Ziwig
INNOVATING FOR WOMEN'S HEALTH

Diagnosis of endometriosis:

ENDOTEST®
A global innovation

PRESS KIT
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Saliva Test for the Diagnosis of Endometriosis:
A Significant Advancement in Women's Health

"Better detection of disease": is one of the essential focus points of the National Strategy to Combat Endometriosis launched by the President of France, Emmanuel Macron, on January the 11th 2022.

Women suffering from endometriosis, for the most part, go through a long medical journey (8 to 12 years on average), during which time they see multiple physicians and undergo numerous tests and examinations before a diagnosis, which most often requires surgery, is finally reached.

A global innovation now allows women suffering from endometriosis to avoid this tortuous medical journey. A French team, made up of expert doctors in endometriosis and engineers in artificial intelligence, has recently developed a saliva based diagnostic test that uses microRNA sequencing.

This simple and non-invasive saliva test, called ENDOTEST®, has been validated by the largest clinical trial ever carried out in this field. The ENDOTEST® allows for the early detection of all types of endometriosis, including the most complex forms, with a reliability of close to 100%.

This tool will revolutionize the diagnosis and the care for endometriosis, and is set to constitute a major advancement in women's health in the near future.

The Tortuous Journey of Women with Endometriosis

Pain and Infertility at the Forefront

Endometriosis affects 10% of women of childbearing age in France, i.e. 1.5 to 2.5 million women. It potentially affects all women and can begin as early as from adolescence.

Endometriosis is characterized by the growth of tissue fragments similar to the endometrium (the mucosa which lines the inside of the uterus) outside of the uterine cavity (womb) on different organs: ovaries, vagina, rectum, bladder, intestines, lungs, etc. During menstruation, these tissue fragments respond to hormonal variations, causing inflammation, and leading to **intense and debilitating pain** associated with a wide variety of symptoms.

Intense menstrual pain	**75 - 90%**
Pain during intercourse	**30 - 70%**
Pelvic pain	**40%**
Digestive disorders	**35%**
Intense tiredness	**15%**
Urinary disorders	**3%**
Infertility	**30 - 40%**

Symptoms of endometriosis and approximate proportion of women affected (according to [3])

The disease can also be completely asymptomatic. In such cases, it is generally discovered fortuitously during a consultation prompted by trouble conceiving. A significant proportion of patients with endometriosis also suffer from **infertility.**

Three forms of endometriosis are classically described: superficial or peritoneal endometriosis, ovarian endometriosis, and deep endometriosis. There is no observable correlation between symptoms and the severity of the disease[3].

The Aggravation of Symptoms over Time

Endometriosis can, in about a third of the cases (especially in superficial forms), stabilize or even regress spontaneously or after medical treatment. In general, however, symptoms, notably pain, get worse over time.

The persistence of pain induces a phenomenon of hypersensitivity: pain thresholds decrease, leading to **the onset of chronic pain.**

Chronic pain can emerge at any stage of endometriosis and last even after the endometriotic lesions are no longer visible[4,5].

Hypersensitivity is prompted by the persistence of pain over time, and thus evolving into chronic pain.

Treatments that target pain

Endometriosis remains without a cure and the only therapies available are symptomatic treatments. In addition to the analgesic treatment adapted to each patient, hormonal treatments, which are based on the use of estrogen-progestin contraceptives or continuous administration of progestogens, aim to stop menstrual cycles. Treatments such as Gn-RH agonists can be used as a second line, with the effect of putting patients in a state of temporary menopause. Hormonal treatments have two disadvantages; significant side effects (hot flashes, weight gain, mood disorders, cardiovascular impact, osteoporosis, joint disorders, etc.) have been documented as well as inconsistent efficacy, which widely varies from one patient to another and is difficult to predict[6].

Surgery is considered when medical treatments provide insufficient pain relief. The objective of surgery is to remove endometriotic lesions and correct anatomical abnormalities caused by the disease; in particular, adhesions that reduce the mobility of organs. Endometriosis surgery is most often performed by laparoscopy[6].

In the event of infertility or if pregnancy is desired, assisted reproductive treatments can be offered. This care involves in particular ovarian stimulation (in mild to moderate endometriosis) or in vitro fertilization (in more severe cases).

