A complete view of endometrial health

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EndomeTRIO- Summary & Key Information

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ERA® Endometrial Receptivity Analysis	**ENDOMETRIAL RECEPTIVITY ANALYSIS** **Expression of 248 genes to guide pET***
EMMA Endometrial Microbiome Metagenomic Analysis	**COMPLETE MICROBIOME ANALYSIS** **Percentage of Lactobacilli, pathogens and dysbiotic bacteria** *Microbiological counselling for a personalised treatment* + **CHRONIC ENDOMETRITIS** **Pathogenic bacteria related to CE** *Microbiological counselling for a personalised treatment*
ALICE Analysis of Infectious Chronic Endometritis	**CHRONIC ENDOMETRITIS** **Pathogenic bacteria related to CE** *Microbiological counselling for a personalised treatment*

**pET: personalised embryo transfer*

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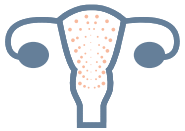
Action Protocol

FOR EMMA

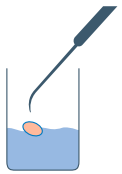
Take a biopsy either:

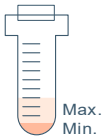
- At the usual ERA timing (below)
- OR between days 15-25 in a natural cycle

Select cycle type



Place the biopsy in the cryotube

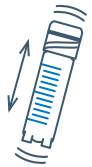




The quantity of tissue must not exceed 1/3 of the total volume of the cryotube.

~7 mm

Label the cryotube and shake vigorously for 10 seconds



Place the sample in the refrigerator at 4-8°C/39-46°F for a minimum of 4 hours

Send the sample at room temperature (<35°C/95°F)

HRT OR NATURAL CYCLE

FOR ERA OR ENDOMETRIO

Take a biopsy during estimated WOI:

- In a HRT cycle, after 5 full days (120 h) of P exposure.
- In a natural cycle, at LH+7 or hCG+7

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Biopsy Timing

TEST PERFORMED	CYCLE TYPE & BIOPSY TIMING				
	NATURAL (Regular 26-32 day cycles)	**NATURAL** (LH surge measured)	**NATURAL** (hCG trigger)	**NATURAL** (ovulation timing)	**HRT** (Start of exog. progesterone)
ERA 'STANDALONE' *(Includes: ERA)*	-	LH+7	hCG+7	-	P+5
ENDOMETRIO *(Includes: ERA+EMMA+ALICE)*	-	LH+7	hCG+7	-	P+5
EMMA 'STANDALONE' *(Includes: EMMA+ALICE)*	Days 15-25 (inclusive; *ONLY patients with regular 26-32 day cycles*)	LH+2 – LH+12 (inclusive)	hCG+2 – hCG+12 (inclusive)	Ov+1 – Ov+11 (inclusive)	P+1 – (inclusive, any time during progesterone treatment)
ALICE 'STANDALONE' *(Includes: ALICE)*					

ANY OTHER CYCLE

Contact Igenomix with details of the cycle (medications, timings etc.) to confirm appropriate biopsy timing

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ERA®

Endometrial Receptivity Analysis

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Rationale

The endometrial factor plays a key role in embryo implantation. When transferring an embryo, the endometrium must be in a receptive state to allow successful implantation - this period of receptivity is called the window of implantation, which varies from one woman to another. 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 personalised 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, Ruiz-Alonso et al. Hum Reprod, 2014; Simon et al. Reprod Biomed Online, 2020).

ERA aims to identify the window of implantation in the endometrial cycle, enabling personalised embryo transfer (pET).

Research by Igenomix has demonstrated that synchronisation between an implantation-ready embryo and a receptive endometrium increases the chances of success during assisted reproductive treatment (Ruiz-Alonso et al. Fertil Steril, 2013; Ruiz-Alonso et al. Hum Reprod, 2014; Clemente-Ciscar et al. Hum Reprod, 2018). Other groups have also published similar results from their own patients after embryo transfer timed 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 synchronising an implantation-ready embryo with a receptive endometrium.

Indications for ERA

ERA is 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.

If your patient requires any intervention at the uterine level, the ERA test should be done after this procedure has been completed, in order to ensure ERA has been performed in the same 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 analyse 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 include a recommendation for personalised embryo transfer according to each patient's specific endometrial profile. In approximately 10% of cases, it may be necessary to validate the personalised window of implantation by performing a second endometrial biopsy on the specific day designated in the report of the patient's first ERA test.

To ensure reproducibility of results, the ERA test must be performed under the same conditions as the planned subsequent embryo transfer cycle (cycle type, treatment, method of administration etc. should be the same) and always during a hormone replacement therapy (HRT) or natural cycle. This test cannot be performed in controlled ovarian stimulated cycles. This is called a "mock cycle"

The first endometrial biopsy for a patient should be taken after 5 full days of progesterone administration (P+5) in an HRT cycle (120 hours of progesterone administration), or 7 days after 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 ERA analyses the endometrium at the time 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.

