eurofins | LifeCodexx

qNIPT PrenaTest®

qPCR-based NIPT
for fetal trisomy 21

02

Information for physicians

The smart qNIPT assay

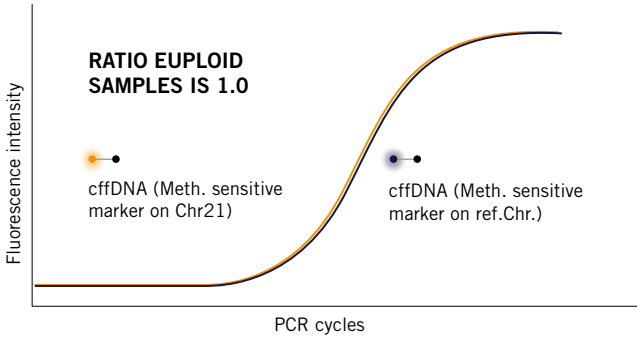
Detection of fetal trisomy 21 based on quantitative real-time PCR

Developed by LifeCodexx, the smart qNIPT assay is based on quantitative real-time polymerase chain reaction (qPCR). The qNIPT determines differences in methylation patterns of specific gene regions on the maternal and fetal DNA. Certain gene regions in maternal DNA are hypo-methylated whereas the same regions are hyper-methylated in fetal DNA. These methylation specific gene regions are used as DNA biomarkers for the determination of fetal trisomy 21.

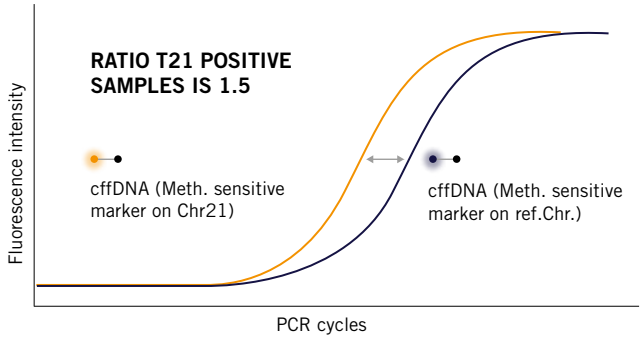
Different qPCR signals for chromosome 21 and reference chromosome

In an euploid case, the CP values[1] detected for chromosome 21 and reference chromosome within the same sample are rather identical, whereas in the T21 positive case the CP value for chromosome 21 is smaller. This means, that in the positive sample the crossing point is reached earlier for chromosome 21 compared to the unaffected reference chromosome. Therefore, for a T21 positive sample, the initial DNA quantity of chromosome 21 is higher compared to the initial DNA quantity of the unaffected reference chromosome. In average, chromosome 21 is 1.5 times higher concentrated, which corresponds to 3 copies of this chromosome.

TRISOMY 21 NEGATIVE



TRISOMY 21 POSITIVE



Comparison of the fetal DNA of chromosome 21 and reference chromosomes for all samples on a single run.

Clinical performance evaluation

Clinical performance evaluation compared to NGS-based PrenaTest®

Following proof-of-principle and feasibility studies with around 1,500 samples, a blinded study was performed to assess the clinical performance in maternal blood samples from singleton pregnancies. The samples were blinded by an independent Contract Research Organization. After extraction of cell-free DNA and methylation-specific digestion of DNA samples, a multiplex qPCR was performed. The primary qPCR data was finally evaluated with the CE-marked PrenaTest® analysis software. Results from the qNIPT analysis and from confirmatory NGS testing were compared with PrenaTest® results using NGS.[2]

Evaluation results

The study results of the performance assessment (n = 1062) show a positive percentage agreement (PPA; corresponds to sensitivity) of 100% (lower 1-sided 95% confidence interval of 93.12%; n = 42/42) as well as a negative percentage agreement (NPA; corresponds to specificity; n = 1019/1020) of 99.9% (lower 1-sided confidence interval of 99.54%; n=1019/1020) in comparison to the NGS-based PrenaTest®. The negative predictive value (NPV) for qNIPT as well as for the confirming NGS analyses was 100% (lower 1-sided 95% confidence interval of 99.71%). The average proportion of cell-free fetal DNA of all blood samples tested was 10.55%.

