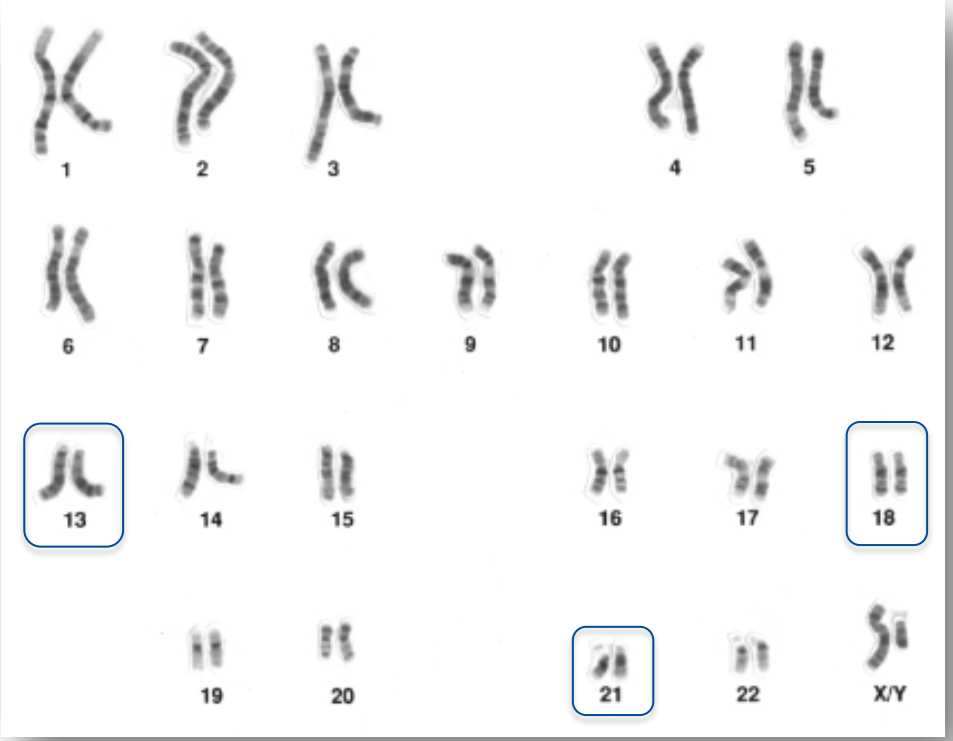
Cell-free DNA Tests for Non-invasive Prenatal Aneuploidy Screening

David G. Grenache, PhD
Professor of Pathology, University of Utah
Medical Director, ARUP Laboratories
Salt Lake City, UT

