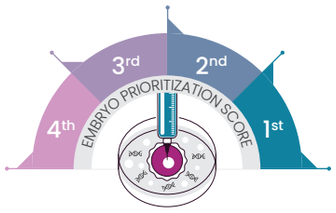
LifeCell® Diagnostics is now mfine /diagnostics

When a woman undergoes IVF treatment,

Her Greatest Wish

is to get pregnant as soon as possible



INTRODUCING

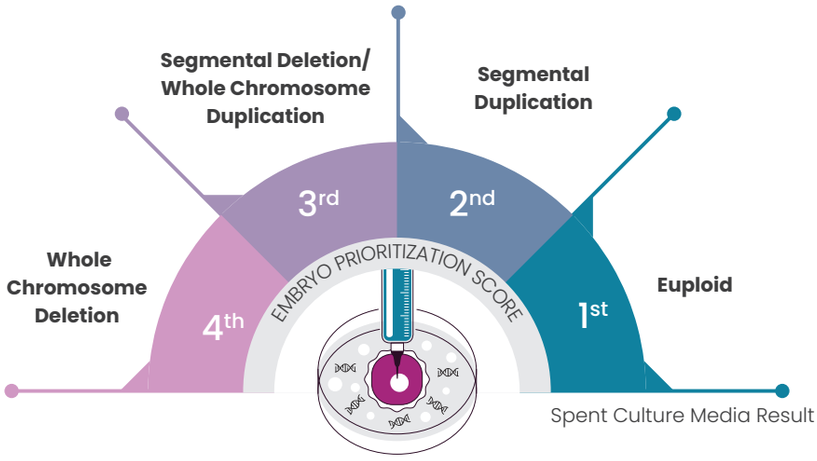
EmbryoScore

A Non-invasive, Easy, Safe and Cost-effective Technique

FOR EMBRYO PRIORITIZATION

Designed to maximize the chances of healthy pregnancy by

HELPING CLINICS CHOOSE EMBRYOS THAT ARE MOST LIKELY TO BE EUPLOID



Spent Culture Media Analysis

What is the EmbryoScore test?

In Vitro Fertilization and Embryo Transfer (IVF-ET) is an effective method for the treatment of infertility, yet it has a relatively low success rate [Kupka M S et al. 2014].

Chromosome abnormalities is a major cause of Implantation failure [Rabinowitz M.et al. 2012; Franasiak J M et al. 2014]. Preimplantation Genetic Testing for Anueploidies (PGT-A) improves the chances of a successful implantation and healthy pregnancy by identifying embryos with correct number of chromosomes. It requires Trophectoderm Biopsy sample for analysis. Spent Culture Media (SCM), in which the embryo is cultured, is routinely discarded at the time of transfer or freezing.

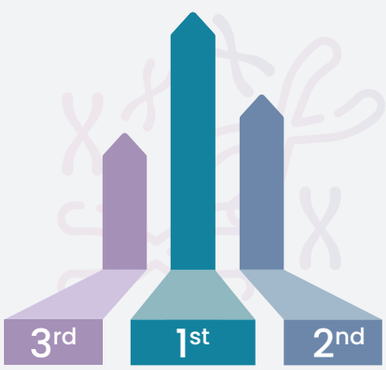
During early embryo development, fetal DNAs are secreted into culture medium

EmbryoScore is a non-invasive Preimplantation Genetic Test for Aneuploidies (niPGT-A) that assesses the chromosomal makeup of the cell free DNA (cfDNA) secreted by an embryo in Spent Culture Media during its evolution [Huang et al. 2019].

For this process the embryos are not biopsied therefore they remain intact and safe in the IVF laboratory.

To accurately analyse the small amount of DNA released in the culture medium, we make use of advanced laboratory methods like Whole Genome Amplification (WGA) and Next Generation Sequencing (NGS) followed by Bioinformatics analysis to screen all 23 pairs of Chromosomes for whole chromosome aneuploidy and sub-chromosomal abnormalities.

Embryo Prioritization Score



A Score Card is then generated with ranking of embryos on the basis of their probability of having correct number of Chromosomes in order to maximize the chances of a healthy pregnancy

The results helps clinician in assessing the quality of embryos and select the best ones with higher Implantation Capability before their transfer to the uterus

By sorting the embryos and selecting the most promising ones, we help to shorten the time for treatment and thus reduce the psychological burden on the couple and the likelihood of spontaneous abortions or other complications in pregnancy.

