A complete view of endometrial health

Igenomix®

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Summary

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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 is also important to determine 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).

Rationale

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 is mainly 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. On the other hand, the application of ERA to patients without RIF has also been explored (Simon et al. Reprod BioMed Online, 2020).

Indications for ERA

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.

Methodology

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 the requires a more personalized recommendation for transfer, beyond what is provided by our algorithm

Methodology

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.

Interpretation of the Results

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.

ERA®
Endometrial
Receptivity Analysis

Interpretation of the Results

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 1.4% 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 (≥35°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 1.6% 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 the a valid result for your patient, trusting on that we are looking for quality and reliability.

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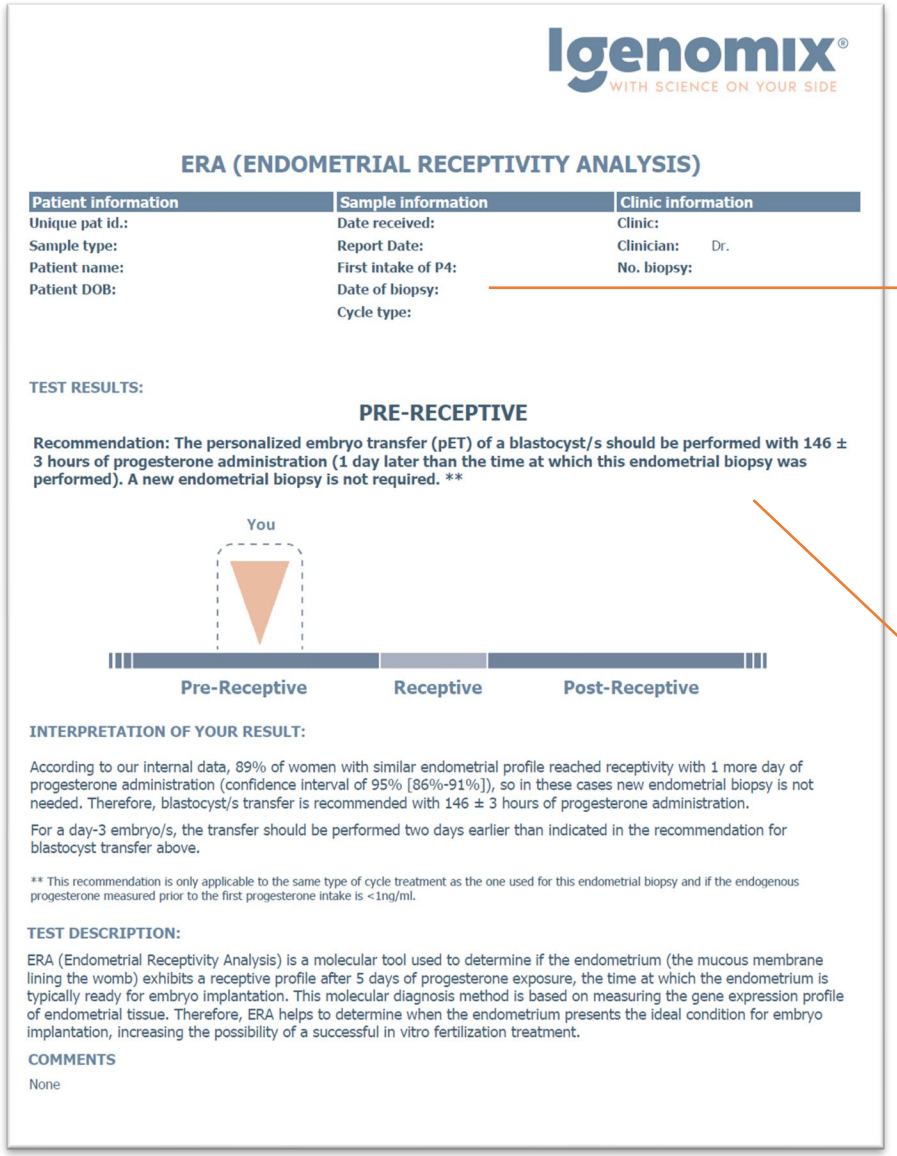
ERA Example Report

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.



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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

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).

