APPLIED GENOMICS

APPLICATION NOTE

Non-invasive Preimplantation Genetic Testing

PG-Seq™ Non-invasive PGT Kit

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Why it's an Important New Frontier for IVF

A significant body of published data has shown the benefit of preimplantation genetic testing for aneuploidy (PGT-A) (Yang 2012, Harton 2013, Forman 2013, Rubio 2017). However, published data has also shown evidence of embryo damage with Day 3 biopsy when comparing implantation rates to un-biopsied embryos (Scott RT 2013). There is concern that whilst trophectoderm biopsy (typically performed on days 5, 6, and/or 7 of embryo development) didn't reduce implantation rates in that same study (Scott L 2013), it may still have an impact on embryo health (Zacchini 2017) and/or neonatal outcomes (Zhang 2019).

It is well known that embryos are impacted by even small changes in pH of the media that can be induced by door openings in the incubator, and certainly by removing a cohort of embryos for assisted hatching on day 3 ahead of trophectoderm biopsy at days 5, 6, and/or 7 (Swain 2012). The time taken to perform trophectoderm biopsy, the number of cells removed during the process, cell damage created during laser assisted opening of the zona pellucida, and/or during the removal of the trophectoderm cells could all create unseen and not very well understood damage to the sample that is being tested and also, importantly to the embryo that is left behind. It has been postulated that biopsy technique and/or skill of the embryologist performing the biopsy may also impact the level of mosaicism. While it seems intuitive that stress on the embryo during culture could have an impact on mosaicism rates and/or aneuploidy rate, a review of the subject published recently shows no concrete evidence of cause and effect between a range of embryo culture condition variables such as media type, its pH and temperature, and the rate of aneuploidy or mosaicism (Swain 2019).

However, in a study presented by Whitney and colleagues at the European Society for Human Reproduction and Embryology (ESHRE) meeting in Barcelona, Spain (Whitney 2018), the authors aimed to 'induce' mosaic aneuploidy through poor biopsy technique. Here they concentrated on inducing cellular lysis through excess laser ablation of cells within the embryo. They found mosaic profiles in samples handled 'poorly' while control samples from the same embryo handled using their normal process remained consistent with the initial biopsy. Interestingly, in one sample handled 'poorly' they also tested the media droplet as a control and found a chaotic abnormal profile in DNA from the media. Lastly the authors observed what they considered to be low level noise in samples handled with the 'poor biopsy technique'. While the study is quite small, it brings a glimpse into how much impact embryo biopsy may have on the outcomes of conventional biopsy-based PGT-A testing results.

In addition to the potential for damage to the biopsy sample and the embryo, one has to consider the amount of time this procedure requires each day. Typically, two highly skilled embryologists are removed from other tasks to prepare embryo biopsy dishes, perform assisted hatching ahead of embryo biopsy, perform embryo biopsy, and tube the cells for genetic testing. Each of these steps must be witnessed to ensure proper technique and to limit the chance of mistakes, sample switches etc. An embryologist performs a great many tasks each day and the move in the field to trophectoderm biopsy with cryopreservation following biopsy has added a substantial burden to the embryology lab and IVF center. Any time one adds new techniques to the laboratory, cost is added at the same time. This cost is typically passed directly to the patient in the form of an embryo biopsy fee which can double or triple the cost of genetic testing of embryos.

Clearly the ability to non-invasively test the chromosome complement of an embryo prior to transfer into the uterus, or non-invasive preimplantation genetic testing for aneuploidy (niPGT-A), would be a great leap forward for the field. To date, no other tool in assisted reproduction has shown the promise that PGT-A has as it relates to improving IVF success rates. Being able to perform PGT-A without biopsy will not only eliminate the risk to the embryo but will also make PGT-A universally accessible.

What We Know so Far

The first report discovering embryo DNA without biopsy was from the blastocoelic fluid (Palini 2013). In routine IVF procedures it is sometimes desirable to remove the fluid inside the cavity that makes up a blastocyst, specifically prior to cryopreservation procedures. In this first report, Palini and colleagues estimated that there was 0.8 to 85 pg of genomic DNA from the embryo in the 0.3 to 0.5 nL volume of blastocoel fluid. It should be noted that each human cell contains approximately 6 pg of DNA. This data suggests that there are up to 14 embryo cells worth of DNA inside the blastocoelic cavity fluid. This group tested the blastocoelic fluid from the two samples which gave the highest yield following whole genome amplification and correctly identified two euploid male embryos, demonstrating that the testing of DNA released from the embryo was possible. Of note, removal of the blastocoelic fluid is less invasive than biopsy but not truly non-invasive to the embryo. Typically, a very thin needle like the needle used to inseminate eggs during intracytoplasmic sperm injection (ICSI) is used to enter through a junction between the trophectoderm cells and withdraw the blastocoele fluid.