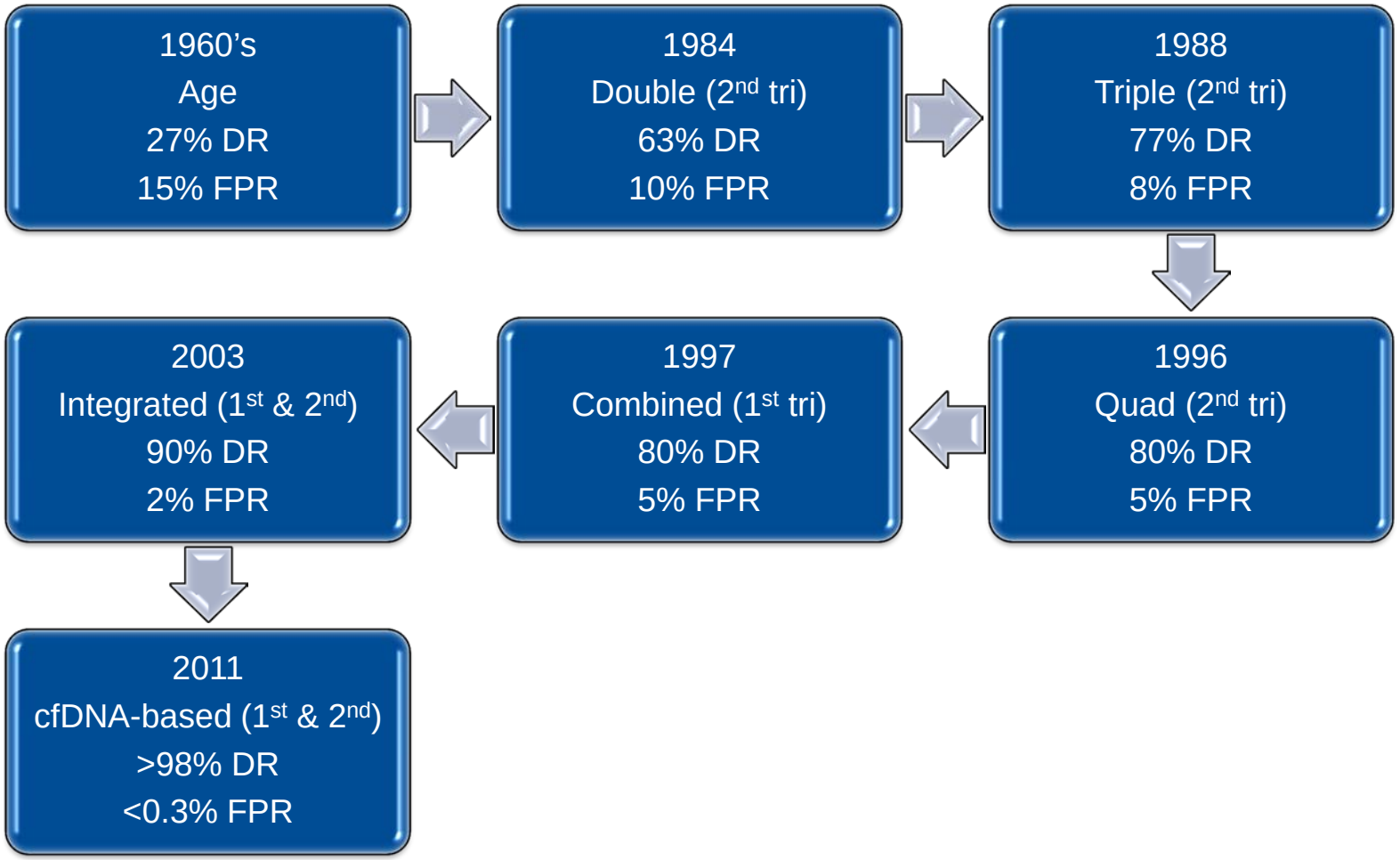
Aneuploidy

- Normal human complement of chromosomes is 46 (23 pairs)
- Aneuploidy is the presence of an abnormal number of chromosomes
- Most common extra autosomal chromosomes among live birth infants are 21, 18 and 13 (trisomies)
  - Multiple phenotypes
  - Intellectual disability common to all



Source: National Institutes of Health

History of Prenatal Screening for Trisomy 21 (Down Syndrome)



Screening for Fetal Trisomies

Method	Fetal Defect	Incidence (live births, approximate)
Biochemical & cfDNA-based screening	Trisomy 21 (Down syndrome)	1 in 700
Biochemical & cfDNA-based screening	Trisomy 18 (Edwards syndrome)	1 in 5,000
cfDNA-based screening only	Trisomy 13 (Patau syndrome)	1 in 16,000

Cell-free Fetal DNA in Maternal Blood

- Reported by Lo, et al. in 1997
- Derived primarily from the placenta and represents ~10% of total DNA circulating in maternal blood by 10th week of pregnancy
- Ushered in screening tests that identify molecular pathology of aneuploidies
  - Non-invasive prenatal testing (NIPT)

cfDNA Aneuploidy Screening Tests (US)

Company	Location	Product	Method
Sequenom	San Diego, CA	MaterniT21™ Plus	MPSS
Illumina	Redwood City, CA	Verifi® Prenatal Test	MPSS
Quest Diagnostics	San Juan Capistrano, CA	QNatal Advanced	MPSS
Natera	San Carlos, CA	Panorama™	Single nucleotide polymorphism sequencing
Ariosa Diagnostics	San Jose, CA	Harmony™ Prenatal Test	Targeted sequencing

