Non-invasive prenatal detection of fetal autosomal aneuploidies using massively parallel sequencing: An European Collaborative Study

PD Dr. rer. nat. Markus Stumm
Center for Prenatal Diagnosis and Human Genetics Kudamm 199
Kurfürstendamm 199, 10719 Berlin, Germany
www.kudamm-199.de

lifecodexx

Non invasive diagnosis - Why?

- 35 years old patient 13+1 GW
- First Trimester Screening: Trisomy 21 risk 1:41
  NT 1,8 mm, free ß-hCG 2,96 MoM, PAPP-A 0,71 MoM
- Quadruple-Test: Trisomy 21 risk >1:10
  hCG 3,1 MoM, Inhibin A 3,12 MoM
- Amniocentesis: 46,XY
- **Amnion rupture and loss of pregnancy!!!**

Non invasive diagnosis - Why?

- Non invasive risk calculations
  - with no additional abortion risk
  - with high risk for wrong positive (5-10%) or wrong negative results (5-10%)
- Invasive genetic testing
  - with additional risk (0.2-1.0%) for abortion
  - with low risk for wrong positive or wrong negative results (<1%)

Prenatal Diagnostics today

Prenatal diagnostics (PND)

Non-invasive PND

- sonographic
- biochemical
- **NEW: molecular genetic**

+ no hazard for mother or child
- assessment of risk for aneuploidies

Invasive PND

- Chorionic villus sampling
- Amniocentesis
- Chordocentesis

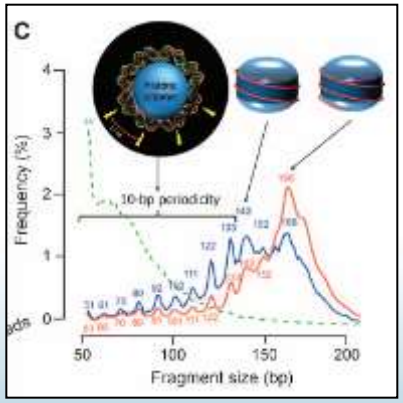
+ diagnostic result
- risk for miscarriage (0.2 – 1 %)

Landmark discovery for non-invasive prenatal diagnostics: circulating cell-free fetal DNA (cffNA)

1997 Discovery of cffDNA fragments in maternal serum and plasma (Lo et. al.)

• Detectable from	4th week of pregnancy on
• Percentage of fetal from total	2-40% / median amount ~ 10%
• Stability	< 2 hours
• Physical characteristics	short fragments mainly 143bp / 163bp mat
• Derived from	trophoblast (placenta) / hematopoetic mat
• Emergence	by apoptosis / necrosis

