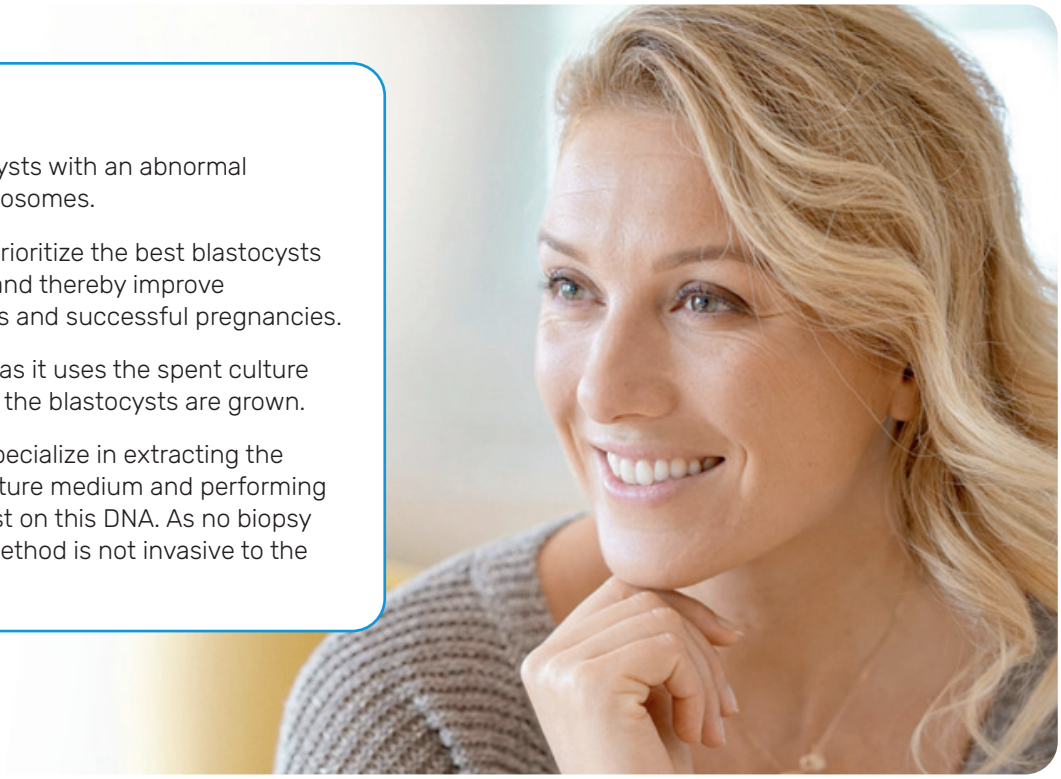
Amplexa Genetics® Providing knowledge

# NON-INVASIVE PGT-A

Non-invasive PGT-A (niPGT-A) is a genetic screening method for blastocysts grown for use in IVF treatment.



## Highlights

- Identifies blastocysts with an abnormal number of chromosomes.
- Helps clinicians prioritize the best blastocysts for implantation and thereby improve implantation rates and successful pregnancies.
- Is "non-invasive" as it uses the spent culture medium in which the blastocysts are grown.
- At Amplexa we specialize in extracting the DNA from the culture medium and performing an aneuploidy test on this DNA. As no biopsy is required, the method is not invasive to the blastocyst.

### THE NEW NON-INVASIVE TECHNIQUE

The most common method employed by clinics for aneuploidy detection is Preimplantation Genetic Testing for Aneuploidy (PGT-A). PGT-A can increase the number of successful pregnancies by prioritizing the blastocysts with the best chromosomal profiles. In conventional PGT-A, a biopsy of the trophectoderm is collected and embryonic DNA extracted from the trophoblasts. However, this biopsy is potentially damaging to the blastocysts and only represents the chromosome count of the trophectoderm and not the embryo itself [3].

Recently, a non-invasive alternative to the trophectoderm biopsy was developed. It was discovered that blastocysts secrete cell-free DNA (cfDNA) into the culture medium in which they are grown. Using this cfDNA as a template for a non-invasive PGT-A (niPGT-A) has proven highly effective. This cfDNA has proven as representative of the actual embryo as the DNA obtained from the trophectoderm biopsy. The origin of the cfDNA is also very likely to originate from healthy blastocyst tissue, representing both the inner-cell mass and the trophectoderm [4].

niPGT-A offers a risk-free alternative to traditional PGT-A with high concordance to trophectoderm biopsies, inner cell mass and the whole blastocyst. The basis for the analysis is the spent culture medium (SCM) from the routine growth of blastocysts in IVF clinics. As SCM is collected and discarded upon vitrification of the blastocysts, incorporation of niPGT-A into the existing workflow is close to seamless.