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

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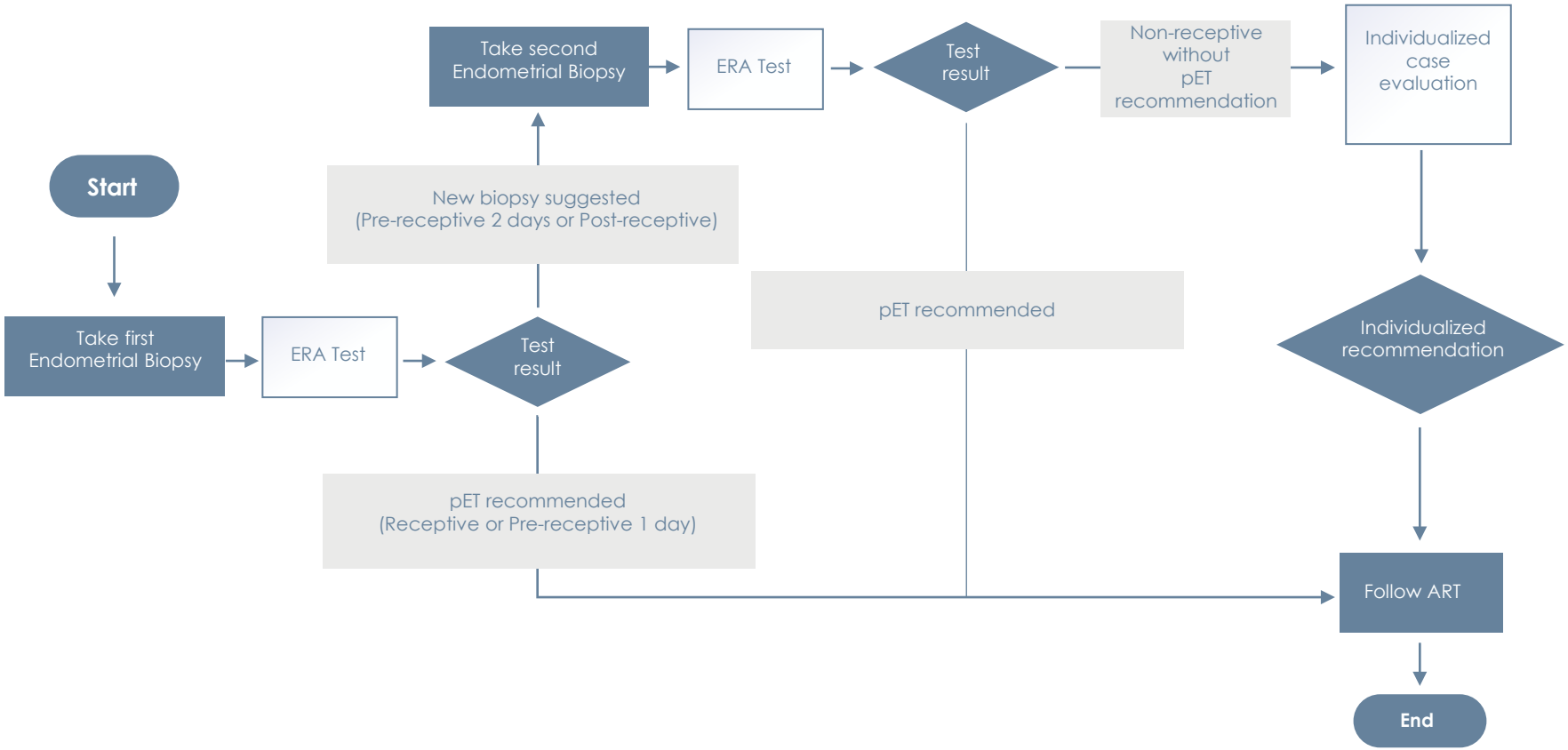
Reproducibility of the Results

The ERA result has been **proven to be reproductible 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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Relevant references

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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).

Rationale

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**.

EMMA

Endometrial Microbiome Metagenomic Analysis

EMMA Example Report

The EMMA report shows 3 tables with the normal ranges† and the values obtained in the endometrial sample for:

4 species of Lactobacilli (*L. crispatus, L. gasseri, L. iners* and *L. jensenii*)

11 species of common reproductive tract pathogens (*Gardnerella vaginalis, Prevotella bivia, Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Megasphaera spp 1, Megasphaera spp 2 Treponema pallidum, Bacteroides fragilis, Bacterial Vaginosis Associated Bacteria 2* and *Haemophilus ducreyi*)

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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 EMMA 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			
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 mullieris	Not detected	N/A	≤ 3.55
Megasphaera spp 1	Not detected	N/A	≤ 4.17
Megasphaera spp 2	Not detected	N/A	≤ 3.43
Treponema pallidum	Not detected	N/A	absent
Bacteroides fragilis	Not detected	N/A	≤ 3.42
BVAB2	Not detected	N/A	≤ 3.51
Haemophilus ducreyi	Not detected	N/A	absent

RESULTS OF ALICE TEST:

PATHOGENS RELATED WITH CHRONIC ENDOMETRITIS			
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	absent
Chlamydia trachomatis	Not detected	N/A	absent

† 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.

EMMA COMMENTS

Pathogens of the reproductive tract, not related to chronic endometritis, have been detected to be out of normal range. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy.

Lactobacillus levels are below normal range. *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.

ALICE COMMENTS

No pathogens related to chronic endometritis have been detected.

ALICE test = 9 species of pathogens most common-causing chronic endometritis (*Streptococcus agalactiae (group B), Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Ureaplasma urealyticum, Mycoplasma hominis, Mycoplasma genitalium, Neisseria gonorrhoeae* and *Chlamydia trachomatis*)

† 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.

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EMMA
Endometrial Microbiome Metagenomic Analysis

EMMA Example Report

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.

Antibiotic dosage recommendations can be found at Igenomix website: https://www.igenomix.com/wp-content/uploads/2021/11/Antibiotic-Dosage-Recommendations-2.png

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[††]:

METRONIDAZOLE is effective against: *Gardnerella vaginalis, Prevotella bivia, Bacteroides fragilis* and *BVAB2.*

CLINDAMYCIN is effective against: *Gardnerella vaginalis, Prevotella bivia, Bacteroides fragilis, BVAB2, Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Streptococcus agalactiae (group B)* and *Staphylococcus aureus.*

TINIDAZOL is effective against: *Gardnerella vaginalis, Prevotella bivia* and *BVAB2.*

AMOXICILLIN-CLAVULANATE is effective against: *Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Streptococcus agalactiae (group B), Bacteroides fragilis, Escherichia coli* and *Enterococcus faecalis.*

AZITHROMYCIN is effective against: *Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, 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.

