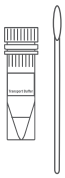
Instructions for use ReceptIVFity

Contents of the packaging



This ReceptIVFity kit contains;
- 1 sterile Copan FLOQswab™
- 1 tube with eNAT buffer
- instructions for use

Intended use

ReceptIVFity is intended to assess the current suitability of an individual woman's embryo implantation acceptation, prior to starting an IVF treatment with or without ICSI, based on vaginal microbiome measurements.

Storage conditions

Store the ReceptIVFity kit before use at 5-25°C. After sampling store the kit at 5 to 25°C. If the product is stored at a different temperature, it will not maintain the stated specifications. After sampling return to the address stated no later than 3 weeks after sampling.

Transport conditions

Samples should be transported between 5 and 25°C. Samples should be transported no later than Wednesday morning during work weeks in Europe. If there are questions please contact ARTPred at info@artpred.com.

Precautions

- Consult instructions for use.
- The pressure applied during sampling should be light since the stick material is breakable
- Do not use if the package is damaged
- Avoid contact with skin and eyes
- Sampling at time of menstrual period is not recommended

Product deterioration

ReceptIVFity test should not be used if (1) there is evidence of damage or contamination to the product, (2) the expiration date has passed, (3) the swab package is open or damaged, (4) the eNAT buffer has leaked or is empty.

If the patient has problems with vision or strength such that sample taking might go wrong, sample taking must be performed under supervision of a medical professional.

Composition of eNAT buffer

Tris-EDTA, HEPES, Guanidine Thiocynate, detergent.

Safety measures related to eNAT-buffer

Precautionary statements: Wash thoroughly after handling; do not eat, drink or smoke when using the product; avoid release to the environment, wear protective gloves/protective clothing/eye protection/face protection.
Ingestion: Harmful if swallowed (H302, Warning, Acute Tox. 4), seek medical care.
Inhalation: Move victim to fresh air. Apply resusciation if victim is not breathing – If trained personnel is available, administer oxygen if breathing is difficult.
Eye contact: If in eyes, rinse cautiously with water for several minutes, ensuring eye lids are held open. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a Poision center/doctor. Causes serious eye damage (H318, Danger, Eye Dam. 1).
Skin contact: If material is splashed onto the skin, remove any contaminated clothing and wash skin thoroughly with water and soap if available. If irritation persists transport to hospital or doctor. Causes skin irritation (H315, Warning, Skin Irrit. 2).
First Aid Facilities: Eyebath/eyewash, Safety shower & general washroom facilities.
Medical Attention & Special Treatment: Treat symptomatically.
Environment: Avoid release to the environment. Dispose of contents/container in accordance with applicable regulations. Adverse physiochemical, human health and environmental effects. Harmful to aquatic life with long lasting effects (H412, Aquatic Chronic 3).
Mixtures: hazardous components within the meaning of the CLP regulation and related classification:

ACCIDENTAL RELEASE MEASURES
Person-related safety precautions:
Take basic hygiene precautions. Gloves, a lab coat and eye protection should be worn.
Measures for cleaning/collecting:
Absorb spilled liquid with paper towels, wash contaminated area with soap and water.

Expiry date

Expiry date is indicated on the package.

Performances

– This test is based on the scientific observation that the presence of certain bacteria in the vagina are an indicator of the suitability of a woman for embryo implantation as part of an IVF treatment with or without ICSI. The test is performed on a vaginal swab, obtained according to the instructions for use as supplied in Appendix 1.
– This test is a reliable predictor for a period of up to two months after sample collection. This test has a sensitivity of 26% and a specificity of 97% for the LOW score.
– The **repeatability** (within run values) was tested with one bacterium per phylum in 15 fold. Results were within the range as described in *Repeatability per phylum log2 transformed*.

Repeatability per phylum log2 transformed

	-2StDev (log2intensity)	Average (log2intensity)	+2StDev (log2intensity)
Firmicutes	14.78	14.94	15.10
Bacteroidetes	14.75	14.89	15.04
Proteobacteria + IC	14.78	14.92	15.05

– For the **reproducibility**, the effects of the variables were evaluated for two values (i.e. two different run dates, two different operators, two different PCR machines and two different ABI machines). Different variables were tested simultaneously. The repeatability was tested with one bacterium per phylum group and was evaluated fifteen-fold. Results were within the range of 2SD (1.86 log2RFU) which has been set as a reference for reproducibility as described i 'IS-pro: high-throughput molecular fingerprinting of the intestinal microbiota'.