In 2016, a new report was published (Shamonki 2016) which reported initial proof of concept results testing embryo-released DNA from spent culture media. In this study, individual embryos were cultured in 15 µL drops of G2 media (Vitrolife) from Day 3 onwards with assisted hatching. Of the 57 media samples tested, 55 of them amplified with yields between 2 and 642 ng/µL. The 6 highest yield samples underwent array comparative genomic hybridization (aCGH), 2 passed QC and were concordant with the embryo biopsy. Since these initial studies demonstrating the possibility of non-invasive PGT, there have been other publications showing variable success. For a thorough review of the topic see Leaver and Wells (2020).

There have been a small number of labs who have switched from biopsy-based testing to non-invasive testing. For example, Fang et al (2019) reported that 170 blastocysts were screened non-invasively, 79 were euploid (46%), 52 were aneuploid (31%), 33 were mosaic (19%), and 6 gave no result. Of the 52 euploid embryos transferred to 43 women, there were 36 (72%) biochemical pregnancies, 29 (58%) clinical pregnancies, and 3 spontaneous miscarriages all without evidence of aneuploidy and 27 (54%) live births. These results are not unlike what is seen in a biopsy-based PGT-A program, but they were achieved without biopsy.

Presuming spent culture media proves to be a reliable source of DNA from the embryo, this presents a truly non-invasive method to test the genetic makeup of an embryo prior to implantation. In theory, non-invasive PGT-A would (should) have the same implantation benefits of biopsy-based PGT-A without the biopsy risk on the growing embryo. In addition, biopsy-based PGT-A requires embryos to reach a certain state of development (the blastocyst stage). Sadly, not all embryos survive to reach this stage of development. Typically, only 45% to 55% of developing embryos make it to the blastocyst stage. In addition, not all laboratories are resourced to be able to handle the large numbers of biopsies necessary to offer PGT-A to all IVF patients. As mentioned earlier, the skills around embryo growth, biopsy, tubing of samples, and cryopreservation are limited to only some IVF centers in the world. Spent culture media testing would allow laboratories to test all embryos in a cohort which could help better understand the genetic makeup of all embryos, not just the embryos with the best morphology. A truly non-invasive method requiring only the collection of spent culture media for each embryo during growth would revolutionize the IVF industry worldwide, making PGT-A universally accessible.

This document is broken down into four main categories with various questions under each category described and answered followed by our recommendations for implementing niPGT-A into routine practice. For quick reference, they are:

What are the Key Considerations & Questions?

1. Questions About the Cell Free DNA in Spent Media

1.1 Where does the DNA come from and is it representative of the entire embryo?

It is known that during early embryo development there is a certain amount of programmed cell death, or apoptosis that occurs in both the inner cell mass and the trophectoderm (Hardy 1997, 1999). It is thought that this apoptosis may occur due to embryo self-correction (Taylor 2015), leading to DNA from abnormal cells being discarded from the embryo into the spent culture media and blastocoelic fluid. Spent culture media would then be expected to contain a higher amount of aneuploid cell DNA, generating an aneuploid genetic profile that is inconsistent with that of the growing euploid embryo (Brouillet 2020). This model could lead to false positive results and misdiagnosis of an otherwise chromosomally healthy embryo that had self-corrected.

However, the high concordance rates between spent media, biopsy, and the rest of the embryo being reported in the literature, even for euploid embryos, suggests that apoptosis is occurring in both euploid and aneuploid cells.

As apoptosis occurs in cells from across the entire embryo, the DNA found in the blastocoelic fluid and spent culture media could more accurately reflect the whole embryo genetic status when compared to the trophectoderm biopsy (Huang 2019). However, there are possibly some differences between the blastocoelic fluid and spent media. Magli et al (2018) found that WGA failed to amplify DNA from the blastocoele more often in euploid embryos (55%) than in aneuploid embryos (19%), suggesting there is more DNA in the blastocoelic cavity of aneuploid embryos. There are still a large number of open questions on this topic and a lot more research to be done.