For more details on biopsy timing, please refer to page 40.

Report and interpretation of results

The ERA report will indicate the optimal time to perform personalised embryo transfer (pET), or when to perform a new ERA biopsy (if required).

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

Late Receptive: The gene expression profile is consistent with an endometrium at the end of the receptive stage. The recommendation for the patient's transfer cycle is to administer 12 hours **LESS** progesterone treatment (HRT) or rest (natural cycle) 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, and the ERA report will provide timings for a personalised embryo transfer. 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 consistent 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.

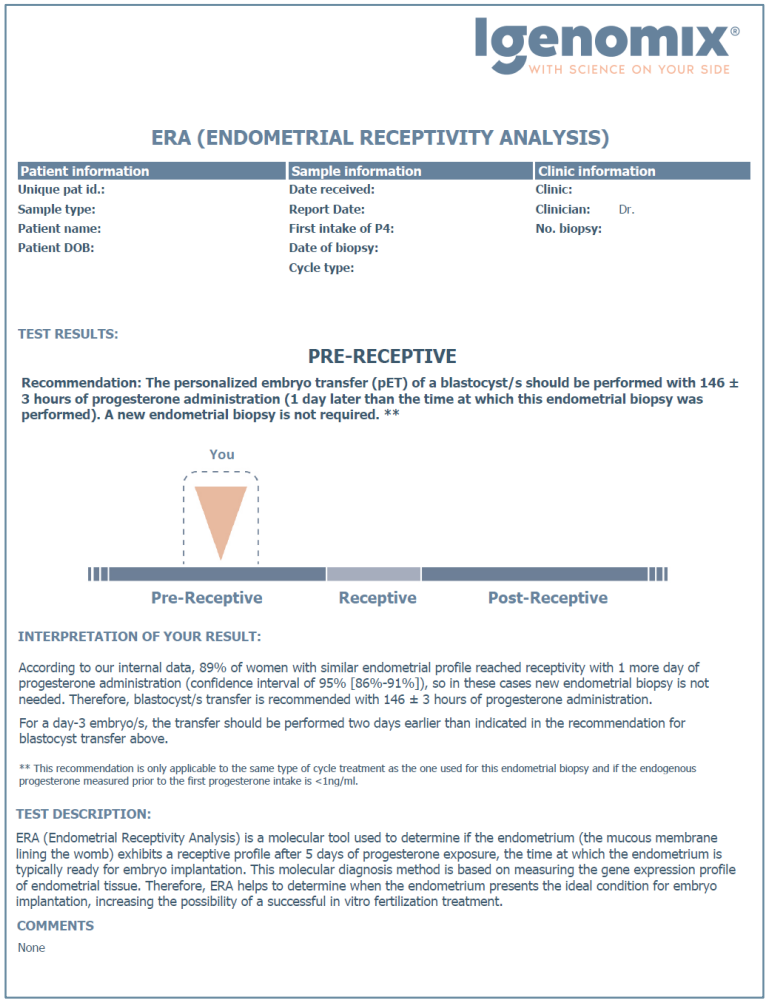
In approximately 5% of samples received, a result cannot be obtained. This is due to a non-informative profile or to the low quantity/quality of the genetic material extracted.

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

Report format

The aim of this test is to provide clinicians with an objective molecular diagnosis of the timing of a patient's window of implantation.

The report must be received and interpreted by the referring clinician.



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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.

