# A complete view of endometrial health

# Summary

# Summary

# ERA®

## Endometrial Receptivity Analysis

Igenomix®

# 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. 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).

Identifying the window of implantation in the endometrial cycle, allows for a 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).

**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 was initially indicated for RIF patients, 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. However, a new randomized clinical trial (Simon et al. Reprod BioMed Online, 2020) demonstrates the benefit of the ERA test for all patients undergoing assisted reproductive treatment.

If your patient requires any intervention at the uterine level, the ERA test should be done after this procedure, in order to replicate the conditions under which embryo transfer will take place.

In the case of an atrophic (< 6 mm) or hypertrophic endometrium (> 12 mm), ERA can be performed as long as the endometrial appearance is consistent for all cycles for this patient.

# 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.

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), or 7 days after the hCG triggering (hCG+7) in a natural cycle (168 hours after hCG triggering). 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.

## Report and Interpretation of the Results

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

## 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.

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

**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.

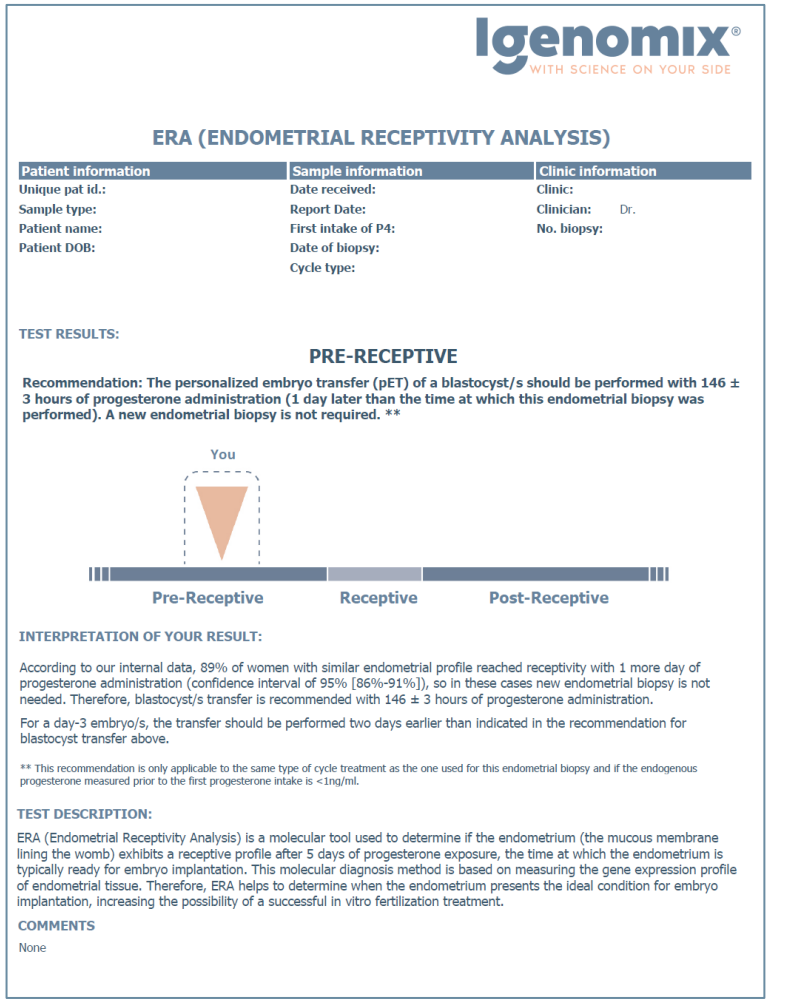
* In approximately 5% of samples received, a result cannot be obtained. This is due to a non-informative profile or to the low quantity/quality of the genetic material extracted.

* Following ERA report recommendations does not guarantee implantation. Failed implantation may be caused by other factors.