– The **limit of detection** (LoD) of the IS-pro was determined using quantified stock cultures (by counting CFU). Five replicates were tested at the concentrations of 0.1, 1, 10, 100 and 1000 CFU. For Firmicutes the bacteria Streptococcus cristatus (DSM 8249T), for Bacteroidetes the bacteria Bacteroides fragilis (NCTC 9343/DSM 2151) and for Proteobacteria the bacteria Stenotrophomas maltophilia (DSM 5017/ATCC 13637) was used. Subsequently, the appropriate dilution was analysed 55-fold, adding up to 60 separate analyses of the LoD, therefore enabling the determination of the LoD with at least 95% confidence.

— Limit of Detection per phylum

Phylum	Bacteria	LoD	Confidence
Firmicutes	Streptococcus cristatus (DSM 5017 / ATCC 13637)	10 CFU	>95%
Bacteroidetes	Bacteroides fragilis (NCTC 9343 / DSM 2151)	10 CFU	>95%
Proteo-bacteria	Stenotrophomas maltophilia (DSM 5017 / ATCC 13637)	100 CFU	>95%

To determine **dynamic range** of a bacterium in the presence of another bacterium from the same phylum, two different bacteria from the same phylum were tested in a dilution series from undiluted to 1:10.000.000 with dilution steps of 1:10. Each dilution was tested with the complete dilution series of the other bacteria and vice versa. This resulted in the following conclusion. The dynamic range of the FAFV group was 1:1.000.000, the dynamic range of the *Bacteroidetes* was 1:100 and the dynamic range of the *Proteobacteria* was 1:1000. For the overall dynamic range the lowest measured dynamic range was taken. This was 1:100.

— Interfering Substances
The presence of PCR inhibitors may cause false negative results. To check whether the internal control adequately monitors PCR inhibition, one µl of the following substances was added to the PCR.
- Magnetic Silica
- Metronidazol (5mg/mL)
- DNAse/RNAse free water
- EDTA 0.5M
- Lysis buffer of EasyMAG
- Ethanol 96%
- NaCl 5M

Metronidazole and Magnetic Silica interfered only with the Firbac PCR. The Proteo PCR was not inhibited by these two interfering substances. DNAse/RNAse free water was no interfering substance. EDTA inhibited the Firbac PCR up to a dilution of 1:10.000.000 and the Proteo PCR for up to 1:100. Lysisbuffer of the easyMAG inhibited the Firbac PCR up to a dilution >1:10.000.000 and for Proteo PCR up to dilution 1:100. Ethanol 96% inhibited the Firbac PCR up to a dilution of 1:10.000.000 and for the Proteo PCR up to 1:10. NaCl 5M inhibited the Firbac PCR up to dilution >1:10.000.000 and NaCl inhibited Proteo PCR up to dilution 1:10. The data showed that the internal control is able to monitor inhibition well.

— Limitations of the procedure (excluding sampling and software)

1. Only specimen described in intended use can be used. Other specimen types have not been validated and may result in false positive or false negative results.

2. Specimen collection, transport and storage may affect the number of organisms and their associated

DNA present in the specimen, affecting the outcome of the result (causing a false positive or a false negative result).

3. Bacteria from other phyla than *Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia Bacteroidetes* and *Proteobacteria* are not detected.

Clinical limitations

Performance is not validated in case of:
-endometriosis pre-treated with a Gn-RH analogue
-use of hormonal contraceptives 3 months prior to start IVF or IVF/ICSI (exclusive 3 weeks use of oral contraceptive pill for the purpose of cycle regulation)

Test result

The outcome of this test shows one factor of the success rate of the fertility treatment; other factors are embryo quality, body mass index, age and several others. Also, the outcome of this test when performed later might be different. The result is interpreted by a professional.