1.2 Does the culture medium need to be lysed?

Generally, it is a requirement of whole genome amplification to perform an initial cell lysis step to break down the cell membranes and create a readily accessible DNA template. During the apoptosis process in embryo development, cells are broken down and the DNA is released into the surrounding medium, leading to the hypothesis that cell lysis is not necessary for non-invasive PGT-A. However, if any DNA is released from the embryo in vesicles, cell lysis could allow this DNA to be amplified.

To test whether cell lysis is required to make DNA available for amplification, spent culture media samples from two different IVF centres were split into replicate samples before whole genome amplification using the PG-Seq™ Non-invasive PGT kit with and without cell lysis.

All samples with and without cell lysis showed strong DNA amplification (Figure 1), generating the expected product size for the kit. There was no decrease in WGA yield in the media samples amplified without lysis, suggesting the DNA is accessible for amplification.

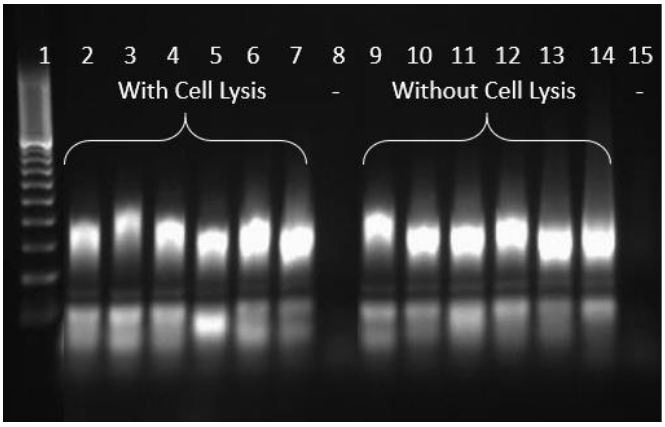

Figure 1: Agarose gel electropherogram of duplicate spent media samples processed with cell lysis (lanes 2-7) and without cell lysis (lanes 9-14). Lanes 8 and 15 represent negative controls and lane 1 is a 100 bp DNA size marker (DMW-100 M, Geneworks).

1.3 Is the cell free DNA in spent embryo culture media fragmented?

One of the main theories for non-invasive prenatal testing is that fetal DNA in maternal circulation is more fragmented than maternal DNA with an abundance of fragments that are shorter than 160 bp. Similarly, it has been suggested that the embryo DNA released into the media may be fragmented as part of the unique DNA degradation process of early human cells. It is also likely that degradation occurs once the embryo DNA has been released into the spent media, further reducing the fragment size.

To determine the size of DNA in spent culture media and investigate whether it is fragmented, an experiment was performed to establish whether WGA can create DNA fragments that are longer than the starting template DNA. Genomic DNA was sheared to an average of 150 bp and 550 bp using a Covaris® instrument. Sheared and un-sheared genomic DNA was diluted to 30 pg before amplification with the DOPlify® WGA kit according to standard protocol.

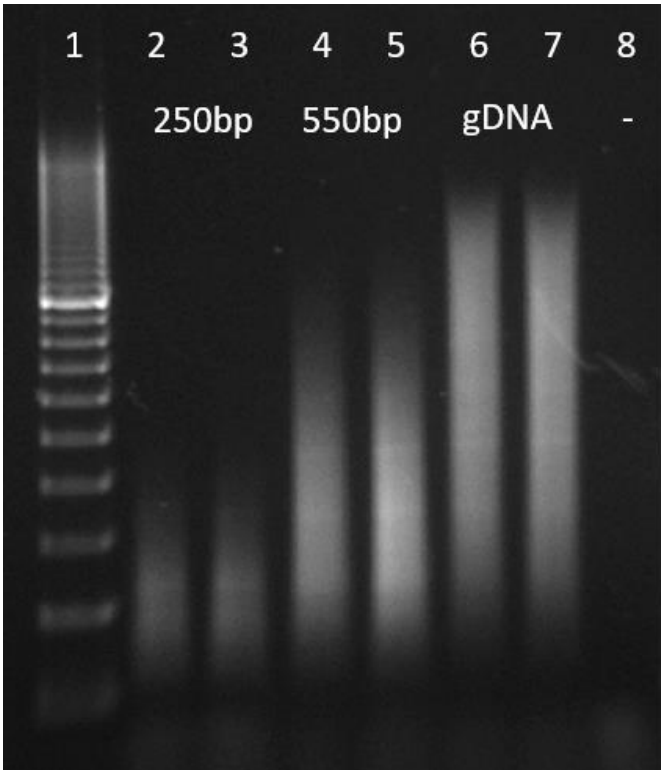

Figure 2: Agarose gel electropherogram of whole genome amplification products from genomic DNA template sheared to 250 bp (lanes 2-3) and 550bp (lanes 4-5) along with unsheared genomic DNA (lanes 6-7). Lane 1 represents a 100 bp DNA marker (DMW-100M) and lane 8 is a negative control.