# 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 referring physician.



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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: Dr. |
| Patient name: | First intake of P4: | No. biopsy: |
| Patient DOB: | Date of biopsy: | |
| | Cycle type: | |

**TEST RESULTS:**

**PRE-RECEPTIVE**

**Recommendation: The personalized embryo transfer (pET) of a blastocyst/s should be performed with 146 ± 3 hours of progesterone administration (1 day later than the time at which this endometrial biopsy was performed). A new endometrial biopsy is not required. ****



**INTERPRETATION OF YOUR RESULT:**

According to our internal data, 89% of women with similar endometrial profile reached receptivity with 1 more day of progesterone administration (confidence interval of 95% [86%-91%]), so in these cases new endometrial biopsy is not needed. Therefore, blastocyst/s transfer is recommended with 146 ± 3 hours of progesterone administration.

For a day-3 embryo/s, the transfer should be performed two days earlier than indicated in the recommendation for blastocyst transfer above.

** 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.

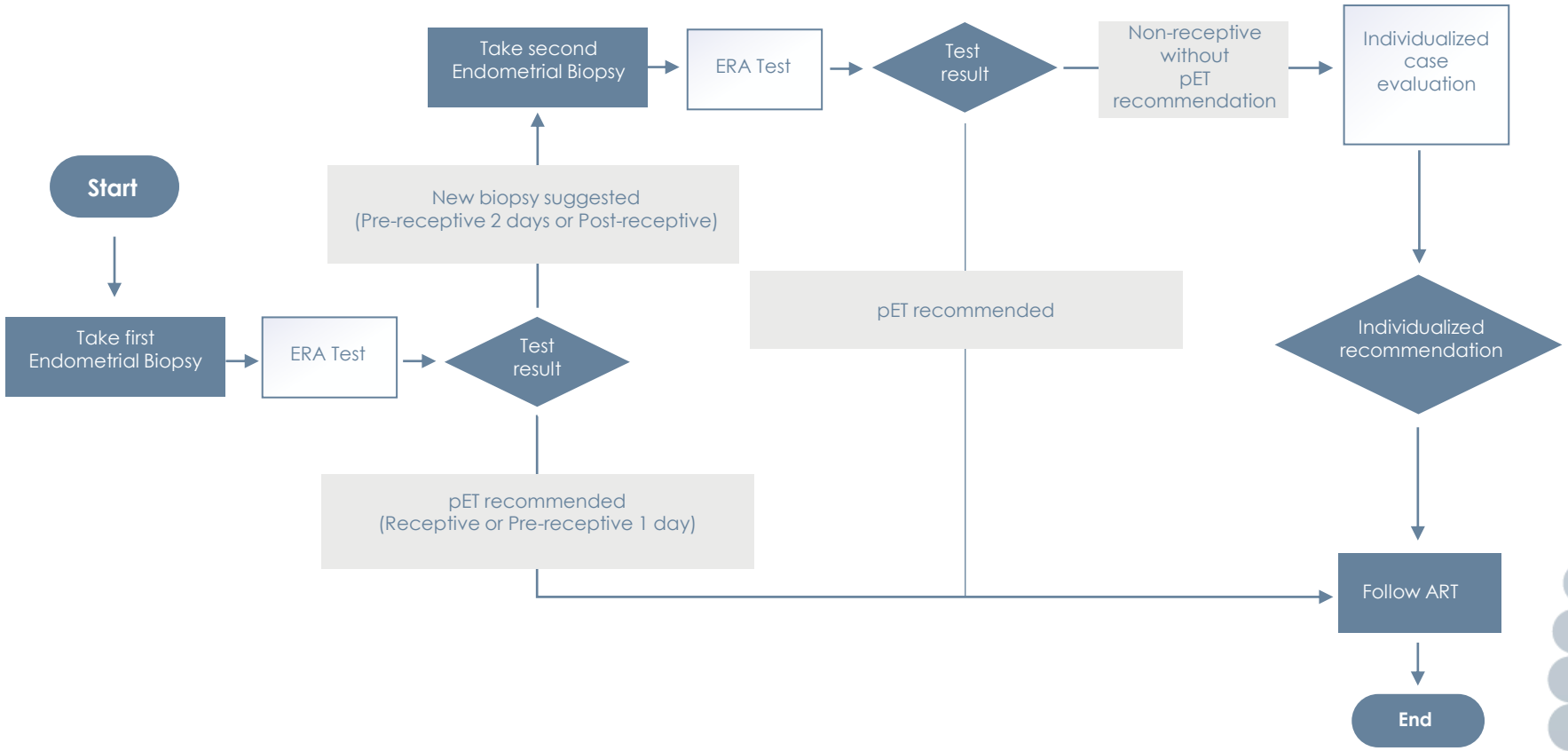
**TEST DESCRIPTION:**

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

# ERA Decision Tree



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# EMMA

## Endometrial Microbiome Metagenomic Analysis

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# 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 information about 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 test utilizes RT-PCR to provide microbiota information in endometrial tissue by analyzing 4 *Lactobacillus* species: *L. crispatus, L. gasseri, L. iners* and *L. jensenii*, 11 bacterial pathogens of the reproductive tract and 9 bacteria most commonly causing CE. 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.

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 endometrial microbiota. EMMA thus provides a microbiological view of the endometrium, to improve clinical management of patients.

# Report and Interpretation of the Results

The EMMA report will provide information about the overall microbial health of the uterine cavity. This includes:

- One table showing the normal ranges† for 4 species of Lactobacilli (*L. crispatus, L. gasseri, L. iners* and *L. jensenii)* and the values obtained in the endometrial sample.
- One table showing the normal ranges† for 11 species of common reproductive tract pathogens (*Gardnerella vaginalis, Prevotella bivia, Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Megasphaera spp, Treponema pallidum, Bacteroides fragilis, Bacterial Vaginosis Associated Bacteria 2 and Haemophilus ducreyi)* and the values obtained in the endometrial sample.
- One table with ALICE results, showing the normal ranges† for 9 species of pathogens causing chronic endometritis (CE) (*Streptococcus agalactiae (group B), Staphylococcus aureus, Enterococcus faecalis, Mycoplasma hominis, Mycoplasma genitalium, Escherichia coli, Ureaplasma urealyticum, Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and the values obtained in the endometrial sample.