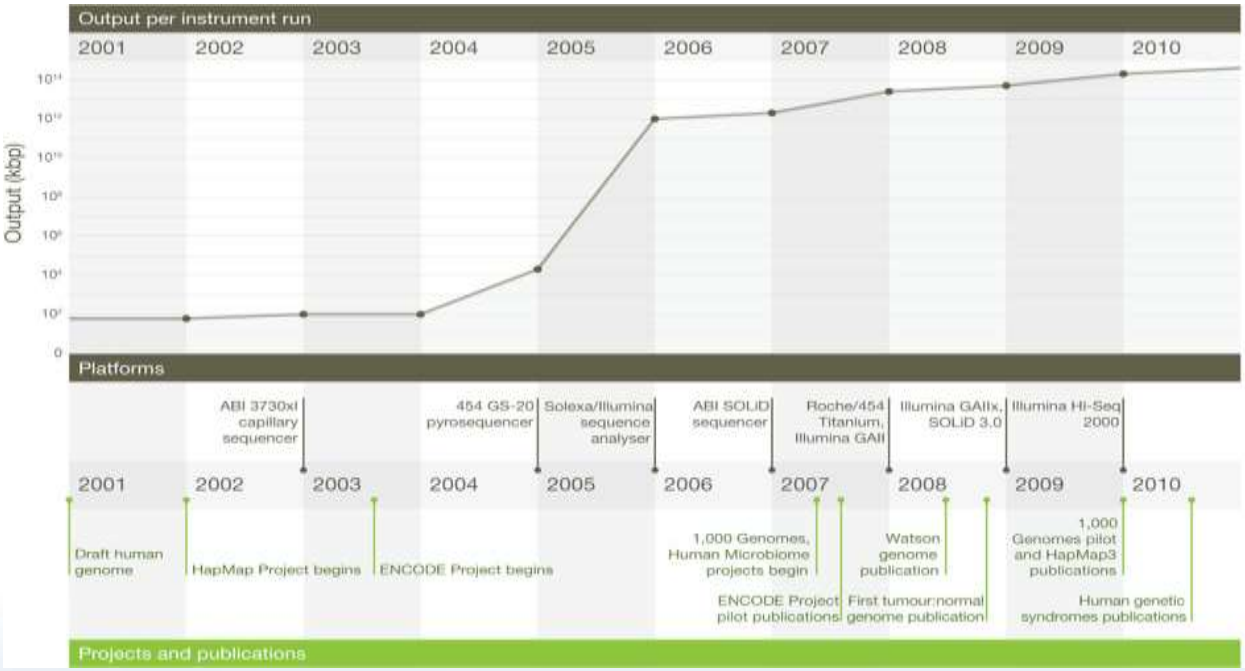
Dennis Lo, (www.cuhk.edu.hk)



Basis for NIPD – New Sequencing Technologies

Since 2005 Next Generation Sequencing (NGS) =
Massively Parallel (Shotgun) Sequencing (MPS)

- Miniaturized operational sequences
- High throughput



ER Mardis. *Nature* **470**, 198-203 (2011) doi:10.1038/nature09796

Method

1. Blood sampling

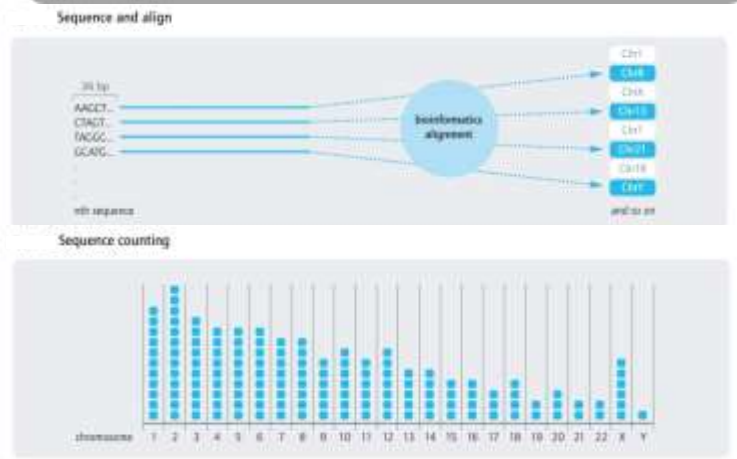
2. Extraction of plasma

3. Isolation of cell-free DNA

4. Library preparation

5. Next Generation Sequencing

6. Bio IT data analysis



7. z-score calculation

$$z(Chr21)_{sample} = \frac{\%(Chr21)_{sample} - \mathrm{Median}(Chr21)_{reference}}{\mathrm{MAD}(Chr21)_{reference}}$$

