Clinical diagnostics in Non-Invasive Prenatal Testing

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Non-invasive prenatal testing (NIPT) based on cell-free DNA analysis from maternal blood is a screening test; it is not diagnostic. Test results must not be used as the sole basis for diagnosis. Further confirmatory testing is necessary prior to making any irreversible pregnancy decision

The VeriSeq NIPT Solution v2 is an in vitro diagnostic test intended for use as a screening test for the detection of genome-wide fetal genetic anomalies from maternal peripheral whole blood specimens in pregnant women of at least 10 weeks gestation. VeriSeq NIPT Solution v2 uses whole genome sequencing to detect partial duplications and deletions for all autosomes and aneuploidy status for all chromosomes. The test offers an option to request the reporting of sex chromosome aneuploidy (SCA). This product must not be used as the sole basis for diagnosis or other pregnancy management decisions.

The VeriSeq NIPT Solution v2 includes: the Workflow for the VeriSeq NIPT Microlab STAR, the VeriSeq NIPT Sample Prep Kits, and the VeriSeq Onsite Server v2 with the VeriSeq NIPT Assay Software v2. The VeriSeq NIPT Solution v2 is intended to be used with a next generation sequencer.

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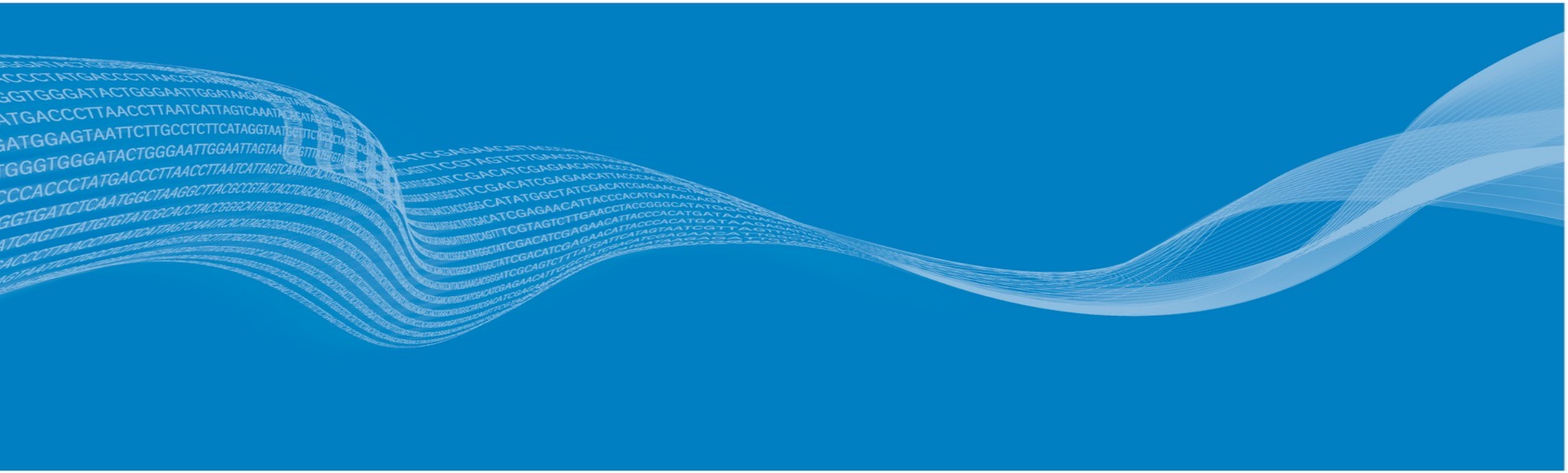
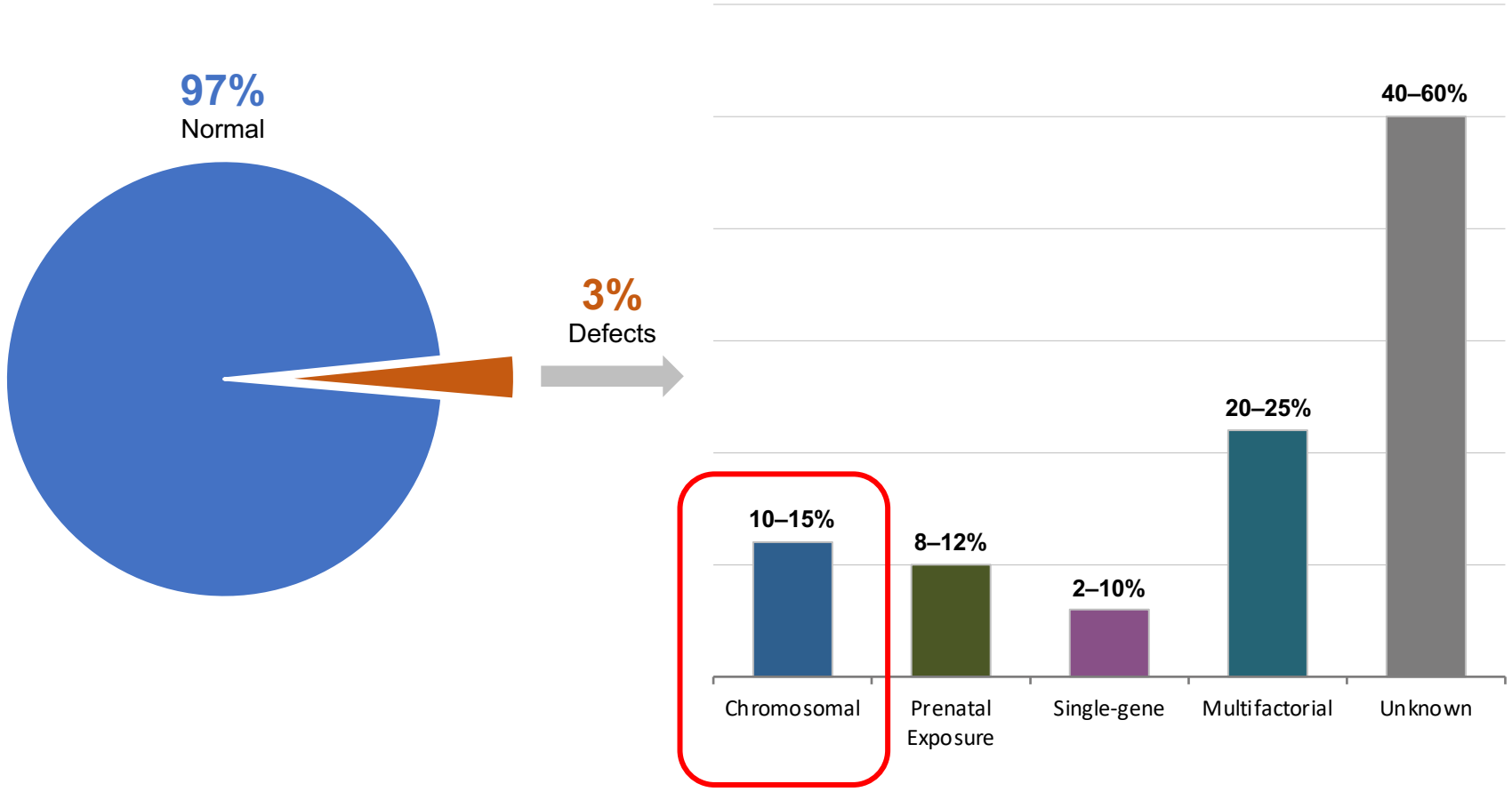
Prenatal Testing Options



Birth Defects

Rates and causes in live births

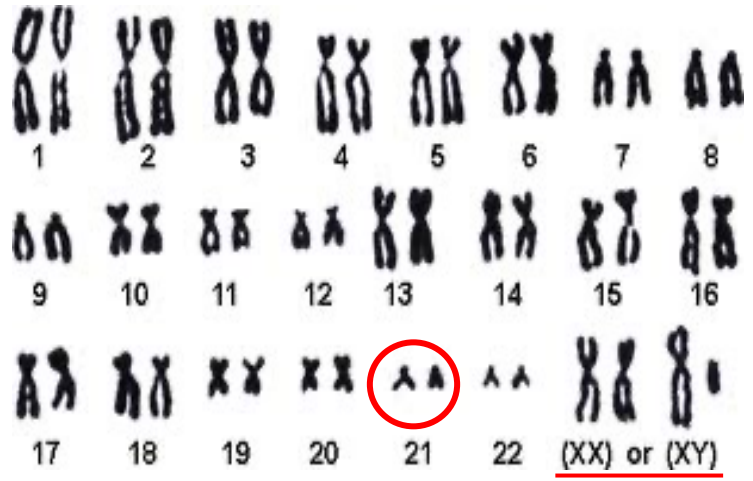


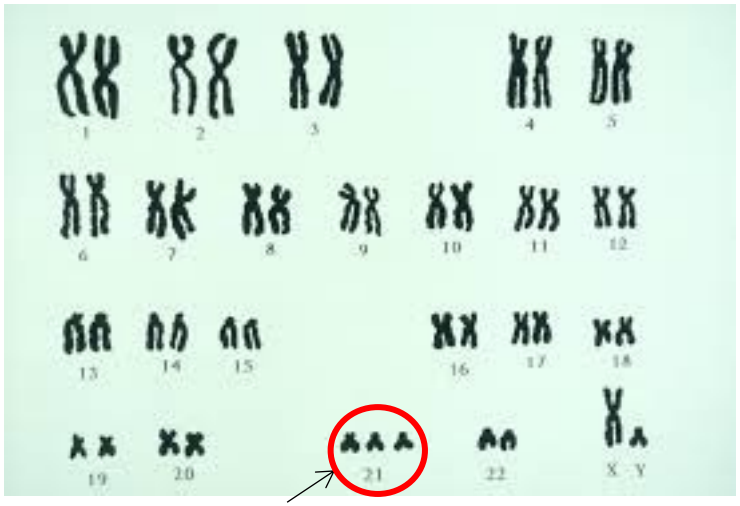
Adapted from Stevenson, RE and Hall, J. *Human Malformations and Related Anomalies*, 2nd ed. Oxford University Press, Oxford, 2006