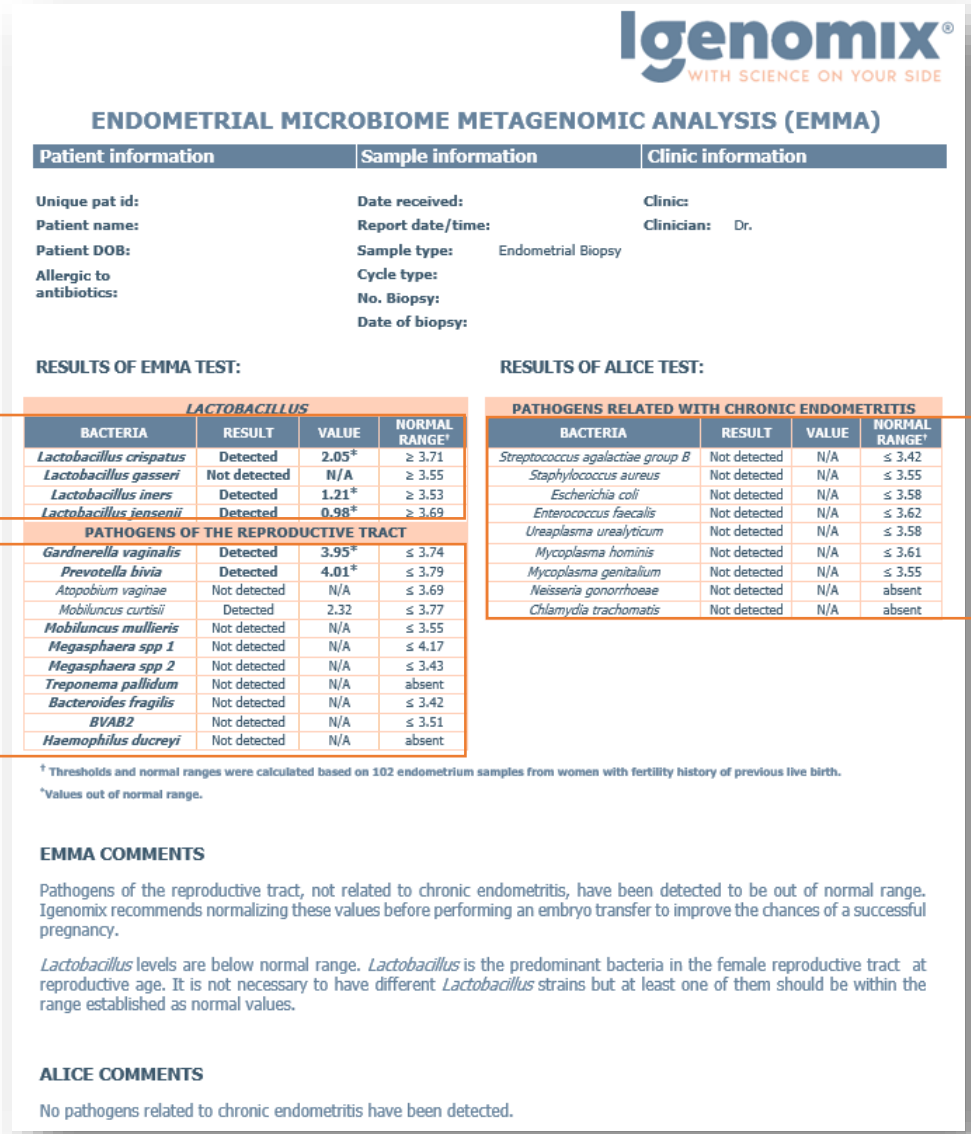
EMMA

Endometrial Microbiome Metagenomic Analysis

EMMA Interpretation of the Results

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.*

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



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	No. Biopsy:	
	Date of biopsy:	

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Neisseria gonorrhoeae	Not detected	N/A	absent
Chlamydia trachomatis	Not detected	N/A	absent

† 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.

EMMA COMMENTS

Pathogens of the reproductive tract, not related to chronic endometritis, have been detected to be out of normal range. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy.

Lactobacillus levels are below normal range. *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.

ALICE COMMENTS

No pathogens related to chronic endometritis have been detected.

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

Note: If any of the **pathogens associated with Sexually Transmitted Infections (*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.

