

# EndomeTRIO Manual

## A complete view of the endometrial health

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# ERA<sup>®</sup>

## Endometrial Receptivity Analysis

# Rationale

**The endometrial factor plays a key role in embryo implantation. In addition to evaluating malformations or anomalies in the uterine cavity, it also determines when the endometrium is receptive, i.e. the window of implantation (WOI).** Recurrent implantation failure (RIF) patients may have a displaced window of implantation, leading to embryo transfer into a non-receptive endometrium (Ruiz-Alonso et al. Fertil Steril, 2013).

**The endometrial gene expression signature allows evaluation of endometrial receptivity, identifying a personalized window of implantation for each patient.**

This analysis is carried out by a tool designed, developed and patented in 2009 (PCT/ES2009/000386) by Igenomix, after more than 10 years of research (Diaz-Gimeno et al. Fertil Steril, 2011; 2013).

To identify the window of implantation in the endometrial cycle, enabling personalized embryo transfer (pET).

**Research by Igenomix has demonstrated that synchronization between an implantation-ready embryo and a receptive endometrium increases the chances of success in an assisted reproductive treatment** (Ruiz-Alonso et al. Fertil Steril, 2013; Ruiz-Alonso et al. Hum Reprod, 2014; Clemente-Ciscar et al. Hum Reprod, 2018; Simon et al. Reprod BioMed Online, 2020).

Other groups have also published similar results from their own patients after guided embryo transfer according to ERA results (Mahajan J Hum Reprod, 2015; Hashimoto et al. Reprod Med Biol, 2017; Findikli et al. Hum Reprod, 2018; Pasternak et al. Fertil Steril, 2018; Taguchi et al. Fertil Steril, 2018; Jia et al. Med Sci Monit, 2022).

**ERA (Endometrial Receptivity Analysis), determines the optimal time in the endometrial cycle to perform embryo transfer.** Thus, ERA can increase the chances of pregnancy by synchronizing an implantation-ready embryo with a receptive endometrium.

# Indications for ERA

ERA is indicated for **patients with recurrent implantation failure (RIF)**, since they are at higher risk of having a displaced window of implantation (Ruiz-Alonso et al. Fertil Steril, 2013). Therefore, this analysis could be beneficial for patients with 2 previous failed cycles with their own oocytes or 1 previous failed cycle with ovum donation, in which good-quality embryos were transferred. On the other hand, the application of ERA to patients without RIF has also been explored (Simon et al. Reprod BioMed Online, 2020).

Our studies have shown there are other circumstances in which patients are at higher risk of having a displaced WOI. In these cases, ERA could help to find the optimal moment for the embryo transfer:

- Patients with **BMI > 30** (Comstock et al, 2017; Bellver et al, 2021)
- Patients with **endometrial atrophy** (endometrial thickness < 6 mm) (Valbuena et al, 2016)
- Patients with **adenomyosis** (Mahajan et al, 2018)
- Patients with **recurrent biochemical pregnancies** (Diaz-Gimeno et al, 2017)

# Methodology

This test uses **Next Generation Sequencing (NGS) technology to analyze the expression of 248 genes related to endometrial receptivity status.**

The results from this test are based on the expression analysis of these 248 genes with a computational predictor designed and developed by Igenomix. After sequencing the genetic material (RNA) from an endometrial biopsy, it is possible to evaluate if the endometrium is Receptive or Non-receptive at any specific time during the endometrial cycle. **This result will be coupled to a recommendation for personalized embryo transfer according to each patient's specific endometrial profile.** In 10% of cases, it may be necessary to validate the personalized window of implantation by performing a second endometrial biopsy on the specific day designated by the first ERA test.

**To enable reproducibility of results, the ERA test must be performed under identical conditions as the subsequent embryo transfer cycle** (cycle type, treatment, method of administration...), **and always during a hormone replacement therapy (HRT) or natural cycle.** This test can not be performed in controlled ovarian stimulated cycles.

**For certain subsequent ERA mock cycles, to finalize diagnostics, our team may contact you to collect information about the patient's mock cycle.** This is to understand if the protocols were replicated exactly in order to know if the results from the cycles can be correlated or not. **Correlation of the results helps our diagnostic team to review both results and provide a more personalized recommendation,** for example in the case of a narrow WOI. Without confirming this information and considering correlation, we may be missing a special observation that requires a more personalized recommendation for transfer, beyond what is provided by our algorithm.

The **first endometrial biopsy should be taken after 5 full days with progesterone administration (P+5) in an HRT cycle (120 hours with progesterone administration). In Natural cycles, the first endometrial biopsy should be taken 7 days (168 hours) after the hCG triggering (hCG+7) or after the LH peak (LH+7). It could be also taken 6 days after ovulation confirmed by ultrasound** (although this last option is not optimal because it is difficult to ensure reproducibility of the results).

**If day-3 embryos are to be transferred, the biopsy should be performed at P+5 or hCG+7, since the ERA checks the endometrium at the moment of implantation.** This way, if you have a receptive result at P+5, you will transfer a blastocyst at P+5 or a day-3 embryo two days earlier, i.e. at P+3.

# Interpretation of the results

**Receptive:** The gene expression profile is concordant with a receptive endometrium. The recommendation is to perform a blastocyst(s) transfer following the same protocol and timings utilized during the ERA test.

**Late Receptive:** The gene expression profile is concordant with an endometrium at the end of the receptive stage. The recommendation is to administer progesterone (HRT) or rest (natural cycle) for 12 hours less relative to when the biopsy was taken before performing a blastocyst(s) transfer.

**Pre-receptive:** The gene expression profile is concordant with an endometrium at a pre-receptive stage. This could be due to a displacement of the window of implantation. In around 5% of cases (when this displacement implies 2 days) a new endometrial biopsy is required for validation.

**Post-receptive:** The gene expression profile is concordant with an endometrium at a post-receptive stage. This could be due to a displacement of the window of implantation. To confirm this result, the analysis of a second biopsy on the recommended day is needed.

**Proliferative:** The gene expression profile is concordant with an endometrium at a proliferative stage. It is recommended to contact the ERA laboratory to evaluate the protocol in which the endometrial biopsy was performed.

\* In approximately 3.3% of the samples received, a diagnosis cannot be obtained, this is due to obtaining a non-informative profile or the low quantity/quality of genetic material obtained.

\* Following the recommendations of the ERA report does not guarantee implantation. Implantation failures may be caused by other factors.

We follow strict quality criteria ensuring that the RNA integrity and quantity are adequate avoiding potential artefactual results which could negatively affect the clinical outcome of your patients.

**Invalid RNA.** In transcriptomics analysis (whatever the technique) it is needed a proper RNA integrity to ensure reliability of the result. In cases in which the RNA is highly degraded the obtained gene expression profile wouldn't be trustable. This occurs in approximately 1.2% of samples received. In these cases, it is necessary to evaluate a new endometrial biopsy (but no charge will be applied). Possible causes: sample size too large, contamination, and/or high temperature ( $\geq 35^{\circ}\text{C}$ ) during shipment.

**Insufficient RNA.** Although with NGS the minimum quantity of RNA necessary to proceed with the analysis is very low, sometimes a low RNA concentration can lead to an inaccurate result. Our strict control systems allow us to identify the reliability of the obtained result. Just in around 1.5% of received samples it is not possible to determine an accurate gene expression profile because there is not enough genetic material. In these cases, it is necessary to evaluate a new endometrial biopsy (but no charge will be applied). Possible causes: low quantity of proper tissue.