When the resulting WGA products were visualised on an agarose gel (Figure 2), it was clear that the size of whole genome amplified DNA was concordant with the size of the starting DNA template, suggesting WGA is unable to create fragments longer than the starting template. With this in mind, a comparison was performed using whole genome amplified product from intact cells versus spent culture media (Figure 3).

It was found that the WGA products from spent media samples are approximately the same size as product obtained from intact cells, suggesting that it is not fragmented. There was no difference in the WGA product size obtained from a media sample found to have maternal cell contamination compared with a contamination free sample. There is some evidence of a small peak of fragmented DNA at 110 bp in the spent culture media with and without maternal DNA contamination.

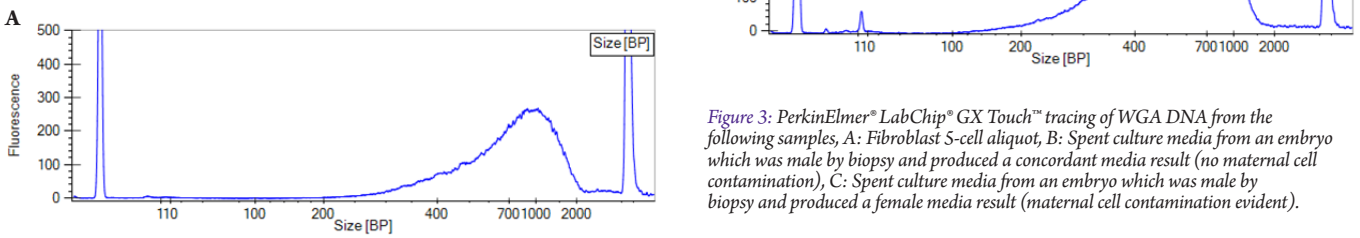

Figure 3: PerkinElmer® LabChip® GX Touch™ tracing of WGA DNA from the following samples, A: Fibroblast S-cell aliquot, B: Spent culture media from an embryo which was male by biopsy and produced a concordant media result (no maternal cell contamination), C: Spent culture media from an embryo which was male by biopsy and produced a female media result (maternal cell contamination evident).

2. Technical Considerations for Spent Media Testing

2.1 What else is in the spent culture media?

Spent culture media is not an ideal starting template for whole genome amplification for many reasons. There is the possibility of contaminating DNA, 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. We recommend that laboratories test each new batch of media to ensure there is no contaminating DNA.

Additionally, the spent culture media sample will contain amplification inhibitors originating from the embryo culture media itself, including salts, proteins, and enzymes. Due to the very low amount of DNA present in the spent media sample, it is not feasible to remove these inhibitors with an extraction and purification process prior to amplification.

As a result of the apoptosis process, DNA contained in the spent culture media may be degraded, fragmented and bound in tangled complexes with proteins. This leads to difficulties when using standard amplification protocols and should be taken into consideration when choosing or optimising an amplification system.

In order to overcome the challenging starting template, changes needed to be made to the whole genome amplification chemistry. The PG-Seq™ Non-Invasive PGT kit was developed to overcome these undesirable PCR conditions while also being accepting of a range of different starting templates and DNA concentrations.

2.2 How should the spent media be collected?

When sampling the media, the entire volume should be collected as soon as possible after the embryo has been removed from culture using a new, clean pipette tip for every sample. Immediately store the collected media at -20°C in sterile PCR-grade tubes. To assist the understanding of spent culture media testing, we recommend that information such as the day of collection, culturing time, type of media used, grade of embryo, and whether it underwent assisted hatching is documented.

2.3 Can the same kit be used to test blastocoel fluid?

The PG-Seq™ Non-Invasive PGT kit can be used according to standard protocol, using the entire volume of blastocoel fluid.

It is also possible to combine the blastocoelic fluid with the spent media by collapsing the embryo in the culture drop before removing the embryo from the media and collecting the media sample. An example of this type of protocol was first published by Kuznyetsov et al (2018).