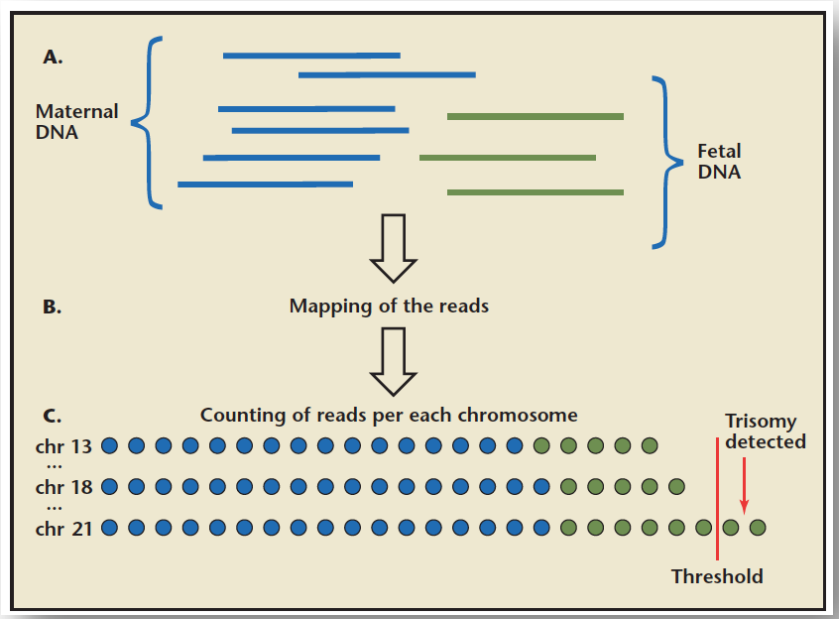
MPSS: Massively parallel shotgun sequencing

- Methods may differ but goal is the same
  - Identify extra copies of a specific chromosome

Massively Parallel Shotgun Sequencing

(Sequenom, Illumina, Quest)

- 1st 25-36 bases of random DNA fragments sequence
- Sequences mapped to a specific chromosome
- Number of sequences are counted and compared to a reference counts from a normal genome
- Excess (or deficiency) number of counts for a specific chromosome indicates aneuploidy
- No distinction between maternal and fetal sequences

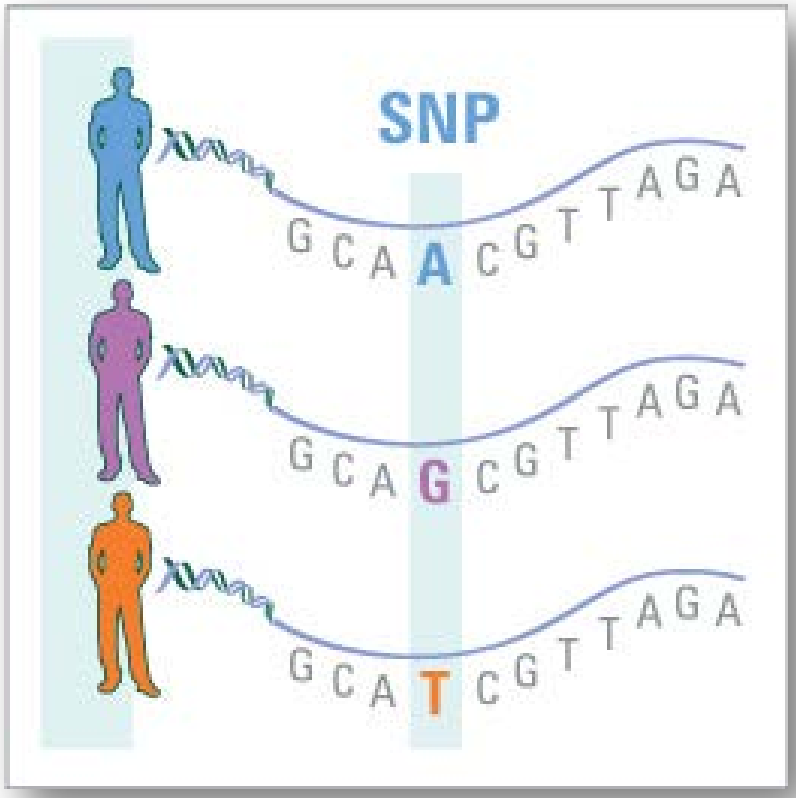


Norwtiz ER, et al. *Rev Obstet Gynecol* 2013;6:48-62

Single Nucleotide Polymorphism (SNP) Sequencing

(Natera)