z-score	**≥3 T21 positive** **<3 T21 negative**

NGS 12plex
15 million 36 bp reads / sample

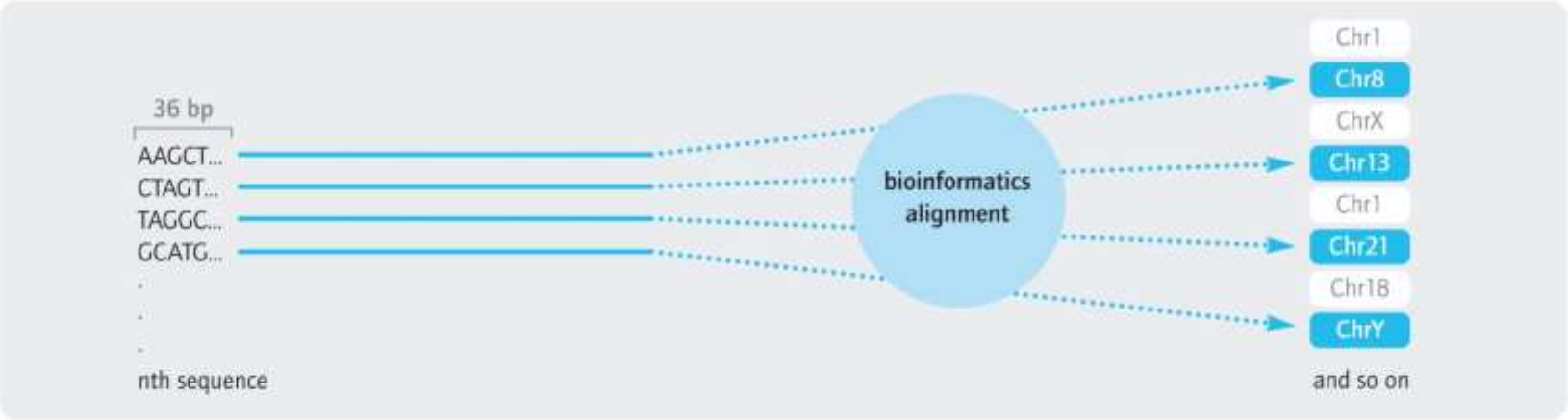
Delivery time of at least 10 working days / two weeks

Method

6. Bio IT data analysis

Alignment / Mapping of reads to human reference genome (hg18)