**Non-Informative.** This result is obtained when the profile analyzed does not match the control gene expression profiles present in the ERA predictor. In these cases, our ERA team will contact you to evaluate the protocol in which the endometrial biopsy was performed. It just happens in  $< 0.7\%$  of analyzed samples and in  $>95\%$  of cases, it is related to the sample itself, not the endometrium, since with a new biopsy (without charge) it is possible to obtain a valid result.

In any of these cases our ERA team will support and guide you, ensuring that we can find a valid result for your patient, trusting on that we are looking for quality and reliability.

# ERA Example Report

The aim of this test is to provide physicians with an objective molecular diagnosis of the patient's endometrial reproductive health.

This test must be prescribed and interpreted by the physician who will perform the subsequent embryo transfer.

CLIA #9902146167





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### ERA (ENDOMETRIAL RECEPTIVITY ANALYSIS)

Patient information	Sample information	Clinic information
Unique pat id.:	Date received:	Clinic:
Sample type:	Report Date:	Clinician:
Patient name:	First intake of P4:	No. biopsies:
Patient DOB:	Date of biopsy:	
	Cycle type:	

TEST RESULTS:

POST-RECEPTIVE

Recommendation: To perform a new endometrial biopsy 1 day earlier than the time at which this endometrial biopsy was performed (125 ± 3 hours of progesterone administration)\*\*



INTERPRETATION OF YOUR RESULT:

The gene expression profile is concordant with an endometrium that has passed its receptive stage, meaning this patient may have a displaced window of implantation. A new endometrial biopsy is recommended to validate this displacement and to guide the personalized embryo transfer. The new biopsy should be performed 1 day earlier than the time at which this endometrial biopsy was performed.

\*\* This recommendation is only applicable to the same type of cycle treatment as the one used for this endometrial biopsy and if the endogenous progesterone measured prior to the first progesterone intake is <1ng/ml.

TEST DESCRIPTION:

ERA (Endometrial Receptivity Analysis) is a molecular tool used to determine if the endometrium (the mucous membrane lining the womb) exhibits a receptive profile after 5 days of progesterone exposure, the time at which the endometrium is typically ready for embryo implantation. This molecular diagnosis method is based on measuring the gene expression profile of endometrial tissue. Therefore, ERA helps to determine when the endometrium presents the ideal condition for embryo implantation, increasing the possibility of a successful in vitro fertilization treatment.

COMMENTS

None

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In order to obtain a pET recommendation expressed in hours, we need the date and time of the endometrial biopsy and one of the following (depending on the cycle type):

- Date and time of the first P4 intake (HRT cycle)
- Date and time for hCG injection, LH surge or ovulation (Natural cycles)

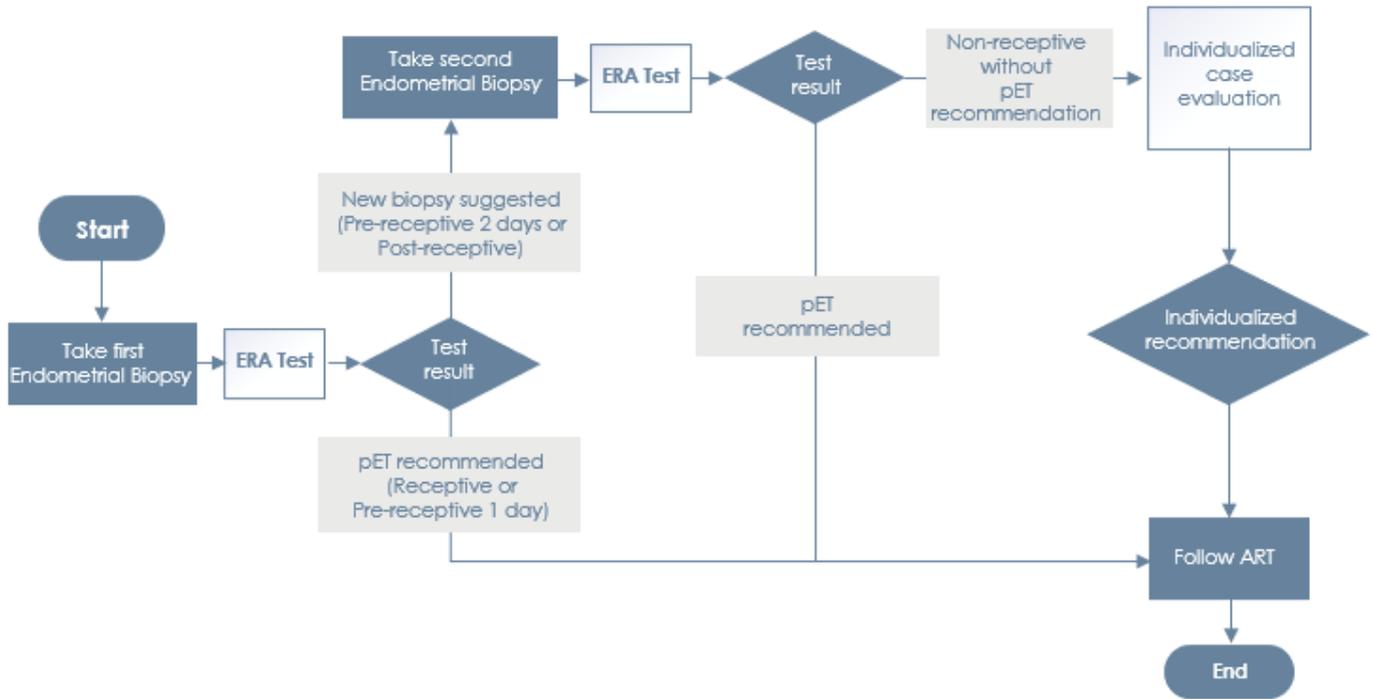
The ERA report will indicate the optimum time to perform personalized embryo transfer (pET), or when to perform a new ERA biopsy (as appropriate).

# Reproducibility of the results

The ERA result has been **proven to be reproducible for at least 36 months**, always that the following is accomplished:

- **Endometrial preparation protocol** must be exactly replicated for biopsy and transfer cycles.
- **Endometrial thickness** must be within the same range from one of the following three: **<6mm, 6-12mm, >12mm**; in both, biopsy and transfer cycles.
- **Changes in the BMI might be accompanied by a shift in the window of implantation.** The ERA test might need to be repeated after significant BMI changes (changing from > 30 to < 30) to ensure accuracy of the results.
- **Intervention at the uterine level** may affect the WOI. After this type of intervention, it should be evaluated if a new ERA needs to be performed. Indeed, if your patient requires any **intervention at the uterine level** prior the embryo transfer, the ERA test should be done after this procedure.
- **Endogenous progesterone** properly controlled in biopsy and transfer cycles, **it must be < 1 ng/ml** within the 24 hours prior the first progesterone intake (HRT cycles) or at LH+0/hCG+0 (Natural cycles).

# ERA decision tree



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# EMMA

## Endometrial Microbiome Metagenomic Analysis



# Rationale

The Human Microbiome Project (HMP) has highlighted **the importance of different microorganisms and their genomes in human health and disease** (Human Microbiome Project Consortium, 2012).

Identification of dysbiotic or pathogenic microbiomes may be key to improving clinical outcomes in various areas of medicine.

Recent research has **identified the existence of an endometrial microbiome** and has demonstrated that dysbiosis of the uterine cavity is associated with poor reproductive outcomes in assisted reproductive treatment patients. This suggests that pathogenic variations of endometrial Lactobacilli levels could play a role in infertility (Moreno et al. Am J Obstet Gynecol, 2016).

EMMA (Endometrial Microbiome Metagenomic Analysis) can determine if the uterine microbial environment is optimal for embryo implantation.

EMMA provides a complete view of the endometrial bacterial composition, including pathogens causing chronic endometritis (CE) that can be specifically investigated in ALICE.