Igenomix®

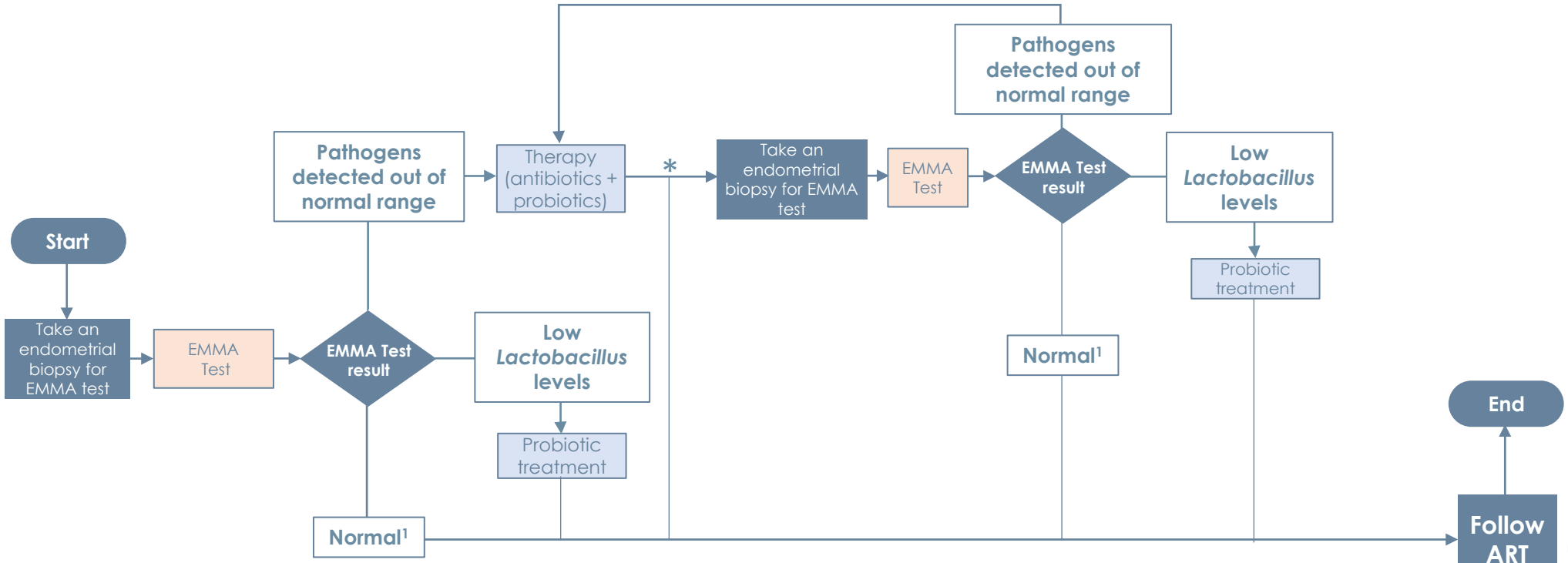
Benefits of Molecular Analysis of the Microbiome *vs* Microbial Culture

Microbial culture is the current gold-standard method for assessment of bacterial populations and infection. However, it has been demonstrated that, depending on the species, 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.

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

*With OpenArray we detect the species and are able to recommend an empiric antibiotic treatment against the pathogens detected, but with culture, it's possible to determine antibiotic resistances and therefore the recommended treatment is specific for the isolated strain/s

EMMA Decision Tree



[1] Normal: no pathogens detected and *Lactobacillus* within the normal 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.

Relevant References

Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature **2012**; 486:207–14.

Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazan J, Alonso R, Alama P, Remohi J, Pellicer A, Ramon D, Simon C. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol. **2016**; 215:684-703.

Inmaculada Moreno; Jason M Franasiak. Endometrial microbiota - new player in town. Fertility and Sterility. **2017;**108, pp. 32 – 39.

Jason M Franasiak; Inmaculada Moreno; Carlos Simon. Microbiome in Embryonic Implantation and Implantation Failure. In: Recurrent Implantation Failure, Etiologies and Clinical Management. **2018**; Chapter 11, pp. 175 - 195. Springer, Cham. ISBN 978-3-319-71966-5.

Carlos Simon; Inmaculada Moreno. Deciphering the effect of reproductive tract microbiota on human reproduction. Reproductive Medicine and Biology. **2018**;18 - 1, pp. 40 – 50.

Inmaculada Moreno; Carlos Simon. Relevance of assessing the uterine microbiota in infertility. Fertility and Sterility. **2018** Aug;110(3):337-343. doi: 10.1016/j.fertnstert.2018.04.041.

Relevant References

Garcia-Grau I, Simon C, Moreno I. Uterine microbiome-low biomass and high expectations. Biol Reprod. **2018** Dec 13; doi: 10.1093/biolre/ioy257. PMID: 30544156.

Inmaculada Moreno and Carlos Simon. Screening the Uterine Microbiome Prior to Embryo Transfer. In: How to Prepare the Endometrium to Maximize Implantation Rates and IVF Success Edited by G. Kovacs & L. Salamonsen; **2019**. Chapter 6 (pp. 54-64). Cambridge: Cambridge University Press.doi:10.1017/9781108236263.007.

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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Moreno, I., Garcia-Grau, I., Bau, D., Perez-Villaroya, D., Gonzalez-Monfort, M., Vilella, F., Romero, R., Simón, C. The first glimpse of the endometrial microbiota in early pregnancy. American Journal of Obstetrics and Gynecology. **2020** Apr;222(4):296-305.