Figure 1 **Sequence and align**

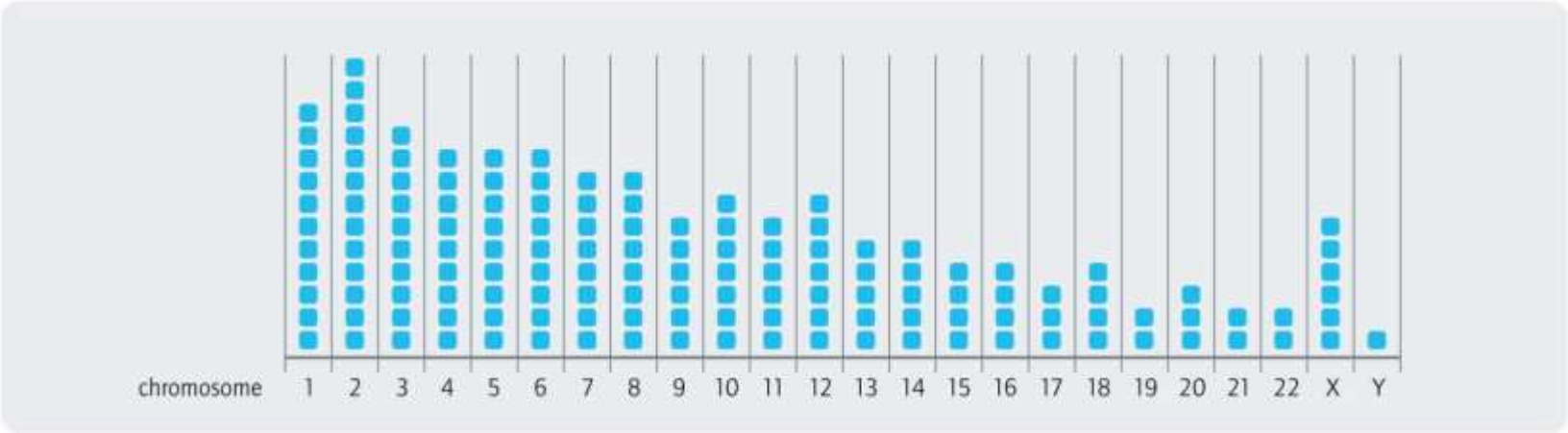


Method

6. Bio IT data analysis

Filtering / counting of reads

Figure 2 **Sequence counting**



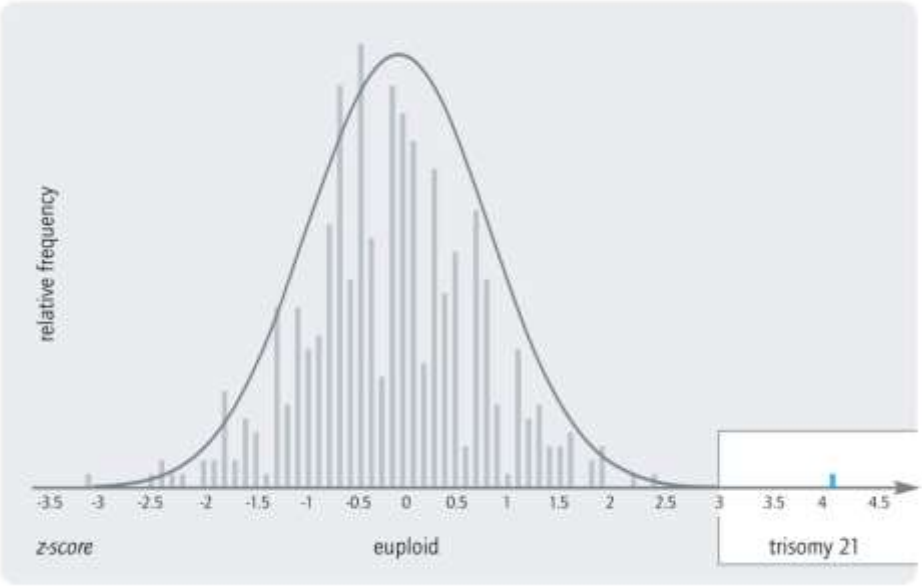
- %chr21 (euploid): 1.25% vs. **%chr21 (T21): 1.32% (only 1.05-fold higher)**

Method

7. z-score calculation

$$z(Chr21)_{\text{sample}} = \frac{\%(Chr21)_{\text{sample}} - \text{Median}(Chr21)_{\text{reference}}}{\text{MAD}(Chr21)_{\text{reference}}}$$

Figure 3 **Example for a frequency distribution of *z-scores***



- Represents the deviation of a given measured variable from the mean value of all measured variables in multiples of the standard deviation
- Z-score of 1 = exactly one standard deviation ; z-score of 3 = 3 standard deviations

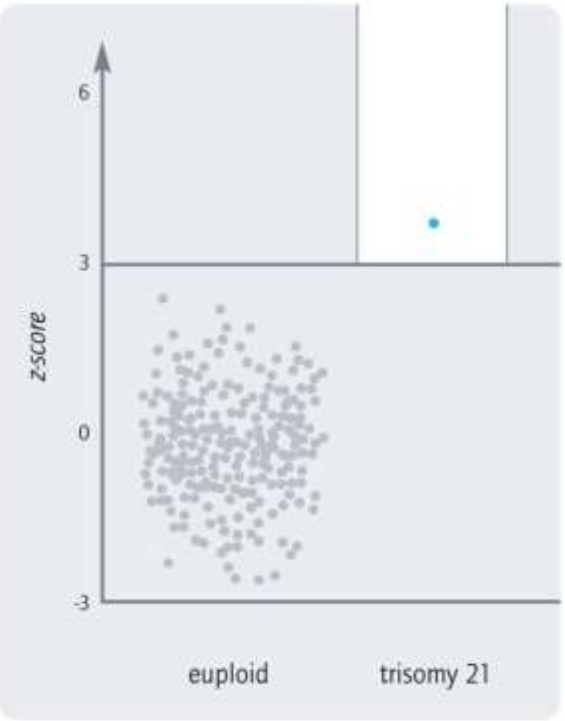
Method

7. z-score calculation

z-score ≥3 T21 positive
<3 T21 negative

In cases of z-scores between 2.5 and 3.5: repeat of analysis

Figure 4 Representation of *z-scores* for a euploid reference set and a case of trisomy 21 as dot plot