A Significant Impact on Personal and Social Life

Endometriosis leads to a significant deterioration in quality of life. Pain is responsible for sleep disorders that induce chronic fatigue and psychological disorders (irritability, depression, etc.), which are the cause of a deterioration in private and public relationships. Patients' sex lives are also greatly affected, often with major repercussions on a couple's wellbeing. Infertility and the uncertainties related to assisted reproductive treatments also are a source of significant stress[3].

The impact of endometriosis on a patients' working life is significant with many missed school/work days. In Western countries, the average annual cost of endometriosis is €9,579 per patient, with an average of 33 days of sick leave per year. The overall annual societal cost of endometriosis is estimated at 10.6 billion Euros in France[7,8].

A Long Diagnostic Delay

The time between the onset of the first symptoms and the diagnosis of endometriosis is generally very long (8 to 12 years on average). There are several reasons for this delay:

- Endometriosis is a multifactorial and **complex disease** the origins of which remain partly unknown.
- It is also a polymorphic disease, which can take several variable forms from one patient to another. It is not characterized by any specific symptom, which makes it difficult to diagnose.
- It is a pathology often **unknown to frontline healthcare professionals** who tend to minimize the pain described by patients and delay referral to a specialist.
- Finally, diagnosis most often requires the intervention of expert doctors who generally have long waiting times[3].

It takes 8 to 12 years on average before a diagnosis of endometriosis is made.

• 7 medical consultations
• 3 ultrasounds
• 4 MRIs
• 1 surgery

This is the number of steps included in an average diagnostic pathway, for a cost of approximately €5,000.

Patients with endometriosis thus suffer a diagnostic delay of 8 to 12 years on average[9], during which time they see multiple practitioners and undergo numerous medical examinations. During this diagnostic delay, symptoms tend to worsen, the disease becomes chronic, and quality of life inevitably deteriorates.

Currently, diagnosis is based on **medical imaging.** The most commonly used examinations are endovaginal pelvic ultrasound and pelvic MRI. In all cases, these examinations must be performed and interpreted by a referring radiologist with extensive experience in endometriosis[6].

The diagnostic performance of medical imaging is sufficient for certain forms of endometriosis (ovarian cysts, deep endometriosis) but insufficient for others; in particular, for the detection of peritoneal endometriosis[6], which constitutes an early stage of the disease.

In many cases, it is therefore necessary to turn to **laparoscopy.**

Laparoscopy, a surgical technique that involves the insertion of a miniature camera through a small incision in the navel, makes it possible to directly visualize the lesions, take biopsies, and perform curative surgical procedures[6].

Laparoscopy, an invasive procedure, performed under general anesthesia, is currently considered to be the primary examination for the diagnosis of endometriosis.

Circulating MicroRNAs: a New Class of Biomarkers

The Need for a Non-Invasive Diagnostic Test

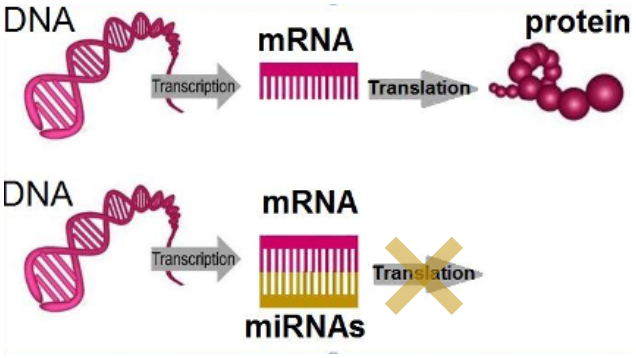
Laparoscopy is currently recognized as the primary examination for the diagnosis of endometriosis[10]. It is nevertheless an invasive procedure that must be performed under general anesthesia and can, like any surgical procedure, be accompanied by per or post-operative complications.

The development of a non-invasive diagnostic test for endometriosis has therefore been a major medical priority for many years. More than a hundred potential biomarkers (angiogenesis factors, growth factors, hormonal, immune, and inflammatory markers, etc.) have been studied over the past decades[10].

Among these biomarkers, a new class of molecules discovered in 2000, microRNAs, has emerged as a promising option, supported by a growing quantity of evidence from studies of cancer and degenerative disorders[11,12].