Aneuploidy

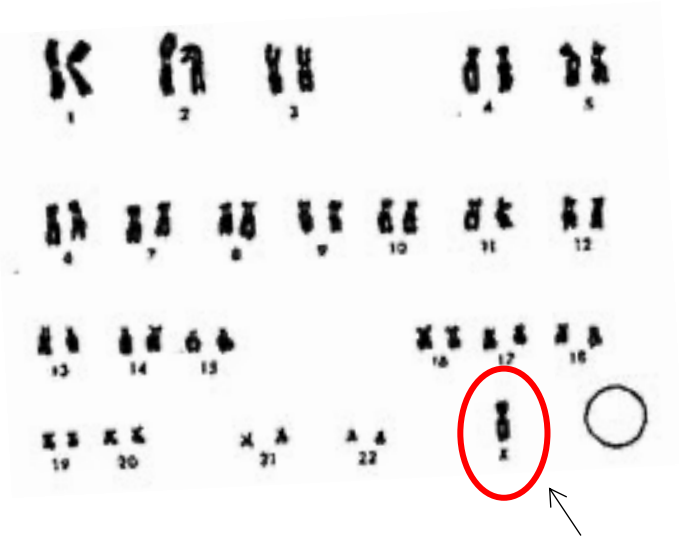
Loss or Gain of Chromosomes

(Euploid) Normal





Down Syndrome -Trisomy 21 (Extra chromosome)



Turner Syndrome Monosomy X (less chromosome)

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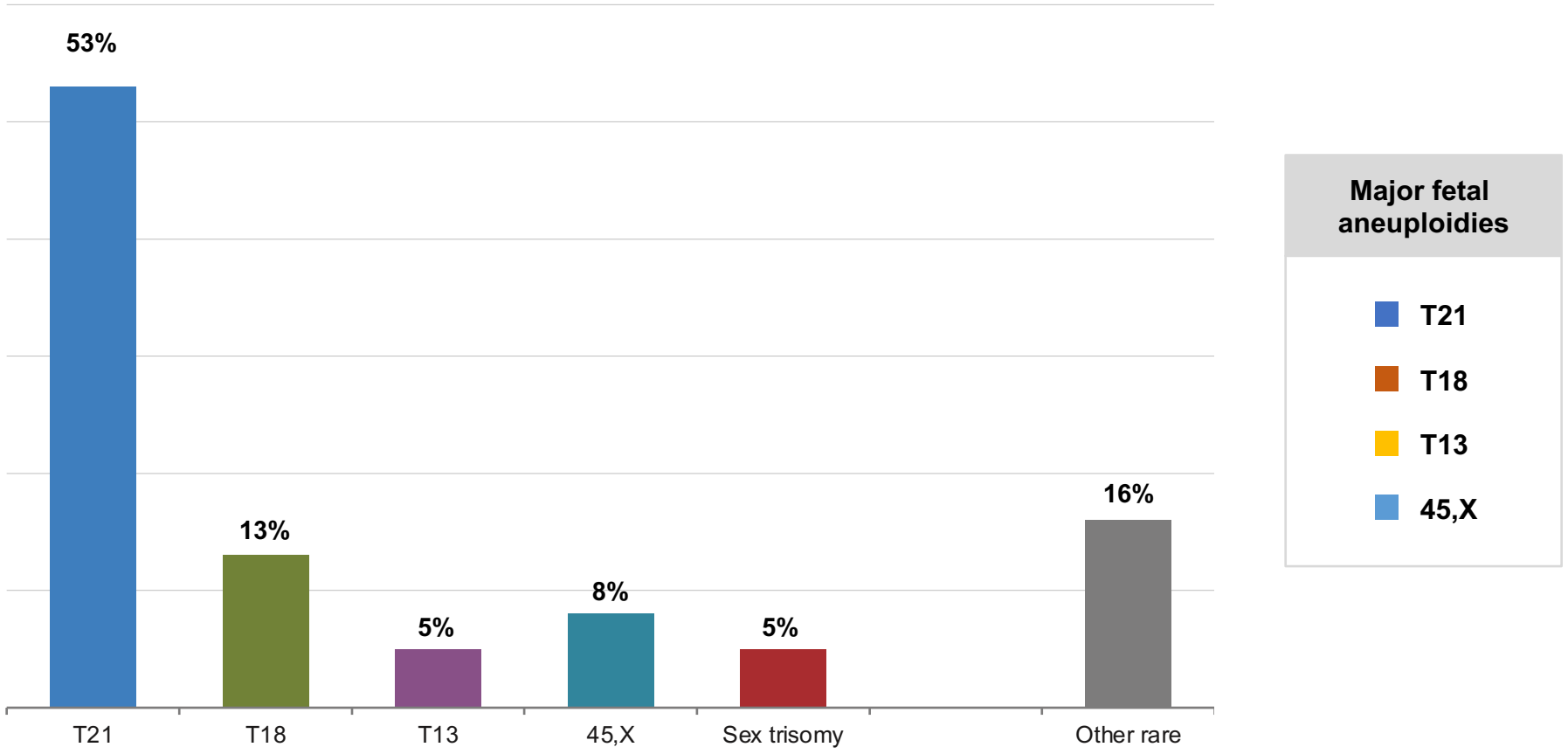
Prenatal Prevalence

Of reported chromosomal abnormalities



Data adapted from Wellesley, D, et al., Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. *Eur J of Hum* Gen 11 January 2012.

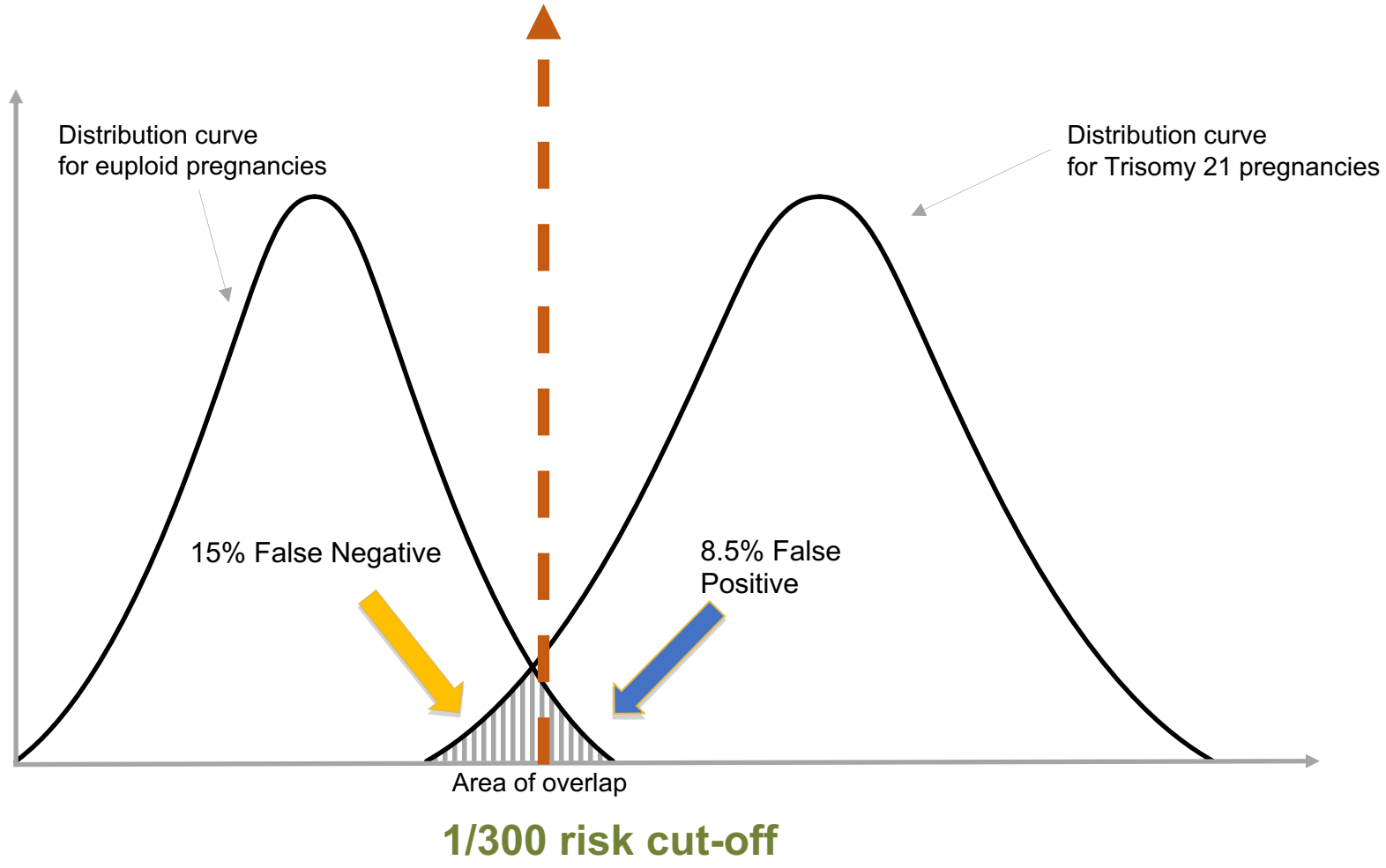
Conventional Prenatal Screening Options

Detection rates for Trisomy 21

		Detection rate (%)
1st Trimester:	**NT Ultrasound**	64–70
1st Trimester:	**1st Trimester blood screen** › **NT Ultrasound**	82–87
2nd Trimester:	**Triple screen**	69
2nd Trimester:	**Quadruple screen**	81
Integrated screen:	**1st Trimester blood screen** › **NT Ultrasound** › **2nd Trimester blood screen**	94–96
Serum integrated:	**1st Trimester blood screen** › **2nd Trimester blood screen**	85–88
Sequential screen:	**1st Trimester blood screen** › **NT Ultrasound** › **2nd Trimester blood screen**	95
	False positive rate:	**~5%**

American College of Obstetricians and Gynecologists. Screening for fetal aneuploidy. Practice Bulletin No. 163. *Obstet Gynecol.* 2016; 127(5):e123-37.