Moreno, I., Garcia-Grau, I., Perez-Villaroya, D., Gonzalez-Monfort, M., Bahçeci, M., Barrionuevo, M.J., Taguchi, S., Puente, E., Dimattina, M., Lim, M.W., Meneghini, G., Aubuchon, M., Leondires, M., Iquierdo, A., Perez Olgiati, M., Chavez, A., Seetharm K., Bau, D., Gomez, C., Valbuena, D., Vilella, F., Simón, C. Endometrial microbiota composition is associated with reproductive outcome in fertile patients. Microbiome. **2022** Jan 4;10(1):1. doi: 10.1186/s40168-021-01184-w

ALICE

Analysis of Infectious Chronic Endometritis

Igenomix®

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.**

Rationale

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 screening 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 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 9 afore mentioned pathogens.

ALICE
Analysis of Infectious Chronic Endometritis

ALICE Example Report

The ALICE report shows 1 table with the normal ranges† and the values obtained in the endometrial sample for:

ALICE test = 9 species of pathogens most common-causing chronic endometritis *(Streptococcus agalactiae (group B), Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, Ureaplasma urealyticum, Mycoplasma hominis, Mycoplasma genitalium, Neisseria gonorrhoeae and Chlamydia trachomatis)*

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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**	**3.65***	≤ 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	absent
Chlamydia trachomatis	Not detected	N/A	absent

†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.

COMMENTS

Pathogens related to chronic endometritis have been detected to be out of normal range. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy.

† 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.

ALICE
Analysis of Infectious Chronic Endometritis

ALICE Example Report

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.

Antibiotic dosage recommendations can be found at Igenomix website: https://www.igenomix.com/wp-content/uploads/2021/11/Antibiotic-Dosage-Recommendations-2.png

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[††]:

METRONIDAZOLE is effective against: *Gardnerella vaginalis, Prevotella bivia, Bacteroides fragilis* and *BVAB2*.

CLINDAMYCIN is effective against: *Gardnerella vaginalis, Prevotella bivia, Bacteroides fragilis, BVAB2, Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Streptococcus agalactiae (group B)* and *Staphylococcus aureus.*

TINIDAZOL is effective against: *Gardnerella vaginalis, Prevotella bivia* and *BVAB2*.

AMOXICILLIN-CLAVULANATE is effective against: *Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, Streptococcus agalactiae (group B), Bacteroides fragilis, Escherichia coli* and *Enterococcus faecalis*.

AZITHROMYCIN is effective against: *Atopobium vaginae, Mobiluncus curtisii, Mobiluncus mullieris, 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.

ALICE
Analysis of Infectious Chronic Endometritis

ALICE Interpretation of the Results

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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
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Neisseria gonorrhoeae	Not detected	N/A	absent
Chlamydia trachomatis	Not detected	N/A	absent

†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.

COMMENTS

Pathogens related to chronic endometritis have been detected to be out of normal range. Igenomix recommends normalizing these values before performing an embryo transfer to improve the chances of a successful pregnancy.

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

*Note: 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.

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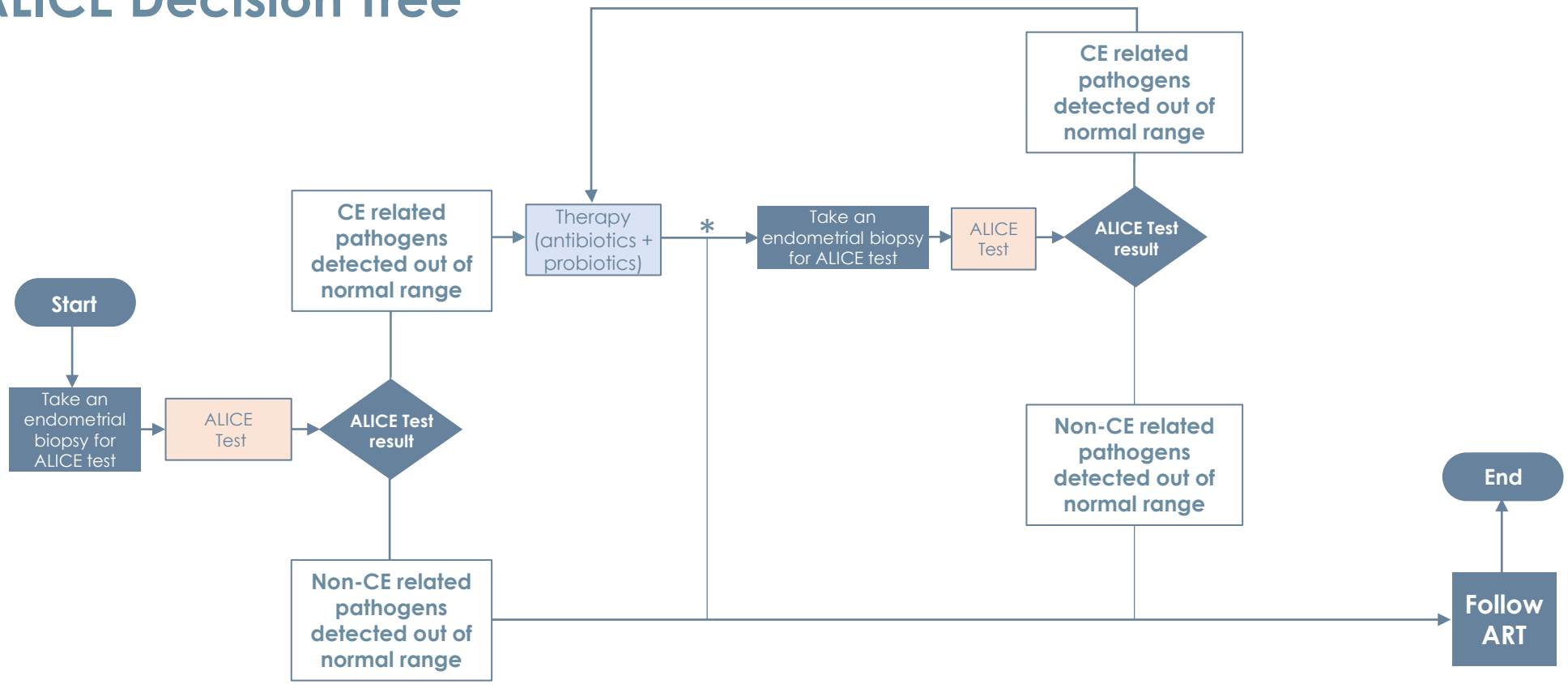
ALICE
Analysis of Infectious
Chronic Endometritis