2.4 Can I combine PG-Seq™ Rapid Non-Invasive samples with PG-Seq™ biopsy samples in the same sequencing run?

Samples from all PG-Seq™ kits including the PG-Seq™ 2.0, PG-Seq™ Rapid and PG-Seq™ Rapid Non-Invasive kits can be run together in a single sequencing run if required. The PG-Seq™ Rapid and PG-Seq™ Rapid Non-Invasive kit barcodes and indexes share the same index sequences and care should be taken when pooling samples from these kits to ensure index sequences are not duplicated. If pooling PG-Seq™ Rapid Non-Invasive kit samples with PG-Seq 2.0 samples, a slight modification to the sample sheet is required.

Please contact support.au@perkinelmer.com for further information.

3. Considerations for the Introduction of a Non-invasive PGT Program

During the development of the PG-Seq™ Non-invasive PGT kit, PerkinElmer collaborated with 15 laboratories all using different embryology protocols. A selection of this data is presented below 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%

The results of this global collaboration have given us some insight into the topics discussed below.

3.1 Which brand of media can I use?

There are many different brands of commercially available media manufacturers and types of media. As well as commercially available embryo culture media, some labs manufacture their own media.

In our study, we have tested media from Vitrolife, Origio, Sage and Global. Our data suggests that there is no one brand or manufacturer that performs better or worse than another. Whilst there does not seem to be a need to use one specific media for non-invasive PGT, additional data is needed. It is possible that in the future, embryo culture media specifically suited to non-invasive PGT may be developed.

3.2 Is it best to use two-step or continuous culture media?

As part of our global collaboration project, both two-step and continuous culture media were tested. The majority of labs used two-step media and it was observed that this method produced higher biopsy concordance rates with less maternal DNA contamination.

As long as the cumulus cells are carefully and thoroughly removed, concordance is possible when using continuous media. We observed that refreshing the media on day 3-4 can help to reduce or remove the maternal DNA contamination.

3.3 What droplet size should we culture our embryos in?

It has been suggested that culturing embryos in smaller media droplets could increase the concentration of DNA in the spent media sample and the concordance. Based on the samples processed as part of our global collaboration project, there was no significant difference found in biopsy concordance when culturing in lower volumes (10 µL) versus higher volumes (50 µL or greater). However, small volume droplet sizes were more common, and more data is needed for higher volume droplet sizes. It is recommended that labs evaluate the concordance rate using their current drop size before considering changes.

3.4 How important is DNA contamination?

Maternal contamination is the leading cause of non-concordance between media and embryo biopsy. Laboratories not willing to denude their embryos will not achieve accurate non-invasive PGT-A results. If maternal DNA is present, the media sample will give a euploid female result. Whilst this seems obvious, it is an important consideration for laboratories prior to embarking on a non-invasive PGT program. The risk of maternal DNA contamination can be reduced by changing the media during embryo culture. This is typically performed on either day 3 or day 4 of embryo culture with sequential media systems. If labs prefer to use single step media, denuding the embryo becomes even more critical because the released maternal DNA isn't removed if the media isn't changed. It is possible that refreshing the media on day 3 of culture could help.

Labs will need to evaluate their own maternal DNA contamination baseline rate and overall concordance rate by performing in-house validations comparing their non-invasive PGT results from male embryos by biopsy. The rate of maternal DNA contamination is determined by looking at the proportion of male embryos by biopsy that give a female embryo result from the culture media. Once a lab has determined their own level of maternal contamination, they can either progress with non-invasive PGT if their maternal DNA contamination rate is zero, or work towards a solution for minimizing this risk by focusing on the procedure of embryo denuding and the media change during culture. This could include introducing an embryo washing step in fresh media, for example.

Labs also need to consider other sources of DNA contamination, including from operators, consumables including the media, and the lab work environment. No template controls should always be included during sample preparation to monitor DNA contamination.

3.5 On what day of embryo culture should we collect the spent media?

The amount of DNA released from early stage embryos is relative to the number of apoptotic cells, which progressively increases as the total number of embryonic cells increase (Hardy et al., 1989). It is difficult to accurately quantify the amount of DNA present in the spent media due to inaccuracies in quantification techniques at such low concentrations. It is also influenced by different embryology techniques including drop volume, different growth rates of embryos and inhibitors present in the sample which confound quantification methods.

Between fertilisation and day 3 in culture, a typical embryo will increase in size from 1 to 8 cells. Since there are so few cells by day 3, it is unlikely that there will be very much, if any, DNA in the spent culture media. The DNA yield and biopsy concordance are higher when culture media is collected on day 5 or later. This is to be expected as in the later stages of embryo development there are more cells in the embryo mass, more cellular turnover and therefore more embryonic DNA released into the spent media to become available template for WGA.