## Indications for EMMA

The impact of the endometrial microbiome in patients with Repeated Implantation Failure (RIF) has been demonstrated (Moreno et al. Am J Obstet Gynecol, 2016). However, **EMMA can be beneficial for any patient wishing to conceive**, by assessing the microbiological environment that the embryo will encounter at implantation.

## Methodology

The **EMMA is a molecular test** that provides microbiota information in endometrial tissue by analyzing a customized panel of bacteria including information about the most frequent *Lactobacillus* species and potentially pathogenic bacteria of the reproductive tract (some of them related to with chronic endometritis). This method is **based on detecting bacterial DNA through RT-PCR, which translates into different profiles that have been linked to the success of pregnancy. DNA extraction followed by microorganism-specific amplification** enables the quantification of targeted bacteria present in a sample.

A single endometrial sample contains both endometrial and bacterial cells. These can be analyzed to predict both endometrial receptivity (using ERA test) and endometrial microbiome. **EMMA thus provides a microbiological view of the endometrium, to improve clinical management of patients.**

# EMMA Example Report

The EMMA report shows 3 tables with the reference ranges and the values obtained for each bacteria of the panel through the analysis of an endometrial sample:



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### ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

Patient information	Sample information	Clinic information
<b>Unique pat id:</b>	<b>Date received:</b>	<b>Clinic:</b>
<b>Patient name:</b>	<b>Report date/time:</b>	<b>Clinician:</b>
<b>Patient DOB:</b>	<b>Sample type:</b> Endometrial Biopsy	
<b>Allergic to antibiotics:</b>	<b>Cycle type:</b>	
	<b>Cycle day:</b>	
	<b>No. Biopsy:</b>	
	<b>Date of biopsy:</b>	

**RESULTS OF EMMA TEST:**

LACTOBACILLUS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Lactobacillus crispatus</i>	Not detected	N/A	≥ 3.71
<b><i>Lactobacillus gasseri</i></b>	<b>Detected</b>	<b>2.71*</b>	≥ 3.60
<i>Lactobacillus iners</i>	Not detected	N/A	> 3.57
<b><i>Lactobacillus jensenii</i></b>	<b>Detected</b>	<b>3.00*</b>	≥ 3.70

PATHOGENS OF THE REPRODUCTIVE TRACT			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Actinomyces israelii</i>	Not detected	N/A	absent
<i>Atopobium vaginae</i>	Not detected	N/A	≤ 3.57
<i>Bacteroides fragilis</i>	Not detected	N/A	≤ 3.57
<i>Bifidobacterium spp</i>	Not detected	N/A	≤ 4.22
<i>Clostridium sordelii</i>	Not detected	N/A	absent
<i>Dialister spp</i>	Not detected	N/A	≤ 3.57
<i>Fusobacterium nucleatum</i>	Not detected	N/A	absent
<i>Gardnerella vaginalis</i>	Not detected	N/A	≤ 3.72
<i>Haemophilus ducreyi</i>	Not detected	N/A	absent
<i>Mycobacterium tuberculosis</i>	Not detected	N/A	absent
<i>Mobiluncus spp</i>	Not detected	N/A	≤ 3.57
<i>Peptostreptococcus anaerobius</i>	Not detected	N/A	≤ 3.57
<i>Porphyromonas asaccharolytica</i>	Not detected	N/A	≤ 3.57
<i>Prevotella bivia</i>	Not detected	N/A	≤ 3.57
<i>Prevotella disiens</i>	Not detected	N/A	≤ 3.57
<i>Sneathia spp</i>	Not detected	N/A	≤ 3.57
<i>Treponema pallidum</i>	Not detected	N/A	absent

**RESULTS OF ALICE TEST :**

PATHOGENS RELATED TO CHRONIC ENDOMETRITIS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Chlamydia trachomatis</i>	Not detected	N/A	absent
<b><i>Enterococcus faecalis</i></b>	<b>Detected</b>	<b>3.82*</b>	≤ 3.63
<i>Escherichia coli</i>	Not detected	N/A	≤ 3.58
<i>Klebsiella pneumoniae</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma genitalium</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma hominis</i>	Not detected	N/A	≤ 3.57
<i>Neisseria gonorrhoeae</i>	Not detected	N/A	absent
<i>Staphylococcus aureus</i>	Not detected	N/A	≤ 3.57
<i>Streptococcus agalactiae group B/ Streptococcus viridans</i>	Not detected	N/A	≤ 3.57
<i>Ureaplasma urealyticum</i>	Not detected	N/A	≤ 3.58

\*Values out of reference range.

### RECOMMENDATION

Igenomix recommends performing the embryo transfer in a Lactobacillus-dominated endometrium to increase the chances of achieving a successful pregnancy (1,2,3). According to the standard Microbiology Guides (4,5), in case of possible infection by bacteria of which we have detected DNA, the suggested treatment would be amoxicillin 500mg/8h for 7 days, orally. To subsequently recolonize the reproductive tract, it is also suggested the use of vaginal probiotics only made by Lactobacillus strains, following the manufacturer's instructions regarding dose and duration of the treatment. The analysis of a new biopsy is also recommended after treatment. The new sample must be taken following the standard test protocol.

The **reference ranges** for all bacteria included in the EMMA test have been calculated analyzing samples from women with a live birth that belong to a clinical study which led in the scientific publication *Moreno et al, Microbiome 2022*.

The EMMA test results include information about:

1) **Table 1**: the **amounts of *Lactobacillus*** most frequently found in the reproductive tract.

<b>LACTOBACILLUS</b>			
<b>BACTERIA</b>	<b>RESULT</b>	<b>VALUE</b>	<b>REFERENCE RANGE</b>
<i>Lactobacillus crispatus</i>	Not detected	N/A	≥ 3.71
<b><i>Lactobacillus gasseri</i></b>	<b>Detected</b>	<b>2.71*</b>	≥ 3.60
<i>Lactobacillus iners</i>	Not detected	N/A	> 3.57
<b><i>Lactobacillus jensenii</i></b>	<b>Detected</b>	<b>3.00*</b>	≥ 3.70

**\*Values out of reference range.**

Values **out of the reference range** are identified in bold and with an asterisk:

- **If at least one of the *Lactobacillus* species is within the reference range, this is considered a normal result.**
- *Lactobacillus* levels will be considered out of the reference range when all the targeted species are not detected or present values below the established reference range.

2) **Table 2:** common **reproductive tract pathogens** with clinical relevance and **not related to chronic endometritis**:

<b>PATHOGENS OF THE REPRODUCTIVE TRACT</b>			
<i>Actinomyces israelii</i>	Not detected	N/A	absent
<b><i>Atopobium vaginae</i></b>	<b>Detected</b>	<b>4.02*</b>	≤ 3.57
<i>Bacteroides fragilis</i>	Not detected	N/A	≤ 3.57
<i>Bifidobacterium spp</i>	Not detected	N/A	≤ 4.22
<i>Clostridium sordelii</i>	Not detected	N/A	absent
<i>Dialister spp</i>	Not detected	N/A	≤ 3.57
<i>Fusobacterium nucleatum</i>	Not detected	N/A	absent
<i>Gardnerella vaginalis</i>	Not detected	N/A	≤ 3.72
<i>Haemophilus ducreyi</i>	Not detected	N/A	absent
<i>Mycobacterium tuberculosis</i>	Not detected	N/A	absent
<i>Mobiluncus spp</i>	Not detected	N/A	≤ 3.57
<i>Peptostreptococcus anaerobius</i>	Not detected	N/A	≤ 3.57
<i>Porphyromonas asaccharolytica</i>	Not detected	N/A	≤ 3.57
<i>Prevotella bivia</i>	Not detected	N/A	≤ 3.57
<i>Prevotella disiens</i>	Not detected	N/A	≤ 3.57
<i>Sneathia spp</i>	Not detected	N/A	≤ 3.57
<i>Treponema pallidum</i>	Not detected	N/A	absent

**\*Values out of reference range.**

Values of **pathogens out of the reference range** are identified in bold and with an asterisk.