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.

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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).

Benefits of Molecular Analysis of the Microbiome *vs* Histology, Hysteroscopy, and Microbial Culture

	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	**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**

*With OpenArray we detect the species and are able to recommend an empiric antibiotic treatment against the pathogens detected, but with culture, it's possible to determine antibiotic resistances and therefore the recommended treatment is specific for the isolated strain/s

Relevant references

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.

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.

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.

Relevant references

I. Moreno, 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

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.

Day of Endometrial Biopsy for ERA Alone or Coupled with EMMA/ALICE

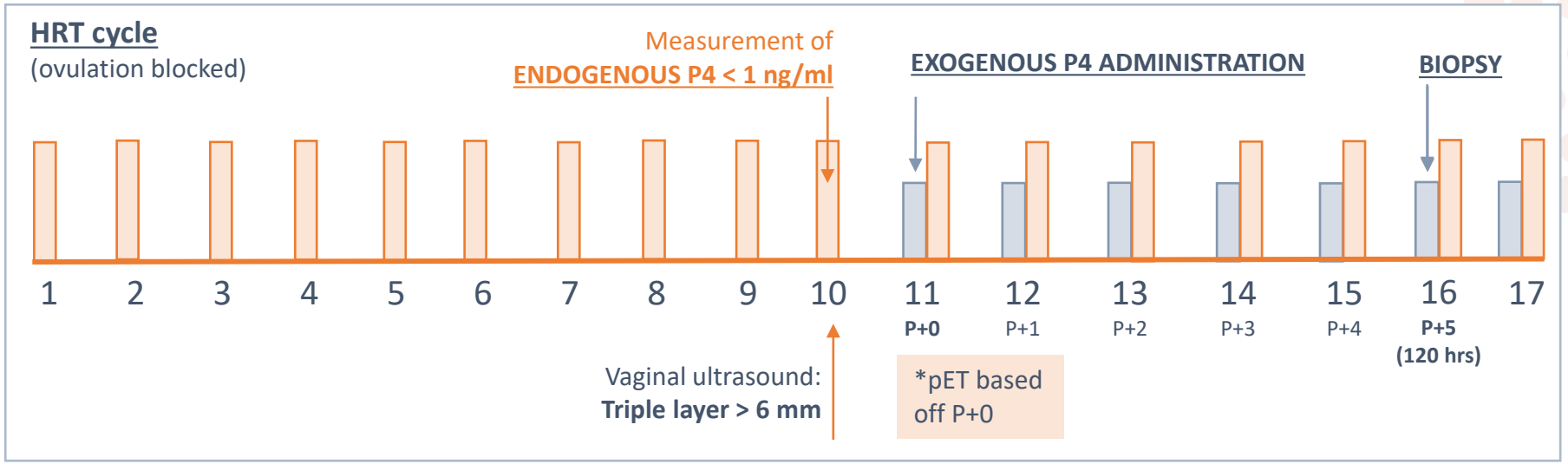
2a) Hormone Replacement Therapy cycle: involves treatment with estrogen and progesterone to inhibit endogenous production of these hormones, using the routine protocol at the clinic or our standard protocol:

Patient starts estradiol therapy from the 1st or 2nd day of the menstrual cycle. Ultrasound assessment is performed 7 to 10 days later. 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).

EndomeTRIO
The endometrium matters
by Igenomix

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 off P+0 (i.e. recommendation based on the total exogenous progesterone exposure time)**

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Day of Endometrial Biopsy for ERA Alone or Coupled with EMMA/ALICE