- Selective sequencing of 13,392 SNPs on chromosomes 13, 18, 21, X, and Y
  - Determines number and identity of each allele
- Algorithm incorporates maternal genotype and recombination frequencies to construct billions of theoretical fetal genotypes
- Probability of each fetal genotype determined and a risk score determined for each chromosome evaluated



http://www.broadinstitute.org

Targeted Sequencing

(Ariosa)

- Counting method similar to MPSS
- Limits mapping of sequences to chromosomes of clinical interest
  - Reduces number of sequences needed (and associated costs)
- Detection rates highest for trisomy 21

Clinical Performance of cfDNA Aneuploidy Screening Tests

Company	Product	Detection rate (%) / False-positive rate (%)		
		T21	T18	T13
Sequenome	MaterniT21™ Plus	99.1	>99.9	91.7
		0.2	0.3	0.9
Illumina, Inc.	Verifi® Prenatal Test	>99.9	97.3	87.5
		0.2	0.4	0.1
Ariosa Diagnostics, Inc.	Harmony™ Prenatal Test	>99	98	80
		0.1	0.1	0.05
Natera, Inc.	Panorama™	>99	>99	>99
		0	<0.1	0

AJOG 2012;206:322.e1-5
Genet Med 2012;14:296-305
Obstet Gynecol 2012;119:890-901
Prenat Diagn 2013;33:591-597
Ultrasound Obstet Gynecol 2013;207:1.e1-6
Prenat Diagn 2013;33:575-579
Prenat Diagn 2013;33:643-649

Pre-test Risk and Post-test Positive Predictive Values Are Important

- Positive predictive value (PPV)
  - PPV = True positives / True positives + False positives
  - What is the probability of an affected fetus given a positive result?

Age (y)	Pre-test risk (1 in x)	Post-test positive predictive value (%)		
		T21	T18	T13
25	1,000	55	39	32
30	730	64	48	40
35	280	81	68	61
40	65	95	90	87

Wax JR, et al. *Am J Obstet Gynecol* 2015;212:548-549

- Compare to the Combined (biochemical) test for T21
  - Sensitivity 93%; False-positive rate 5%
  - At prevalence of 1:280 the PPV is only 6%

High-risk vs. Low-risk Women

- Most professional practice guidelines recommend cfDNA aneuploidy screening tests for “high risk” pregnancies

Box 1. Indications for Considering the Use of Cell Free Fetal DNA ⇦

- Maternal age 35 years or older at delivery
- Fetal ultrasonographic findings indicating an increased risk of aneuploidy
- History of a prior pregnancy with a trisomy
- Positive test result for aneuploidy, including first trimester, sequential, or integrated screen, or a quadruple screen.
- Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.

ACOG. *Obstet Gynecol* 2012;120:1542-1534

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The following protocol options are currently considered appropriate:

(1) cfDNA screening as a primary test offered to all pregnant women (Completed weeks (e.g. 10 = 10 weeks 0 days to 10 weeks 6 days)).