Note: If any of the **pathogens associated with Sexually Transmitted Infections (*Haemophilus ducreyi* and/or *Treponema pallidum*)** are out of the reference range, an additional confirmatory test will be recommended. Infections caused by these bacteria are of mandatory notification to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.

3) **Table 3:** pathogens most common-causing chronic endometritis (ALICE test)

## RESULTS OF ALICE TEST :

PATHOGENS RELATED TO CHRONIC ENDOMETRITIS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Chlamydia trachomatis</i>	Not detected	N/A	absent
<i>Enterococcus faecalis</i>	Not detected	N/A	≤ 3.63
<i>Escherichia coli</i>	Not detected	N/A	≤ 3.58
<i>Klebsiella pneumoniae</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma genitalium</i>	Not detected	N/A	≤ 3.57
<b><i>Mycoplasma hominis</i></b>	<b>Detected</b>	<b>3.71*</b>	≤ 3.57
<i>Neisseria gonorrhoeae</i>	Not detected	N/A	absent
<i>Staphylococcus aureus</i>	Not detected	N/A	≤ 3.57
<i>Streptococcus agalactiae</i> group B/ <i>Streptococcus viridans</i>	Not detected	N/A	≤ 3.57
<i>Ureaplasma urealyticum</i>	Not detected	N/A	≤ 3.58

**\*Values out of reference range.**

Values of **pathogens out of the reference range** are identified in bold and with an asterisk.

Note: If any of the **pathogens associated with Sexually Transmitted Infections (*Chlamydia trachomatis* and/or *Neisseria Gonorrhoeae*)** are out of the reference range, an additional confirmatory test will be recommended. Infections caused by these bacteria are of mandatory notification to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.

The EMMA report also includes a recommendation (if necessary) to achieve a *Lactobacillus*-dominated reproductive tract, increasing the chances of achieving pregnancy, as described in the scientific literature. Furthermore, a section with interpretation of all results obtained is also included. Examples:

### RECOMMENDATION

Igenomix recommends performing the embryo transfer in a *Lactobacillus*-dominated endometrium to increase the chances of achieving a successful pregnancy (1,2,3). According to the standard Microbiology Guides (4,5), in case of possible infection by bacteria of which we have detected DNA, the suggested treatment would be azithromycin 500mg/24h day 1 and then 250mg/12h for 4 days, orally. To subsequently recolonize the reproductive tract, it is also suggested the use of vaginal probiotics only made by *Lactobacillus* strains, following the manufacturer's instructions regarding dose and duration of the treatment.

The analysis of a new biopsy is also recommended after treatment. The new sample must be taken following the standard test protocol.

### INTERPRETATION OF EMMA RESULTS:

No DNA from reproductive tract potentially pathogenic bacteria, not related to chronic endometritis, has been detected.

No DNA from *Lactobacillus* has been detected in the endometrial sample. *Lactobacillus* is the predominant bacteria in the female reproductive tract at reproductive age. It is not necessary to have different *Lactobacillus* strains but at least one of them should be within the range established as normal values.

### INTERPRETATION OF ALICE RESULTS:

DNA from potentially pathogenic bacteria related to chronic endometritis has been detected out of reference range in the endometrial sample. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy according to scientific evidence (1,2,3).

In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

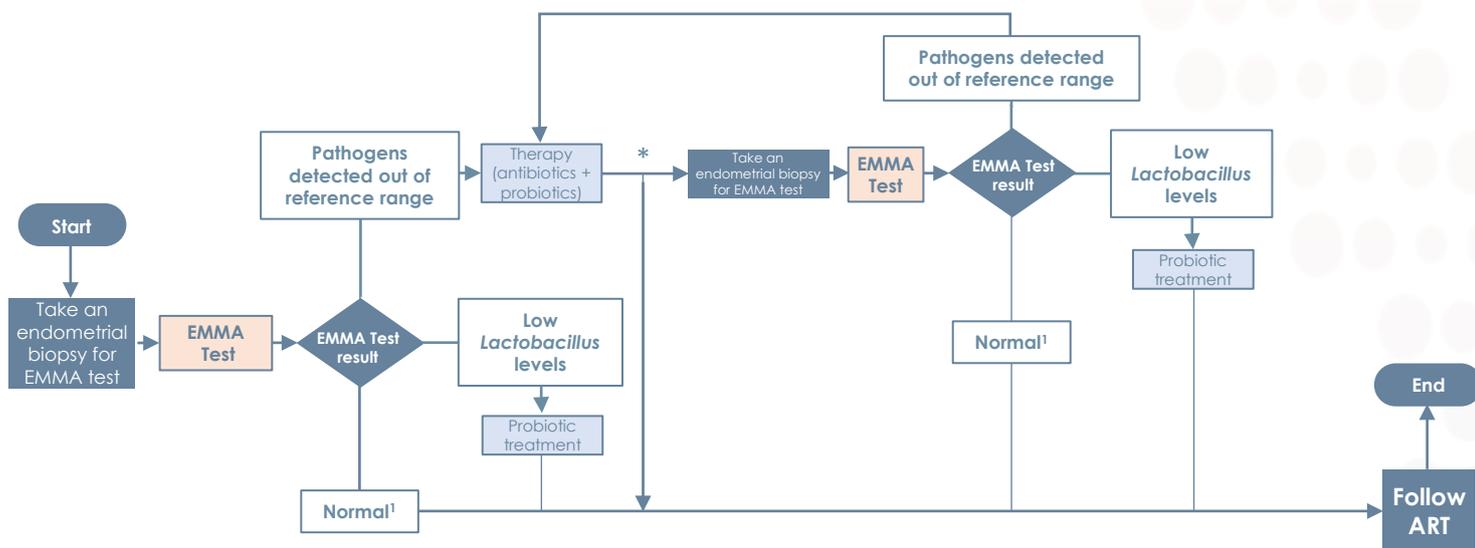
Note: the numbers in brackets in the text of the report refer to scientific publications, which are listed at the end of the report.

# Benefits of molecular analysis of the microbiota vs microbial culture

Microbial culture is the current gold-standard method for assessment of bacterial populations and infection. However, it has been demonstrated that, depending on location, between 20% and 60% of bacteria cannot be cultured. Molecular assessment of the microbiome allows detection of culturable and non-culturable bacteria present in a sample.

	<b>CULTURE</b>	<b>MOLECULAR</b>
<b>BASED ON</b>	The identification of culturable endometrial pathogens	The use of RT-PCR to detect all bacteria (including difficult-to-culture)
<b>OBJECTIVE RESULTS</b>	<b>YES</b>	<b>YES</b>
<b>SPECIFIC (TARGETED AB TREAT.)</b>	<b>YES</b>	<b>YES</b>
<b>DETECTS NON-CULTURABLE BACT</b>	<b>NO</b>	<b>YES</b>
<b>SHORT TURNAROUND TIME</b>	<b>NO</b>	<b>YES</b>

# EMMA decision tree



<sup>1</sup> Normal: no pathogens detected and *Lactobacillus* within the reference range

\* After treatment, it must be decided if going directly to the embryo transfer or repeating the EMMA test to ensure the clearance of pathogens.