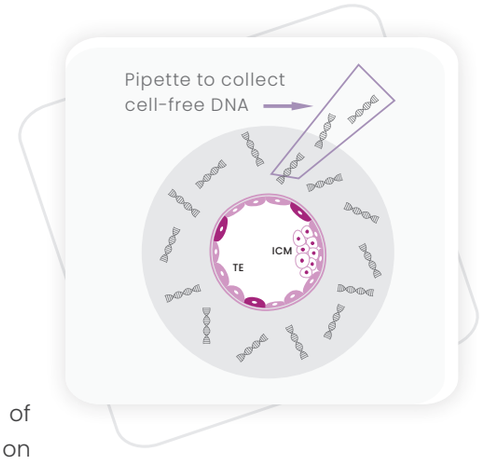
What are the advantages of choosing EmbryoScore?

Multiple studies have reported a high success rate of cfDNA amplification and detection, ranging from 77.3% to 100% [Kuznyetsov V et al 2018; Huang L et al. 2019; Jiao J et al. 2019; Li X et al. 2021; Chen L et al. 2021; Rubio C et al 2020].

Below are the unparalleled benefits of EmbryoScore:

NON INVASIVE

Simply requires a sample of the culture media for embryonic DNA analysis. Without the need for an invasive embryo biopsy the process is easier which ensures zero damage risk to the embryo.



SAFER

Use of Spent Culture Media decreases the risk of biopsy associated complications as the embryos remain intact.

EASYLY ADAPTABLE

Eliminates the technological hurdle of embryo biopsy and the need of specialized equipment like laser machines. Less strict requirement on equipment, training and techniques makes it easily adaptable for the IVF Clinic.

MORE EFFECTIVE

Offers one more criteria of prioritization on the top of morphological examination.

DNA REPRESENTS THE WHOLE EMBRYO

EmbryoScore uses the DNA released by the embryo into the media during its development, due to various physiological mechanisms such as apoptosis and cellular necrosis. The sample is therefore representative of the ICM as well, not only the trophectoderm [Lei Huang et al. 2019].

ACCESSIBLE TO ALL IVF JOURNEYS

Embryo Biopsy and PGT-A is not a routine procedure in all IVF Clinics. Availability of non-invasive cost effective option can make it easily accessible to all.

ACCESSIBLE TO ALL EMBRYOS

Spent culture media testing would allow laboratories to test all embryos in a cohort which could help better understand their genetic makeup, not just the embryos with the best morphology [Hiroki Sonehara et al. 2022].

How is EmbryoScore clinically relevant?

- Provides a reference for Embryo Transfer
- Increases the chances of healthy pregnancy by helping to prioritize embryos that the most likely to be euploid
- As an alternative to PGT-A, it provides a second chance for embryos excluded by PGT-A or in cases where biopsy cannot be performed
- Saves the time required to do biopsy procedures

When is EmbryoScore recommended?

The non-invasive nature of the test makes it universally accessible to all patients.
Some clinical indications are:

- Advanced maternal age
- History of recurrent miscarriage Repeated failure in IVF cycles (>2)
- History of recurrent miscarriages
- Men with chromosomal anomalies in sperm
- Families requiring Single Embryo Transfer

Note: It is not for Patients requiring PGT-M, PGT-SR, such patients should pursue testing based on trophectoderm biopsy

To introduce EmbryoScore, what Adaptations are required in the IVF Lab?

Avoiding contamination of the spent culture media is critically important to the test result.

Extreme caution must be exercised to prevent the introduction of foreign DNA contaminants including cumulus cells, sperm, potential contamination with operator DNA, and exogenous DNA from additives in the media such as human albumin. Each one of these DNA sources has the potential to lead to false results for the embryo, predominantly false negatives.