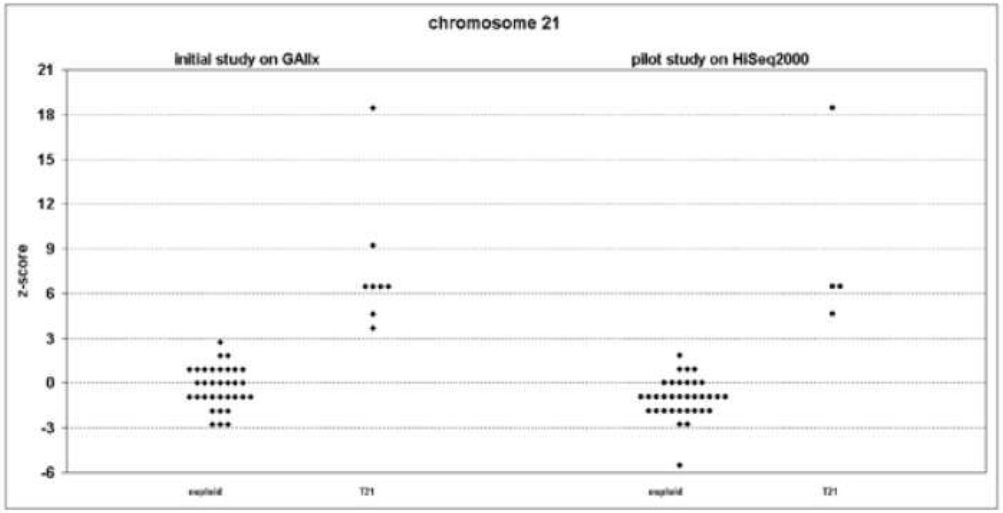


Proof of concept study for non-invasive detection of trisomy 21

Noninvasive prenatal detection of chromosomal aneuploidies using different next generation sequencing strategies and algorithms

Markus Stumm[1], Michael Entezami[1], Nastasja Trunk[2], Martina Beck[2], Julia Löcherbach[2], Rolf-Dieter Wegner[1], Andreas Hagen[1], Rolf Becker[1] and Wera Hofmann[3]*

[1]Zentrum für Pränataldiagnostik und Humangenetik, Kurfürstendamm 199, 10719 Berlin, Germany
[2]GATC Biotech AG, Jakob-Stadler-Platz 7, 78467 Konstanz, Germany
[3]LifeCodexx AG, Jakob-Stadler-Platz 7, 78467 Konstanz, Germany
*Correspondence to: Wera Hofmann. E-mail: w.hofmann@lifecodexx.com



WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Massively parallel sequencing of cell-free fetal DNA allows noninvasive prenatal detection of trisomy 21.

WHAT DOES THIS STUDY ADD?

- New algorithm for a more precise detection of fetal trisomy 21.
- New strategy to detect fetal trisomy 13 and trisomy 21 using a combination of target enrichment approach and massively parallel sequencing.

Prenatal Diagnosis 2012, **32**, 569–577

Figure 1 z-score calculation of chromosome 21 of 42 DNA samples sequenced on GAIIx and 38 DNA samples sequenced on HiSeq2000 using algorithm hg18_rm_0mm and a GAIIx reference set

Clinical validation study for non-invasive detection of trisomy 21

Prospective blinded multi-center study :