†Data obtained from the analysis of samples of 234 women of which 102 had a Live Birth (LB). The normal ranges were calculated with the results obtained from the 102 women with LB.

# Report and Interpretation of the Results

- Values of pathogens out of the normal range are identified with an asterisk.
- *Lactobacillus* is the *predominant bacteria in the reproductive tract of women at reproductive age. If at least one of the Lactobacillus species is within the normal range, this is considered a normal result. Lactobacillus levels will be considered out of the normal range when all the targeted species are not detected or present values below the established normal range.*
- The report includes a list of antibiotics that can be applied to each specific bacterium detected that is out of the normal range. This list is provided as a general reference, it is the doctor's responsibility to prescribe the antimicrobial therapy.
- In case *Haemophilus ducreyi, Treponema pallidum, Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* are out of the normal 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.

# EMMA

Endometrial Microbiome
Metagenomic Analysis

## Example of Report

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### ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

| 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:** | |
| | **No. Biopsy:** | |
| | **Date of biopsy:** | |

**RESULTS OF THE TEST**

| *LACTOBACILLUS* | | | |
|---|---|---|---|
| **BACTERIA** | **RESULT** | **VALUE** | **NORMAL RANGE†** |
| *Lactobacillus crispatus* | Detected | 2.05* | ≥ 3.71 |
| *Lactobacillus gasseri* | Not detected | N/A | ≥ 3.55 |
| *Lactobacillus iners* | Detected | 1.21* | ≥ 3.53 |
| *Lactobacillus jensenii* | Detected | 0.98* | ≥ 3.69 |
| **PATHOGENS OF THE REPRODUCTIVE TRACT** | | | |
| **BACTERIA** | **RESULT** | **VALUE** | **NORMAL RANGE†** |
| *Gardnerella vaginalis* | Detected | 3.95* | ≤ 3.74 |
| *Prevotella bivia* | Detected | 4.01* | ≤ 3.79 |
| *Atopobium vaginae* | Not detected | N/A | ≤ 3.69 |
| *Mobiluncus curtisii* | Detected | 2.32 | ≤ 3.77 |
| *Mobiluncus mulieris* | Detected | 3.69* | ≤ 3.55 |
| *Megasphaera spp 1* | Detected | 4.77* | ≤ 4.17 |
| *Megasphaera spp 2* | Detected | 3.49* | ≤ 3.43 |
| *Treponema pallidum* | Detected | 3.86*[1] | ≤ 3.73 |
| *Bacteroides fragilis* | Detected | 3.49* | ≤ 3.42 |
| *Bacterial vaginosis-associated bacterium type 2 (BVAB2)* | Detected | 4.00* | ≤ 3.51 |
| *Haemophilus ducreyi* | Detected | 3.84*[1] | ≤ 3.68 |
| **ANALYSIS OF INFECTIOUS CHRONIC ENDOMETRITIS (ALICE)** | | | |
| **BACTERIA** | **RESULT** | **VALUE** | **NORMAL RANGE†** |
| *Streptococcus agalactiae (group B)* | Not detected | N/A | ≤ 3.42 |
| *Staphylococcus aureus* | Not detected | N/A | ≤ 3.55 |
| *Escherichia coli* | Not detected | N/A | ≤ 3.58 |
| *Enterococcus faecalis* | Not detected | N/A | ≤ 3.62 |
| *Ureaplasma urealyticum* | Not detected | N/A | ≤ 3.58 |
| *Mycoplasma hominis* | Not detected | N/A | ≤ 3.61 |
| *Mycoplasma genitalium* | Not detected | N/A | ≤ 3.55 |
| *Neisseria gonorrhoeae* | Not detected | N/A | ≤ 3.37 |
| *Chlamydia trachomatis* | Not detected | N/A | ≤ 3.65 |