## References

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- Inmaculada Moreno, Iolanda Garcia-Grau, Carlos Simon. Microbiota and Pathogen Screening in the Female Reproductive Tract. In: *Encyclopedia of Reproduction.* 2018; Chapter 9, vol. 4, pp. 36 - 44. Academic Press: Elsevier. DOI: 10.1016/B978-0-12-801238-3.64730-X
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# ALICE

## Analysis of Infectious Chronic Endometritis



**ALICE**

Detects the bacteria  
causing chronic  
endometritis

## Rationale

The best example of pathology caused by an altered endometrial microbiota is chronic endometritis (CE). **CE is a persistent inflammation of the endometrial lining, caused by infection of the uterine cavity, mainly by bacterial pathogens.** Because it is usually asymptomatic and current classical diagnosis methods (histology, hysteroscopy and microbial culture) are unsatisfactory, CE is often overlooked, although it affects approximately 30% of infertile women, and prevalence in patients with Recurrent Implantation Failure (RIF) and Repeated Pregnancy Loss (RPL) may reach 66%.

A study carried out by Igenomix has demonstrated that molecular assessment of CE is a reliable diagnostic method compared to classical methods (Moreno et al. Am J Obstet Gynecol, 2018). **This new approach should improve detection of this often-undiagnosed endometrial pathology, by identifying specific microorganisms and enabling guided, personalized treatment.**

**ALICE (Analysis of Infectious Chronic Endometritis), detects the most frequent bacteria that cause chronic endometritis.** This expands the service offered by Igenomix, to evaluate the endometrium at the microbiological level, with the aim of improving the clinical management of patients with this silent disease.

## Indications for ALICE

ALICE can be beneficial for any patient wishing to conceive, by assessing the microbiological environment that the embryo will encounter at implantation. ALICE may also be beneficial for patients with a history of RPL and/or RIF, because CE has been linked to these events.



## Methology

The ALICE test utilizes RT-PCR to provide a molecular screening of CE in endometrial tissue by analyzing the bacteria most commonly causing the disease (*Streptococcus agalactiae* (group B) & *Streptococcus viridans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*). The technology used for these purposes is based on DNA extraction followed by microorganism-specific amplification that enables the quantification of targeted bacteria present in a sample. After receiving the endometrial biopsy and extracting the genetic material (DNA), sample minimum quality requirements are evaluated before use of the screening tools.

A single endometrial sample contains both endometrial and bacterial cells. These can be analyzed using deep sequencing to predict endometrial receptivity and RT-PCR for the study of the afore mentioned pathogens.

# ALICE report example

The EMMA report shows a table with the reference ranges and the values obtained for each bacteria of the panel through the analysis of an endometrial sample:



**Igenomix**<sup>®</sup>  
PART OF VITROLIFE GROUP<sup>®</sup>

### ANALYSIS OF INFECTIOUS CHRONIC ENDOMETRITIS (ALICE)

Patient information	Sample information	Clinic information
Unique pat id:	Date received:	Clinic:
Patient name:	Report date/time:	Clinician: Dr.
Patient DOB:	Sample type: Endometrial Biopsy	
Allergic to antibiotics:	Cycle type:	
	Cycle day:	
	No. Biopsy:	
	Date of biopsy:	

**RESULTS OF ALICE TEST :**

BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Chlamydia trachomatis</i>	Not detected	N/A	absent
<i>Enterococcus faecalis</i>	Detected	3.81*	≤ 3.63
<i>Escherichia coli</i>	Not detected	N/A	≤ 3.58
<i>Klebsiella pneumoniae</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma genitalium</i>	Not detected	N/A	≤ 3.57
<i>Mycoplasma hominis</i>	Not detected	N/A	≤ 3.57
<i>Neisseria gonorrhoeae</i>	Not detected	N/A	absent
<i>Staphylococcus aureus</i>	Not detected	N/A	≤ 3.57
<i>Streptococcus agalactiae</i> group B/ <i>Streptococcus viridans</i>	Not detected	N/A	≤ 3.57
<i>Ureaplasma urealyticum</i>	Not detected	N/A	≤ 3.58

\*Values out of reference range.

### RECOMMENDATION

Igenomix recommends performing the embryo transfer in a Lactobacillus-dominated endometrium to increase the chances of achieving a successful pregnancy (1,2,3). According to the standard Microbiology Guides (4,5), in case of possible infection by bacteria of which we have detected DNA, the suggested treatment would be amoxicillin 500mg/8h for 7 days, orally. To subsequently recolonize the reproductive tract, it is also suggested the use of vaginal probiotics only made by Lactobacillus strains, following the manufacturer's instructions regarding dose and duration of the treatment. The analysis of a new biopsy is also recommended after treatment. The new sample must be taken following the standard test protocol.

### INTERPRETATION OF ALICE RESULTS

DNA from potentially pathogenic bacteria related to chronic endometritis has been detected out of reference range in the endometrial sample. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy according to scientific evidence (1,2,3).

In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

### ADDITIONAL COMMENTS

None.



Values of **pathogens out of the reference range** are identified in bold and with an asterisk.

PATHOGENS RELATED TO CHRONIC ENDOMETRITIS			
BACTERIA	RESULT	VALUE	REFERENCE RANGE
<i>Chlamydia trachomatis</i>	Not detected	N/A	absent
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\*Values out of reference range.

Note: If any of the **pathogens associated with Sexually Transmitted Infections (*Chlamydia trachomatis* and/or *Neisseria Gonorrhoeae*)** are out of the reference range, an additional confirmatory test will be recommended. Infections caused by these bacteria are of mandatory notification to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the doctor's responsibility to declare these infections.

The ALICE report also shows, a recommendation (if needed) to increase the chances of achieving a healthy pregnancy, as described in the scientific literature, an interpretation of the result obtained.

#### RECOMMENDATION

Igenomix recommends performing the embryo transfer in a Lactobacillus-dominated endometrium to increase the chances of achieving a successful pregnancy (1,2,3). According to the standard Microbiology Guides (4,5), in case of possible infection by bacteria of which we have detected DNA, the suggested treatment would be amoxicillin 500mg/8h for 7 days, orally. To subsequently recolonize the reproductive tract, it is also suggested the use of vaginal probiotics only made by Lactobacillus strains, following the manufacturer's instructions regarding dose and duration of the treatment. The analysis of a new biopsy is also recommended after treatment. The new sample must be taken following the standard test protocol.

#### INTERPRETATION OF ALICE RESULTS

DNA from potentially pathogenic bacteria related to chronic endometritis has been detected out of reference range in the endometrial sample. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy according to scientific evidence (1,2,3).

In the case of prescribed treatment, it is also recommended to analyze a new biopsy after its completion to confirm normalized values of pathogens. The new sample must be taken following the standard test protocol.

#### ADDITIONAL COMMENTS

None.

# Benefits of molecular analysis of the microbiota vs histology, hysteroscopy and microbial culture

Current diagnosis of CE is traditionally based on histology, hysteroscopy and/or microbial culture. However, **these three classical methods provide inconclusive or misleading results in 80% of cases.** While histology usually underdiagnoses CE, hysteroscopy usually overdiagnoses the disease. These methods cannot accurately identify the pathogens causing the disease, and broad-spectrum antibiotics are often prescribed.

Microbial culture is able to isolate the causative pathogen; however, between 20% and 60% of bacteria cannot be cultured in standard laboratory conditions or are not usually assessed in clinical practice.

**Molecular microbiology presents equivalent results to the combined results obtained by using histology, hysteroscopy and microbial culture** (Moreno et al. Am J Obstet Gynecol, 2018).