MicroRNAs at the Heart of the Disease

MicroRNAs (miRNAs) are small non-coding RNAs: unlike messenger RNAs (mRNAs, nowadays used in several COVID vaccines), they are not converted into proteins by the cellular machinery. On the contrary, their role is to suppress gene expression: when a miRNA binds to its target (a specific messenger RNA) it blocks its transduction of proteins and/or induces its degradation[12].



The regulation of gene expression by miRNAs

miRNAs are also present in the extracellular medium within different transport structures, that protect them from circulating RNases enzymes and gives them remarkable stability. These circulating miRNAs are found in varying amounts in most biological fluids (blood, urine, breast milk, tears, saliva, etc.)[11,12].

miRNAs play an important role in vital biological pathways (such as differentiation, proliferation, and cell death, or the metastasis process) as wall as in the pathophysiology of many diseases (type 2 diabetes, cancers, cardiovascular diseases, etc.).

In recent years, increasing evidence has supported the **involvement of miRNAs in the cellular mechanisms of endometriosis.** A direct link between the deregulation of certain miRNAs and the development of endometriosis[13,14] lesions has been established.

Several preliminary studies have also shown the benefit of using miRNAs in the diagnosis of the disease.

Unfortunately, due to methodological weaknesses (limited number of patients, limited amount of miRNAs studied, etc), these studies have not made it possible to develop a diagnostic test usable in the general population[13,14,15].

There is a direct link between the deregulation of certain miRNAs and the development of endometriosis lesions.

A Global Innovation Based on 2 Disruptive Technologies

Next-Generation Sequencing and Artificial Intelligence

In January 2021, a French research team combining endometriosis expert doctors and artificial intelligence engineers from the start-up ZIWIG, launched the Endo-miRNA clinical trial[16].

This study stood out from the previous trials through the use of two advanced technologies:

- **Next-generation Sequencing** (NGS), or massive parallel sequencing, which allows for the simultaneous acquisition of data relating to millions of DNA or RNA fragments.
- **Artificial intelligence** (combined with machine learning), which allows for the analysis of the large volumes of data produced by next-generation sequencing.

Next-generation sequencing and artificial intelligence:

2 cutting-edge technologies to assess the microARNome.

The use of this combination of these two technologies provides a holistic approach, analyzing the entire human capital of miRNA (the microARNome), i.e. more than 2600 miRNAs.

A Reliable Signature for the Efficient Detection of Endometriosis

The objective of **the prospective Endo-miRNA clinical trial**[16,17] was to analyze the human microARNome in order to distinguish patients with endometriosis from non-affected patients, and to develop a diagnosis based on blood and saliva miRNAs.

This study, the largest in the world in terms of the number of patients, included 200 women with chronic pelvic pain alluding to endometriosis.

The diagnosis of endometriosis was established, either on the basis of a laparoscopy with biopsy, or on the basis of an MRI showing images characteristic of endometriosis. It was confirmed through a blind test by a multidisciplinary committee of experts.

This radiological or surgical diagnosis allowed for the identification of a group of 153 patients with various forms of endometriosis from a control group of 47 women who did not have the disease. Blood and saliva samples were collected from all women. The analysis of the miRNAs in these samples was carried out double-blind without knowledge of diagnostics.

109 miRNAs from more than 2600 human miRNAs involved in the pathophysiology of endometriosis have been identified in saliva, and 86 in blood, providing a reliable signature of the disease.

The diagnostic performance of the saliva test (sensitivity of 97%, specificity of 100%, AUC of 98%) proved to be superior

A reliable test almost up to 100% to detect all types of endometriosis, even complex ones (inconsistent/ contradictory)

to that of all currently available diagnostic tools (including laparoscopy, MRI, and pelvic ultrasound).

Saliva testing detects all types of endometriosis, from superficial to deep forms, and it is an efficient diagnostic tool for complex cases among patients with pain suggestive of endometriosis but with a negative clinical examination and medical imaging.

A Simple Saliva Sample

Saliva offers a high concentration of miRNA and excellent stability, even after long storage[11,18]. Saliva sampling is non-invasive and inexpensive method that can easily be carried out, anywhere, by patients themselves. The idea of a saliva test therefore naturally prevailed over a blood test: an invasive procedure requiring complex and expensive logistics of collection, storage, and transport. **Thus, a global innovation was created: the Endotest® Saliva Test.**

The Endotest® is particularly simple to use : women collect saliva samples themselves at home using a self-collection kit. Then, they send the kit back to the laboratory, which confirms or disproves the diagnosis of endometriosis in a matter of a few days, with a precision close to 100%.