A study by Lane et al (2017) analysed spent embryo media collected during the day 4-6 window and the day 5-7 window. It was observed that amplification success and concordance with the embryo biopsy improved with the later collection window (Figure 4).

The late stage of embryo development needed for media collection means that embryo freezing is still going to be necessary. In the future, as we learn more about non-invasive PGT and the optimal embryology protocol, earlier media sampling and fresh transfers may become possible.

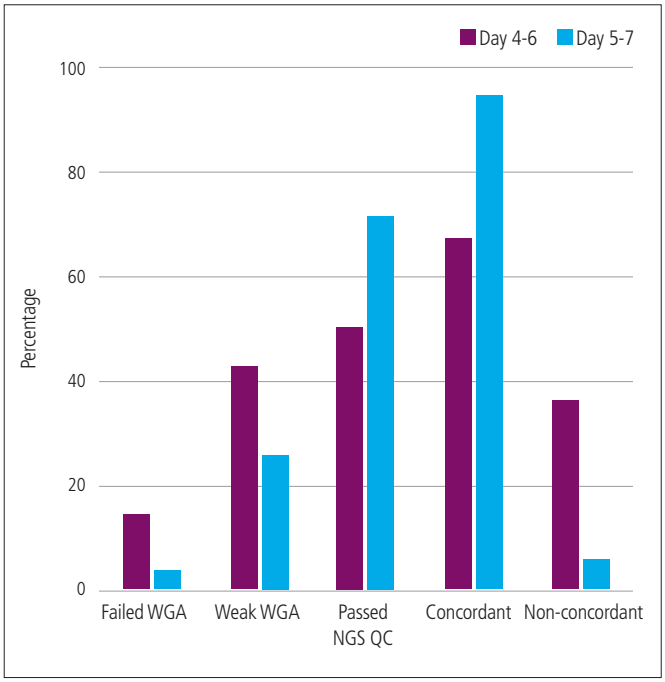

Figure 4: The percentage of failed WGA, weak WGA, passed NGS QC, concordant and non-concordant results of spent embryo culture media collected from embryos grown to day 4-6 compared with day 5-7.

3.6 To hatch or not to hatch?

At this stage, it is unclear whether embryos need to undergo assisted hatching and when. Also, do embryos need to be left in media until they hatch?

It has long been known that embryo DNA can be found in the blastocoel fluid. It is also known that the zona pellucida forms a protective layer around the embryo which would limit the exchange of substances between the embryo and its surrounding media. Therefore, it could be anticipated that a hatched embryo would release more DNA template into the media than an unhatched embryo.

We have found a trend towards an increase in WGA yield when embryos have undergone assisted hatching. However, there is a high degree of variability in the yield from each spent media sample, even when the samples are from a single lab and the same embryology protocol has been used for all embryos. This variability between embryo media samples makes it difficult to clearly determine the effect of hatching on yield. More research is needed on this topic. Ideally, embryos should be minimally handled, and assisted hatching should not be needed for NI-PGT.

3.7 Do embryology protocols need to change?

The focus needs to remain firmly on using an embryology protocol that gives the embryo the best chance of success, and then ascertaining whether a non-invasive PGT protocol can be incorporated.

The most critical factor for achieving accurate results from spent media comes upstream of the PG-Seq™ Rapid Non-invasive PGT kit. It is all the subtleties in embryology protocols that cascade down and affect the amount and quality of DNA in the media sample available for amplification and testing. Our global collaborative project has narrowed the focus down to three key areas, avoiding the introduction of any contaminating DNA, allowing the embryo to develop until there has been enough cellular turnover for sufficient DNA to be released into the media and confirming your own concordance with embryo biopsy result before introducing a non-invasive option into your lab.

3.8 Confirming Concordance

To determine whether an embryology protocol is suitable for non-invasive PGT, a side by side comparison study should be completed using spent culture media from embryos that will also have biopsy-based PGT-A results. Ideally, the spent media should be collected prior to embryo biopsy as part of a routine PGT-A methodology. Alternatively, embryos previously biopsied and resulted for PGT-A can be warmed or thawed, placed into individual media droplets and allowed to re-expand for at least 24 hours prior to sampling the culture media. The second approach has limitations as the freeze, thaw, sample embryology procedure is not typical in IVF and results from this method should be interpreted with caution.