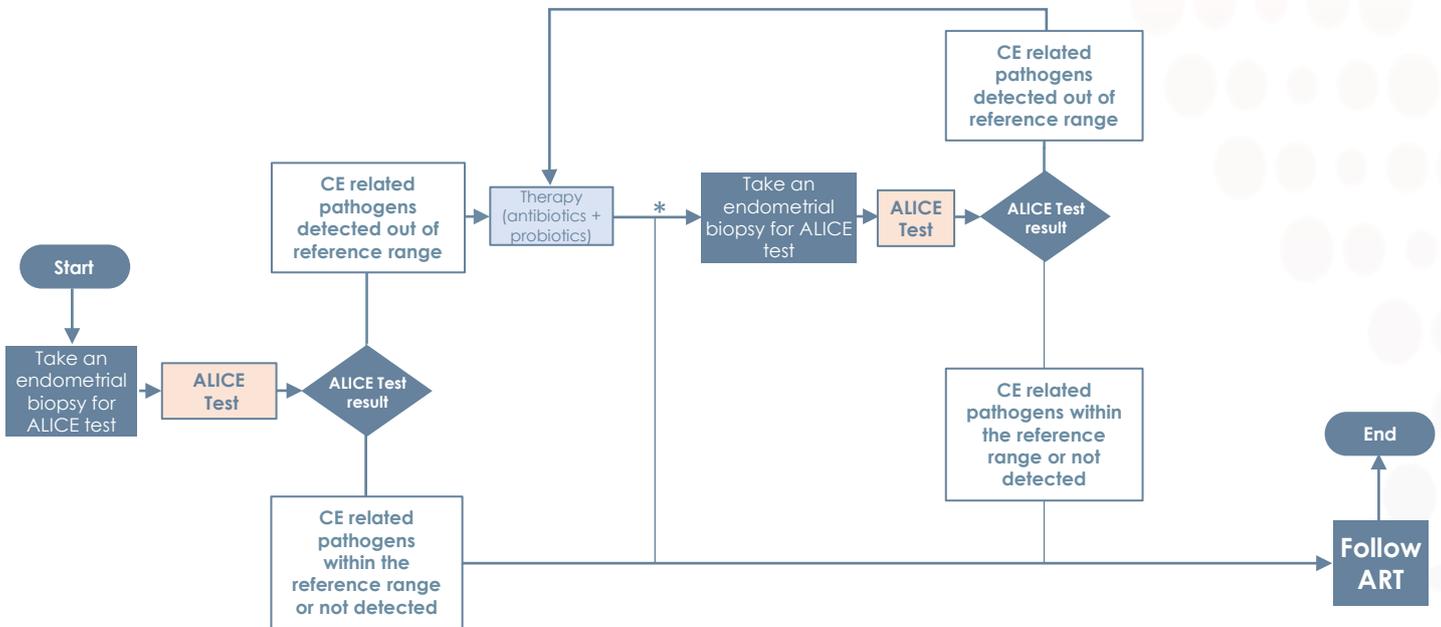
	HISTOLOGY	HYSTEROSCOPY	CULTURE	MOLECULAR
BASED ON	The identification of CD138+ Plasma Cells in the endometrial stroma	The identification of stromal edema, focal or diffuse epithelial hyperemia, and/or the presence of micropolyps	The identification of culturable endometrial pathogens	The use of RT-PCR to detect all bacteria (including difficult-to-culture)
OBJECTIVE RESULTS	NO	NO	YES	YES
SPECIFIC (TARGETED AB TREAT.)	NO	NO	YES	YES
DETECTS NON-CULTURABLE BACT	NO	NO	NO	YES
SHORT TURNAROUND TIME	NO	YES	NO	YES



**ALICE**

Detects the bacteria causing chronic endometritis

# ALICE decision tree



\* After treatment, it must be decided if going directly to the embryo transfer or repeating the ALICE test to ensure the clearance of pathogens.



## References

- Cicinelli E, Matteo M, Tinelli R, Lepera A, Alfonso R, Indraccolo U, Marocchella S, Greco P, Resta L. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. *Hum Reprod*, 2015; 30(2):323-30.
- Cicinelli E, Matteo M, Tinelli R, Pinto V, Marinaccio M, Indraccolo U, De Ziegler D, Resta L. Chronic endometritis due to common bacteria is prevalent in women with recurrent miscarriage as confirmed by improved pregnancy outcome after antibiotic treatment. *Reprod Sci* 2014; 21(5):640-7.
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- Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez M, Bau D, Vilella F, De Ziegler D, Resta L, Valbuena D, Simon C. The diagnosis of chronic endometritis in infertile asymptomatic women: a comparative study of histology, microbial cultures, hysteroscopy, and molecular microbiology. *Am J Obstet Gynecol*. 2018; 218(6):602.e1-602.e16

# Endometrial Biopsy



# Requirements for the endometrial biopsy taking

- Endometrial biopsy for ERA, EMMA, ALICE or EndomeTRIO tests should be taken following all this manual's indications.
- The endometrial biopsy can be taken in the gynaecologist consultant, without using anesthetic. **If a biopsy is going to be taken during a hysteroscopy, we recommend taking it at the beginning of the procedure**, before distending the uterine cavity and without antibiotic treatment before, during or after the procedure. The biopsy only can be taken if the hysteroscopy is observational, and any intervention will be performed.
- **To perform the EMMA or ALICE tests (alone or with ERA test), antibiotic intake should be avoided at least the 7 days prior to taking the sample, during the procedure and until receiving the test results.** In this way, the microbiome of the biopsy day will be representative of the patient's microbiome the day in which the test results are received. **Any drugs that may alter the patient's microbiota or immunological status should also be included in the test requisition form**, including all the data related to taking antibiotics during the month prior to the biopsy (active principle, route of administration, dose and duration of treatment).



# Day of Endometrial Biopsy for ERA alone or coupled with EMMA/ALICE

In the case of an ERA test is requested (alone or coupled with other tests) the endometrial biopsy should be performed according to the indications described below:

- 1. The ERA diagnosis is valid for the type of cycle in which the test was performed,** and therefore the embryo must be transferred in the same type of cycle and the personalized window of implantation within which a 'Receptive' diagnosis was obtained. Therefore, the type of cycle for biopsy should match to the type of cycle planned for the embryo transfer.
- 2. Cycle type:** Hormonal Replacement Therapy (P+5) or Natural cycle (hCG+7/LH+7/Ovulation+6) as explained as follows. Note: If Day-3 embryos are to be transferred, the biopsy should still be performed at P+5 or hCG+7/LH+7/Ovulation+6, since the ERA checks the endometrium at the moment of implantation. In this way, if you have a receptive result at P+5, you will transfer a blastocyst at P+5 or a Day-3 embryo two days earlier, i.e. at P+3.