2nd Trimester Quadruple Serum screening



Distribution curves not to scale.

Malone FD, Canick JA, Ball RH, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med.* 2005;353(19):2001–2011.

Factors Affecting Analyte Levels in Traditional Screening

Gestational age[1]

- First trimester:
  - Human chorionic gonadotropin (hCG) levels fall rapidly; Pregnancy-associated plasma protein A (PAPP-A) increases rapidly
- Second trimester:
  - Alpha-feto protein (AFP) and unconjugated estriol increase; hCG declines; Inhibin-A remains stable
- Errors in estimation can lead to false positive or false negative results

Maternal Weight[2,3]

- Inversely correlated to analyte levels due to the larger blood volume of heavier women

Racial/Ethnic background[4]

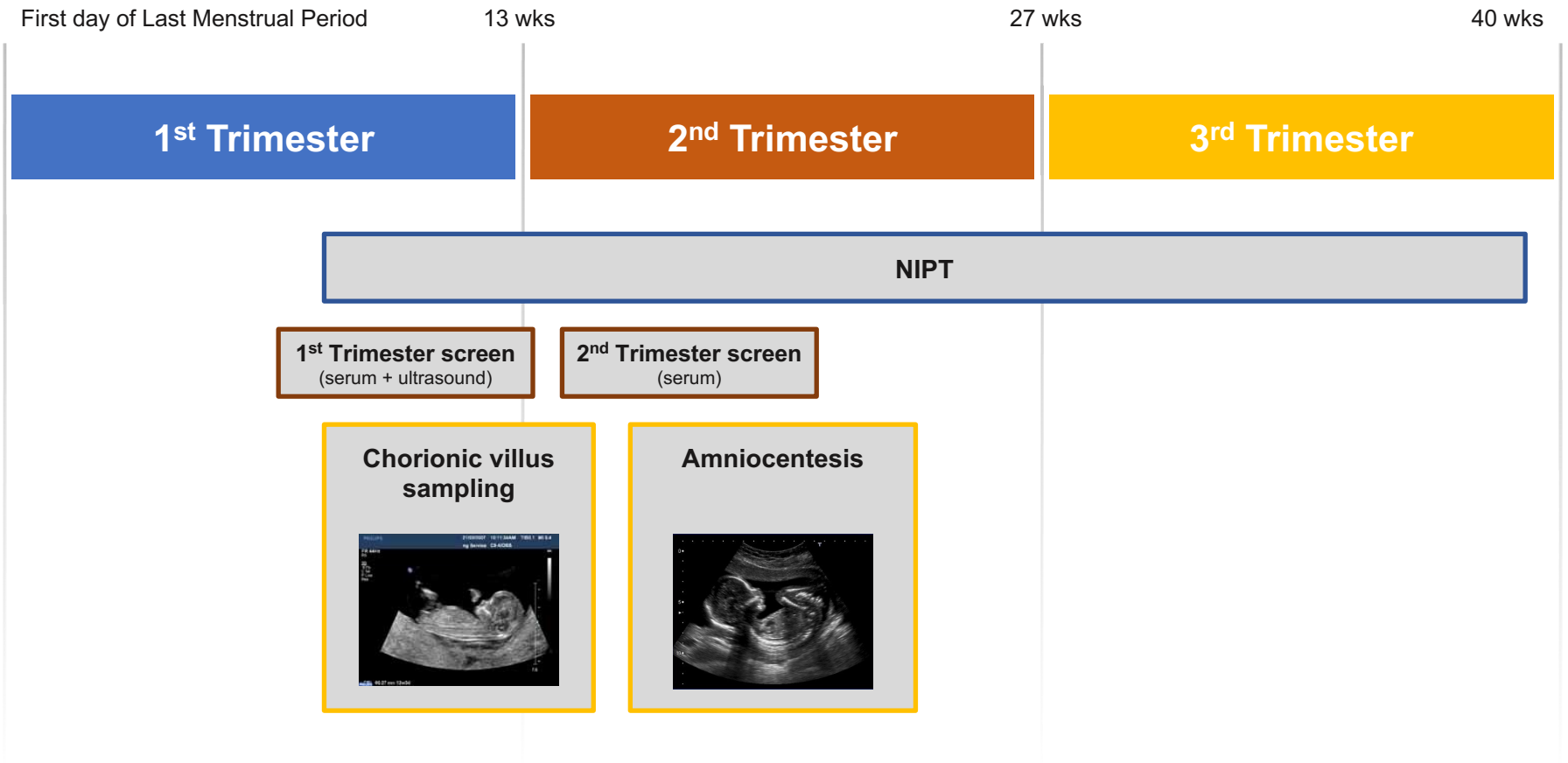
- African American women tend to have higher AFP, PAPP-A, and hCG levels and lower Inhibin-A levels than Caucasian women

Diabetes Mellitus[5]

- Women with insulin dependent diabetes mellitus have lower levels of AFP and unconjugated estriol compared to pregnant women without diabetes

1. American College of Medical Genetics. *Standards and Guidelines for Clinical Genetics Laboratories.* Second Edition, 1999
2. Lambert-Messerlian G, Palomaki GE, and Canick JA. Adjustment of serum markers in first trimester screening. *J Med Screen*. 2009;16(2):102-3.
3. Wald NJ, Kennard A, Hackshaw A, and McGuire A. Antenatal screening for Down's syndrome. *J Med Screen*. 1997;4(4):181-246. Review. Erratum in: *J Med Screen* 1998;5(2):110. *J Med Screen* 1998;5(3):166.
4. Spencer K, Heath V, El-Sheikhah A, Ong CY, and Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenat Diagn*. 2005;25(5):365-9.
5. Baumgarten A and Robinson J. Prospective study of an inverse relationship between maternal glycosylated hemoglobin and serum alpha-fetoprotein concentrations in pregnant women with diabetes. *Am J Obstet Gynecol*. 1988;159(1):77-81.

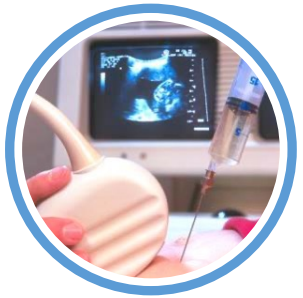
Current Paradigm of Prenatal Screening & Diagnostic Testing



Diagnostic Testing Options

Trimester–Test	Sensitivity	Specificity	Fetal Loss Rate
1st–CVS	99.25%[1]	98.65%[1]	1.1%[3]
2nd–Amniocentesis	99.4%[2]	99.5%[2]	0.4%[3]

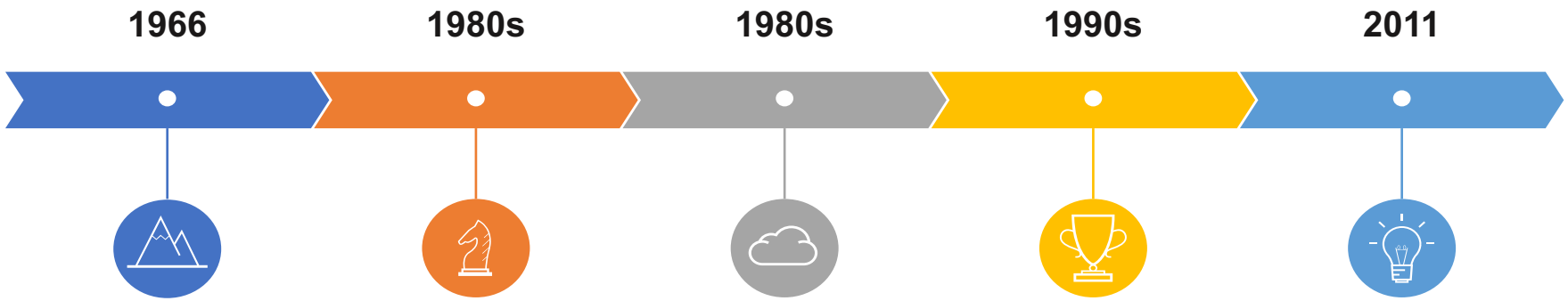
Even the gold standard is not 100% sensitive and specific. Additionally, there are risks of maternal infection, bleeding, fluid leakage, and fetal loss.



1. Hahnemann JM, Vejerslev LO. Accuracy of cytogenetic findings on chorionic villus sampling (CVS)—diagnostic consequences of CVS mosaicism and non-mosaic discrepancy in centres contributing to EUCROMIC 1986-1992. *Prenat Diagn*. 1997;17(9):801-820.
2. Mid-trimester amniocentesis for prenatal diagnosis. Safety and accuracy. *JAMA*. 1976; 236(13): 1471-1476.
3. Enzensberger C, Pulvermacher C, Degenhardt J, et al. Fetal loss rate and associated risk factors after amniocentesis, chorionic villus sampling and fetal blood sampling. *Ultraschall Med*. 2012;33(7):E75-9.