Following amplification, sequencing and result analysis, performance metrics as per the table below (table 2) should be calculated and compared to biopsy.

Table 2: Recommended performance metrics to calculate as a measure of success of concordance studies

Type of Error	Total
False Positive	# false pos./total
False Negative	# false neg./ total
No Call Rate	# no result/# samples
Sensitivity	# true pos./(true pos. + false neg.)
Specificity	# true neg./(true neg. + false pos.)

False positive results are likely to be caused by insufficient or degraded DNA in the spent media sample, resulting in sub-optimal WGA and poor-quality results. False negative results, especially euploid female results are usually attributed to maternal cell contamination but could also be from other contamination sources. Embryo mosaicism also has the potential to cause differences between the spent media result in comparison to the biopsy. Where possible, the whole embryo should be used in the side by side testing. No call results can be due to failed amplification, or samples which do not pass quality measures. Again, this is likely to be attributed to insufficient or degraded DNA in the spent media sample.

4. Considerations for Interpreting Results

4.1 What quality metrics should I use?

Based on our experience, we recommend adhering to less stringent QC metrics when analysing results from spent embryo culture media in comparison to biopsies. In particular, metrics such as WGA yield and the quality score are influenced by the challenging starting material of DNA in spent culture media.

The small and variable amount of DNA template found in spent media can result in WGA yields that are often lower compared with embryo biopsies. However, correlations with biopsy and the rest of the embryo can still be high regardless of the WGA yield and should be considered when implementing a yield QC cut off.

DNA amplified from spent culture media generally produces sequencing data with increased genome coverage variability (noise). In the PG-Find™ software, the noise is reflected in the quality score metric which measures the variance of each data point across the genome, comparable to a standard deviation. Higher quality scores indicate lower quality DNA or issues with the whole genome amplification. To reduce the amount of noise in the analysis, we recommend reducing the number of data points analysed across the genome by using a larger bin width of 1,000,000 bp or higher.

Once a concordance study has been completed, end users should develop their own quality metrics in response to their results.

4.2 Can I analyse segmental aneuploidies and mosaicism using this kit?

While we have seen that the detection of segmental aneuploidies and mosaicism is possible when analysing spent culture media, we recommend starting with the analysis of whole chromosome aneuploidies first to determine the concordance of the spent media with the biopsy result. Poor quality DNA can lead to noisy NGS results, which may be interpreted as small CNVs or mosaicism. Whilst the media may appear mosaic, we would caution against reporting it at this stage of validation.

Once laboratories are able to achieve high (>90%) concordance rates between spent media and embryo biopsies for whole chromosome aneuploidy and sex of the embryo, they can consider trying to increase the resolution for the detection of smaller CNVs and mosaicism.

5. Recommendations Before Implementing NI as Standard Practice

Before implementing non-invasive PGT-A as standard practice, we recommend that laboratories benchmark their spent embryo media result against their biopsy results by running both methods in parallel. This will provide a biopsy concordance baseline level and will give an estimate of the degree of maternal contamination to determine if the current culturing protocols are suitable for use with routine non-invasive PGT-A.

Depending on the biopsy concordance baseline level, labs can choose various methods to optimize their culturing protocols to improve results. For example, if the WGA yield is low, the embryos should be cultured in the media for longer to increase the amount of template available for WGA. If the sex concordance is low and a high proportion of male embryos by biopsy are giving euploid female results from the culture media, a change to embryology protocol to improve removal of cumulus cells and contaminating maternal DNA should be introduced. Lab staff should use personal protective equipment to reduce the risk of their own DNA contaminating the embryo culture media.