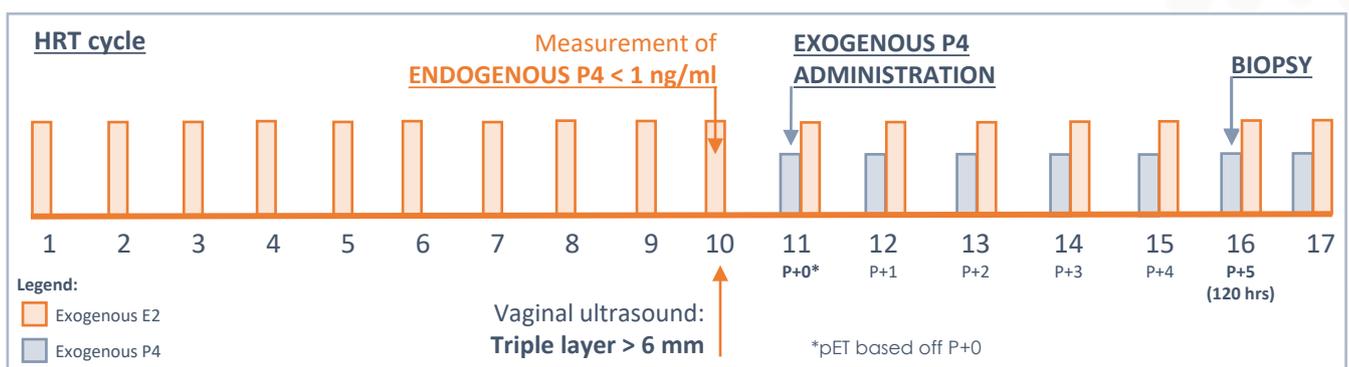
**2a) Hormone Replacement Therapy cycle (HRT):** involves treatment with estrogen and progesterone to prepare the endometrium in a controlled manner, similar to a natural cycle for embryo transfer, using the routine protocol at the clinic or our standard protocol:

Patient starts estradiol therapy from the 1st or 2nd day of the menstrual cycle. Ultrasound assessment is performed 7 to 10 days later. Please note that we don't recommend the estradiol therapy to be longer than 17 days before the start of the progesterone intake.



Start progesterone (P4) intake when a trilaminar endometrium >6 mm is reached with a serum P4 <1 ng/ml (within 24 hours prior to starting exogenous P4), continuing with estradiol treatment. The day on which the P4 treatment starts is referred to as P+0, and the biopsy is taken on day P+5, after 5 full days (120 hours from the first intake to biopsy collection).

### HRT Routine Protocol:



- In an HRT cycle it is very important to ensure that there is no ovulation, and therefore endogenous P4 level should always be measured within the 24 hours prior to the first P4 intake. The level should be <1ng/ml, otherwise the recommendation is to cancel the cycle and start a new one. Failure to properly control for endogenous P4 may result in an endogenous P4 artifact that can affect the accuracy and reproducibility of the ERA results.
- **Personalized embryo transfer time (pET) will be based on the total exogenous progesterone exposure time** (the reference point will be day P+0).



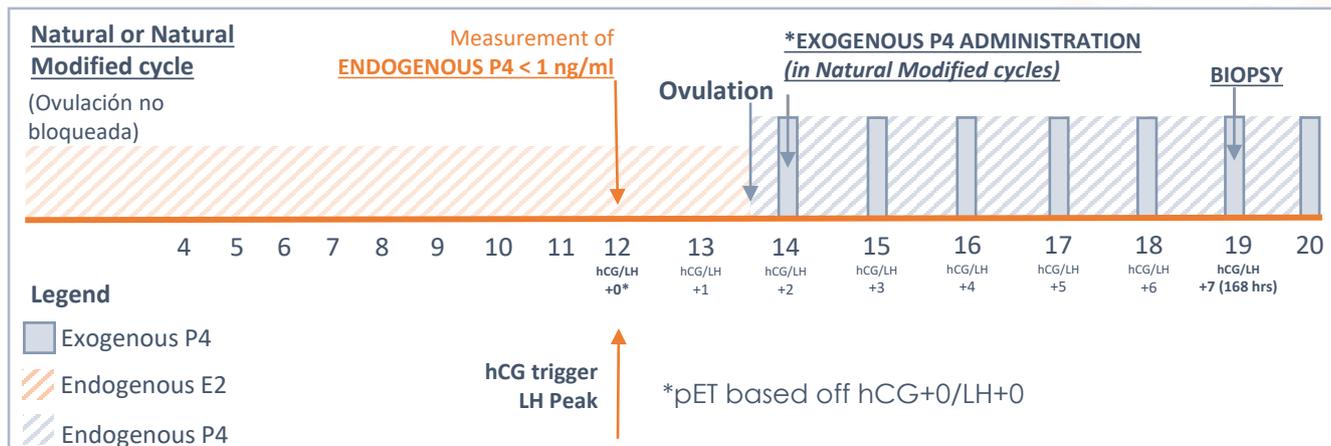
**2b) Natural cycle:** For Natural Cycles we always need to have a reference date regarding ovulation timing, which could be one of the following three options:

- i. hCG (recombinant or urinary) date:** hCG is administered according to routine parameters in a natural cycle (follicle size >17 mm). The day of the hCG administration is considered as hCG+0 and the biopsy will be taken 7 days later, at hCG+7 (168 hours after hCG triggering).
- i. LH surge date:** to properly detect the LH peak, the LH levels in urine or blood must be measured during several followed days (from day 9 in a regular cycle) obtaining at least one positive flanked by two negative results. The day of the LH surge is considered as LH+0 and the biopsy will be taken 7 days later, at LH+7.
- i. Ovulation date:** The sample can be also collected in a natural cycle, during secretory phase, because ovulation induces the production of estrogens and progesterone. The day of ovulation determined by ultrasound will be considered as Ov+0 and the biopsy will be collected 6 days later, at Ov+6.

In Natural cycles, progesterone supplementation can be administered, being then referred to as Modified Natural cycles. In these cycles the reference date for the pET recommendation still is the hCG/LH/Ovulation date. The progesterone supplementation can start from LH+1/hCG+1/Ov+0 at the moment in which it is usually done in the routine clinical practice of your center (never prior hCG triggering or LH surge). It must be considered that the moment in which progesterone supplementation is started, should be replicated also in transfer cycle (i.e. if a patient starts progesterone supplementation at hCG+2 for the biopsy cycle, it should be started also at hCG+2 in the transfer cycle, independently of the result obtained).



### Natural Routine Protocol:



To ensure that there is no endogenous progesterone escape by the time of hCG trigger/LH surge, the endogenous P4 level should always be measured at hCG+0/LH+0 and this should be <1ng/ml, otherwise the recommendation is to cancel the cycle and start a new one. Failure to properly control for endogenous P4 may result in an endogenous P4 artifact that can affect the accuracy and reproducibility of the ERA results.

**The personalize embryo transfer (pET) will be based off hCG/LH/ovulation.** Please note that if we do not have this information in Natural cycles, our recommendation is to cancel the analysis. The ERA test cannot be performed without this reference date since the result would not be reproducible.



## Day of Endometrial Biopsy for EMMA and/or ALICE (without ERA)

For EMMA/ALICE tests the patient should **avoid antibiotics at least 7 days before biopsy collection, during the procedure, and after the procedure until results are received.**

The **endometrial biopsies for EMMA&ALICE tests must be always collected in secretory phase** because this is the period of maximum stability of the reproductive tract microbiota due to the influence of estrogens and progesterone. **A sample taken outside the phase indicated below, could give us a non-reliable result.**

If an EMMA or ALICE test is requested alone, the endometrial biopsy can be taken following the same protocol as for ERA. If ERA protocol is not followed, the biopsy must be taken as follows:

- a) **HRT cycles:** the samples must be taken during the progesterone intake days (P+1 onwards), preferably on day P+5.
- b) **Natural or Modified Natural cycle:** The biopsy must be taken between days 15 and 25 of the menstrual cycle if the patient has regular cycles (between 26 to 32 days).