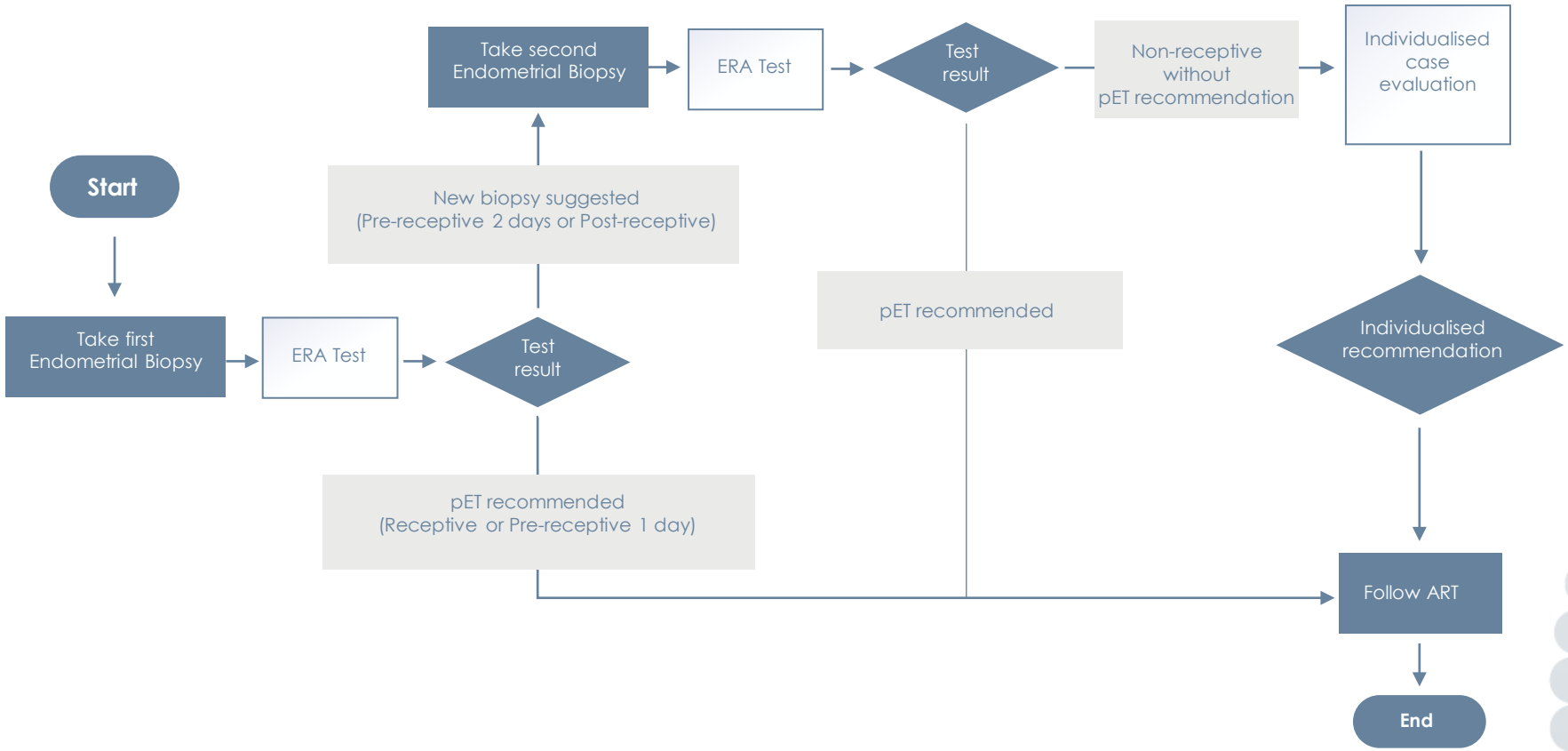
TEST DESCRIPTION:

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; Moreno et al. Microbiome, 2022).

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

This test uses the latest Next Generation Sequencing (NGS) technology to provide microbiome information for endometrial tissue by analysing the **complete endometrial microbiome profile**. The technology is based on DNA extraction followed by amplification and barcoded sequencing of the bacterial 16S ribosomal RNA gene.

This bacterial gene, observed in all bacteria, presents nine variable regions with species-specific DNA sequences. This enables the taxonomic assignment and relative quantification of each bacteria present in a sample.

A single endometrial sample contains both endometrial and bacterial cells. Analysis of these cells using deep sequencing can provide a complete profile of the endometrial microbiome, thus improving clinical management of patients.

Report and interpretation of results

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

- Percentage of *Lactobacilli* in the endometrial sample.
- Percentages of other bacteria detected in the endometrial sample (for those present in a significant amount).
- Classification of the endometrial microbiota profile: normal (high percentage of *Lactobacillus*), abnormal (significant presence of pathogenic or dysbiotic bacteria), dysbiotic (low percentage of *Lactobacillus*) or ultralow biomass (the amount of endometrial flora is extremely low)
- Suggested probiotic/antibiotic therapy. Recommendations for antibiotic therapy will always be guided by an expert clinical microbiologist, who will counsel the doctor on an individual basis.
- ALICE test results: because **EMMA includes ALICE**, the results of CE diagnosis and abundance of CE-causing bacteria are also shown in the EMMA report.