References

1. *Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, Peck AC, Sills ES, Salem RD. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet. 2012 May2;5(1):24.*
2. *Harton GL, Munné S, Surrey M, Grifo J, Kaplan B, McCulloh DH, Griffin DK, Wells D; PGD Practitioners Group. Diminished effect of maternal age on implantation after preimplantation genetic diagnosis with array comparative genomic hybridization. Fertil Steril. 2013 Dec;100(6):1695-703.*
3. *Forman EJ1, Upham KM, Cheng M, Zhao T, Hong KH, Treff NR, Scott RT Jr. Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer. Fertil Steril. 2013 Sep;100(3):718-24.*
4. *Rubio, C., Bellver, J., Rodrigoa, L., Castillón, G., Guillén, A., Vidal, C. et al. In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized controlled study. Fertil Steril. 2017; 107: 1122–1129.*
5. *Scott RT Jr, Upham KM, Forman EJ, Zhao T, Treff NR. Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. Fertil Steril. 2013 Sep;100(3):624-30.*
6. *Zacchini F, Arena R, Abramik A, Ptak GE. Embryo biopsy and development: the known and the unknown Reproduction (2017) 154 R143–R148.*
7. *Cimadomo D, Capalbo A, Ubaldi FM, Scarica C, Palagiano A, Canipari R, Rienzi L. The Impact of Biopsy on Human Embryo Developmental Potential during Preimplantation Genetic Diagnosis Hindawi Publishing Corporation BioMed Research International Volume 2016, Article ID 7193075, 10 pages.*
8. *Zhang WY, von Versen-Hoynck F, Kapphahn KI, Fleischmann RR, Zhao Q, Baker VL. Maternal and neonatal outcomes associated with trophectoderm biopsy. Fertil Steril. 2019 Aug;112(2):283-290.*
9. *Swain JE. Is there an optimal pH for culture media used in clinical IVF? Hum Reprod Update 2012;18(3):333–339.*
10. *Swain JE. Controversies in ART: can the IVF laboratory influence preimplantation embryo aneuploidy? Reprod. Bio. Med. Online. Vol 39;Issue 4, 2019.*
11. *Whitney JB, Rios CM, Balloch K, Anderson RE, Schiewe MC. Induced Cellular Lysis and Increased Laser Exposure During Trophectoderm Biopsies Can Generate Low-level Mosaic Profiles: a Prospective Study. ESHRE, Barcelona, Spain. 2018.*
12. *Yang Z, Zhang J, Salem SA, Liu X, Kuang Y, Salem RD, Liu J. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. BMC Medical Genomics 2014, 7:38.*
13. *Shamonki MI, Jin H, Haimowitz Z, Liu L. Proof of concept: preimplantation genetic screening without embryo biopsy through analysis of cell-free DNA in spent embryo culture media. Fertility and Sterility (2016) 106(6) 1312-1318.*
14. *Leaver M, Wells D. Non-invasive preimplantation genetic testing (niPGT-A): the next revolution in reproductive genetics? Hum Reprod Update. 2020 Jan 1;26(1):16-42.*
15. *Fang R, Yang W, Zhao X, Xiong F, Guo C, Xiao J, Chen L, Song X, Wang H, Chen J, Xiao X, Yao B, Cai L-Y. Chromosome screening using culture medium of embryos fertilised in vitro: a pilot clinical study. J Transl Med 2019 17:73*
16. *Hardy K. Cell death in the mammalian blastocyst. Mol. Hum. Reprod. 1997; 3: 919-925*
17. *Hardy K. Apoptosis in the human embryo. Rev. Reprod. 1999; 4: 125-134*
18. *Taylor TH, Gitlin SA, Patrick JL, Crain JL, Wilson JM, Griffin DK. The origin, mechanisms, incidence and clinical consequences of chromosomal mosaicism in humans. Hum. Reprod. Update. 2014; 20: 571-581*
19. *Brouillet S, Martinez G, Coutton C, Hamamah S. Is cell-free DNA in spent embryo culture medium an alternative to embryo biopsy for preimplantation genetic testing? a systematic review RMB Online Published:February 19, 2020DOI:https://doi.org/10.1016/j.rbmo.2020.02.002*
20. *Huang L, Bogale B, Tang Y, Lu S, Xie XS, Racowsky C. Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy Proc Natl Acad Sci U S A. 2019 Jul 9;116(28):14105-14112*
21. *Magli MC, Albanese C, Crippa A, Tabanelli C, Ferraretti AP, Gianaroli L. Deoxyribonucleic acid detection in blastocoelic fluid: a new predictor of embryo ploidy and viable pregnancy. Fertil Steril. 2019 Jan;111(1):77-85*
22. *Hardy K, Handyside AH, Winston RM. The human blastocyst: cell number, death and allocation during late preimplantation development in vitro. Development. 1989; 107: 597-604*
23. *Lane M, Zander-Fox DL, Hamilton H, Jasper M, Hodgson B, Fraser M, Bell F. Ability to detect aneuploidy from cell free DNA collected from media is dependent on the stage of development of the embryo. Fertility and Sterility 2017 108 (3)*
24. *Kuznyetsov V, Madjunkova S, Antes R, Abramov R, Motamedi G, Ibarrientos Z, Librach C. Evaluation of a novel non-invasive preimplantation genetic screening approach. PLoS One 2018 May 10 ; 13(5)*

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