Benn P, et al. *Prenat Diag* 2015;35:1-10

ACOG
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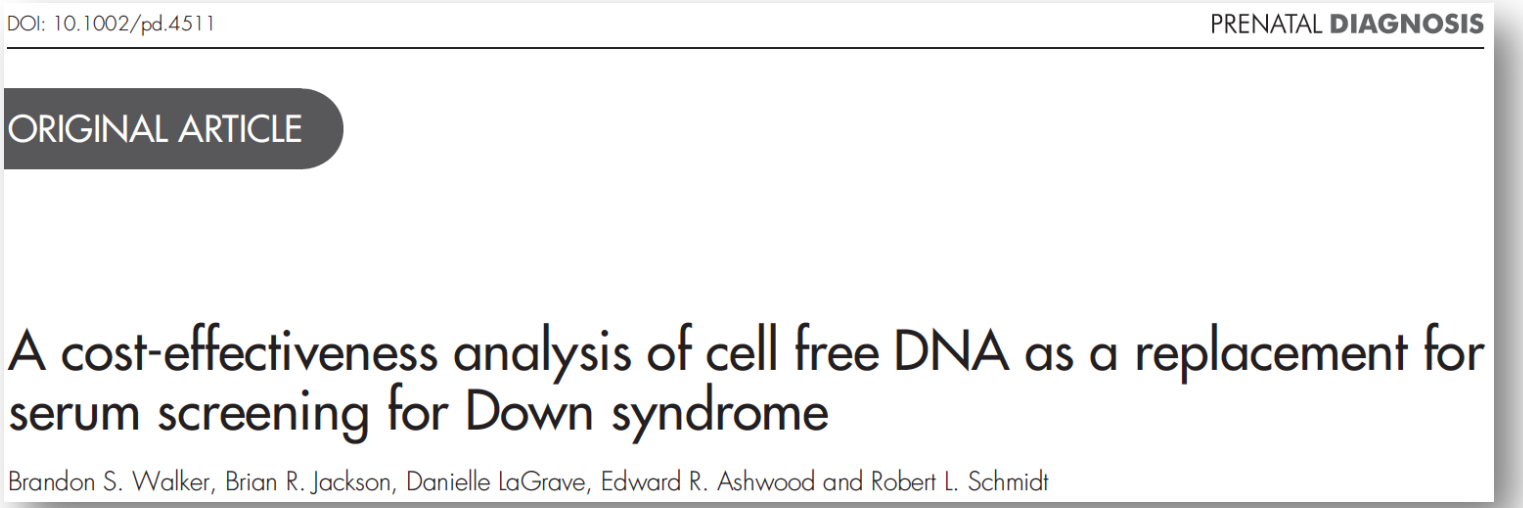
“Although any patient may choose cfDNA screening…conventional screening methods remain the most appropriate choice for first-line screening for most women in the general obstetric population.”

ACOG. *Obstet Gynecol* 2015; published ahead of print (doi: 10.1097/AOG.0000000000001007)

Cost Effectiveness of cfDNA Aneuploidy Screening Tests

- cfDNA aneuploidy screening costs more than conventional screening ($>1,000 vs. $<200)
- cfDNA is more accurate than conventional tests