For patients with non-regular cycles, we recommend performing an HRT cycle or monitoring ovulation. In this case, the biopsy can be taken on days:

- LH+2 to LH+12 (both inclusive)
- hCG+2 to hCG+12 (both inclusive)
- Ov+1 to Ov+11 (both inclusive)



c) **Oral Contraceptive Pills (OCPs):** only for OCPs with certain compositions. Please, confirm always with Igenomix before scheduling the biopsy. The biopsy must be taken between **days 14 to 21 of OCPs (days of active pills intake when patient takes the placebo pills), or on day 14 and onwards (if the patient doesn't take placebo pills neither have a rest, and is under continuous use).**

Day of endometrial biopsy: summary table

Cycle type	ERA	EMMA&ALICE	Comments
HRT	P+5 (120hrs)	P+0 Onwards	
Natural or Modified Natural	hCG+7 (168 hrs) LH+7 (168 hrs) Ov+6 (144 hrs)	hCG+2 to hCG+12 LH+2 to LH+12 Ov+1 to Ov+11 Cycle Days 15 to 25	The times recommended for EMMA&ALICE apply to patients with regular cycles 26-32 days. Otherwise, we recommend performing an HRT cycle or to monitor ovulation.  For each period, the first and last dates are included.
During OCPs	NO	14 - 21 (days of active intake pills if patient has also placebo pills) 14 onwards (continuous intake of active pills)	Not all OCPs will be suitable for EMMA/ALICE. We recommend pre-approving OCP prior to patient biopsy cycle.  For each period, the first and last dates are included.



### Day of endometrial biopsy: non-valid protocols

Cycle type	Cycle Day	ERA	EMMA&ALICE	Comments
Controlled ovarian stimulation	NA	NO	NO	Samples cannot be collected in a stimulated cycle as conditions cannot be replicated during the pET cycle. The microbiome is not representative because hormone levels are not comparable to a Natural or HRT cycle.
Biopsy during the follicular phase	NA	NO	NO	Samples must only be collected during the secretory phase to ensure microbiome stability.



# Endometrial Biopsy protocol

A single endometrial biopsy is sufficient for an individual test or for EndomeTRIO (ERA, EMMA, and ALICE). Igenomix will supply a cryotube for each biopsy. The cryotube contains 1.5 ml of a transparent solution to preserve the genetic material.

- 1. Clean cervix with sterile, dry gauze** (avoid the use of betadine) and do not introduce fluid into the endometrium.
- 2. Label tube** with: patient name, DOB and date of biopsy.
- 3. The endometrial biopsy must be taken from the uterine fundus using a Pipelle catheter** (Genetics, Hamont Achel, Belgium) or similar.
- 4. Collect at least 70mg of tissue** (corresponds to a cubic piece of tissue with sides of approximately 7 mm). The **sample volume must not exceed the white line marked on the cryotube (corresponding to 1/3 of the total cryotube volume; see picture below)**. For bigger amounts of tissue there will not be sufficient stabilizing in the cryotube (which will lead to genetic material degradation).



Document: SPA\_L1\_ERA\_013\_EN: Manual EndomeTRIO Custom OA (EN) v1.0  
Authorised on: 08-May-2023. Authorised by: diana.valbuena.



5. **Ensure that the sample is made up of endometrial tissue, not solely blood or mucus.** Excessive amounts of blood or mucus should also be avoided.
6. **Avoid the contact of the sample with any solution other than the buffer in the tube** (don't wash the sample).
7. **After the biopsy has been performed, the sample should be transferred immediately to the supplied cryotube avoiding touching the tube with the Pipelle and shaking vigorously for at least 10 seconds** (to ensure that the buffer penetrates the tissue and stabilizes the RNA of the sample).
8. **The cryotube containing the sample should be immediately transferred to a refrigerator (4-8°C /39-46°F) and stored there for at least 4 hours** (do not place in the freezer before completing these 4 hours).
9. **After refrigerating for at least 4 hours, samples may be sent to Igenomix at room temperature.** If samples are going to be exposed to >35°C/95°F, we recommend shipping them with a cold gelpack. Deliveries at room temperature should never exceed 5 days.
10. **Samples may also be kept in a refrigerator for up to 3 weeks or may be frozen at -20°C/-4°F** (after the first 4 hours at 4-8°C/39-46°F) if not being sent to Igenomix straightaway. However, in the case of an EMMA, ALICE or EndomeTRIO test, as the microbiome can fluctuate over time, the recommendation is to process the sample as soon as possible after collection. We do not recommend delaying the shipment of samples for more than a week.



# Logistics

## Sample and documents:

- Read and complete properly the “Test Requisition Form” and “Informed Consent.”
- Place the cryotube containing the biopsy inside the rigid plastic blister and close it. Introduce the secondary container inside the kit and introduce it in the plastic (courier) return bag (provided by Igenomix). Insert the receptacle inside the kit box and the box inside the return bag.
- Introduce “Test Requisition Form” and “Informed Consent” previously filled inside the return bag.
- Depending on the geographic area, it should be attached the provided courier documents and UN3373 sticker to the included courier bag to return the sample.
- Shipments at room temperature should not exceed 5 days in order to ensure the preservative action of the liquid in the cryotube. We recommend shipping the samples with a cold gelpack if outside temperatures exceed 35°C/95°F. For further details, please contact us.

## Shipment:

- Please inform us by email about each shipment indicating the number of samples and their clinical or reference record number.
- You may use your usual courier, or alternatively ask us about our pick up service.



# A complete view of the endometrial health

REQUESTED TEST	TESTS INCLUDED AND APPLICATION		
<b>EndomeTRIO</b> The endometrium matters	<b>ENDOMETRIAL RECEPTIVITY ANALYSIS</b> Expression of 248 genes to guide pET*	<b>ENDOMETRIAL MICROBIOME ANALYSIS</b> Lactobacilli and pathogenic bacteria of the reproductive tract <i>Molecular detection of bacteria present to allow for more personalized treatment</i>	<b>CHRONIC ENDOMETRITIS</b> Pathogenic bacteria related to CE <i>Molecular detection of CE pathogens to allow for more personalized treatment</i>
<b>ERA®</b> Endometrial Receptivity Analysis	<b>ENDOMETRIAL RECEPTIVITY ANALYSIS</b> Expression of 248 genes to guide pET*		
<b>EMMA</b> Endometrial Microbiome Metagenomic Analysis		<b>ENDOMETRIAL MICROBIOME ANALYSIS</b> Lactobacilli and pathogenic bacteria of the reproductive tract <i>Molecular detection of bacteria present to allow for more personalized treatment</i>	<b>CHRONIC ENDOMETRITIS</b> Pathogenic bacteria related to CE <i>Molecular detection of CE pathogens to allow for more personalized treatment</i>
<b>ALICE</b> Analysis of Infectious Chronic Endometritis			<b>CHRONIC ENDOMETRITIS</b> Pathogenic bacteria related to CE <i>Molecular detection of CE pathogens to allow for more personalized treatment</i>

\*pET: personalized embryo transfer



## List of abbreviations

<b>ALICE</b>	Analysis of Infectious Chronic Endometritis
<b>BMI</b>	Body Mass Index
<b>CE</b>	Chronic Endometritis
<b>DNA</b>	Deoxyribonucleic Acid
<b>E<sub>2</sub></b>	Estrogens
<b>EMMA</b>	Endometrial Microbiome Metagenomic Analysis
<b>ERA</b>	Endometrial Receptivity Analysis
<b>hCG</b>	Human Chorionic Gonadotropin
<b>HMP</b>	Human Microbiome Project
<b>HRT</b>	Hormonal Replacement Therapy
<b>LH</b>	Luteinizing Hormone
<b>NGS</b>	Next Generation Sequencing
<b>OCPs</b>	Oral Contraceptive Pills
<b>Ov</b>	Ovulation
<b>P<sub>4</sub></b>	Progesterone
<b>pET</b>	Personalized Embryo Transfer
<b>RIF</b>	Recurrent Implantation Failure
<b>RNA</b>	Ribonucleic Acid
<b>RPL</b>	Recurrent Pregnancy Loss
<b>RT-PCR</b>	Real Time Polymerase Chain Reaction
<b>WOI</b>	Window of Implantation