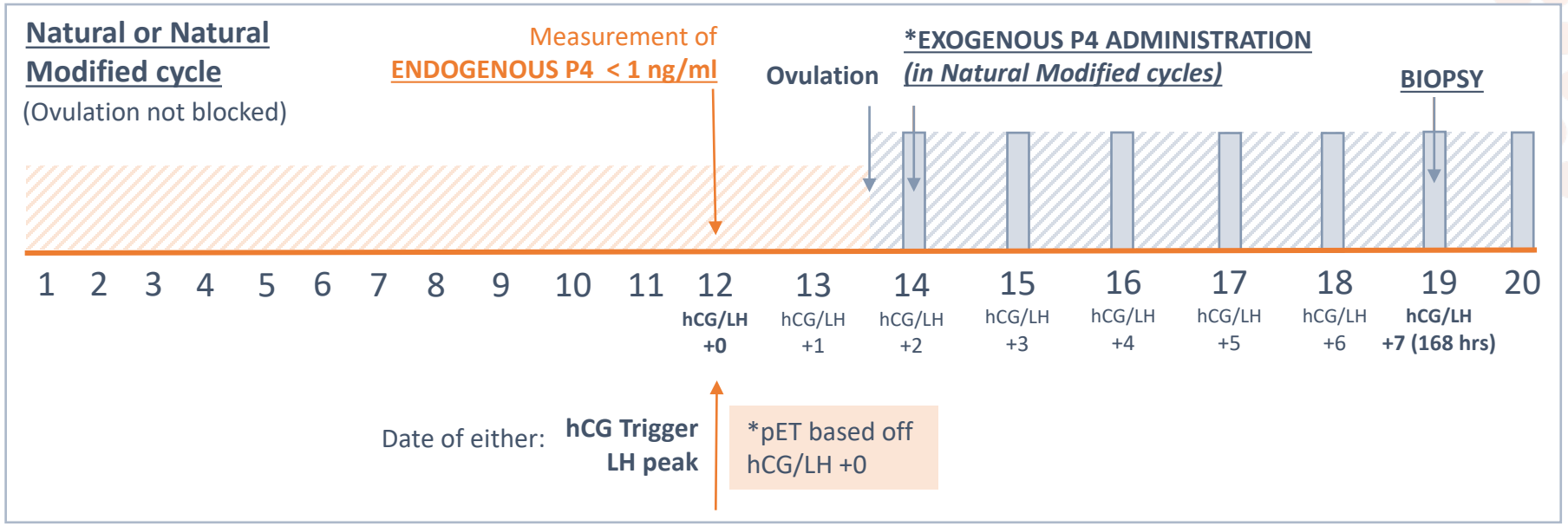
2b) Natural cycle: For Natural Cycles we always need to have a reference date regarding ovulation timing, which could be one of the following three options:

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).
ii. **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.
iii. **Ovulation date**: 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).

EndomeTRIO
The endometrium matters
by Igenomix

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.

- **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.

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EndomeTRIO
The endometrium matters
by Igenomix

Day of Endometrial Biopsy for EMMA and/or ALICE alone

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 only an EMMA or ALICE test is requested, the endometrial biopsy should be taken following the same protocol as for ERA or as follows:

a) **HRT cycles**: the samples must be taken during the progesterone intake days, preferably on day P+5.

b) **Natural or Modified Natural cycle**: The natural cycle is only indicated for patients with regular cycles (between 26 and 32 days). The biopsy must be taken between days 15 and 25 of the cycle. For patients with non-regular cycles, we recommend performing an HRT cycle.

c) **Oral Contraceptive Pills (OCPs):** only for OCPs with certain compositions (please check approved OCPs on Igenomix website or ask us in case you don't find it in the list: *https://www.igenomix.com/wp-content/uploads/2021/09/Acceptable-OCP-for-EA-Testing-3-1.png*). The biopsy can occur between day 14-21 of OCPs (days of active pills intake) when patient takes the placebo pills, or after 14 and onwards if she is under continuous use.

Igenomix

EndomeTRIO
The endometrium matters
by Igenomix

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. For each period, first and last date are included.
During OCPs	NO	14 - 21 (days of active intake pills if patient has also placebo pills) 14 onwards (continuos 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, first and last date are included.

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Day of Endometrial Biopsy: Not Valid Protocols

Cycle type	Cycle Day	ERA	EMMA&ALICE	Comments
Controlled ovarian stimulation	NA	**NO**	**NO**	Samples can't be collected in a stimulation cycle as conditions could not be replicable during the pET cycle and the microbiome is not representative due to suprafisiological E2 levels compared to those found in a Natural or HRT cycle.
Biopsy during the follicular phase	NA	**NO**	**NO**	Samples can only be collected during the secretory phase to ensure microbiome stability and findings representative of time at embryo transfer

EndomeTRIO
The endometrium matters
by Igenomix

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)**. For bigger amounts of tissue there will not be sufficient stabilizing in the cryotube (which will lead to RNA degradation).
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)

Igenomix

Endometrial Biopsy Protocol

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). Ensure that the cryotube actually contains endometrial tissue before sending it (not only blood and/or mucus).

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. Ship to Igenomix Monday – Thursday only. 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. Please take note of FedEx tracking number.

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 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.**

EndomeTRIO
The endometrium matters by Igenomix

Endometrial Biopsy Protocol

Notes:

- **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**. 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.
- **EMMA/ALICE testing can not be added on to a sample processed for ERA only. The request needs to be made before receiving the sample at Igenomix (indicated on the requisiton form)** since the lab protocol for Endometrio (ERA + EMMA + ALICE) is different than for ERA alone.
- **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 before, during or after the procedure. Other drugs that may alter the patient's microbiota or immunological status should also be included in the form.
- **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.**