DOI: 10.1002/pd.4511

PRENATAL **DIAGNOSIS**

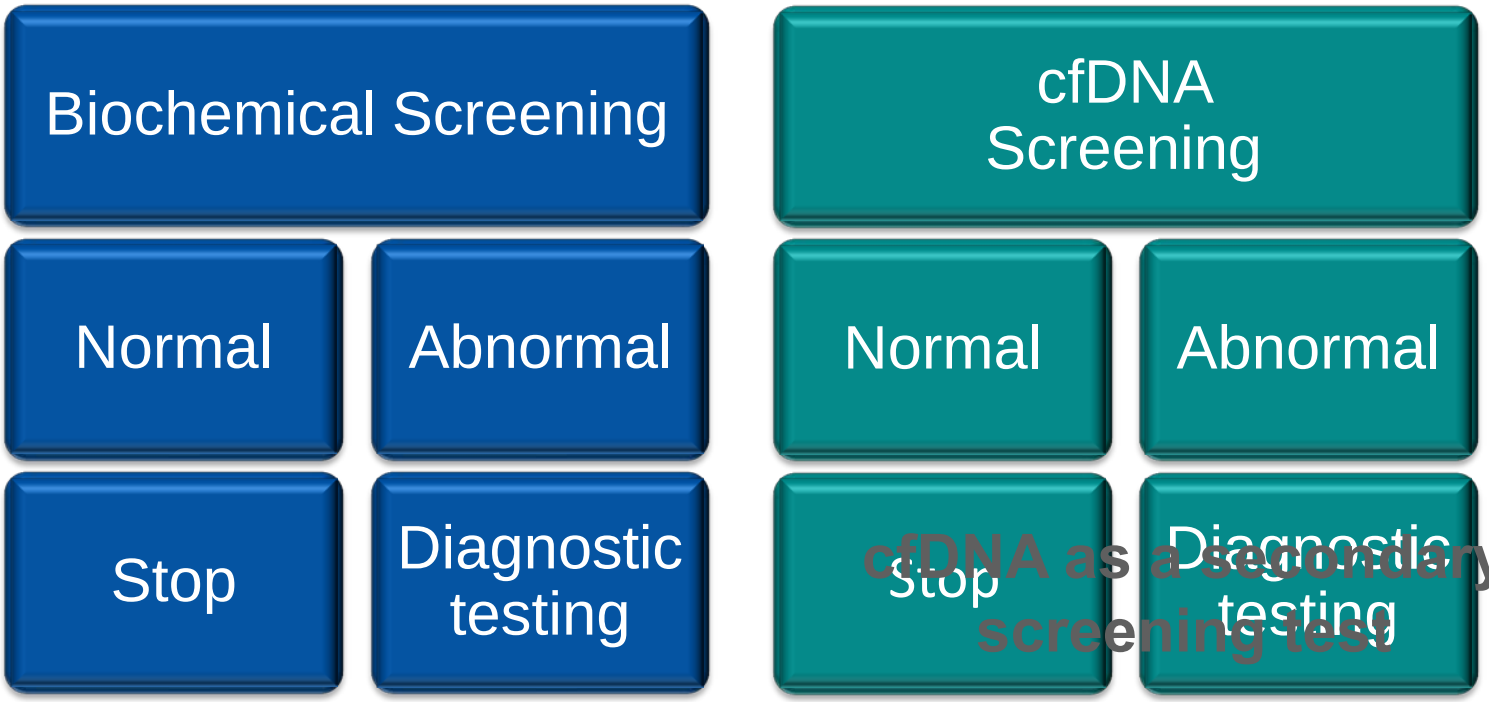
ORIGINAL ARTICLE

A cost-effectiveness analysis of cell free DNA as a replacement for serum screening for Down syndrome