† Thresholds and normal ranges were calculated based on 102 endometrium samples from women with fertility history of previous live birth.

*Values out of normal range.

[1] Additional confirmatory test and follow-up by a physician is highly recommended. Infections caused by *Haemophilus ducreyi*, *Treponema pallidum*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are of mandatory notification to the local Health Authorities in different countries. In the case that these pathogens

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# Benefits of Molecular Analysis of the Microbiome *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 using RT-PCR allows detection of culturable and non-culturable targeted bacteria present in a sample.

# References

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# ALICE

## Analysis of Infectious Chronic Endometritis

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# 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 diagnostic 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 RIF and Recurrent Pregnancy Loss (RPL) may reach 60%.

A recent 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.

# Methodology

The ALICE test utilizes RT-PCR to provide a molecular diagnosis of CE in endometrial tissue by analyzing the 9 bacteria most commonly causing the disease (*Streptococcus agalactiae* (group B), *Staphylococcus aureus, Enterococcus faecalis, Mycoplasma hominis, Mycoplasma genitalium, Escherichia coli, 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 diagnosis 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 9 aforementioned pathogens.

# Report and Interpretation of the Results

The ALICE report, shows a table with the normal ranges† for 9 species of reproductive tract pathogens most often related with chronic endometritis (*Streptococcus agalactiae* (group B), *Staphylococcus aureus, Enterococcus faecalis, Mycoplasma hominis, Mycoplasma genitalium, Escherichia coli, Ureaplasma urealyticum, Chlamydia trachomatis* and *Neisseria gonorrhoeae*) and the values obtained in the endometrial sample. Values of pathogens out of the normal range are identified with an asterisk.

† Data obtained from the analysis of samples of 234 women of which 102 had a Live Birth (LB). The normal ranges were calculated with the results obtained from the 102 women with LB.

# Report and Interpretation of the Results

- ALICE report includes a list of antibiotics that can be applied to each specific bacterium detected out of the normal range. This list is provided as a general reference, it is the doctor's responsibility to prescribe the antimicrobial therapy.

- In case *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* are out of the normal 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.

# Example of Report

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## 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:** | |
| | **No. Biopsy:** | |
| | **Date of biopsy:** | |

### RESULTS OF THE TEST

**REPRODUCTIVE TRACT PATHOGENS MOST OFTEN RELATED WITH CHRONIC ENDOMETRITIS**

| BACTERIA | RESULT | VALUE | NORMAL RANGE† |
|---|---|---|---|
| *Streptococcus agalactiae (group B)* | Not detected | N/A | ≤ 3.42 |
| *Staphylococcus aureus* | Detected | 3.51 | ≤ 3.55 |
| *Escherichia coli* | Detected | 3.56 | ≤ 3.58 |
| *Enterococcus faecalis* | Not detected | N/A | ≤ 3.62 |
| *Ureaplasma urealyticum* | Not detected | N/A | ≤ 3.58 |
| *Mycoplasma hominis* | Not detected | N/A | ≤ 3.61 |
| *Mycoplasma genitalium* | Not detected | N/A | ≤ 3.55 |
| *Neisseria gonorrhoeae* | Not detected | 3.45*[1] | ≤ 3.37 |
| *Chlamydia trachomatis* | Not detected | N/A | ≤ 3.65 |

†Thresholds and normal ranges were calculated based on 102 endometrium samples from women with fertility history of previous live birth.

