



❖ A complete
view of
endometrial
health



EMMA Endometrial Microbiome
Metagenomic Analysis



Rationale

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

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

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

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

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

Indications for EMMA

The impact of the endometrial microbiome in patients with Recurrent 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 analyzing 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, conserved 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. These can be analyzed using deep sequencing to predict both endometrial receptivity and the endometrial microbiome. EMMA thus provides a microbiological view of the endometrium, to improve clinical management of patients.

Samples

EMMA can be performed on a small piece of endometrial biopsy.

If the patient is undergoing an ERA test, a small portion of the same biopsy can be used, so **no additional sample will be required.**



Day of biopsy

EMMA can be performed between days 15 and 25 of the natural cycle, or during the uterine secretory phase in a HRT cycle.

If the patient is having an endometrial biopsy for ERA, EMMA can be analyzed in the same endometrial biopsy. In this case, the sample should be taken according to ERA timings.



Report

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 the most abundant bacteria detected in the endometrial sample.
- Whether the endometrial microbiome is normal (high percentage of Lactobacilli), or abnormal (presence of dysbiotic or pathogenic bacteria).
- Recommended probiotic/antibiotic therapy, if necessary.

ENDOMETRIAL MICROBIOME METAGENOMIC ANALYSIS (EMMA)

Patient information	Sample Information	Clinic information
Unique pat id.: XXX	Date received: XX/XX/XXXX	Clinic: XXX
Sample type: Endometrial biopsy	Report Date: XX/XX/XXXX	Clinician: Dr. XXX
Patient name: XXX	Cycle type: Natural	No. biopsy: XXX
Patient DOB: XX/XX/XXXX	Day of cycle: XX/XX/XXXX	Date of biopsy: XX/XX/XXXX

TEST RESULT: XXX MICROBIOME PROFILE

YOUR ENDOMETRIAL MICROBIOTA

NORMAL	DYSBIOTIC	PATHOGENIC
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Percentage of Lactobacilli: 92%

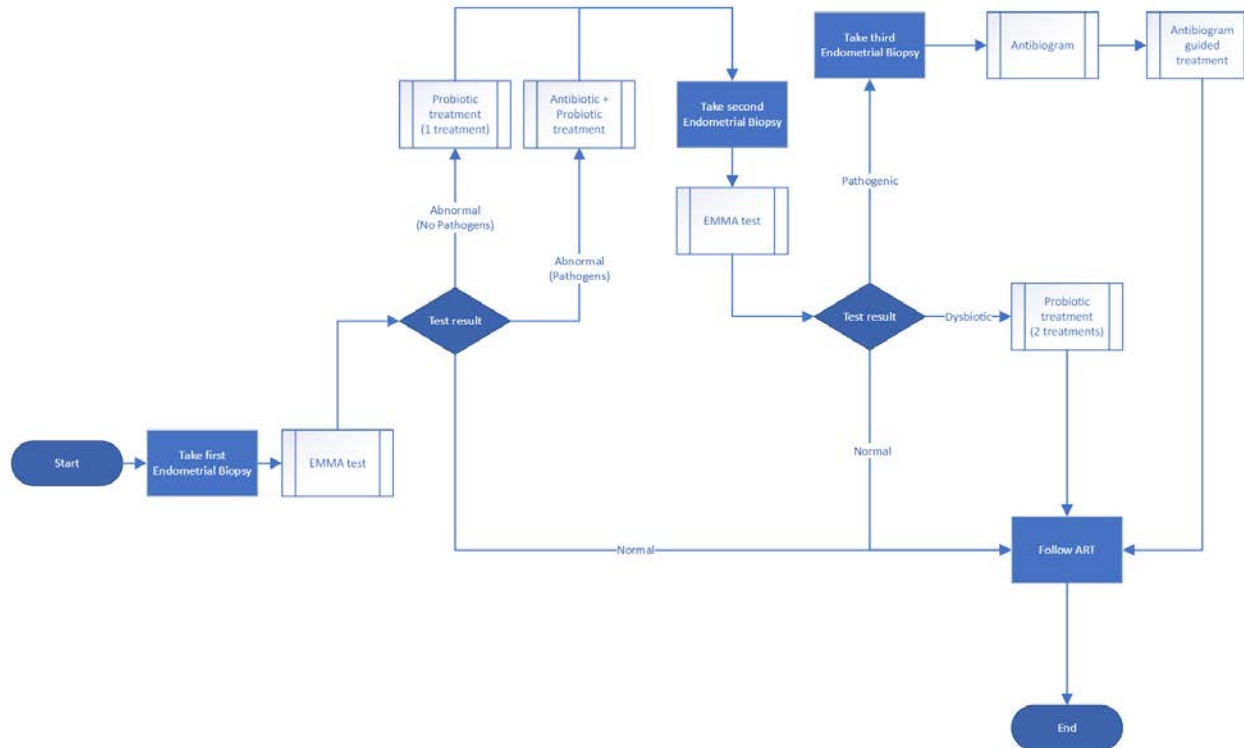
10 most abundant bacteria:

10 most abundant bacteria	%
<i>Lactobacillus</i>	92%
<i>Bifidobacterium</i>	3%
<i>Propionibacterium</i>	2%
Others	3%

Chronic endometritis-causing bacteria:

ALICE pathogens	%

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

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-Almázan J, Alonso R, Alamá P, Remohí 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.