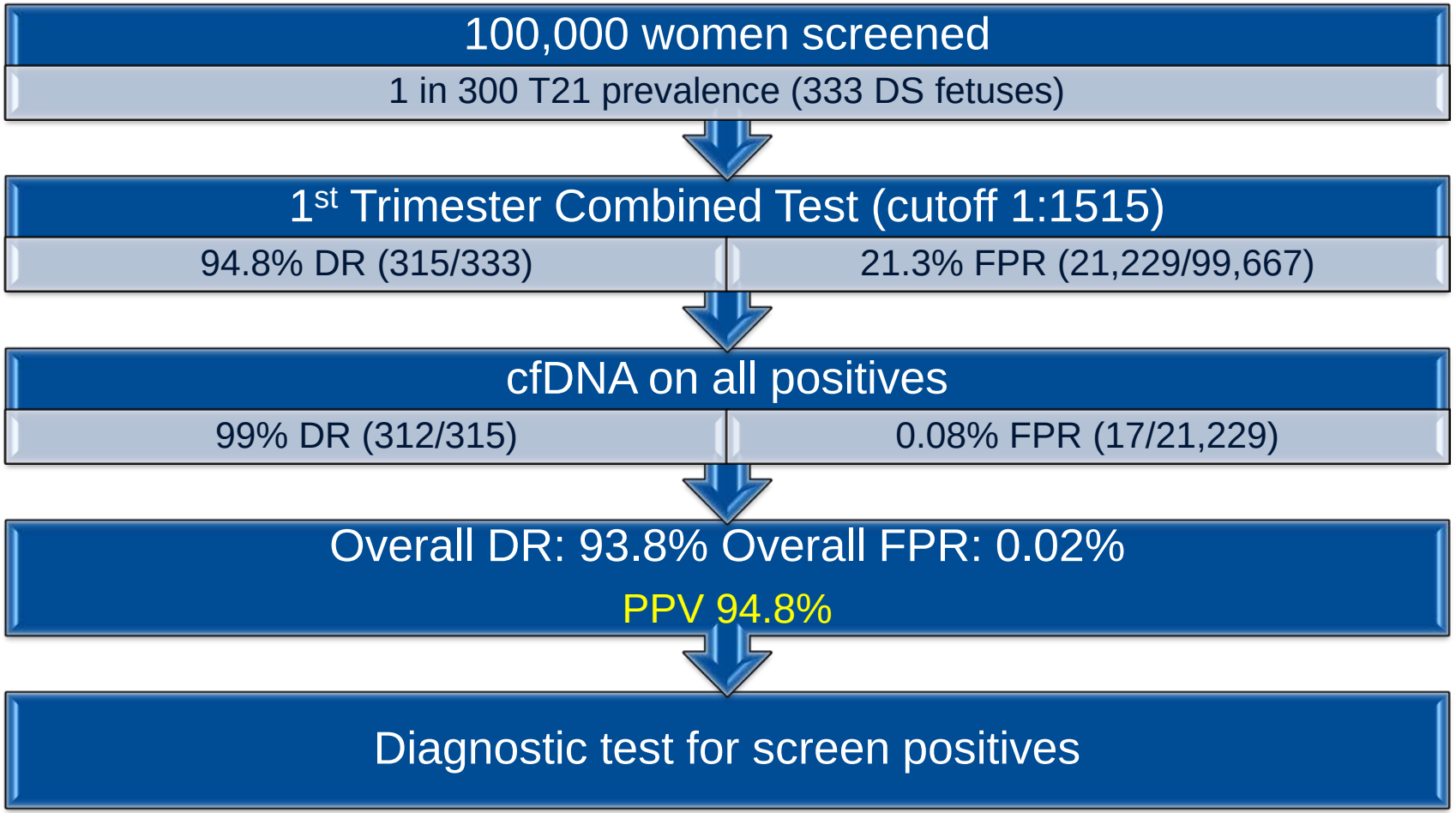
Brandon S. Walker, Brian R. Jackson, Danielle LaGrave, Edward R. Ashwood and Robert L. Schmidt

- cfDNA screening is cost effective to society at <$550/test
- Cost-effective to payers at <$217/test

Contingent Screening



Contingent Screening



Walker BS, et al. *PLoS* 2012;10:e0131402

The “No result” Problem

- Test fails to produce a result in 1-8% of samples due to low fetal fraction of DNA (e.g. obese patients, fetus with aneuploidy)
- Some studies fail to account for these “no calls” when reporting detection rates
- Repeat testing successful in ~50% of women
- A low fetal DNA fraction increases risk of having affected fetus
- Women should receive genetic counseling and be offered diagnostic testing (ACOG)

Misperceptions of cfDNA Aneuploidy Screening Tests

- cfDNA outperforms conventional biochemical screening tests
- Better performance has created the *perception* that cfDNA screening tests produce conclusive results. Not true!

The Boston Globe

By Beth Daley | NEW ENGLAND CENTER FOR INVESTIGATIVE REPORTING DECEMBER 14, 2014

Oversold prenatal tests spur some to choose abortions

- Abnormal cfDNA screening tests should be followed by invasive diagnostic testing (e.g. fetal karyotype, FISH, microarray)

Summary

- cfDNA screening tests identify the molecular pathology of aneuploidies whereas conventional biochemical tests rely on the determination of a biochemical phenotype

- cfDNA screening tests have high aneuploidy detection rates (highly sensitive) and very low false-positive rates (highly specific)

- An abnormal positive cfDNA screening test result must be interpreted with an understanding of the positive predictive value

- Contingent aneuploidy screening is a logical protocol to be followed until cfDNA screening as a primary test becomes cost-effective

ARUP LABORATORIES

UNIVERSITY OF UTAH
SCHOOL OF MEDICINE

Department of Pathology

Multiple Gestations

- All fetuses contribute cfDNA to maternal blood
- No ability to distinguish a differential risk between multiple fetuses
- Very limited data on cfDNA aneuploidy screening test performance in twin gestations and no data on higher-order multiples
- cfDNA screening tests are not recommended for women with multiple gestations