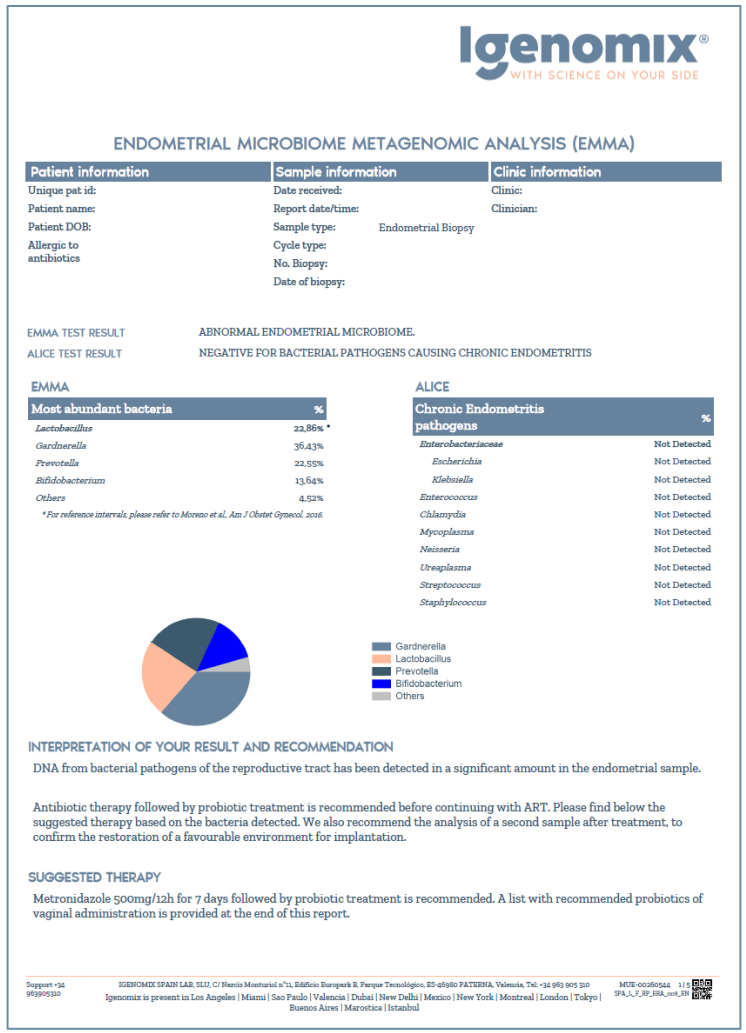
EMMA

Endometrial Microbiome
Metagenomic Analysis

Report

Recommendations for antimicrobial therapy will always be guided by an expert clinical microbiologist, who will counsel the doctor on an individual basis.

The report must be received and interpreted by the referring clinician.



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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:
Patient DOB:	Sample type: Endometrial Biopsy	
Allergic to antibiotics	Cycle type:	
	No. Biopsy:	
	Date of biopsy:	

EMMA TEST RESULT — ABNORMAL ENDOMETRIAL MICROBIOME.
ALICE TEST RESULT — NEGATIVE FOR BACTERIAL PATHOGENS CAUSING CHRONIC ENDOMETRITIS

EMMA

Most abundant bacteria	%
Lactobacillus	22,86% *
Gardnerella	36,43%
Prevotella	22,55%
Bifidobacterium	13,64%
Others	4,52%

* For reference intervals, please refer to Moreno et al, Am J Obstet Gynecol. 2016.

ALICE

Chronic Endometritis pathogens	%
Enterobacteriaceae	Not Detected
Escherichia	Not Detected
Klebsiella	Not Detected
Enterococcus	Not Detected
Chlamydia	Not Detected
Mycoplasma	Not Detected
Neisseria	Not Detected
Ureaplasma	Not Detected
Streptococcus	Not Detected
Staphylococcus	Not Detected

INTERPRETATION OF YOUR RESULT AND RECOMMENDATION

DNA from bacterial pathogens of the reproductive tract has been detected in a significant amount in the endometrial sample.

Antibiotic therapy followed by probiotic treatment is recommended before continuing with ART. Please find below the suggested therapy based on the bacteria detected. We also recommend the analysis of a second sample after treatment, to confirm the restoration of a favourable environment for implantation.

SUGGESTED THERAPY

Metronidazole 500mg/12h for 7 days followed by probiotic treatment is recommended. A list with recommended probiotics of vaginal administration is provided at the end of this report.