ALICE Analysis of Infectious
Chronic Endometritis



Rationale

The best example of pathology caused by an altered endometrial microbiota is **chronic endometritis** (CE). CE is a persistent inflammation of the endometrial lining, caused by infection of the uterine cavity, mainly by bacterial pathogens. Because it is usually asymptomatic, and current classical diagnosis methods (histology, hysteroscopy and microbial culture) are unsatisfactory, CE is often overlooked, although **it affects approximately 30% of infertile women, and prevalence in patients with RIF and Recurrent Pregnancy Loss (RPL) could reach 60%.**

A recent study carried out by Igenomix has demonstrated that molecular assessment of CE is a reliable diagnostic method compared with 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

ALICE uses the latest NGS technology to provide information of the abundance of the specific bacteria causing 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.

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

Samples

ALICE can be performed on a small piece of endometrial biopsy.

If the patient is undergoing an ERA test, a small portion of the same biopsy can be used, so **no additional sample will be required.**



Day of biopsy

ALICE can be performed between days 15 and 25 of the natural cycle, or during the uterine secretory phase in a HRT cycle.

If the patient is having an endometrial biopsy for ERA, ALICE can be analyzed in the same endometrial biopsy. In this case, the sample should be taken according to ERA timings.



Report

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

These bacteria are: *Enterococcus* spp., Enterobacteriaceae, *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 antibiotic therapy for the pathogens that are detected.

Report

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

ANALYSIS OF INFECTIOUS CHRONIC ENDOMETRITIS (ALICE)

Patient information	Sample information	Clinic information
Unique pat id.: XXX	Date received: XX/XX/XXXX	Clinic: XXX
Sample type: Endometrial biopsy	Report Date: XX/XX/XXXX	Clinician: Dr. XXX
Patient name: XXX	Cycle type: Natural	No. biopsy: XXX
Patient DOB: XX/XX/XXXX	Day of cycle: XX/XX/XXXX	
	Date of biopsy: XX/XX/XXXX	

TEST RESULT: XXX FOR CHRONIC ENDOMETRITIS

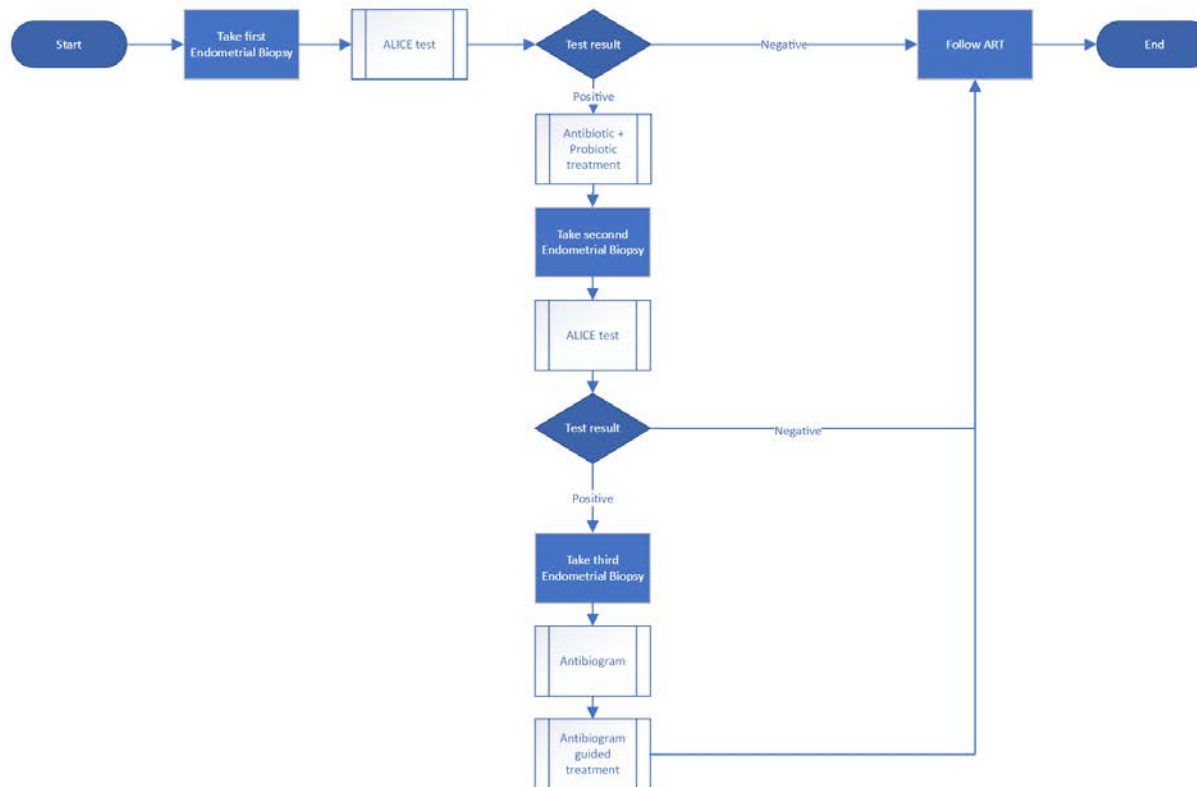
Chronic endometritis-causing bacteria:

ALICE pathogens	%
Enterococcus	1%
<i>Staphylococcus</i>	ND
Streptococcus	10%
Enterobacteriaceae	0.2%
<i>Mycoplasma</i>	ND
<i>Ureaplasma</i>	ND
<i>Chlamydia</i>	ND
<i>Neisseria</i>	ND

INTERPRETATION OF YOUR RESULT AND RECOMMENDATION:

EJEMPLO: Chronic endometritis-causing bacteria were detected in a significant amount in the endometrial sample. Antibiotic therapy followed by probiotic treatment is recommended before continuing with assisted reproductive treatment. Then, a second sample should be analyzed to confirm the restoration of a favorable environment for implantation.

ALICE Decision tree



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.

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, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almázan J, Alonso R, Alamá P, Remohí 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.

Moreno I, Cicinelli E, Garcia-Grau I, Gonzalez-Monfort 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.

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