*Values out of normal range.

[1] Additional confirmatory test and follow-up by a physician is highly recommended. Infections caused by *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are of mandatory notification to the local Health Authorities in different countries. In the case that these pathogens are identified, it is the clinic's responsibility to declare these infections.

### ANTIBIOTICS INFORMATION

Antimicrobial therapy for bacterial pathogens is regulated by the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC). Following the recommendation of the Sanford guide††: Clindamycin is effective against: *Streptococcus agalactiae (group B)* and *Staphylococcus aureus*; Amoxicillin-clavulanate is effective against: *Streptococcus agalactiae (group B), Escherichia coli* and *Enterococcus faecalis*; Azithromycin is effective against: *Ureaplasma urealyticum, Mycoplasma hominis* and *Mycoplasma genitalium*; Trimethoprim-sulfamethoxazole is effective against: *Streptococcus agalactiae (group B), Staphylococcus aureus* and *Escherichia coli*; Dicloxacillin is effective against: *Staphylococcus aureus*; Ciprofloxacin is effective against: *Escherichia coli*; Moxifloxacin is effective against: *Enterococcus faecalis, Ureaplasma urealyticum, Mycoplasma hominis* and *Mycoplasma genitalium*; Fosfomycin tromethamine is effective against: *Enterococcus faecalis*; Doxycycline is effective against: *Ureaplasma urealyticum, Mycoplasma hominis* and *Mycoplasma genitalium.*

†† The Sanford Guide to Antimicrobial Therapy 2020. Editors, DN. Gilbert, M.D., HF. Chambers, M.D., MS. Saag, M.D., AT. Pavia, M.D. Sperryville, VA, USA: Antimicrobial Therapy, Inc., 2020.

# Benefits of Molecular Analysis of the Microbiome *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 inconsistent results in 80% of cases. While histology usually underdiagnoses CE, hysteroscopy usually overdiagnoses the disease. Histology and hysteroscopy 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).

# References

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# Endometrial Biopsy

# Endometrial Biopsy

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. The cryotube must be labeled with the patient's name, date of birth, and date of biopsy.

After the biopsy has been performed, the sample should be transferred immediately to the supplied cryotube and shaken vigorously for 10 seconds.

# Endometrial Biopsy

The endometrial biopsy must be taken from the uterine fundus using a pipelle catheter (Genetics, Hamont Achel, Belgium) or similar. When taking the endometrial biopsy it is very important to take the correct quantity of tissue, around 70 mg, which corresponds to tissue with sides of approximately 7 mm. 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. It is important not to exceed the white line marked on the cryotube, in order to avoid possible degradation of the genetic material. In the case that an EMMA or ALICE tests are requested (alone or coupled with ERA test) the use of prophylactic antibiotics during and after the procedure should be avoided.

# Endometrial Biopsy

After the biopsy has been performed, the sample should be transferred immediately to the supplied cryotube and shaken vigorously for at least 10 seconds. Ensure that the cryotube actually contains endometrial tissue before sending it (not only blood and/or mucus).

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. After this time, samples may be sent to Igenomix at room temperature. If samples are going to be exposed to >35°C/95°F, we recommend shipping the samples with a cold gelpack. Deliveries at room temperature should never exceed 5 days.

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 or ALICE 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.

# Day of Endometrial Biopsy

**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 and during the procedure**. If the patient has taken any antibiotic in the previous three months, it must be documented on the "Test Requisition Form": name of the active ingredient, dose, way of administration and duration of the treatment. This includes any prophylactic antibiotic such as those used for oocyte retrievals. Likewise, if a biopsy is 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 during or after the procedure. Other drugs that may alter the patient's microbiota or immunological status should also be included in the form.