Study 2019	
Correctly classified samples	1061/1062 (99.9%)
Trisomy 21 positive	42/42 (100%)
Trisomy 21 negative	1019/1020 (99.9%)
Sensitivity (lower 1-sided 95% CI)	100% (93.12%)
Specificity (lower 1-sided 95% CI)	99.9% (99.54%)
NPV (lower 1-sided 95% CI)	100% (99.71%)

qNIPT – very reliable and robust

Our results show that the qNIPT is an extremely reliable test method which can be used in routine clinical practice according to the recommendations of the national and international professional associations[3].

The relative proportion of fetal DNA to all cell-free DNA is not crucial for the qNIPT. Only the absolute quantity of fetal DNA needs to be sufficient for a valid result.

1 Definition of CP value of the Lightcycler (Roche): This method identifies the crossing point (CP) of a PCR reaction as the point where the reaction's fluorescence reaches the maximum of the second derivative of the amplification curve, which corresponds to the point where the acceleration of the fluorescence signal is at its maximum.

2 LifeCodexx internal data from laboratory routine (August 2012 to September 2016).

3 ACOG 2015; ESHG/ASHG 2015; ACMG 2016; ISPD 2015; Austrian-German-Swiss Recommendations for NIPT 2016; available at www.lifecodexx.com.

4 Internal data from lab routine and clinical data based on qPCR and NGS available at www.lifecodexx.com.

5 After sample receipt. Monday to Friday, except public holidays.

qNIPT PrenaTest®
Safe. Rapid. Reliable.

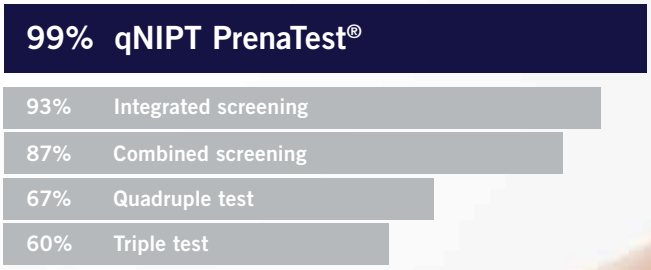
Starting from the ninth week of pregnancy (9+0 weeks since LMP) the qNIPT PrenaTest® determines fetal trisomy 21 from maternal blood with a very high test performance. qNIPT PrenaTest® is suitable for singleton pregnancies and can be used following assisted reproduction – even if donor eggs are used. If desired, the gender of the fetus may also be determined (when legally authorised). Results are usually available in 2 to 6 working days after sample receipt in the LifeCodexx laboratory and successful quality control.

99%	0.1%	<0.6%	2 – 6 DAYS
Detection rate [4]	False positive rate [4]	No-call rate [4]	Turnaround time [5]

In accordance with the In-Vitro Diagnostic Directive 98/79/EC

First NIPT with CE-marked data analysis software

Comparison of screening methods[6]



Detection rates of screening methods for the determination of fetal trisomy 21 compared to qNIPT PrenaTest® [6,7]

NIPT can be used as a primary screening method for fetal trisomy 21 in pregnant women of every age and risk group.

Austrian-German-Swiss Recommendations for NIPT 2016[8]

[...] any patient may choose cell-free DNA analysis as a screening strategy for common aneuploidies regardless of her risk status [...]
ACOG 2015[8]

[...] NIPT offers improved accuracy when testing for common autosomal aneuploidies compared with existing tests such as cFTS [...]
ESHG/ASHG 2015[8]

[...] informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies [...]
ACMG 2016[8]

[...] NIPT as a primary test can be offered to all pregnant women [...]
ISPD 2015[8]

6 Internal data from lab routine and clinical data available at www.lifecodexx.com.
7 Cuckle H, Benn P, Wright D (2005). Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. Seminars in Perinatology 29, 252-257.
8 Recommendations of medical associations available at www.lifecodexx.com.

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Eurofins LifeCodexx
First NIPT provider in Europe

We have been developing clinically validated, non-invasive prenatal tests (NIPT) since 2010. In 2012, the PrenaTest® was launched on the market as Europe's first NIPT. It has now become firmly established in many gynecological practices in Europe, the Middle East, and Asia as a reliable, fast and safe test method. Since the start of 2018, LifeCodexx has belonged to Eurofins Scientific, a leading international laboratory group, and has strengthened the technology portfolio of the Clinical Diagnostics business unit.

Expert hotline: +49 (0) 7531-97694844

www.lifecodexx.com

Eurofins LifeCodexx GmbH
Line-Eid-Straße 3, 78467 Konstanz, Germany
Phone +49 (0) 7531-9769460, Fax +49 (0) 7531-9769480, info@lifecodexx.com

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