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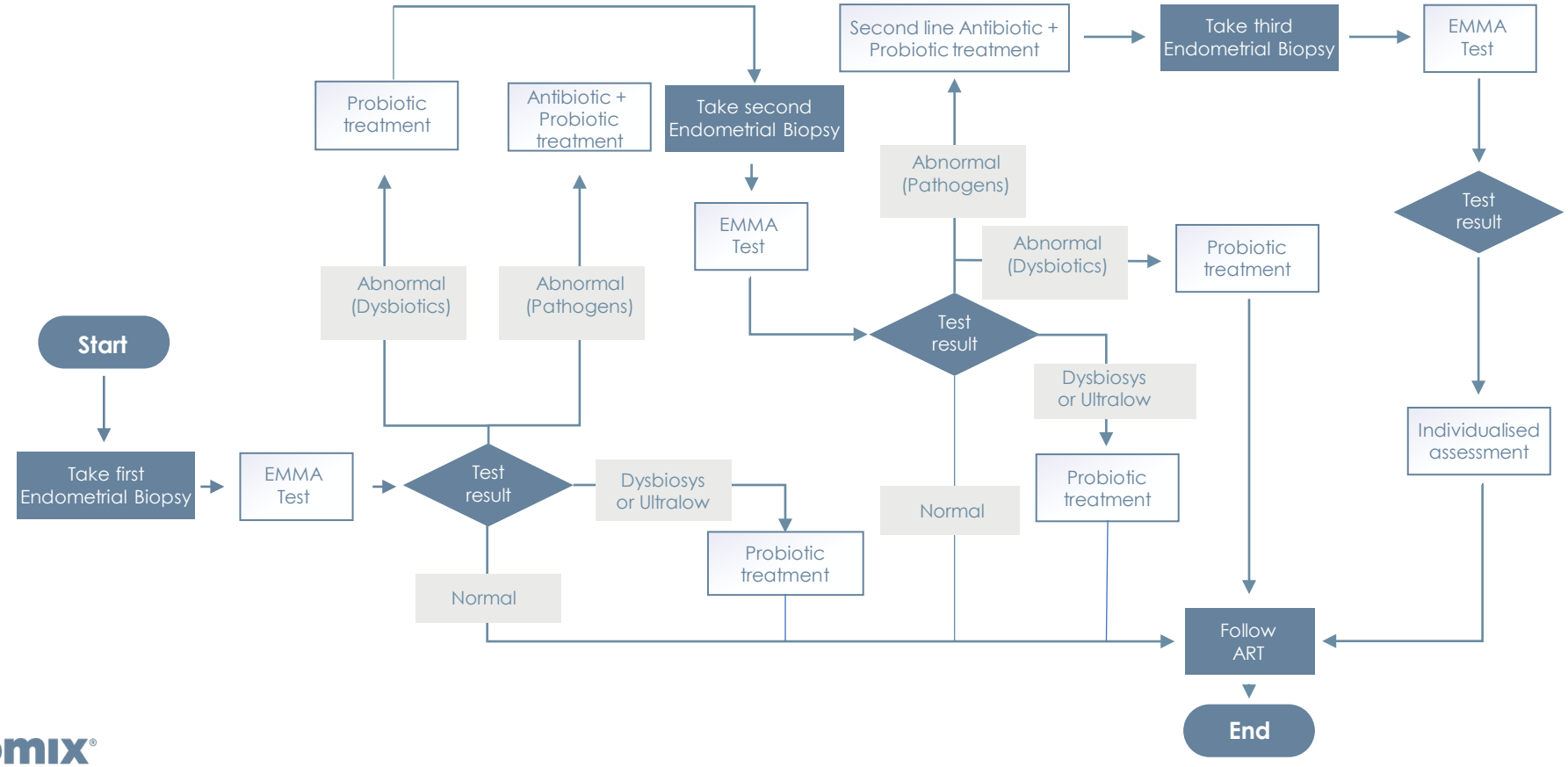
EMMA Decision tree



Benefits of NGS 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 the location of sampling, between 20% and 60% of bacteria cannot be cultured. Molecular assessment of the microbiome using NGS allows detection of culturable and non-culturable bacteria.

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ALICE

Analysis of Infectious Chronic Endometritis

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Rationale

The best example of a 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 CE is usually asymptomatic and because of known limitations of current classical diagnostic methods (histology, hysteroscopy and microbial culture), CE is often overlooked, although may affect 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, personalised 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 outcomes.

Methodology

ALICE uses the latest NGS technology to provide information about the abundance of specific bacteria that cause CE in an endometrial sample.

The technology is based on DNA extraction followed by amplification and barcoded sequencing of the bacterial 16S ribosomal RNA gene from the most-frequently-CE-causing bacteria.

A single biopsy contains both endometrial and bacterial cells. ALICE test can differentiate the bacterial genes from human genes present in the DNA extracted from the sample. The 16S rRNA gene is observed in all bacteria and presents nine variable regions with species-specific DNA sequences. **This enables the taxonomic assignment and relative quantification of CE bacteria present in a sample.**

Report and interpretation of results

The ALICE report will focus on the detection and abundance of those specific bacteria that cause CE.

These bacteria are: *Enterococcus* spp., Enterobacteriaceae (*Escherichia* and *Klebsiella*), *Streptococcus* spp., *Staphylococcus* spp., *Mycoplasma* spp, and *Ureaplasma* spp. In addition, other pathogens associated with sexually transmitted infections (STI), such as *Chlamydia* and *Neisseria* spp. will be reported.