## HOW IS niPGT-A NON-INVASIVE?

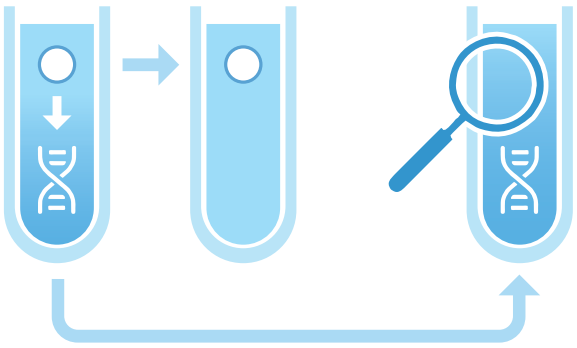
**1.** The blastocyst naturally releases DNA into its culture medium.

**Fresh blastocysts**
Spend culture medium (SCM) is collected on day 5 in sterile DNase and RNase free tubes.

**Frozen blastocysts**
Strip the blastocysts thoroughly immediately after thawing. Transfer to growing trays. Cultivate for 24 hours.

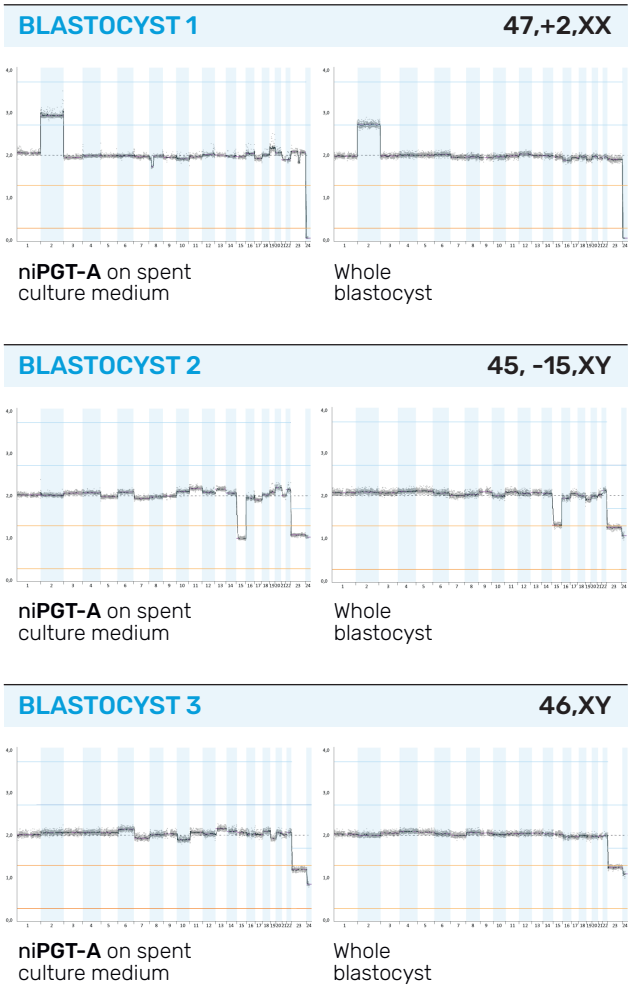
**2.** The blastocyst is transferred to a new pure liquid to be preserved at the clinic.

**3.** Upon vitrification of the blastocyst, Place all the samples in a freezer (-20°C) for at least one hour before the spent culture medium can be sent to Amplexa Genetics for niPGT-A analysis.



## IS cfDNA REPRESENTATIVE OF THE EMBRYO?

cfDNA was previously thought to come from apoptotic aneuploid cells within the blastocysts, however, aneuploid blastocysts do not secrete more cfDNA than aneuploid blastocysts [4].




## HOW ACCURATE IS niPGT-A COMPARED TO PGT-A?

Recent studies into the concordance rate of niPGT-A compared to PGT-A have achieved great results. Part of the improvement is due to the removal of maternal contamination in the form of cumulus cells. Additionally, it has been found that the best concentration of cDNA in the SCM is found on day 5 of blastocyst growth. Taking these factors into account, niPGT-A has been found to have higher concordance rates for embryo ploidity than PGT-A (94% vs 82%) [5].

**FIGURE 1** Chromosomal profiles based on cfDNA from spent culture medium (SCM) and DNA extracted from the whole blastocyst. Each column represents the same blastocyst, first tested with niPGT-A on the SCM and then with a regular PGT-A on the entire blastocyst rather than a trophectoderm biopsy. The whole blastocyst represents the ideal chromosomal profile as it is based on the entire genetic profile of the inner cell mass and trophectoderm. Our results show that the niPGT-A based on the cfDNA from the SCM has high concordance with the whole blastocyst. Blastocyst 1 has a trisomy on chromosome 2, Blastocyst 2 is missing one chromosome 15 and Blastocyst 3 has a healthy chromosomal profile.