OPTIMIZATION RECOMMENDED TO MINIMIZE DNA CONTAMINATION:

- **Each embryo is to be cultured individually in a two-step culture process**
  Individualised culture is essential to ensure that the cfDNA has originated from the Embryo to be tested.
- **Denudation of the Oocytes**
  Each oocyte should be denuded thoroughly before being placed into culture. This cleaning can include both physical methods and chemical methods.
- **ICSI technique recommended for fertilization over conventional insemination**
  This is to reduce the risk of contamination of the SCM by extraneous sources.
- **Change of culture media on Day 3 or Day 4**
  The embryo is to be washed on day 3 or day 4 and rinsed in fresh culture media and then moved to an individual 10-20 microlitre drop. Culture until Day 5 or Day 6 for at least 40 hours to maximize the DNA availability.
- **Sample Collection & Transportation**
  Spent culture media should be collected as soon as possible after the embryo has been removed from culture using a new, clean pipette tip for each and every media sample.
  The samples should be transferred to the bottom of a clean, sterile 0.2 mL PCR tube and immediately frozen at -20˚ C if shipped with dry ice.
- **Negative Control**
  Each batch of SCM samples for a patient should include a negative control sample consisting of a 10-20 microlitre drop of medium undergoing the same protocol but without embryo exposure to Rule out any media contamination.

*It is recommended that sample collection, storage and shipping conditions are validated before use.

PRECAUTIONS AT THE SITE OF COLLECTION:

In order to reduce the risk of contamination from external sources, all activities surrounding embryology and sampling of SCM should be undertaken with strict adherence to good aseptic laboratory practices.

- To prevent contamination, protective clothing should be worn, including full surgical gown (clean, not sterile and changed regularly), hair cover/hat, face mask (covering nose and mouth) and preferably shoe covers or dedicated shoes.
- For areas within the IVF Centre, protective clothing, preferably with low particle-shedding and non-powdered gloves and masks should be considered.
- Specific care to be taken for handling of reaction tubes to reduce cross-contamination:
  - It is recommended to avoid touching the inside or the lid of the tubes with your fingers.
  - It is recommended to avoid touching the outside or the cap of the tubes with the tip of the pipette. If this happens, the pipette tip should be changed immediately.
  - It is recommended to keep the reaction tubes open not longer than necessary.

OPTIMIZATION AT AT MFine/Diagnostics LABORATORY:

- **Advanced Protocol for Embryonic cfDNA analysis**

  PG Seq™ Rapid Non-Invasive PGT Kit is specifically developed to analyse picogram quantities of DNA (low template DNA) from spent embryo culture media and assessed for its accuracy by the degree of correlation to embryo biopsies.

- **Use of DOPlify® kit with advanced DOP-PCR Technology**

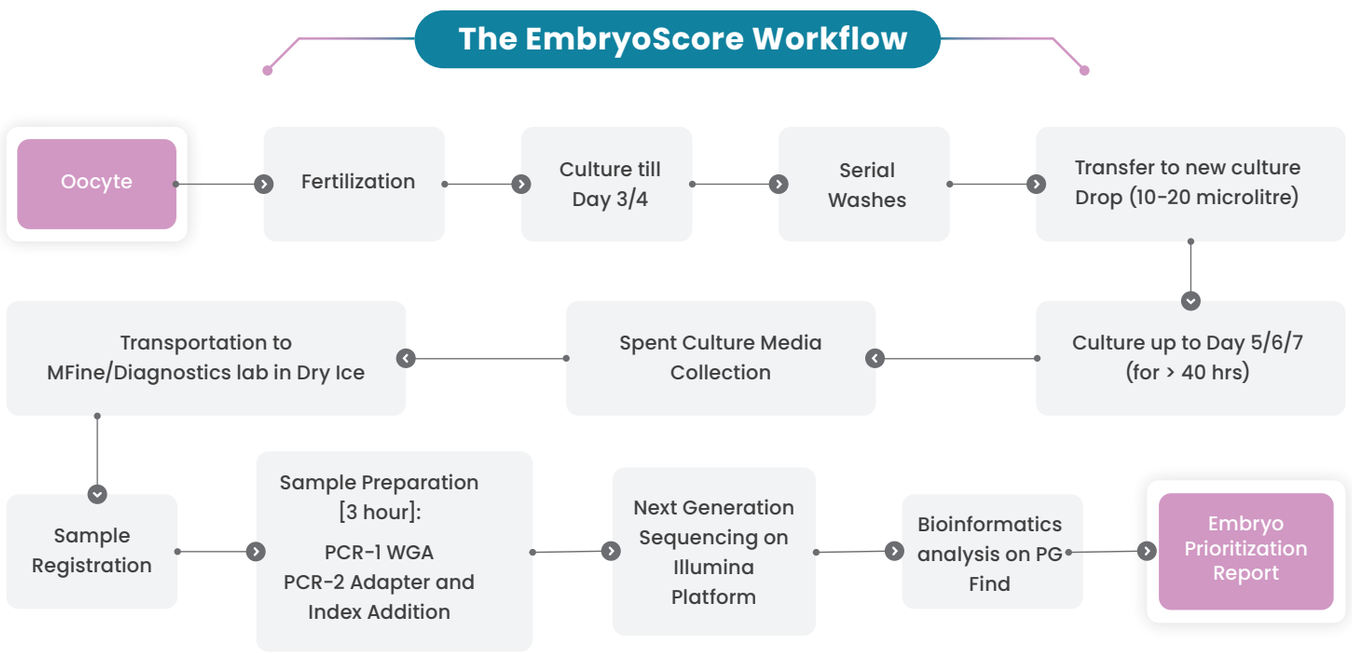
  The kit uses a two-step PCR to whole genome amplify and then attach indexes and sequencer-specific adapters to the template DNA, in a fast, 3-hour sample preparation workflow. Highly multiplex PCR based **DOPlify®** WGA kit improves DNA yield allowing for the chromosome copy number status to be determined.