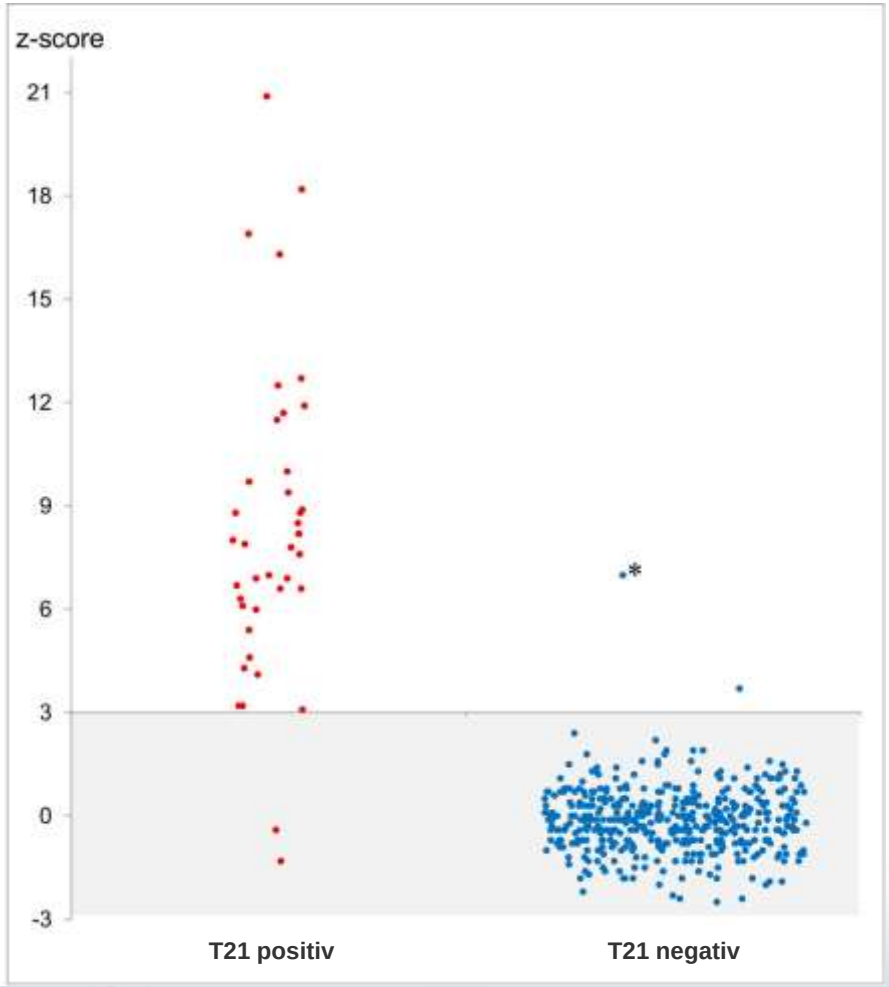
Evaluation of sensitivity and specificity for the established analysis work flow for the detection of fetal trisomy 21 in a large collective of pregnant women at risk for chromosomal aberrations in the fetus

Clinical gold standard:

Karyotyping after chorionic villi sampling, amniocentesis or chordocentesis

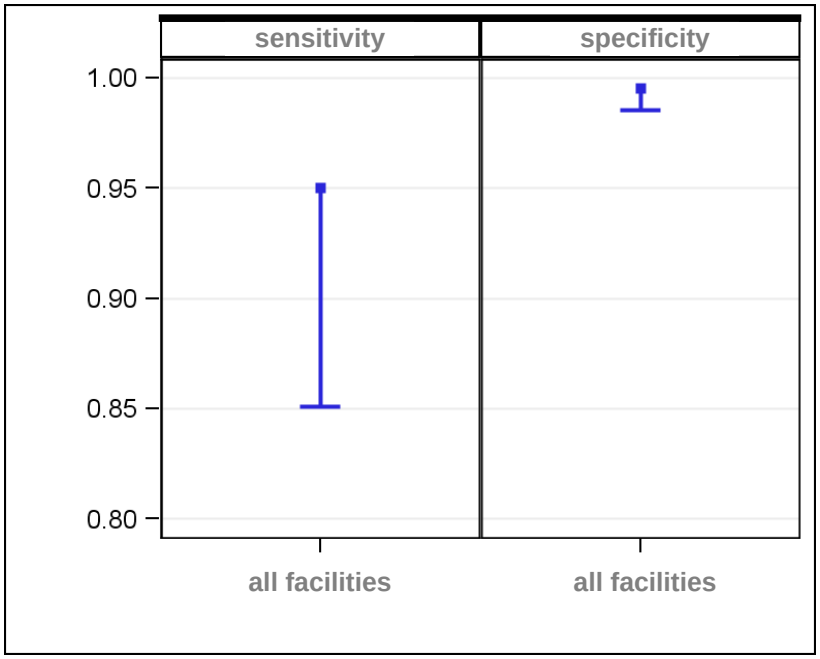
First results

z-scores for 472 samples analysed



- 38 of 40 samples were correctly classified as T21 positive
- 430 of 432 samples were correctly classified as T21 negative
- 2 false positive and 2 false negative samples
- * The karyotype of one sample, that was classified as negative for trisomy 21, had a partial trisomy 21 due to rec(21)dup(21q)inv(21)(p12q21,1)

Clinical Validation – Sensitivity and Specificity

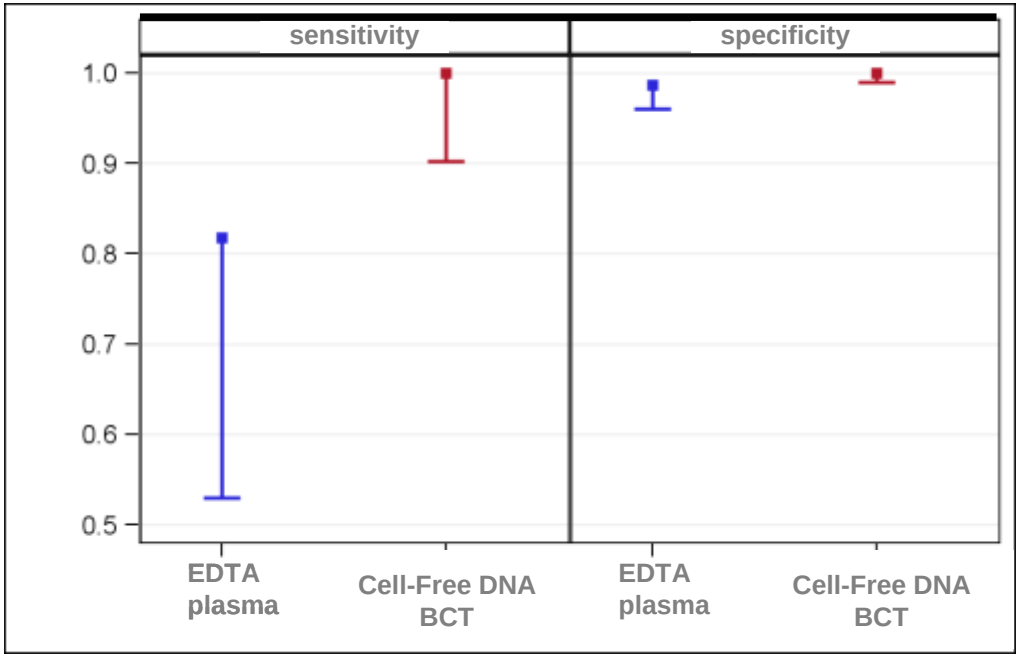


- **Sensitivity of 95 %** (one-sided confidence interval of 85.1%)
- **Specificity of 99.5 %** (one-sided confidence interval of 98.5%)
- **False-negative rate: 5.0%**
- **False-positive rate: 0.5%**

The analysis of 472 samples including 42 trisomy 21 cases showed a sensitivity of 95% and a specificity of 99,5%.