# Endometrial Biopsy

If only an EMMA or ALICE test is requested, the endometrial biopsy should be taken following the same protocol as for ERA or between days 15 and 25 of a natural cycle (only for patients with regular cycles between 26-32 days). If the patient does not cycle regularly, we recommend performing an HRT cycle and take the biopsy on P+5.

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) and 2)].

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

a) **Hormone Replacement Therapy cycle:** involves treatment with estrogen and progesterone to inhibit endogenous production of these hormones, 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.

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).

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.

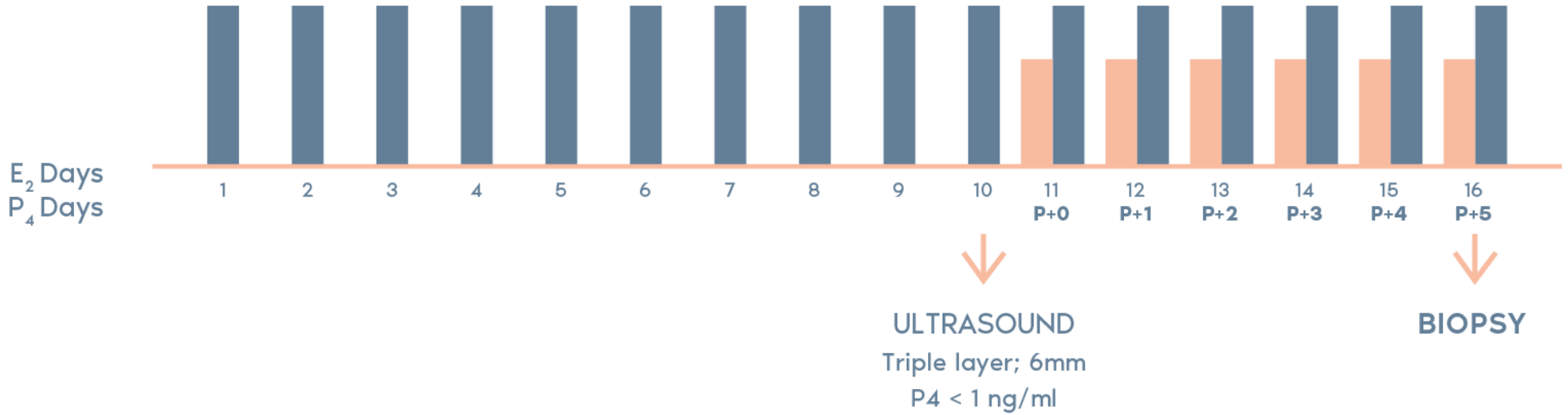
b) **Natural cycle**: hCG (recombinant or urinary) is administered according to routine parameters in a natural cycle (follicle size >17 mm). The day of the hCG administration is considered as hCG+0 and the biopsy will be taken 7 days later, at hCG+7 (168 hours after hCG triggering).

c) **Controlled ovarian stimulation:** The endometrial biopsy cannot be performed in a controlled ovarian stimulated cycle. Therefore, it should be performed in a subsequent HRT or natural cycle as indicated above.

The first biopsy should always be performed at P+5, hCG+7 or LH+7, since the ERA checks the endometrium at the moment of implantation. In that 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.

EndomeTRIO
The endometrium matters
by Igenomix

# HRT Routine Protocol




Igenomix

# Logistics

**Sample and documents**:

- Read and properly fill all the information required in the "Test Requisition Form" and "Consent Form".
- Place the cryotube containing the biopsy inside the rigid plastic blister and close it. Next, place the plastic blister inside the biohazard bag. Then, place the biohazard bag in the kit provided by Igenomix. Lastly, place the kit in the plastic (courier) return bag (also provided by Igenomix).
- Place the filled-out "Test Requisition Form" and "Consent Form" inside the return bag.

- Attach the provided courier documents to the included courier bag to return the sample.
- Transit 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 our Customer Support Department.

**Shipment**:

- Please inform us by email about each shipment, indicating the number of samples and their clinical or reference record number. Please take note of tracking information prior to sending.

# A Complete View of Endometrial Health

## Endometrial Health Solutions

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

**pET: personalized embryo transfer*

Igenomix®