Evolution of Prenatal Testing

Diagnostic & Screening Test Options



Amnio
Amniocentesis for fetal chromosomes

Serum Screen
For neural tube defects & aneuploidy (T21, T18)

CVS
Chorionic Villus Sampling for placental/fetal chromosomes

NT
Nuchal Translucency for aneuploidy

NIPT
Noninvasive Prenatal Testing for fetal chromosomes & Prenatal Microarrays

Non-Invasive Prenatal Testing (NIPT)

Technology Overview

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Noninvasive Prenatal Testing (NIPT)

Objectives of NIPT

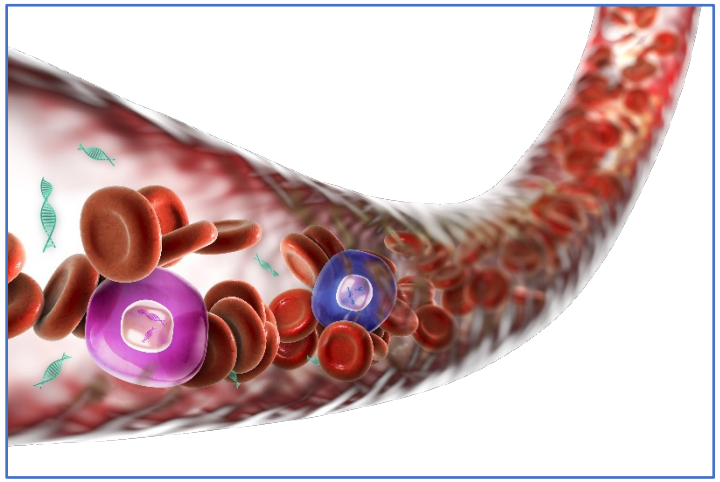
- Reduce exposure of fetus to risk
- Reduce false positives
- Testing that can easily be offered to pregnant women
- Enable a high detection rate

Cell-Free DNA (cfDNA) in Maternal Blood

An ideal analyte for aneuploidy testing

Maternal blood contains both maternal and fetal cfDNA

- 2–20% of total cfDNA in maternal blood is placental (cytotrophoblastic)[1,2]
- Released into bloodstream through apoptosis (cell death)



Detected after 7+ weeks gestation and undetectable within hours postpartum[2]

1. Barrett, A, Zimmerman BG, Wang D, Holloway A, Chitty L. Implementing prenatal diagnosis based on cell-free fetal DNA: Accurate identification of factors affecting fetal DNA yield. *PLoS One*. 2011;6(10):e25202..
2. Nigam A, Saxena P, Prakash A, Acharya A.Detection of fetal nucleic acid in maternal plasma: A novel noninvasive prenatal diagnostic technique. *J Interntl Med Sci Acad.*2012; 25(3): 119-120.

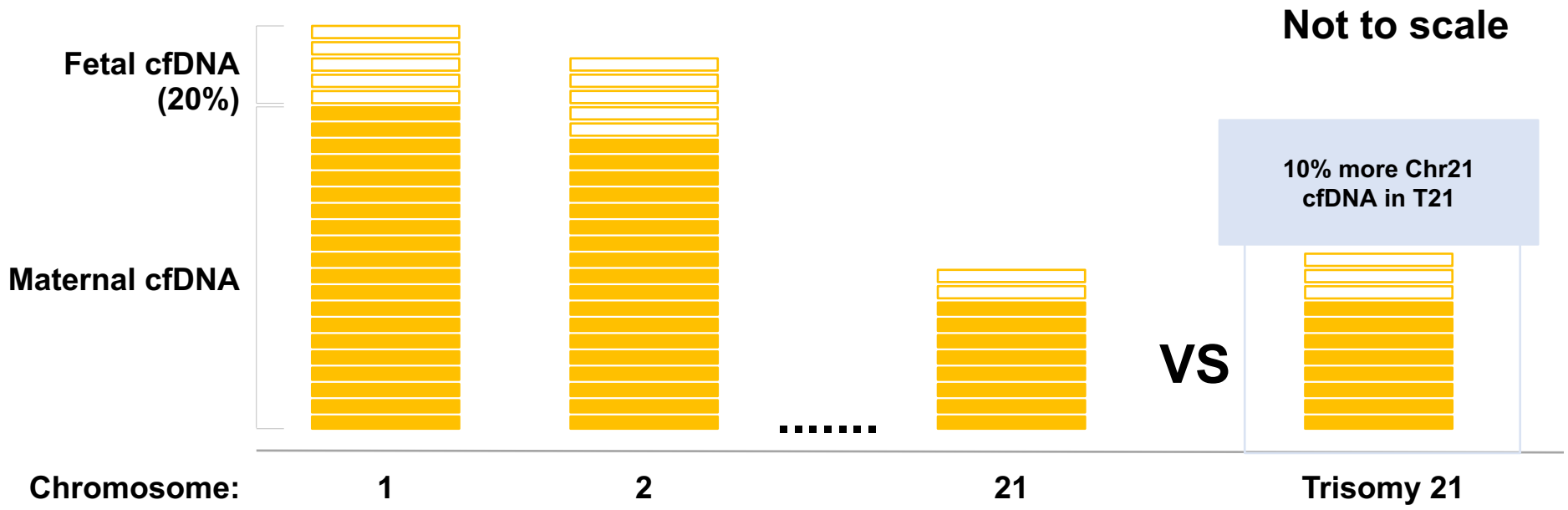
Method of Analysis for Illumina NIPT

1. Extract and Prepare cfDNA

2. Whole-Genome Sequencing

3. Alignment

4. Counting



Evidence for NIPT Performance

Updated meta-analysis

Review of the clinical validation and implementation studies for cfDNA screening for fetal aneuploidies

35 publications on NIPT for detection of aneuploidies between 2011–2016*

	Pooled Weighted Detection Rate (%)	95% Confidence Interval	False Positive Rate (%)	95% CI
Singleton Pregnancies				
Trisomy 21	99.7	99.1-99.9	0.04	0.02-0.07
Trisomy 18	97.9	94.9-99.1	0.04	0.03-0.07
Trisomy 13	99.0	65.8-100	0.04	0.02-0.07
Monosomy X	95.8	70.3-99.5	0.14	0.05-0.38
Other sex aneuploidies**	100.0	83.6-100	0.004	0-0.08
Twin Pregnancies				
Trisomy 21***	100.0	95.2-100	0	0-0.003

* Includes papers describing three different sequencing methodologies used for NIPT: massively parallel shotgun sequencing (MPSS), also known as whole-genome sequencing (WGS); chromosome-specific sequencing (CSS, also known as targeted sequencing); single-nucleotide polymorphism (SNP) sequencing. Note: Samples that resulted in a test failure were excluded from the analysis.

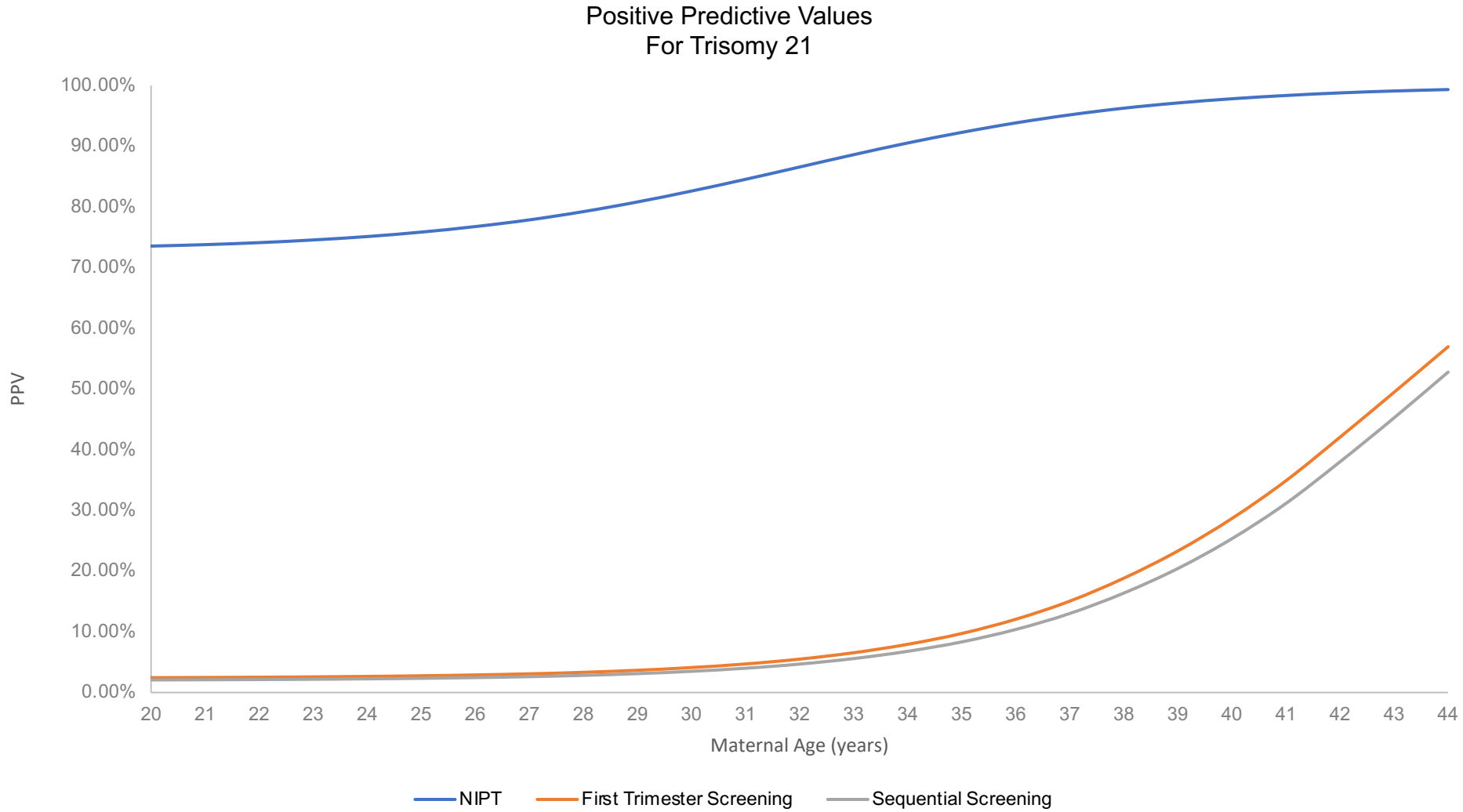
** The authors concluded that the number of reported cases of sex chromosome abnormalities is too small for accurate assessment of performance in screening.