Qty	Name	Ident. Number	Classification
>=40% - <50%	Guanidine Thiocyanate	CAS: 593-84-0 EC: 209-812-1	3.1/4/Oral Acute Tox. 4 H302 3.3/1 Eye Dam. 1 H318 3.2/2 Skin Irrit. 2 H315 4.1/C3 Aquatic Chronic 3 H412 EUH032
>=0.1% - <0.25%	N-Lauroylsarcosine	CAS: 97-78-9 EC: 202-608-3	3.1/2/Inhal Acute Tox. 2 H330 3.2/2 Skin Irrit. 2 H315 3.3/2 Eye Irrit. 2 H319 3.8/3 STOT SE 3 H335

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Instructions for use
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Appendix 1: Instructions for use in different languages

EN: Instructions for use for patient

1. Place the tube filled with eNAT-buffer on a solid and stable surface
2. Open the cap of the tube, which is filled with eNAT-buffer
3. Open the peel and extract the swab, only hold the swab at the side without the tip
4. Spread the labia with one hand, so that the vagina is accessible for insertion of the swab
5. Insert the swab 3-5 cm into the vagina and rotate it 10-15 sec along the vaginal wall
6. Remove the swab from the vagina and break the swab at the breakpoint on the edge of the tube
7. Put the swab directly into the buffer; do not put the swab on a surface
8. Close the tube with the cap. Close the container firmly and do not under tighten
9. Give the kit to your doctor

NL: Instructies voor patiënt

1. Plaats de buis met eNAT-buffer op een stevige en stabiele ondergrond
2. Open de dop van de buis die gevuld is met eNAT-buffer
3. Open de verpakking van de swab (wattenstaafje) en houd de swab alleen vast aan het uiteinde waar geen kop van watten op zit
4. Spreid de lippen van de vagina met één hand, zodat de vagina toegankelijk is voor insertie van de swab
5. Steek de swab 3-5 cm naar binnen in de vagina en roteer de swab gedurende 10-15 seconden langs de vaginale wand
6. Haal de swab uit de vagina en breek de swab bij het breekpunt op de rand van de buis
7. Stop de swab direct in de buffer; laat de swab niet op een ander oppervlak rusten
8. Draai de dop op de buis. Sluit de capsule stevig en zorg ervoor dat de dop niet te zacht is dichtgedraaid
9. Geef het afnamesetje terug aan uw arts

DE: Anweisungen für die Patientin

1. Platzieren Sie das mit dem eNAT-Puffer gefüllte Röhrchen auf eine feste und stabile Unterlage.
2. Öffnen Sie die Kappe des mit dem eNAT-Puffer gefüllten Röhrchens.
3. Öffnen Sie die Verpackung des Abstrichstäbchens (Wattestäbchen) und halten Sie das Stäbchen nur an der Seite fest, das nicht mit Watte umwickelt ist.
4. Ziehen Sie die Schamlippen mit einer Hand auseinander, sodass die Scheide für das Einführen des Stäbchens zugänglich ist.
5. Führen Sie das Stäbchen 3-5 cm in die Scheide ein und drehen Sie es 10-15 Sekunden lang entlang der Scheidenwand.
6. Nehmen Sie das Stäbchen aus der Scheide heraus und brechen Sie das Stäbchen an der Bruchlinie an der Kante des Röhrchens ab.
7. Geben Sie das Stäbchen sofort in die Puffersubstanz, lassen Sie es nicht auf irgendeiner Fläche liegen.
8. Schrauben Sie die Kappe wieder auf das Röhrchen auf. Drehen Sie die Kappe gut fest und achten Sie darauf, dass sie nicht zu locker angezogen ist.
9. Geben Sie Ihrem Arzt das Entnahmeset zurück.

FR: Instructions pour la patiente

1. Placez le tube de contenant le tampon eNAT dans une base robuste et stable
2. Ouvrez le capuchon du tube rempli de tampon eNAT
3. Ouvrez l'emballage de l'écouvillon (coton-tige) et tenez l'écouvillon uniquement par l'extrémité dépourvue d'embout ouaté
4. Écartez les lèvres vulvaires d'une main pour permettre l'introduction de l'écouvillon dans le vagin
5. Introduisez l'écouvillon d'environ 3 à 5 cm dans le vagin et faites-le tourner pendant 10 à 15 secondes le long de la paroi du vagin
6. Retirez l'écouvillon du vagin et cassez le bâtonnet de l'écouvillon sur le bord du tube au niveau du trait de cassure
7. Plongez immédiatement l'écouvillon dans le tampon ; ne le laissez pas reposer sur une autre surface
8. Revissez le capuchon sur le tube. Fermez soigneusement le tube et assurez-vous que le capuchon est suffisamment serré
9. Rapportez le kit de prélèvement à votre médecin