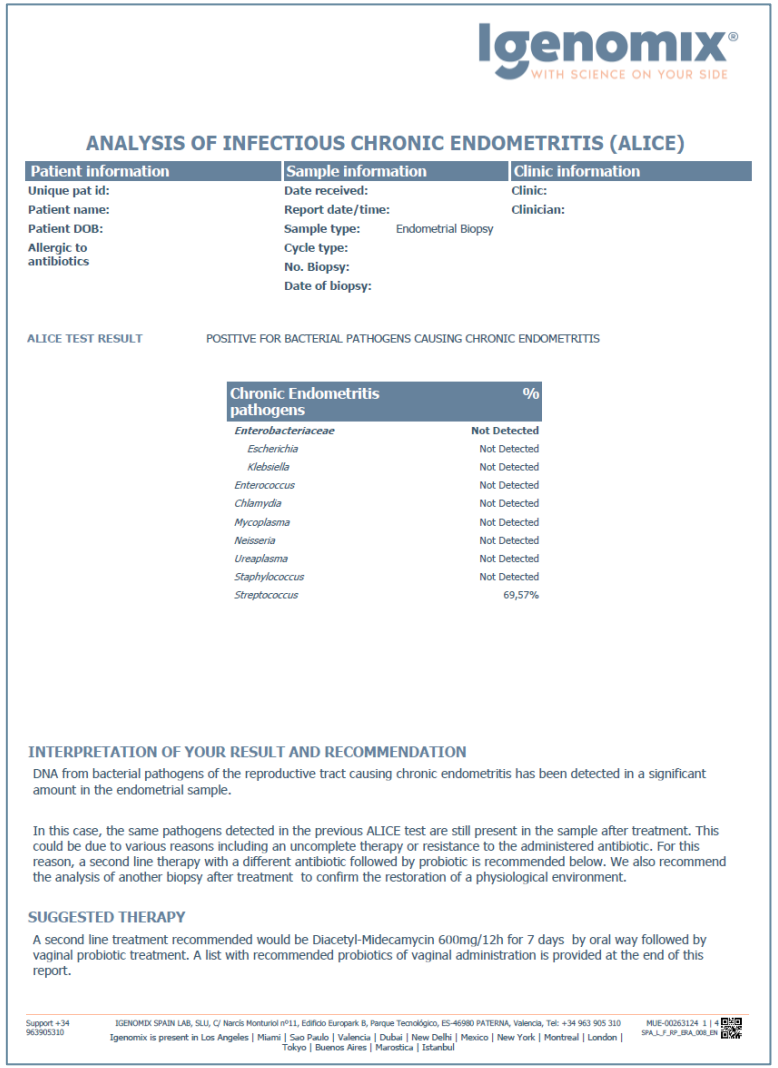
The report will recommend individualised treatment with the appropriate antibiotics and probiotics.

Note that ALICE IS INCLUDED IN THE EMMA TEST.

Report

Recommendations for antimicrobial therapy will always be guided by an expert clinical microbiologist, who will counsel the clinician on an individual basis.

The report must be received and interpreted by the referring clinician.



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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:**
Patient DOB:	**Sample type:** Endometrial Biopsy	
Allergic to antibiotics	**Cycle type:**	
	No. Biopsy:	
	Date of biopsy:	

ALICE TEST RESULT POSITIVE FOR BACTERIAL PATHOGENS CAUSING CHRONIC ENDOMETRITIS

Chronic Endometritis pathogens	%
Enterobacteriaceae	**Not Detected**
Escherichia	Not Detected
Klebsiella	Not Detected
Enterococcus	Not Detected
Chlamydia	Not Detected
Mycoplasma	Not Detected
Neisseria	Not Detected
Ureaplasma	Not Detected
Staphylococcus	Not Detected
Streptococcus	69,57%

INTERPRETATION OF YOUR RESULT AND RECOMMENDATION

DNA from bacterial pathogens of the reproductive tract causing chronic endometritis has been detected in a significant amount in the endometrial sample.

In this case, the same pathogens detected in the previous ALICE test are still present in the sample after treatment. This could be due to various reasons including an uncomplete therapy or resistance to the administered antibiotic. For this reason, a second line therapy with a different antibiotic followed by probiotic is recommended below. We also recommend the analysis of another biopsy after treatment to confirm the restoration of a physiological environment.

SUGGESTED THERAPY

A second line treatment recommended would be Diacetyl-Midecamycin 600mg/12h for 7 days by oral way followed by vaginal probiotic treatment. A list with recommended probiotics of vaginal administration is provided at the end of this report.

