Non-invasive PGT-A

While biopsy-based testing of embryos for aneuploidy, or preimplantation genetic testing for aneuploidy (PGT-A) has been around for more than 20 years, the collection and screening of spent culture media (SCM) from the media droplet surrounding a growing embryo is a new approach that has shown promising early results. This concept, termed non-invasive PGT-A, allows genetic testing of embryos without the need for an invasive embryo biopsy. In addition, there are other advantages to non-invasive PGT-A including testing of more embryos from each patient cohort since there is no minimum developmental stage an embryo needs to reach for non-invasive testing, and non-invasive PGT-A eliminates the technological hurdle of embryo biopsy, a technique that not all embryology labs globally are able to achieve. These advantages should allow more patients access to genetic testing of their embryos prior to making transfer decisions.

While a great deal is known about invasive PGT-A, only a handful of papers have been published thus far looking at non-invasive PGT-A. For a thorough review, see Lever and Wells (1). To date, the largest single trial looking at SCM and non-invasive PGT-A was published by Rubio and colleagues (2). This study had very specific embryology requirements for the collaborating centers.

During the development of the PG-Seq™ Rapid Non-Invasive PGT-A kit, PerkinElmer worked with a number of IVF centers globally to collect spent culture media for analysis. No specific parameters around the embryology and collection of the media were controlled, instead the collaborating labs provided details of their embryo culturing and handling methods. This has led to great insight into which parameters are critical for generating high concordance with the gold standard of biopsy-based PGT-A.

In this document we discuss what has been learned thus far during the development and early access trials of the PerkinElmer PG-Seq™ Rapid Non-Invasive PGT-A kit.
Considerations when reviewing concordance compared to an invasive biopsy:

Concordance of a SCM result to an invasive biopsy result is a critical measure of the success of a non-invasive PGT-A protocol. Each laboratory that undertakes validation of non-invasive PGT-A should understand their own concordance rate when compared to invasive biopsy and how this concordance ultimately relates to the positive and negative predictive values of the test and, therefore, the likelihood of false positive and false negative results. These values should be calculated from an in-house validation of at least 50 embryos with a range of euploid and aneuploid biopsy-based PGT-A results. The performance of the non-invasive protocol must be shared with clinical staff and genetic counselors so that they can appropriately counsel patients. At this time, we do not recommend offering non-invasive PGT-A as a stand-alone genetic test until a correlation with implantation rates has been assessed locally. The clinical studies, including RCTs, of biopsy-based PGT-A have shown that this approach can improve IVF outcomes, particularly with implantation rates has been assessed locally. The clinical studies, including RCTs, of biopsy-based PGT-A have shown that this approach can improve IVF outcomes, particularly for women over the age of 35 years, so this remains the gold standard until non-invasive PGT-A has been clinically validated in the same way (3, 4, 5, 6, 7).

While the DNA from spent culture media, and from invasive biopsy, originates from the same embryo, there are a number of potential reasons for non-concordance between an embryo biopsy and SCM, including:

- **Maternal DNA contamination**, which can be detected when the media result is 46,XX and the biopsy is not. If there was no biopsy PGT-A result to compare against, it could also be suspected by a disproportionately high number of female embryo results compared to male in one embryo cohort. The biggest risk with maternal DNA contamination is a false negative result, which may lead to the transfer of an aneuploid embryo. We have witnessed varying degrees of maternal DNA contamination, from full contamination where an aneuploid XY biopsy sample produced a euploid 46,XX SCM result, to partial contamination where the SCM from a 46,XY biopsy produces a chromosome X copy number value ranging between 1 to 2 copies, and a Y copy number value ranging from 0 to 1 copy.

- **Poor quality or insufficient DNA template** in the spent media, which will cause a noisy result from the media that could be interpreted as an aneuploid result. Poor quality DNA can be caused by degradation of the DNA before or after it is released from the embryo. Insufficient and/or low template samples are often detected at early stages of embryo development (e.g. day 3 media collection) or if the embryo is only cultured in the media droplet for a short period of time (e.g. less than 24 hours). There does not appear to be a standard quantity of DNA released by developing embryos as seen by examining amplified DNA yield following whole genome amplification (WGA). Further research is needed to understand whether there is any correlation between blastocyst grade and the amount of DNA template in the spent media.

- **Poor biopsy DNA quality**, which will lead to a likely false positive in the biopsy results that could be interpreted as a false negative from the media. Careful side by side analysis is required to ensure that both results were accurately interpreted. Note that this may bring into question the original biopsy result for some embryos, so it must be undertaken with caution.

