriogenoma.eu

Lab and Medical Practice Milan
20161 Milano - Affori Centre,Via Enrico Cialdini, 16
Tel. : + 39 02 3929 7626 - Fax : + 39 02 3929 76261
Web: www.genomamilano.it
E-mail info@genomamilano.it

Limitations and warnings

Cancer is heterogeneous disease that can occur as a result of somatic mutations in various driver genes. **PAPnext™** identifies somatic cancer derived mutations in 30 cancer driver genes (Table 1). This test is not meant to diagnose cancer, and is only meant to screen for a possible malignancy as an adjunct to other medical examinations and interventions. It will not detect all cancers, and has not been designed to find very small tumors. No test can replace a physician's examination, imaging studies, and tissue biopsies as the gold-standard for cancer diagnosis. It is possible that mutations in these or other genes not tested in GENOMA's **PAPnext™** test may be involved in the patient's disease. Therefore, a negative test result, where no mutations are detected, does not eliminate involvement of other genes and/or mutations. Furthermore, a positive test result needs to be interpreted in the context of individual's clinical history including stage of disease, imaging results, therapeutic details, and other laboratory data.
Results could be misinterpreted if clinical information provided is inaccurate or incomplete. Genetic counseling or medical consultation is recommended for the individual tested.

Technical limitations

Gene amplifications, translocations, and insertions or deletions over 25 bases in length are not detectable by this assay. Variants predicted to be non-deleterious (such as synonymous coding changes and common population variants) are not reported.
In validation studies, the analytical sensitivity and specificity of the targeted cancer gene assay were > 99% and > 99.9%, respectively. These may be lower for structural alterations and vary depending on the quality of the specimen. Next generation sequencing approaches may provide incorrect sequence or mutational data due to insufficient coverage in specific regions of the genome, inability to distinguish highly related human sequences, and sequencing errors.
The analysis of sequence specific alterations can also be hampered by three aspects related to the tumor DNA. First, the quality of tumor DNA obtained; second, the quantity of DNA obtained can be very low, limiting the amount of DNA molecules that can be successfully analyzed by next generation sequencing. Third, the purity of tumor DNA can be a factor, as a significant portion of the DNA analyzed in the tumor sample may be derived from contaminating normal tissues. These three aspects can reduce the chance of detecting somatic sequence and copy number alterations and rearrangements.
Genetic alterations are defined as clinically significant based on published literature and other evidence. Literature references are not comprehensive and there may be other studies that relate to the test results. This test, meant to identify somatic mutations, is not intended to detect the presence or absence of germline mutations.

Genetic Counselling

Genetic counselling is essential for any patient. Genoma will provide a genetic counselling session for those patients that screen positive, and the service is included in the cost of the test. It aids the patients in medical comprehension and enhances patient satisfaction by providing access to experts who are skilled at explaining genetics risk in terms patients can understand.

Disclaimer

Results presented in this report are intended for use solely by a qualified health care professional. Any diagnosis, counseling, or treatment determination made as a result of data presented in the report should be made by a qualified health care professional in conjunction with other individual patient health information, including clinical presentation and other test reports. Information contained within the report is current as of the report date; a qualified health professional should reassess these data as relevant literature becomes available.

Bibliography

1) Howlader et al. SEER Cancer Statistics Review, 1975–2014 (National Cancer Institute, 2017).
2) Bray et al.. Int. J. Cancer 10.1002/ijc.27711 (2012).
3) International Agency for Research on Cancer, GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10; http://globocan.iarc.fr.
4) Kinde et alSci Transl Med. 2013 Jan 9;5(167):167ra45)
5) Wang et al. Sci Transl Med. 2018 Mar 21;10(433). pii: eaap8793.
6) Westin et al. 2013 Sci. Transl. Med. 5, 167ps1

Eurofins Genoma Group S.r.l a socio unico

Legal Head Office
00138 Roma - Via di Castel Giubileo, 11
C.F. e P. Iva: 05402921000
REA: 883.955
Iscr. Reg. Impr. 369761/1997

Lab and Medical Practice Rome
00138 Roma - Via di Castel Giubileo, 11
Tel. +39 06 881 1270 (12 linee PBX) - Fax +39 06 6449 2025
Web: www.laboratoriogenoma.eu
E-mail: info@laboratoriogenoma.eu

Lab and Medical Practice Milan
20161 Milano - Affori Centre,Via Enrico Cialdini, 16
Tel. : + 39 02 3929 7626 - Fax : + 39 02 3929 76261
Web: www.genomamilano.it
E-mail info@genomamilano.it