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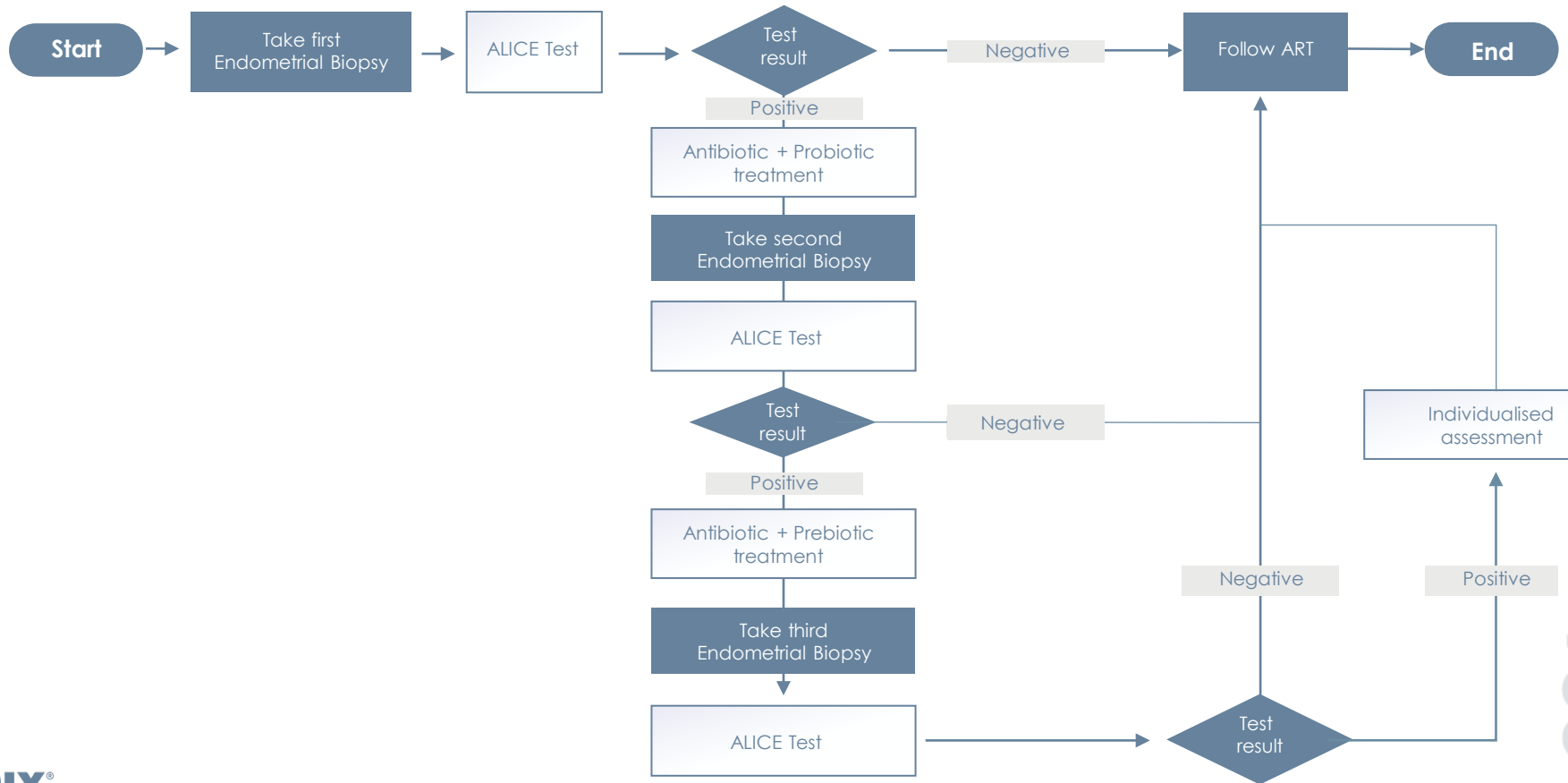
ALICE
Analysis of Infectious Chronic Endometritis

Alice Decision tree



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Benefits of NGS CE pathogen detection vs classical methods

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).

References

Moreno I, Cicinelli E, Garcia-Grau I, et al. 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

Cicinelli E, Matteo M, Tinelli R, et al. 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.

Cicinelli E, Matteo M, Tinelli R, et al. Prevalence of chronic endometritis in repeated unexplained implantation failure and the IVF success rate after antibiotic therapy. Hum Reprod, 2015; 30(2), pp. 323-30.

References

I. Moreno and C. Simón. Microbiological diagnosis: the human endometrial microbiome—Endometritis. In: The Endometrial Factor, A Reproductive Precision Medicine Approach. Edited by Simón C and Giudice L. Taylor & Francis Group; 2017. Chapter 5. DOI: 10.1201/9781315151472.

Endometrial Biopsy

Igenomix®

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 labelled with either:

- The patient's initials and their date of birth or
- The patient's initials and their unique ID/medical record number

After the biopsy has been performed, the sample should be transferred immediately to the supplied cryotube and shaken vigorously for a few seconds. The pipelle catheter used to collect the sample must be discarded as clinical waste in compliance with current legal regulations.

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. If an EMMA or ALICE test is requested (alone or coupled with ERA test), the use of prophylactic antibiotics should be avoided during the procedure.

Endometrial Biopsy

Ensure that the cryotube actually contains endometrial tissue before sending it.

The cryotube containing the sample should be transferred to a refrigerator (4-8°C/39-46°F) immediately, and should be stored there for **at least 4 hours.** After this time, samples may be sent to Igenomix at room temperature (<35°C/95°F). 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 they are 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.