- **Inverse result** from biopsy and media, for example if a biopsy result showed a trisomy for a specific chromosome while the media showed a monosomy for the same chromosome. While the exact mechanisms for this have not been resolved, it has been suggested that it can occur due to the following:
  - During mitosis, a whole chromosome error results in one daughter cell containing an extra chromosome (trisomy) and the other daughter cell missing a chromosome (monosomy). In this example, the trisomic daughter cell would propagate into the blastocyst, which was biopsied, while the monosomic daughter cell would lyse and its DNA would be found in the SCM.
  - There may be selective pressure within the embryo to eliminate or minimize cells with the wrong genotype. These cells may undergo apoptosis and/or they may be ‘relegated’ to the trophectoderm or removed from the embryo altogether.
  - DNA from the polar bodies may have been included in the SCM, which would give the complete opposite result to the embryo.

A concise and important description of the origins of embryo mosaicism and its impact on PGT-A accuracy can be found in Kahmaran et al. (8). These same mechanisms could possibly apply to SCM versus trophectoderm biopsy.

**Embryology recommendations:**

- The oocyte should be denuded thoroughly before being placed into culture. This cleaning can include both physical methods (use of specific sized tips and movement up and down the tip to physically remove cumulus cells) and chemical methods including commercial or home-made solutions containing hyaluronidase.

- The embryo should be removed from the culture media during growth, ideally between day 3 and day 4 of embryo development, rinsed in fresh culture media and then placed into a new drop of culture media to assist in the removal of contaminating DNA. This protocol should be used no matter the brand or type of culture media used for embryo growth, including single step media.

- Some laboratories have reported reduced maternal/paternal cell contamination when using cryopreservation prior to SCM sampling, however more data is needed before we could recommend this protocol.
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. A number of different time points for SCM collection were analyzed during the global collaboration project with varying concordance levels noted:

<table>
<thead>
<tr>
<th>Earliest Time Point</th>
<th>Latest Time Point</th>
<th>Concordance to Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 5</td>
<td>Day 6/7</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>Day 4</td>
<td>Day 5/6</td>
<td>&gt;75%</td>
</tr>
<tr>
<td>Day 3</td>
<td>Day 5/6</td>
<td>&gt;70%</td>
</tr>
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It is recommended to:

- Collect SCM on day 5 or later
- Freeze the media samples as soon as possible at -20°C until processed so that the DNA integrity is preserved.
- Collect as much spent media sample as possible, at least >8µl, so that there is an opportunity to retest if the initial result is inconclusive.

Method of insemination and recommendations:

Most IVF centers use a mixture of conventional insemination and intracytoplasmic sperm injection (ICSI) for their patients. There has been some controversy in the field about the need to use ICSI for all biopsy-based PGT samples. Certainly ICSI is required when testing for specific genetic mutations (PGT-M) and probably best when testing for chromosome rearrangements (PGT-SR), however data does exist that shows that ICSI is not required for biopsy-based PGT-A tests (6).

- As contamination of the SCM by extraneous sources is a risk for non-invasive PGT-A, it is our recommendation that ICSI be performed for all non-invasive PGT-A tests at this time.

Contamination of SCM from extraneous sources and recommendations:

Contamination of the SCM by outside sources in the IVF lab is a risk to non-invasive PGT-A. 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. The small amount of embryonic DNA found in spent culture media makes it particularly susceptible to interference from external contaminating DNA.

- Each batch of SCM samples processed should include the use of ‘no template controls’ (negative controls) of the media being used in the embryology lab to determine media contamination levels.

Level of detection in non-invasive PGT-A:

At the moment there is some debate about the level of detection/reporting for biopsy-based PGT-A, specifically around mosaic embryos. Some have recently called for the field to stop calling embryos ‘mosaic’ but to rather call embryos ‘intermediate’ when the gain or loss of a chromosome (or a small piece of a chromosome) does not reach the threshold set by the laboratory for a full chromosome gain or loss. The data on the transfer of embryos diagnosed as mosaic (or intermediate) is growing and shows that some percentage of embryos with an intermediate gain or loss can implant and lead to live births, however, at least one documented case has led to the birth of a child with mosaicism confirmed by prenatal and postnatal diagnosis (8).

While this debate continues and the evidence around mosaic (intermediate) embryos grows, it is our recommendation that:

- Laboratories that offer non-invasive PGT-A should perform an internal validation of the chosen niPGT-A platform using previously biopsied embryos, cell lines with known chromosome abnormalities, and/or non-selection style studies to determine the false positive and false negative rates of the test in the lab.
- During this validation study, the lab should set specific thresholds for niPGT-A samples and ensure that lab staff are trained to accurately assess niPGT-A samples that may present with noisier and/or more difficult profiles to assess as compared to biopsy-based PGT-A samples.
- It is our recommendation that niPGT-A samples should only be called for full chromosome gains and losses and that mosaic or intermediate calls should not be made until more data is available to better understand this laboratory finding.

A number of other key success factors for the introduction of non-invasive Preimplantation Genetic Testing have already been covered in our earlier Application Note:

References