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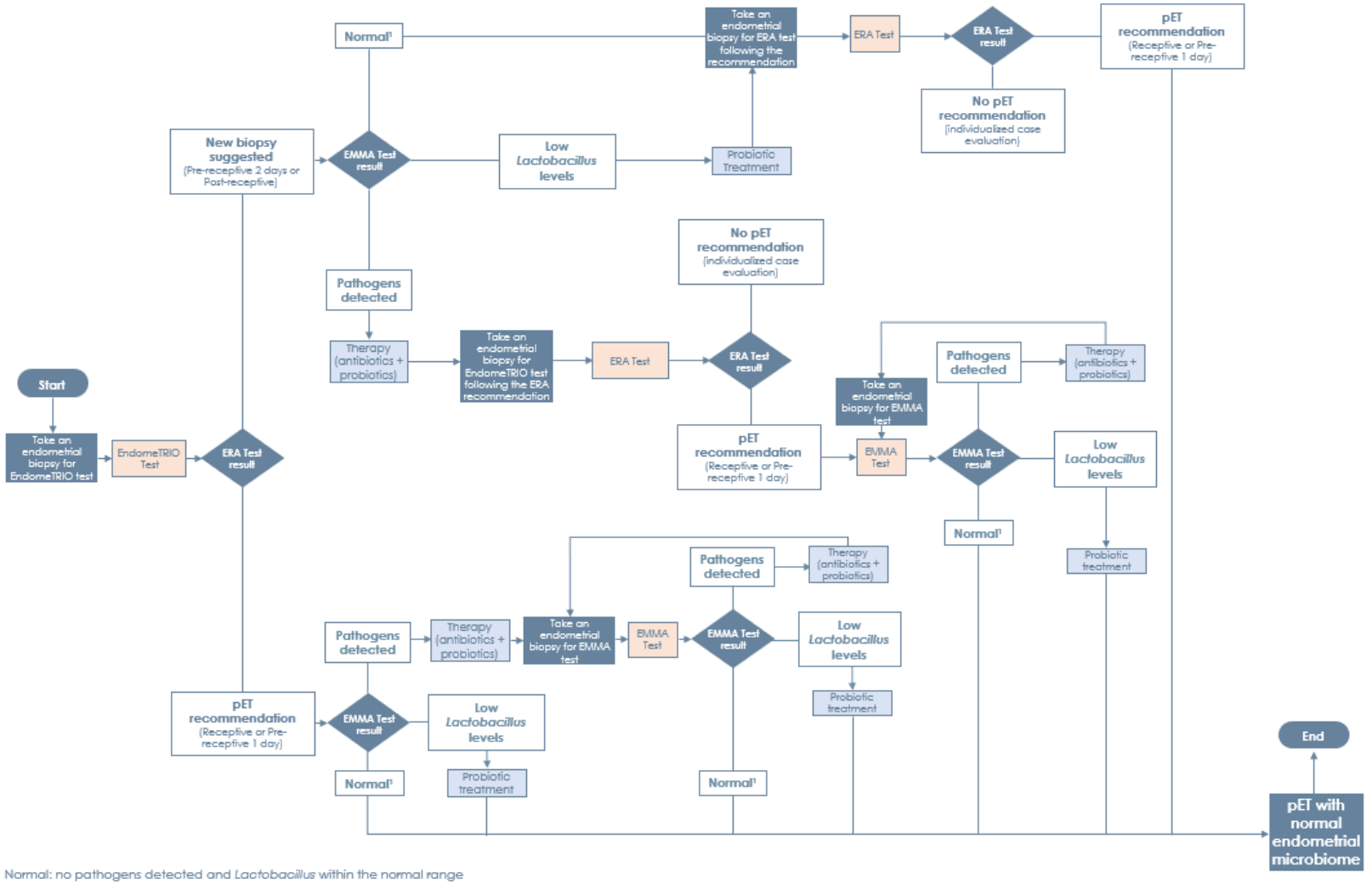
EndomeTRIO
The endometrium matters
by Igenomix

EndomeTRIO Decision Tree (USA)



¹ Normal: no pathogens detected and *Lactobacillus* within the normal range

* Our recommendation for EMMA&ALICE tests his performing these tests in a cycle as close as possible to the programmed embryo transfer cycle. We can't ensure the of validity of the tests results after 3 months, and provided that during that time the patients have not shown signs of infections in the reproductive tract and / or have taken antibiotics for this or other reasons.
On the other hand, for patients who have already perform the tests and have finished their corresponding treatments to restore the normal endometrial flora, we can suggest to continue with probiotics (one cycle after each menses) until the embryo transfer cycle to increase the probability of maintaining the normal flora.

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A Complete View of Endometrial Health

Endometrial Health Solutions

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

**pET: personalized embryo transfer*

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List of Abbreviations

ALICE	Analysis of Infectious Chronic Endometritis
BMI	Body Mass Index
CE	Chronic Endometritis
DNA	Deoxyribonucleic Acid
E_2	Estrogens
EMMA	Endometrial Microbiome Metagenomic Analysis
ERA	Endometrial Receptivity Analysis
hCG	Human Chorionic Gonadotropin
HMP	Human Microbiome Project
HRT	Hormonal Replacement Therapy
LH	Luteinizing Hormone
NGS	Next Generation Sequencing
OCPs	Oral Contraceptive Pills
Ov	Ovulation
P_4	Progesterone
pET	Personalized Embryo Transfer
RIF	Recurrent Implantation Failure
RNA	Ribonucleic Acid
RPL	Recurrent Pregnancy Loss
RT-PCR	Real Time Polymerase Chain Reaction
WOI	Window of Implantation

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