*** The authors concluded that the performance of screening for trisomy 21 in twin pregnancies is encouraging, but the number of cases reported is small.

Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol*. 2017 Apr 11. doi: 10.1002/uog.17484. [Epub ahead of print].

PPV of NIPT and Serum Screening

Across maternal ages



1. Snijders RJ, Sebire NJ, and Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. *Fetal Diagn Ther*. 1995 Nov-Dec;10(6):356-67.
2. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol. 2017 Apr 11. doi: 10.1002/uog.17484.
3. Santorum M, Wright D, Syngelaki A, Karagioti N, and Nicolaides KH. Accuracy of first trimester combined test in screening for trisomies 21, 18, and 13. *Ultrasound Obstet Gynecol*. 2017 Jun;49(6):714-720.
4. Cuckle H, Benn P, and Wright D. Down Syndrome Screening in the First and/or Second Trimester: Model Predicted Performance Using Meta-Analysis Parameters. *Semin Perinatol.* 2005 Aug;29(4):252-7.

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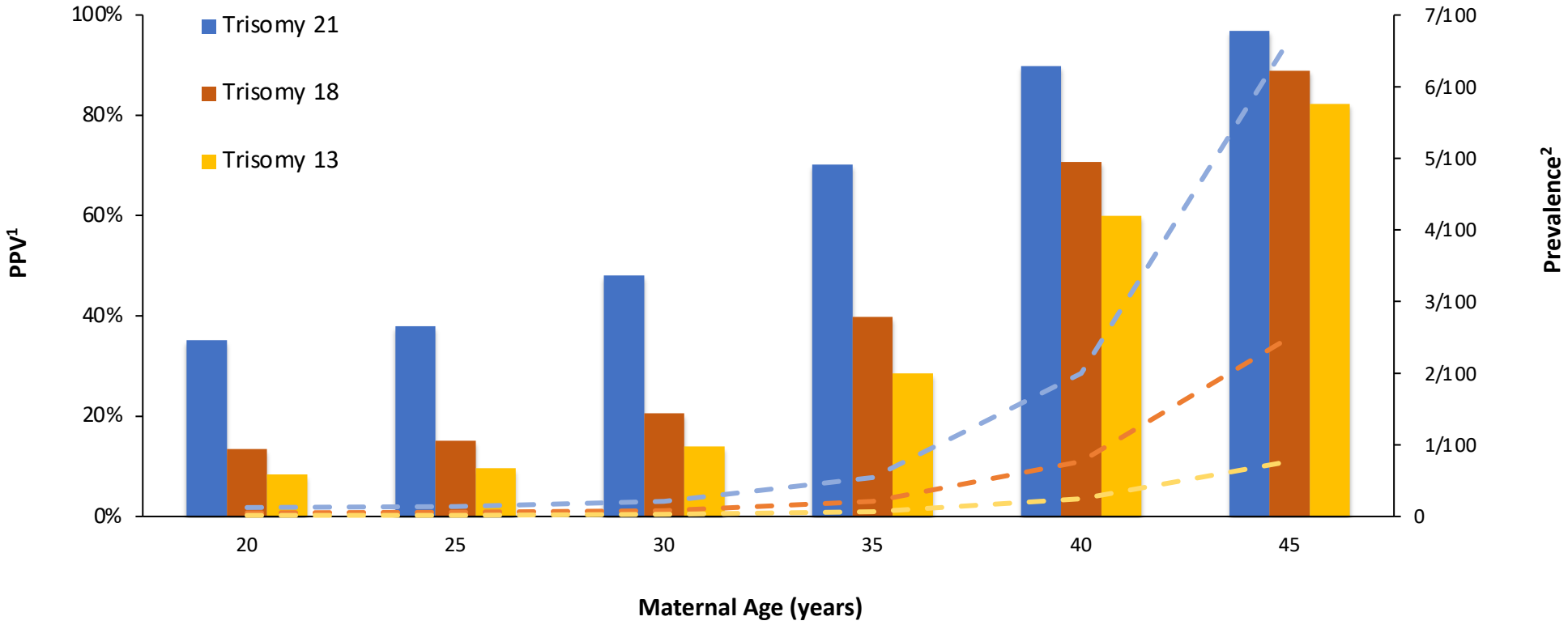
Projected PPVs by Maternal Age

PPV directly related to prevalence of the condition



Taneja PA, Snyder HL, de Feo E, et al. Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85 000 cases. *Prenat Diagn*. 2016; 36(3):237-243.

1. PPV calculated using sensitivities and specificites based on observed clinical outcomes data
2. Estimated prevalences at 10 weeks of gestation derived from: Gardner RJM, Sutherland GR, Schaffer LG. Chromosome Abnormalities and Genetic Counseling 4th ed. New York, NY: *Oxford University Press*; 2012.

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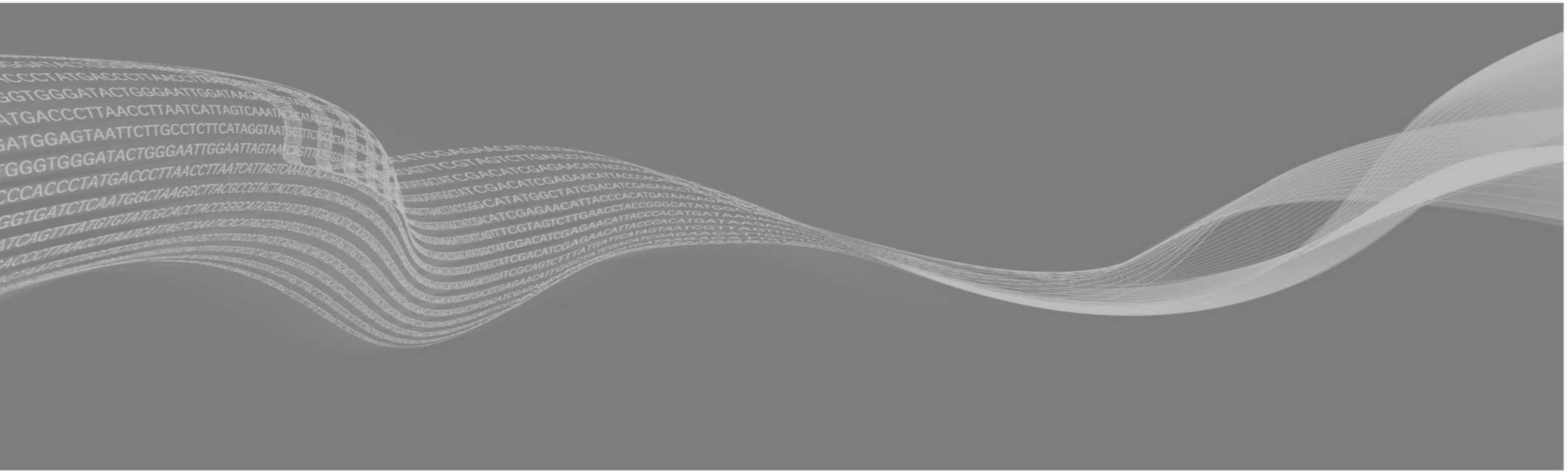
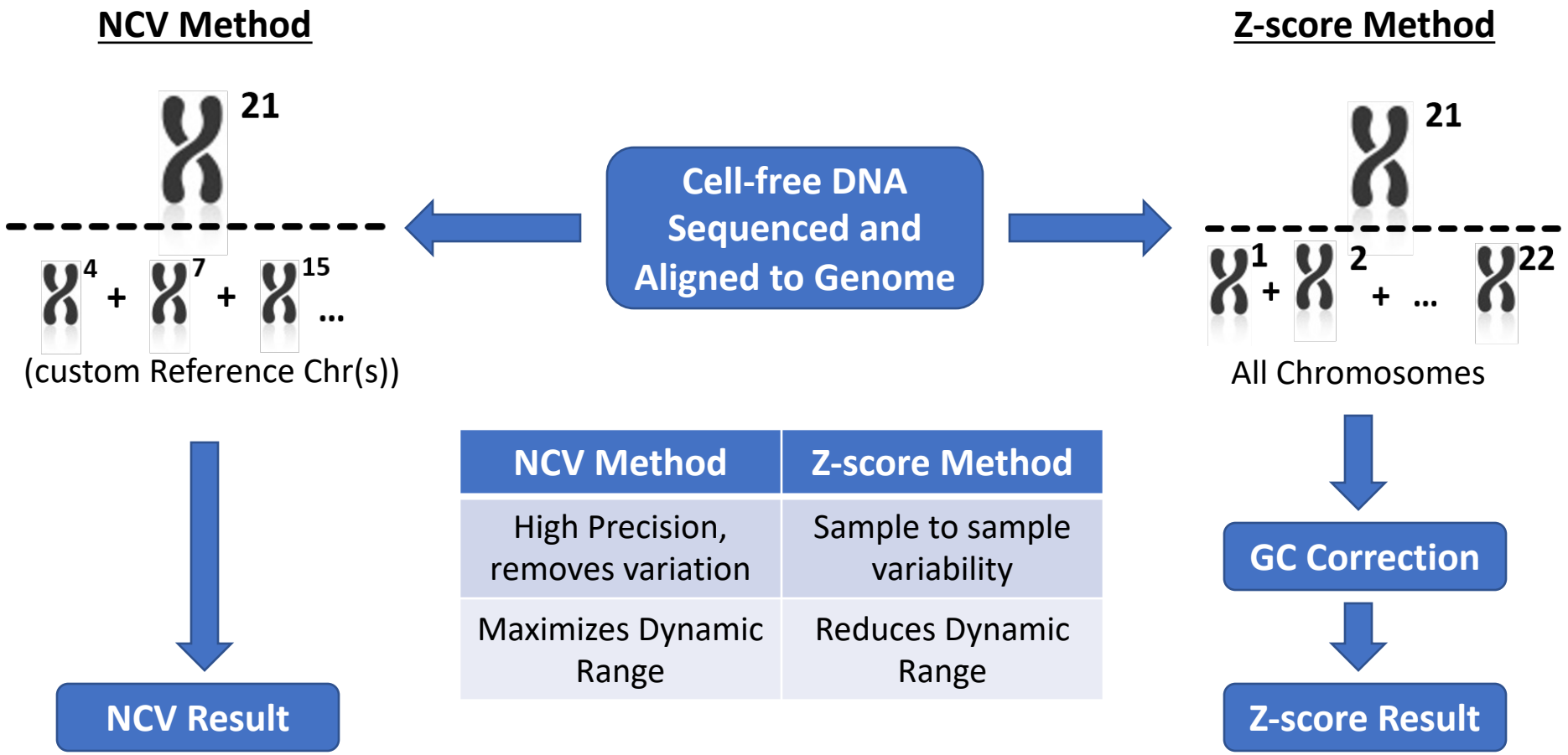
NIPT Statistical Tools