A new study involving 1,000 women was launched at the end of 2021 in five French endometriosis centers. This study should provide new data in particular with respect to patients' suffering from endometriosis with fertility trouble.

A Revolution in Endometriosis Care

Over and above a simple diagnosis, the Endotest® is set to improve overall care for endometriosis.

This major innovation will in fact limit diagnostic delays and **pave the way for early treatment of endometriosis,** making it possible to slow down or even stop the aggravation of pain (by reducing the risk of hypersensitivity) and other symptoms, optimize infertility care, and limit the deterioration of patients' quality of life.

The Ziwig Endotest® Saliva test is set to shorten the average time to diagnosis from 8 years to few days.

The Endotest® is set to make diagnostic laparoscopy obsolete, unnecessary surgeries can now be avoided, especially for patients with symptoms similar to endometriosis but who are not carriers of the disease.

The Ziwig Endotest® has *CE* rating, and its' implementation is currently the subject of a consultation with the French health authorities regarding its inclusion in the care pathway and its possible reimbursement by health insurance.

This revolutionary tool will, in the long term, allow for a significant reduction in the medico-social cost of endometriosis and contribute to the improvement of healthcare by allowing all patients, including those residing in medical deserts, to benefit from a reliable, early and non-invasive diagnostic tool.

Thus, Endotest® will revolutionize endometriosis diagnosis and care, and constitutes an important advancement in women's health.

About Ziwig

ZIWIG is a French company with an ambition to improve women's health.

This goal, constantly guided by the quest for excellence, relies on cutting-edge technologies such as next-generation sequencing of human microRNAs and artificial intelligence in order to develop innovative and effective diagnostic or prognostic tools.

Ziwig contributes to the emergence of precision medicine that is individualized, predictive, and participatory, serving women's well-being and quality of life.

All data collected by Ziwig is secure, anonymized, and encrypted, in accordance with the general data protection regulations of the National Commission for Information and Liberties. The servers that host this data are all located in Europe. They are certified HDS (Health Data Hosts) by the Digital Health Agency.

www.ziwig.com

Bibliographic references

1 *Zondervan KT et al. Nat Rev Dis Primers 2018;4(1):9*

2 *https://solidarites-sante.gouv.fr/soins-et-maladies/prises-en-charge-specialisees/endometriose*

3 *https://www.endomind.eu/wp-content/uploads/2019/02/Reflexions_sur_lendiometriose_en_france.pdf*

4 *https://www.who.int/fr/news-room/fact-sheets/detail/endometriosis*

5 *https://www.endofrance.org/wp-content/uploads/2020/07/Hypersensibilisation_Endofrance-SPloteau.pdf*

6 *Haute Autorité de Santé. Prise en charge de l'endométriose. Fiche de synthèse. Décembre 2017. https://www.has-sante.fr/upload/docs/application/pdf/2018-01/prise_en_charge_de_lendiometriose_-_de-marche_diagnostique_et_traitement_medical_-_fiche_de_synthese.pdf*

7 *Kanj O. Evaluation économique de la prise en charge de l'endométriose. https://tel.archives-ouvertes.fr/tel-02080162v2/document*

8 *Fourquet J et al. Fertil Steril 2010; 93(7): 2424–2428. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2860000/pdf/nihms147074.pdf*

9 *Kiesel L et al. Climacteric 2019;22(3):296-302.*

10 *Nisenblat V et al. Cochrane Database Syst Rev 2016;2016 (5):CD012179.*

11 *Baulande S et al Medsci 2014; 30 : 289-296*

12 *Agrawal S et al.Int J Mol Sci 2018;19(2):599*

13 *Panir K et al. Hum Reprod Update 2018;24(4):497-515.*

14 *Dana PM et al. Biomark Med 2020;14(13):1277-1287.*

15 *Moustafa S et al. Am J Obstet Gynecol 2020;223(4):557.e1-557.e11*

16 *Bendifallah S. et al. J Clin Med 2022 ; 11(3):612. https://doi.org/10.3390/jcm11030612*

17 *Dabi Y. et al. Diagnostics (Basel) 2022 ; 12(1):175. https://doi.org/10.3390/diagnostics12010175*

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