Sensitivity and specificity, depending on type of samples

A differentiated analysis revealed substantial differences between EDTA tubes and Cell-Free DNA™ blood collection tubes



- **Cell-Free DNA™ BCT (n=306)** yielded results with 100% clinical sensitivity and 100% specificity
- **Results of EDTA tubes (n=166)** achieved only a sensitivity rate of 81.8% and a specificity rate of 98.7%

The study results demonstrate high sensitivity and specificity and since August 2012, the PraenaTest® is commercially available.

NGS for non-invasive detection of fetal trisomy 21

Study	cases	Fetal T21	Sensitivity (False neg.)	Specificity (False pos.)
proof-of-concept				
Fan et al. 2008	18	9	100%	100%
Chiu et al. 2008	28	14	100%	100%
Chiu et al. 2010	15	5	100%	100%
Sehnert et al. 2011	47	13	100%	100%
Sparks et al. 2012	298	89	100%	100%
Stumm et al. 2012	42	8	100%	100%
clinical setting				
Chiu et al. *BMJ* 2011	232*2-plex*	86	100%	97,9% (3)
Ehrich et al. *AJOG* 2011	449	39	100%	99,7% (1)
Palomaki et al. 2011	1696*GC*	212	99,1% (2)	99,9% (1)
Bianchi et al. 2012	532	89	100%	100%

Application of NIPD (PraenaTest®)

- Genetic test for pregnant women at risk for trisomy 21 (extension to T13 und T18 in near future)
- Currently, no „stand alone“ test but complements present non-invasive prenatal methods (ISPD 2011)
- Decision guidance for or against invasive PND:
  - Negative test result: relaxation for the pregnant woman and renunciation of invasive testing. Further sonographic monitoring, where appropriate.
  - Positive test result: advise for invasive PND to confirm the diagnose.

Summary NIPD (PraenaTest®)

- Does not replace first trimester screening (FTS) for fetal malformation, but improves the specificity for detection of fetal trisomy 21 (FPR reduced from 5% to about 0.2% = coefficient 25)
- Due to the more precise result, invasive procedures due to false positive FTS can be avoided for non affected fetuses.
- **NIPD does not replace invasive methods, but makes early screening for fetal aneuploidies more precisely and thus reduces the number of invasive diagnostic procedures.**

Acknowledgements

- Zentrum für Pränataldiagnostik und Humangenetik, Berlin
  - PD Dr. Markus Stumm
  - PD Dr. Michael Entezami
  - Prof. Dr. Rolf Becker
  - Prof. Dr. Rolf Wegner
  - Dr. Andreas Hagen
- Pränatal-Medizin und Genetik, Düsseldorf
  - Dr. Karsten Haug
  - Dr. Cornelia Blank
- Zentrum für Pränatalmedizin, Hannover
  - Dr. Max Wüstemann
  - Dr. Robin Schwerdtfeger
- Praxis für Humangenetik, Hannover
  - Dr. Bernt Schulze
  - Dr. Gisela Raabe-Meyer
- Universitätsspital Zürich, Klinik für Geburtshilfe, Zürich
  - Dr. Tilo Burkhardt
  - Prof. Dr. Roland Zimmermann
- Institut für Humangenetik, Technische Universität München
  - Prof. Dr. Meitinger
  - Dr. Maja Hempel
  - Dr. Sabine Langer-Freitag
- Praxis für Pränatale Diagnostik, München
  - Prof. Dr. Marcus Schelling
- Abteilung für Gynäkologie und Geburtshilfe, Technische Universität München
  - Dr. Eva Ostermayer
- LifeCodexx AG
  - Dr. Wera Hofmann
  - Dr. Daniel Schöner
  - Sebastian Grömminger
  - Janet Riedel
- GATC Biotech AG
  - Martina Beck
  - Marina Koßmann
  - Vera Gögele
  - Nastasja Trunk
  - Tina Schleicher
  - Patrick Lang
  - Dr. Yadhu Kumar
  - Marian Thieme

lifecodexx