Different Statistical Approaches



NCV Method	Z-score Method
High Precision, removes variation	Sample to sample variability
Maximizes Dynamic Range	Reduces Dynamic Range

1. Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study Genet Med 2011;13:913–920.
2. Palomaki GE, Deciu C, Kloza EM, et al. DN A sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 2012:14(3):296–305.
3. Bianchi DW, Platt LD, Goldberg JD, et al. Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet Gynecol 2012;119:890–901.*

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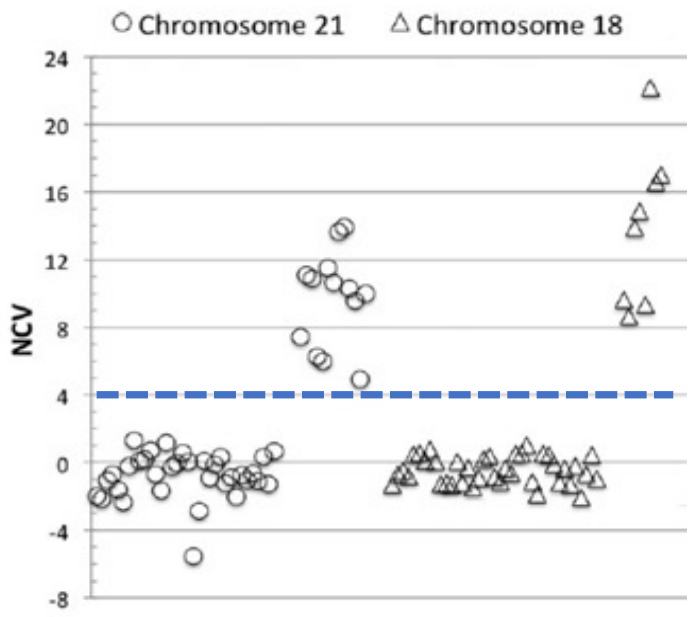
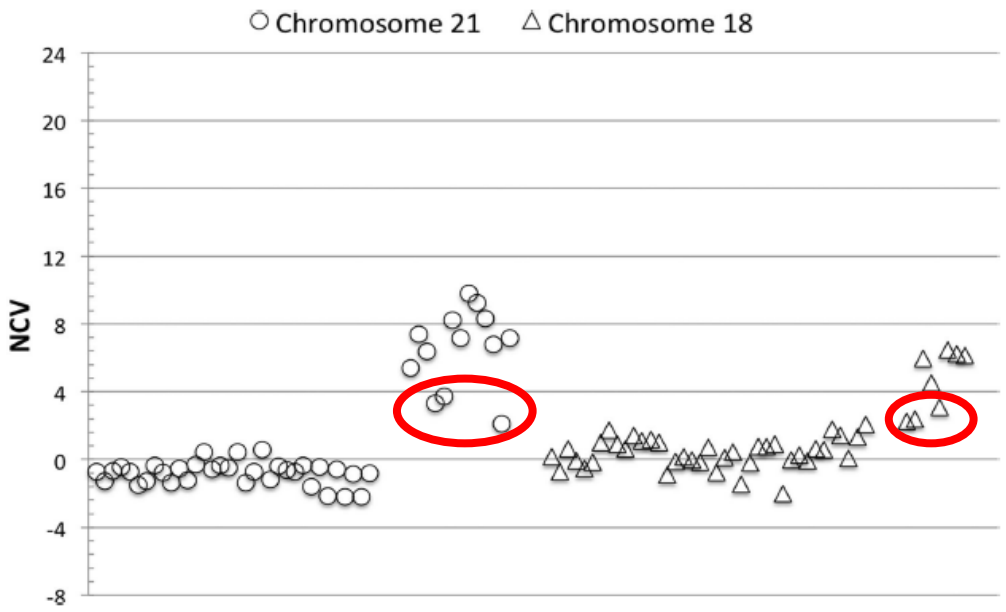
Technical Methods Comparison

Analysis using the same dataset demonstrated diminished separation between affected and unaffected samples and decreased detection rate with uncorrected Z-score method

NCV Method



Z-score Method



Sehnert, et al, *Clin Chem*, Jul;57(7):1042-9.

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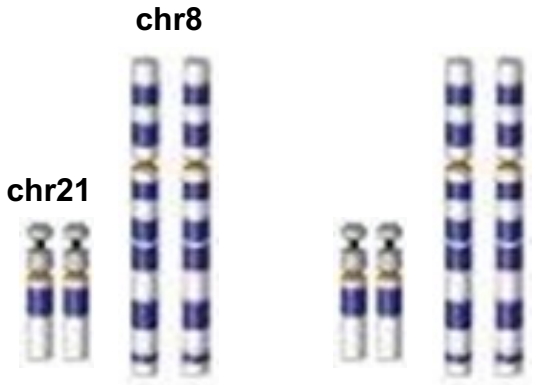
Detection of Aneuploidy Trisomy 21

Normal



Mom (90%) Fetus (10%)

	# of mapped read on Chr21	# of mapped read on Chr8
Total	1000	4000
Mom	900	3600
Fetus	100	400

$$\frac{\text{Total read\# of Chr21}}{\text{Total read\# of Chr8}} = \frac{1000}{4000} = 0.25$$

Trisomy 21

Mom (90%) Fetus (10%)

	# of mapped read on Chr21	# of mapped read on Chr8
Total	1050	4000
Mom	900	3600
Fetus	150	400

$$\frac{\text{Total read\# of Chr21}}{\text{Total read\# of Chr8}} = \frac{1050}{4000} = 0.2625$$

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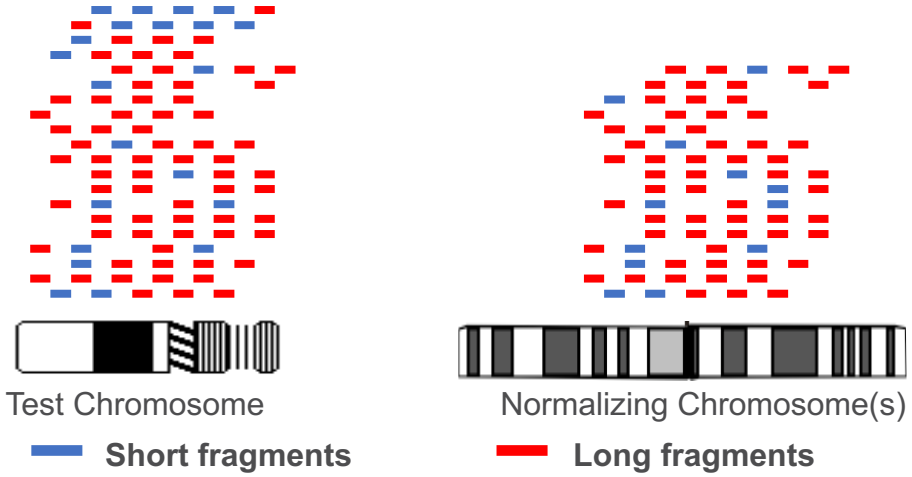
Paired-End Sequencing