Endometrial Biopsy

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**. If the patient has taken any antibiotic in the previous three months, please include the following information in the "Test Requisition Form": name of the active ingredient, dose, method of administration and duration of the treatment. This includes any prophylactic antibiotic such as those used during oocyte retrieval. 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 the natural cycle (for patients with regular cycles between 26-32 days).

In 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 as the mock cycle used for ERA. The transfer should be timed according to the personalised window of implantation provided in the ERA report. **The type of cycle for biopsy should match the type of cycle planned for the embryo transfer.**

Endometrial Biopsy

2) Cycle type

a) Hormone Replacement Therapy cycle: Involves treatment with oestrogen and progesterone to inhibit endogenous production of these hormones, using the routine protocol at the clinic or our standard protocol:

Patient starts oestradiol 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 <1ng/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).

Endometrial Biopsy

In an HRT cycle it is very important to ensure that there is no ovulation, and therefore it is recommended to always measure the endogenous P4 level 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.

b) **Natural cycle**: hCG (recombinant or urinary) is administered according to routine parameters in a natural cycle (follicle size >17 mm). The day of hCG administration is considered hCG+0 and the biopsy will be taken 7 full days later, at hCG+7 (168 hours after hCG triggering). ERA can also be performed based on the measurement of the LH surge; in these cases, the biopsy should be taken at LH+7 (168 hours post-LH surge).

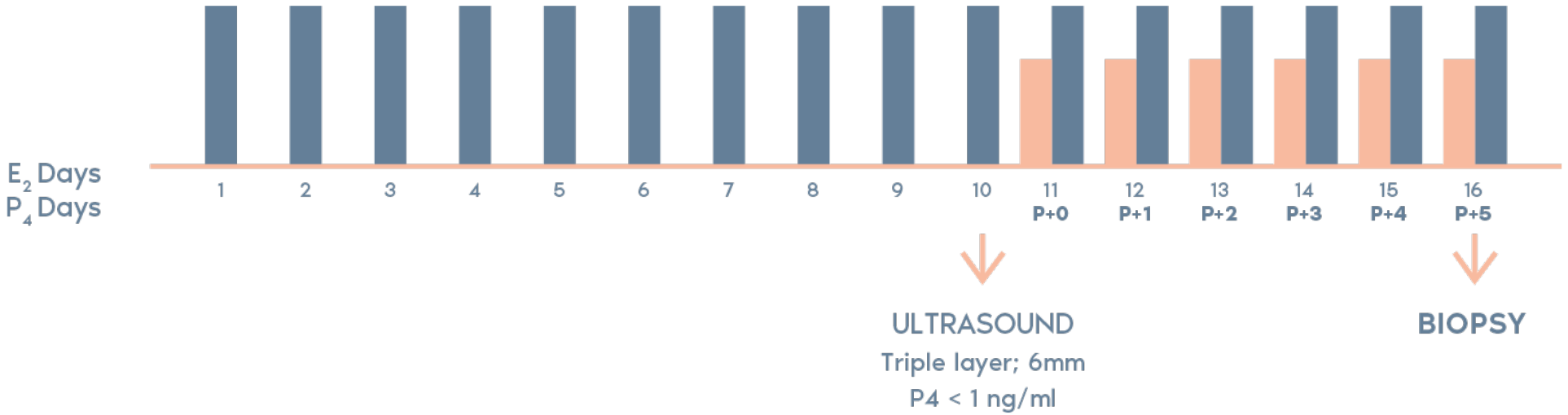
Endometrial Biopsy

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 ERA checks the endometrium at the time of implantation, and these timings are the most frequent timings for frozen embryo transfer. Using the ERA result, 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.

Endometrial Biopsy

HRT Routine Protocol



Igenomix®

Endometrial Biopsy

Logistics

Packaging the sample:

- Read and complete all the information required in the Test Requisition and Consent Form.
- Place the cryotube containing the biopsy inside the rigid plastic blister and close it.
- Package the blister and completed forms inside the kit box
- Place the kit box in the plastic courier return bag provided by Igenomix.

Endometrial Biopsy

Shipment:

- Contact support.uk@igenomix.com up to 5 days in advance to arrange a collection.
- Domestic same-day collection is available Monday-Thursday for requests prior to 13:30.

- You will receive the courier waybill documents by email. There are two of these documents:
  - The package waybill document has three barcodes and should be placed in the dedicated document compartment, which can be found on the courier return envelope. Seal the compartment.
  - The courier waybill document has one barcode and should be handed to the courier when they arrive.
- Transit at room temperature should not exceed 5 days. We recommend shipping the samples with a cold gelpack if outside temperatures exceed 35°C. For further details, please contact our Customer Support team.

Igenomix®