# niPGT-A BIOPSY FREE & GENTLE

The new non-invasive screening method for blastocyst.
No damage.

## HOW ARE THE BEST niPGT-A RESULTS ACHIEVED?

To achieve the best results from an niPGT-A, we recommend the following steps during blastocyst growth:

**Step 1.** Removal of maternal cumulus cells: To avoid maternal DNA contaminating the sample, it is essential to remove any cumulus cells before the oocyte is placed into the growth medium. It can be removed chemically or with a 135 µm denudation pin.

**Step 2.** Cell washing: To ensure no maternal DNA is left over, we recommend washing the oocytes thoroughly the following denudation and transferring them to new growing trays in 20µl droplets.

**Step 3.** Sample quantity: To get sufficient cfDNA in the medium we also recommend that the blastocysts have been grown in said medium for a minimum of 24h. The minimum required SCM for niPGT-A is 6µl, is recommended to collect as much SCM as possible.

## REFERENCES

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3. H. F. Chen, M. Chen, and H. N. Ho, "**An overview of the current and emerging platforms for preimplantation genetic testing for aneuploidies (PGT-A) in in Vitro fertilization programs**," Taiwanese Journal of Obstetrics and Gynecology, vol. 59, no. 4. Elsevier Ltd, pp. 489–495, Jul. 01, 2020. doi:10.1016/j.tjog.2020.05.004.
4. M. Vera-Rodriguez et al., "**Origin and composition of cell-free DNA in spent medium from human embryo culture during preimplantation development**," Human Reproduction, vol. 33, no. 4, pp. 745–756, Apr. 2018, doi: 10.1093/humrep/dey028.
5. L. Huang, B. Bogale, Y.Tang, S. Lu, X. S. Xie, and C. Racowsky, "**Noninvasive preimplantation genetic testing for aneuploidy in spent medium may be more reliable than trophectoderm biopsy**," Proceedings of the National Academy of Sciences of the United States of America, vol. 116, no. 28, pp. 14105–14112, 2019, doi:10.1073/pnas.1907472116.
6. "2018data reported by National Summary Report," **https://www.sartcorsonline.com/rptCSR_Public-MultYear.aspx,2018**.

## WHEN IS niPGT-A RELEVANT?

Since aneuploidy increases significantly with age, an niPGT-A analysis becomes more relevant as The patient grows older. According to data from the 2018 National Summary Report, PGT analysis is most effective in women above the age of 38 [6].

### Additional Information:

- Embryos underwent biopsy for preimplantation genetic testing for genetic disease and/or chromosomal abnormalities.

### Live births of all cycle types

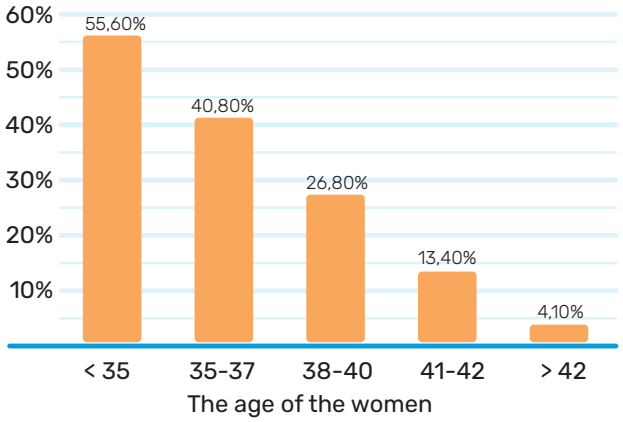


**FIGURE 2** Graphic data was collected on 06-12-2021 and could be updated.

### Live births of cycles with PGT

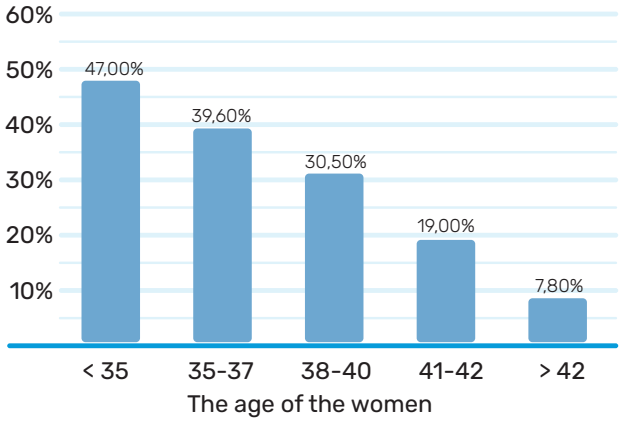


**FIGURE 3** Graphic data was collected on 06-12-2021 and could be updated.