Combine Counting and Fragment Size

- Using counting statistics, determine if more than expected number of reads mapped to the target chromosome
- Using fragment size statistics, determine if fraction of short fragments was higher on the target chromosome
- Combine counts and frag size statistics for final scoring



*Counting statistic compared after within-sample normalization

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Expanded Options and Future directions

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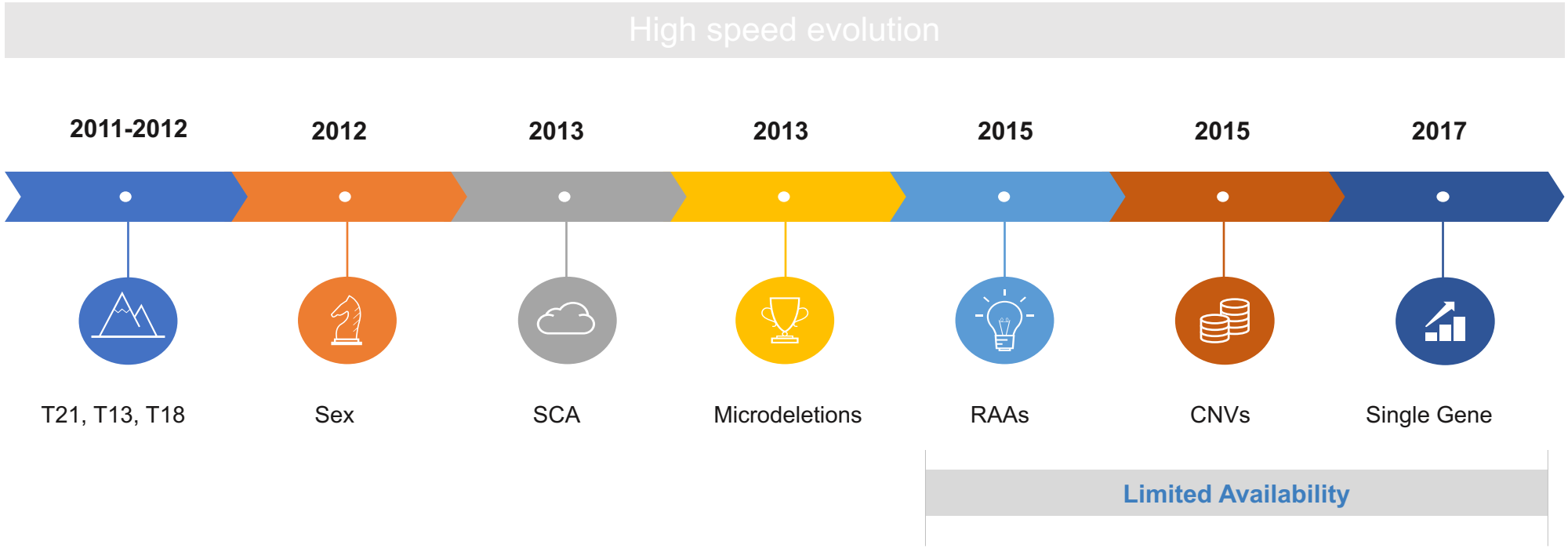
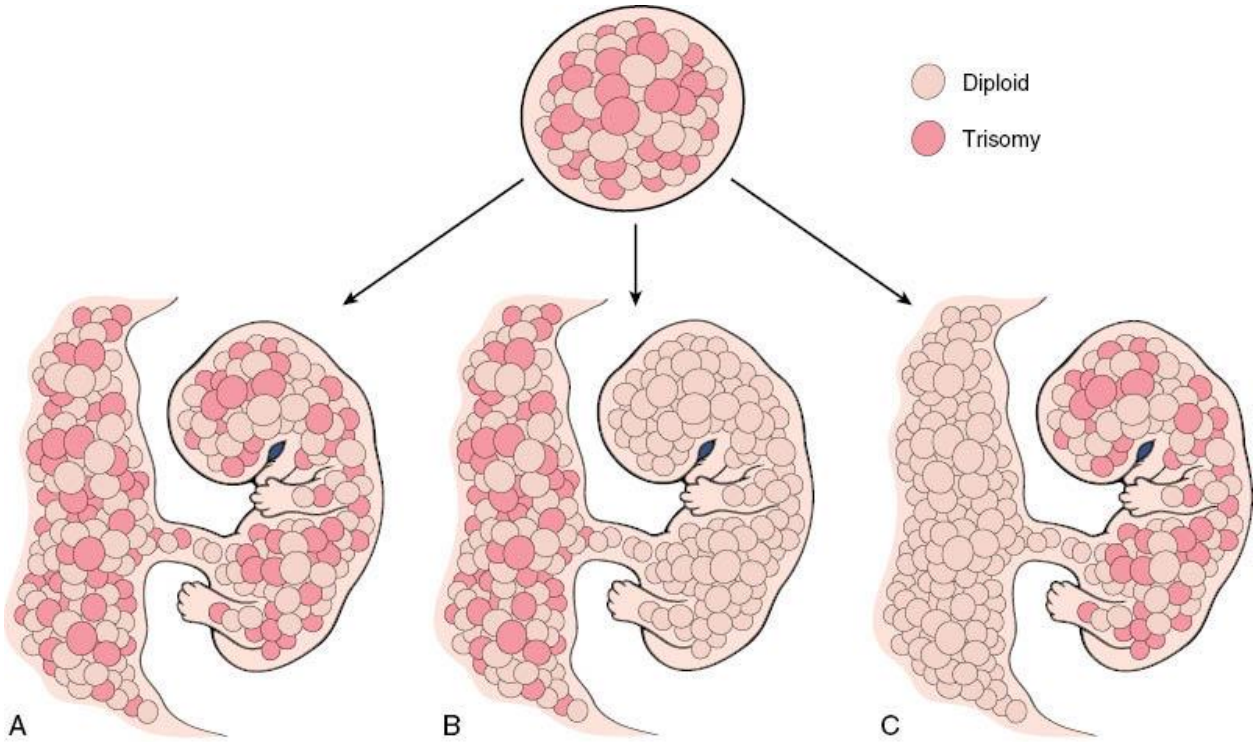
NIPT Progression