- **Optimised Workflow**

  The kit follows a single tube workflow to minimize the pipetting steps needed and requires only two sample tube openings, reducing the risk of sample contamination.

  The sample undergoes Next Generation Sequencing on **Illumina®** instrument.

  PG-Find™ analysis software is used for automatic calling of aneuploidy and copy number variants.



OUR RECOMMENDATIONS FOR IMPLEMENTING EMBRYOSCORE AS A ROUTINE PRACTICE IN IVF LABORATORY:

- Each lab benchmarks its spent culture media results through concordance analysis side by side with biopsy-based analysis by running both systems concurrently.
- This will allow to understand culture conditions and precise measurement of the true concordance rate and false positive/false negative rates.
- It is critical to include negative control/blank in every sequencing run for EmbryoScore to ensure that the media and lab are not additional sources of contamination.
- Depending on the baseline concordance levels and target performance parameters, changes to the culture protocol can be considered to improve concordance levels.

PG-Seq™ Rapid Non-Invasive Kit shows High Correlation to Biopsied Embryos

Optimization of the of the PG-Seq™ Non-invasive PGT kit, by PerkinElmer was performed using non-invasive samples from 15 laboratories all using different embryology protocols. A selection of this data is presented in Table 1.

Table 1: Review of metrics and concordance from 8 collaborator IVF laboratories using differing embryology protocols

	Culture volume (µl)	DNA yield (ng/µl)	Weak WGA #	Failed WG #	Autosome concordance*	Sex concordance*
Two-step Culture						
Lab 1	10	7.5	26% (8/30)	3% (1/30)	90% (26/29)	97% (28/29)
Lab 2	60	6.4	42% (5/12)	8% (1/12)	70% (7/10)	50% (5/10)
Lab 3	20	10.2	26% (5/19)	5% (1/19)	67% (4/6)	83% (5/6)
Lab 4	20	5.5	50% (4/8)	0% (0/8)	75% (6/8)	88% (7/8)
Range/Mean	10-60 µl	5.5-10.2 ng/µl	32%	4%	81%	85%
Continuous Culture						
Lab 5	40	6.0	32% (7/22)	22% (5/22)	35% (6/17)	47% (8/17)
Lab 6	20	10.7	30% (12/40)	8% (3/40)	33% (5/15)	53% (8/15)
Lab 7	20-50	10.8	42% (5/12)	8% (1/12)	29%(2/7)	86% (6/7)
Lab 8	25	4.8	25% (6/24)	4% (1/24)	75% (12/16)	94% (17/18)
Range/Mean	20-50 µl	4.8-10.8 ng/µl	31%	10%	45%	68%

Optimal timing for SCM collection and recommendations:

Spent culture media samples containing higher amounts of DNA are more likely to produce a reliable result with the **PG-Seq™ Rapid Non-Invasive PGT-A kit**

Media Collection Ranges		
Earliest Time Point	Latest Time Point	Concordance to Biopsy
Day 5	Day 6/7	>80%
Day 4	Day 5/6	>75%
Day 3	Day 5/6	>70%

Source: https://perkinelmer-appliedgenomics.com/wp-content/uploads/marketing/RHS/Non-Invasive_PGT-AppNote-AG012006_02_AP.pdf; https://perkinelmer-appliedgenomics.com/home/products/preimplantation-genetic-testing/pg-seq-rapid-non-invasive-kit/