Mosaicism

A static biological limitation



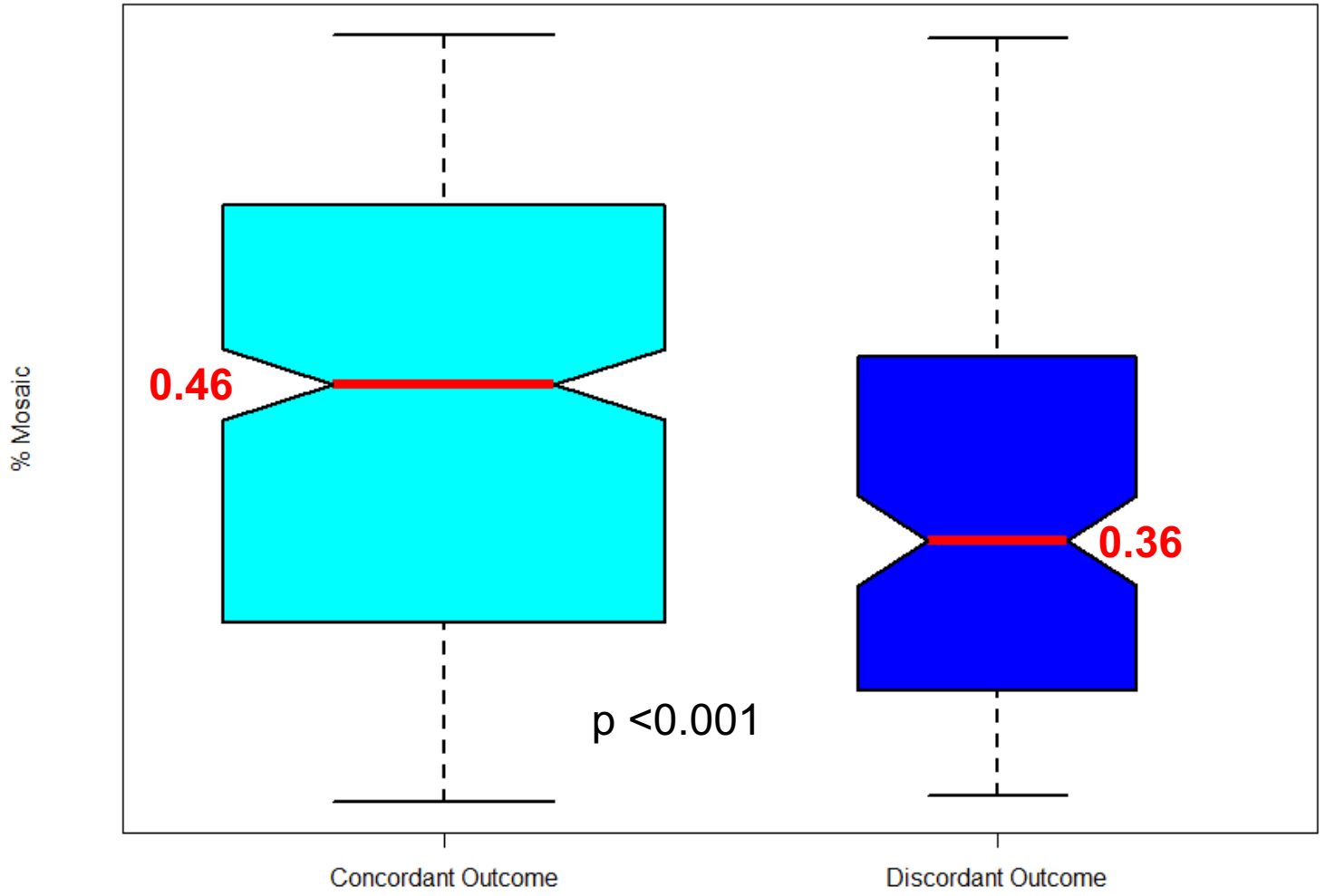
Generalized mosaicism

Confined placental mosaicism

Fetal mosaicism

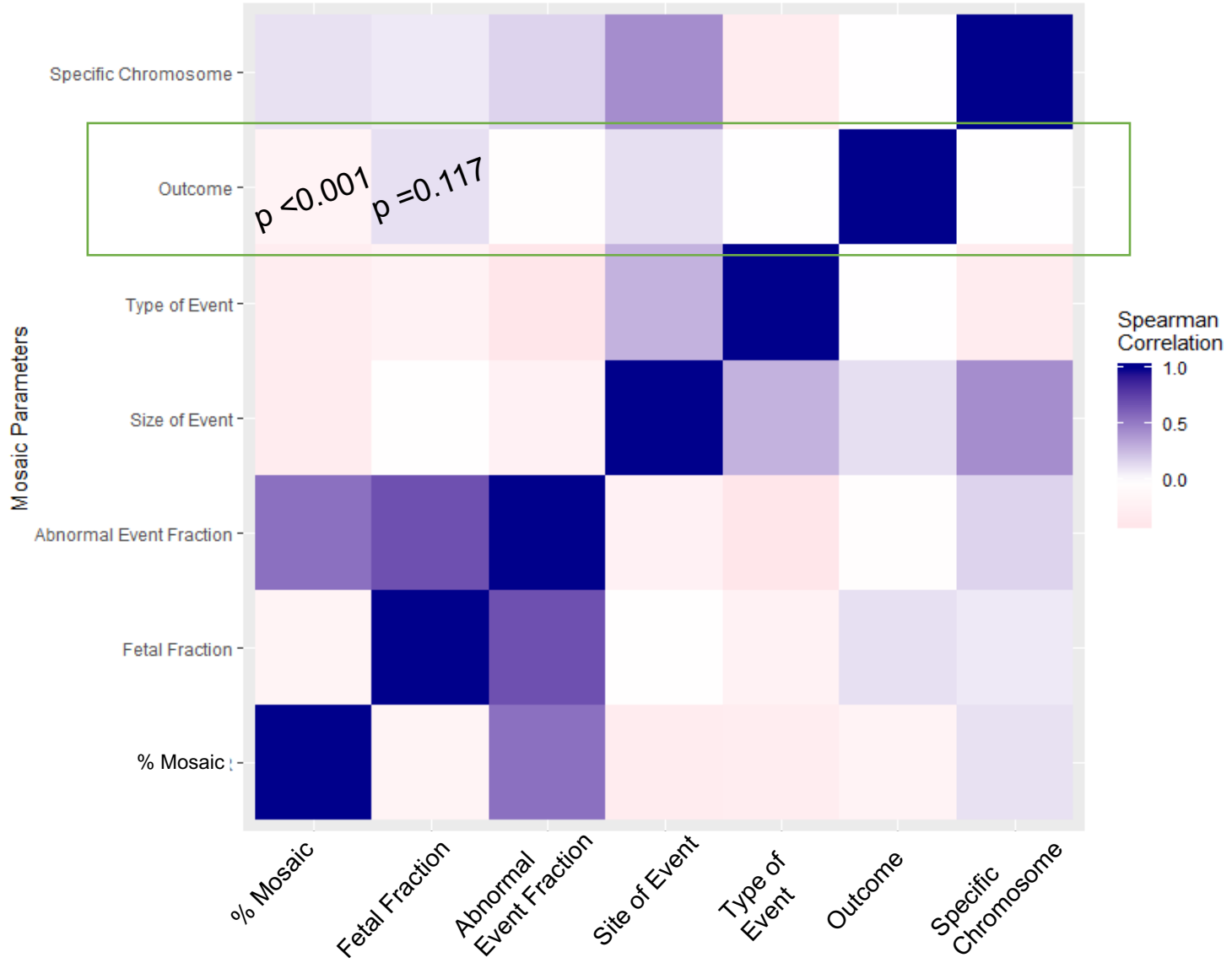
Kumar, et al. *Robbins & Cotran Pathologic Basis of Disease*, 7th Edition. Philadelphia, PA: W.B. Saunders Company; 2004.

Comparison of Mosaicism ~ Outcome



Boomer, T et al. Mosaicism Ratio in cfDNA Prenatal Screening: An invaluable tool for clinical management guidance.
Poster & oral presentation @ ACMG 2019 Annual Meeting, Seattle, WA.

Mosaicism Correlation Heatmap



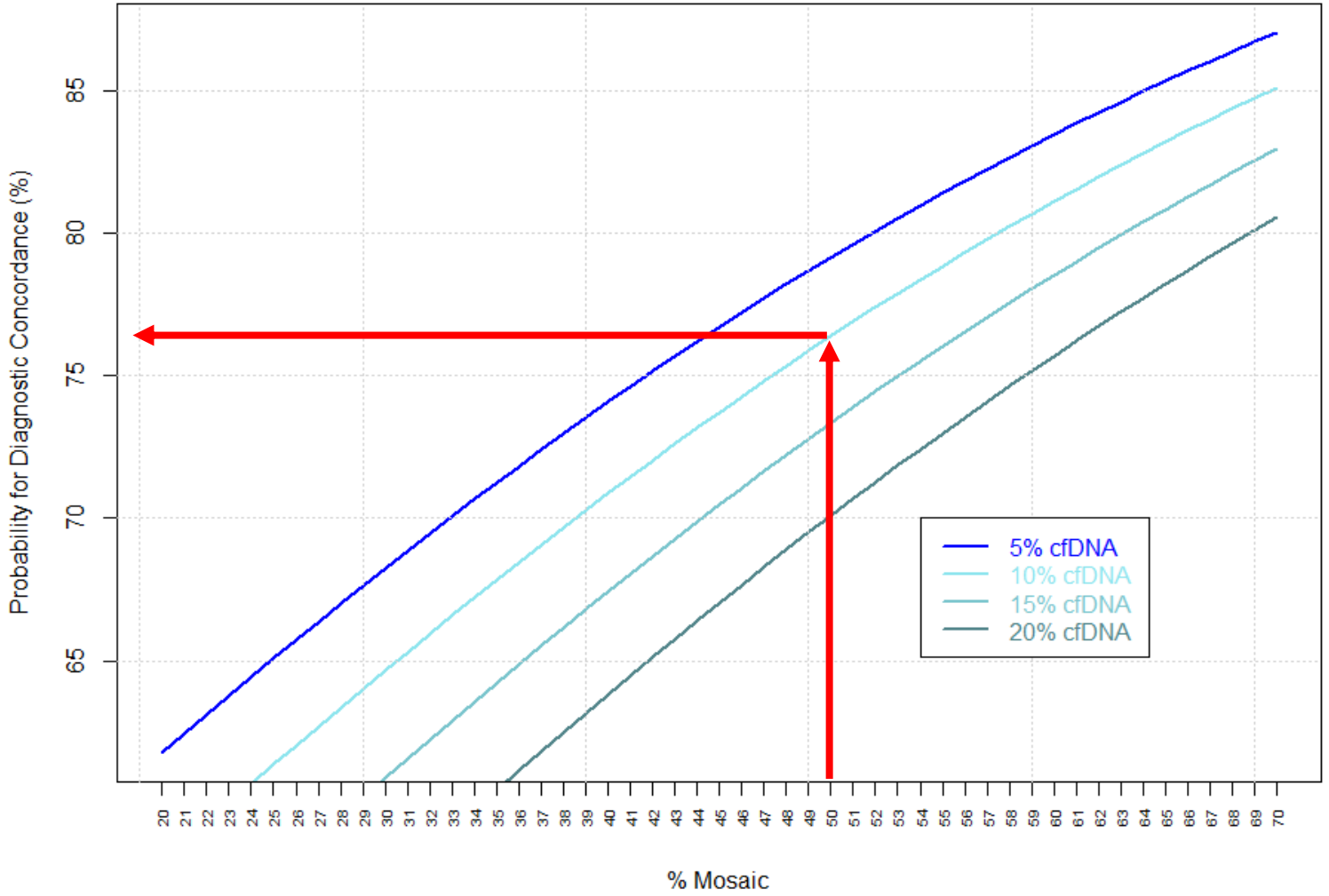
Boomer, T et al. Mosaicism Ratio in cfDNA Prenatal Screening: An invaluable tool for clinical management guidance. Poster & oral presentation @ ACMG 2019 Annual Meeting, Seattle, WA.

‘Positive Predictive’ Logistic Regression Model

$$logit = ln[p/(1-p)] = -a - \beta_1(\text{Ratio of Mosaicism}) + \beta_2(\text{cfDNA.FF})$$

$$p \text{ (aka ‘probability for discordance’)} = e^{logit}/(1+(e^{logit}))$$

$$PPV \text{ (aka ‘probability for concordance’)} = 1-p$$



Boomer, T et al. Mosaicism Ratio in cfDNA Prenatal Screening: An invaluable tool for clinical management guidance.
Poster & oral presentation @ ACMG 2019 Annual Meeting, Seattle, WA.

Summary

NIPT

Non-Invasive Prenatal Testing

01 Transformed prenatal testing

02 Unpredicted rapid adoption & development of an LDT

03 Clinicians faced with learning / adopting statistical tools

04 Future directions will heavily rely on advanced statistical analyses and modeling